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Chapter I

MENDELIAN INHERITANCE

Mendel's laws. Laws of heredity for monogenic expression. Autosomal inheritance.

Gonosomal inheritance.

Mendel's laws

Single-gene inheritance refers to those disorders that are due to the inheritance of a single mutant gene/one pair of mutant alleles.

Mendel made the first descriptions of unifactorial inheritance in 1865, when he published the results of his experiments conducted on pea plants. His work was largely ignored until Bateson republished it in 1901, from which time the term Mendelian inheritance became synonymous with single-gene inheritance or monogenic inheritance.

In 1865 Mendel published the results of his experiments on the garden pea, in which he crossed pure lines differing in one or two clear characteristics and followed the progeny of the crosses for two or three generations. He proposed ' hereditary factors' (genes) were inherited from parents. From those experiments he described the laws of heredity. They may be stated as follows:

1. Genes come in pairs (Mendel termed them factors), one inherited from each parent (the word “factor” used by Mendel is equivalent today with the term *gene*). Mendel stated that blending of the characteristics of the parents did not occur, but, although they might not be expressed in the first offspring generation, the parental traits could reappear unchanged in a later generation. Moreover, individual genes can have different alleles, some of which - dominant traits - exert their effects over other - recessive traits. In Mendel's own words, those characters which are transmitted entirely, unchanged in the hybridization are termed *dominant*, and those which become latent in the process (in phenotype), are termed *recessive*. An individual who has different alleles (one dominant and one recessive) is heterozygous (a heterozygote trait), but when an individual has a pair of identical alleles, she/he is said to be homozygous (a homozygote).

2. Law of Segregation: Each individual has two alleles for each gene. At meiosis, alleles segregate from each other during gamete formation, with each gamete receiving only one allele. The two members of a single gene pair (alleles) always segregate and pass to different gametes. Example: Aa → gametes A or a (50% each).

When each parent is heterozygote in a pair of alleles, the genotype in offspring will be in the following proportions: 1/4 homozygote for dominant gene, 1/2 heterozygote with both genes dominant and recessive, and 1/4 homozygote for recessive genes. In this last case, the recessive trait will be expressed in the phenotype.

Clinical relevance: Explains carriers having affected children.

3. Law of independent assortment: different pairs of alleles assort independently. Inheritance of one gene doesn’t affect another. In other words, there is random recombination of parental and maternal

chromosomes in the gametes; exception to this rule takes place to genes closely linked on the same chromosome (genetic linkage), which tend to remain together when passed from generation to generation.

Patterns of single - gene inheritance

Single-gene traits - often called *Mendelian traits* - segregate within families and, on the average, occur in fixed proportions among the offspring of specific types of matting. The single-gene phenotypes described so far are listed containing more than 8000 loci. A single-gene disorder is one that is determined by a specific allele at a single locus on one or both members of a chromosome pair.

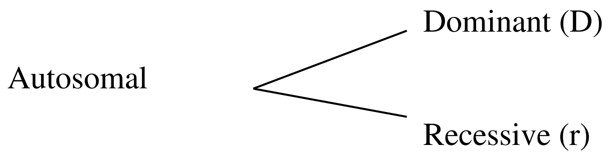
Single-gene disorders are characterized by their patterns of transmission in families. To establish the pattern of transmission, a usual first step is to obtain information about the family history of the patient and to summarize the details in the form of a <u>pedigree</u>, using standard symbols.

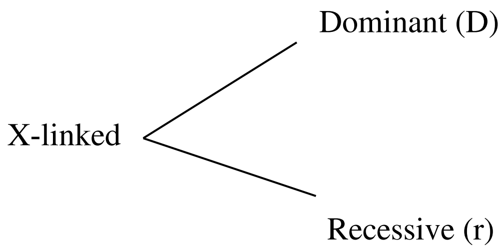
The patterns shown by single-gene disorders in pedigrees depend on two factors:

1. The chromosomal location of the gene locus, which may be <u>autosomal</u> (located on an autosome), or <u>X-linked</u> (located on the X chromosome).

2. Whether the phenotype is <u>dominant</u> (expressed even when only one chromosome of a pair carries a variant allele - heterozygote state), or <u>recessive</u> (expressed only when both chromosomes of a pair carry a variant allele –homozygote state).

Thus there are four basic patterns of a single-gene inheritance:





The other types are Y-linked (holandric) and mitochondrial (maternal).

Fundamental to the understanding Mendelian inheritance are the concepts of *dominant and recessive*. Dominance is not an intrinsic property of a particular allele, but describes the relationships between it and the corresponding allele on the homologous chromosome. By formal definition, a phenotype expressed in the some way in both homozygotes and heterozygotes is *dominant*, and a phenotype expressed only in homozygotes is *recessive*.

Autosomal dominant disorders are typically more severe in homozygotes than in heterozygotes. If expression of each allele can be detected even in the presence of the other, the two alleles are termed *codominant*. Although a recessive phenotype is defined as being clinically undetectable in heterozygotes, many traits classified as recessive do have heterozygous manifestations when examined at the cellular, biochemical, or molecular level.

Autosomal inheritance

Patterns of autosomal dominant inheritance

Among many known Mendelian phenotypes, more than half are autosomal dominant (AD). The incidence of some AD disorders is higher in specific geographic areas than in others. Although many autosomal dominant disorders are much less common, they are so numerous that their total incidence is appreciable. The burden of AD disorders is further increased because of their hereditary nature; they may be transmitted through families and become problems not only for affected persons but also for the entire family.

In classical AD inheritance, every affected person in a pedigree has an affected parent, who also has an affected parent and so on.

Since individuals with AD disorders are heterozygous for a mutant and a wild-type gene, there is a 1 in 2 (50%) chance a gamete will carry the normal allele and a 1 in 2 (50%) chance a gamete will carry the mutant allele. Assuming that the individual ′s partner will contribute a wild-type allele, there is a 1 in 2 (50%) chance that the offspring will inherit the disorder with each pregnancy.

When one parent is heterozygous for a dominant mutation and the other parent is homozygous for the wild-type allele, parents' genotypes can be written as: A / a + a / a

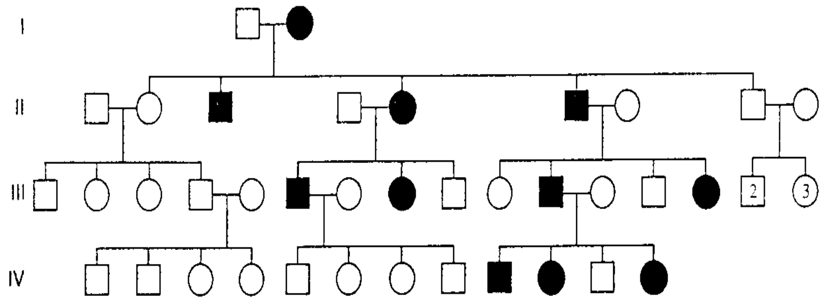
"A" is dominant gene for abnormal phenotype; "a" is wild-type gene. The genotypes of their children can be written as below:

		Healthy parent	
		a	**a**
Affected parent	**A**	**A/a** ***affected***	**A/a** ***affected***
	a	**a/a** ***healthy***	**a/a** ***healthy***

Each child of these parents has a 50 percent chance of receiving the affected parent′s abnormal allele A and thus being affected (A/a), and a 50 percent chance of receiving the wild-type allele ***a*** and thus being unaffected (a/a) (the unaffected parent can transmit only a wild-type ***a*** allele to each child).

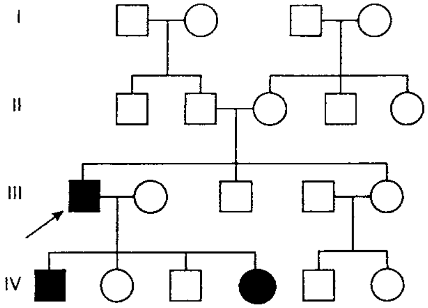
Statistically speaking, each pregnancy is an "independent event", not governed by the outcome of previous pregnancies; thus within a family the distribution of affected and unaffected children may be quite different from the theoretical 1:1, although in the population as a whole the offspring of A/a + a/a *parents* are approximately 50 percent A/a and 50 percent a/a.

One of the AD disorders is *Huntington disease*, characterized by choreic movements and progressive dementia. The onset age of Huntington disease is almost later, by the age 36-40 years. Huntington disease is one of the first diseases in which molecular genetic methods led to the discovery of a DNA marker closely linked to the gene, which in suitable families allows presymptomatic and even prenatal diagnosis of individuals at risk.



Pedigree of a family with Huntington disease, an autosomal dominant disorder with relatively late age of onset.

Much more typical of the kind of AD pedigree that comes to clinical attention is *neurofibromatosis*. It is a common disorder of the nervous system. Since the gene is said to be clinically expressed in everyone who carries it, there is a great variability in its expression and moreover, some individuals with pathological gene are severely affected, whereas others show only mild symptoms; it is appropriate example for reduced penetrance and variable expressivity.



Pedigree of family with neurofibromatosis type1, apparently originating as a new mutation in the proband (arrow).

In medical practice, homozygosity for AD genes is not often seen. Clinically, usually, homozygotes are much more severely affected. For example, patients with homozygous form of achondroplasia are much more severely affected than heterozygotes. Achondroplasia is a skeletal disorder, a short - limbed dwarfism and large head size (normal size); most patients have normal intelligence and live normal lives within their physical capabilities.

Approximately half the cases of neurofibromatosis result from **new mutations**. If the patient has inherited the defect, the risk that any of his or her sibs will also inherit it is 50%, but if the child has a new mutant gene, there is almost no risk that any sib will be affected. The disorder can be detected presymptomatically and even prenatal by molecular analysis.

Another example is *familial hypercholesterolemia (FH)*, an AD disorder of receptors for plasma low-density lipoprotein (over 2,000 mutations identified in LDLR gene alone) leading to premature atherosclerosis and cardiovascular disease, in which the rare homozygous patients have a much more severe disease, than do the relatively common heterozygotes (prevalence of heterozygous FH: 1 in 200–250 individuals and of homozygous FH: 1 in 160,000–300,000). It is the most common monogenic disorder affecting lipid metabolism. The LDLR gene is located on chromosome 19p13.2 and encodes low-density lipoprotein receptor (LDLR), mediates endocytosis of LDL particles in hepatocytes. Heterozygotes have elevated LDL-C (190–400 mg/dL). Clinically: xanthomas (tendinous, tuberous), corneal arcus and premature coronary artery disease (30s–40s).

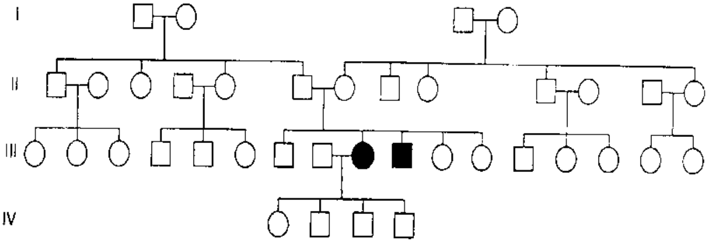
Multiple xanthomas (https://www.wikilectures.eu/w/Xanthoma)

Family Cascade Screening:

- Identify relatives of affected individuals
- Measure LDL-C and perform genetic testing
- Essential for early detection and prevention

Patterns of autosomal recessive inheritance

Autosomal recessive phenotypes represent about one third of the recognized Mendelian disorders. In contrast to AD disorders, in which affected persons are usually heterozygous, autosomal recessive disorders are expressed only in homozygotes, who thus must have inherited a mutant allele from each parent (both parents are heterozygotes=carriers).



Typical pedigree showing autosomal recessive inheritance.

The mutant recessive allele is symbolized ⇒ r

The normal allele ⇒ R

The risk to the offspring in each case listed below:

Parents	***Risk to offspring***
carrier + carrier R/r + R/r	¼ R/R, ½ R/r, ¼ r/r ¾ unaffected, ¼ affected
carrier + affected R/r + r/r	½ R/r, ½ r/r ½ unaffected, ½ affected
affected + affected r/r + r/r	r/r only all affected

The characteristics of an autosomal recessive inherited disorders are:

- both males and females are affected
- the disorder normally occurs in only one generation, usually within a single sibship
- the percent of affected persons along 2-3 generations does not go over 25%
- the parents often are consanguineous

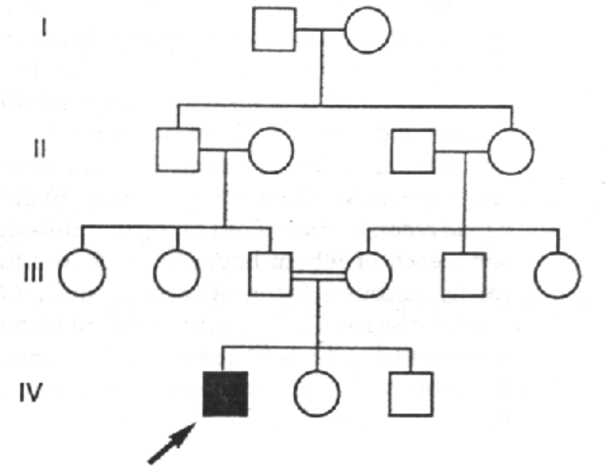
A *consanguineous* couple occurs when the members of the couple have at least one ancestor in common in the precedent few generations. This means that they are more likely to carry identical alleles, inherited from this common ancestor and could both transmit the identical allele to their offspring, who would then be homozygous for that specific allele. A consanguineous couple has an increased risk that their offspring will be affected with a recessive disorder.

Recurrence risk. When two parents carrying the same disease allele reproduce, there is an equal chance that gametes contain the abnormal allele.

Because the increased risk of birth defects in their offspring is well known, consanguineous couples request genetic counseling before they will have children.

In a mating of an affected homozygote with a heterozygote (r / r + R/r), the offspring each have a 50 percent chance of being affected, just like in AD inheritance, showing a quasidominant or pseudodominant pattern.

Most of molecular diseases are autosomal recessive.

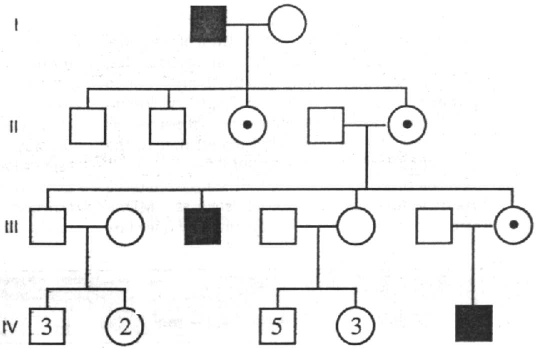


Sex -linked inheritance

Sex-linked inheritance refers to the inheritance patterns shown by genes on the sex chromosomes. If the gene is located on the X-chromosome, it is said to show X-linked inheritance and, if on the Y chromosome, Y-linked or holandric inheritance.

Patterns of X-linked recessive inheritance

This form of inheritance is conventionally referred to as sex-linked inheritance, namely to disorders due to recessive genes on the X chromosome. Males have a single X-chromosome and are therefore, hemizygotes for most of the alleles on the X chromosome; if they have a mutant allele, they will manifest the disorder. Females, on the other hand, while usually only manifest the disorder if they are homozygous for the disease allele and if heterozygous will be unaffected. Since it is rare for females to be homozygous for a mutant allele, X-linked recessive disorders usually only affect males.



Pedigree pattern of an X-linked recessive disorder

The characteristics of X-linked recessive inherited disorders are:

- Males are affected almost exclusively
- Transmission occurs through unaffected or carrier females to their sons
- Male - to - male transmission is never seen
- Affected males are at risk of transmitting the disorder to their grandsons through their obligate carrier daughters

A carrier female has one X chromosome with disease allele and one X chromosome with normal allele; therefore, her sons have 1 in 2 (50%) chance of being affected, while her daughters have an 1 in 2 (50%) chance of being carriers (as their mother).

If a woman would be a carrier and her husband would be affected, they could have a homozygous daughter, affected with an X-linked recessive disorder (e.g. color blindness),

A classic X-linked recessive disorder is *Hemophilia A* in which the blood fails to clot normally because of a deficiency of factor VIII, an anti-hemophilic protein.

If a carrier female mates with healthy male, there are four possible genotypes in their progeny:

$\mathbf{X^H} \quad \mathbf{X^h}$

$\mathbf{X^H} \quad \mathbf{X^H X^H} \quad \mathbf{X^H X^h}$
Daughters: ½ normal, ½ carriers

$\mathbf{Y} \quad \mathbf{X^H Y} \quad \mathbf{X^h Y}$
Sons: ½ healthy, ½ affected

(X^h- the mutant allele and X^H- the wild-type allele).

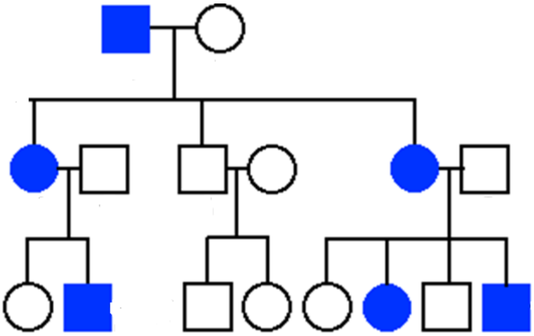
Another X-linked recessive disorder transmitted by mother (carrier females), which affects only boys (her sons, 50%) is *Duchenne muscular dystrophy*, a muscle disease. The disorder is usually apparent by the time the child begins to walk and progresses rapidly.

Patterns of X - linked dominant inheritance

An X-linked phenotype is described as dominant if is regularly expressed in heterozygotes. The distinguishing feature of an X - linked dominant pedigree is that all the daughters and none of the sons of affected males are affected; if any daughter is unaffected or any son is affected, the inheritance must be autosomal, not X – linked.

The pattern of inheritance through females is no different from the AD pattern; because females have a pair of X chromosomes just as they have pairs of autosomes, each child of an affected female has a 50% chance of inheriting the trait, regardless of sex. As a general rule, rare X - linked dominant phenotypes are about twice as common in females as in males, although the expression is usually much milder in females, who are almost always heterozygotes.

Only a few genetic disorders are X-linked dominant. Example: *X - linked hypophosphatemic rickets* (vitamin - D resistant rickets), in which the ability of the kidney tubules to reabsorb filtered phosphate is impaired. Some of the rare genetic defects expressed exclusively or almost exclusively in females appear to be X - linked dominant conditions that are lethal in males before birth. Typical pedigrees of these conditions show transmission by affected females, who produce affected daughters, healthy daughters and normal sons in equal proportions. An example is *Rett syndrome*. The disorder is lethal in hemizigous males, and occurs exclusively in females.



Pedigree pattern of X-linked dominant inheritance

Criteria for X - linked dominant inheritance:

1. Affected males with healthy mates have no affected sons and no healthy daughters.
2. Both male and female offspring of carrier have a 50% risk of inheriting the phenotype. This is the same as the AD pedigree pattern.
3. For rare phenotypes, affected females are about twice as common as affected males, but affected females typically have milder expression of the phenotype.

Y - linked (holandric) inheritance

It refers to genes carried on the Y chromosome. They will be present only in males and the disorder would be passed on only to all their sons. Although a number of traits, such as hairy ears, have been postulated to be inherited in this manner, there is no good evidence for this type of inheritance being associated with disease in humans. Genes involved in spermatogenesis have been also mapped to the Y chromosome; a male with a mutation in a Y-linked gene involved in spermatogenesis will be infertile.

Conclusions:

- *Mendel's laws underpin genetic inheritance (single-gene inheritance)*
- *Recognizing inheritance patterns aids diagnosis*
- *Recurrence risk quantification is vital*
- *Apply principles in counseling and screening*

Chapter II

MECHANISMS OF GENE EXPRESSION IN SINGLE-GENE INHERITANCE. NON-CLASSICAL PATTERNS OF INHERITANCE

Mechanisms of gene expression in single-gene inheritance

Many genetic conditions segregate sharply within families; that is, the abnormal phenotype can be distinguished clearly from the normal one. In clinical experience, however, some disorders are not expressed at all in genetically predisposed persons, and others have extremely variable expressions in terms of clinically severity onset age, or both. Expression of an abnormal genotype may be modified by other genetic loci, by other alleles, or by environmental factors. These differences in expression, which are most particularly characteristic, of autosomal dominant disorders (but also can be seen in recessive disorders), often can lead to difficulties in diagnosis and pedigree interpretation.

From these reasons, we discuss here about:

- penetrance
- expressivity

- onset age
- pleiotropy
- sex influence (the effect of sex on phenotypic expression)
- genetic heterogeneity
- environmental factors

Penetrance and expressivity are characteristics of gene expression with distinct meanings.

Penetrance

Definition: The proportion of individuals with a specific genotype who express the expected phenotype (the probability that a gene will have a phenotypic expression). In other words, the penetrance of a disorder is in an index of the proportion of individuals with an allele who has manifestations of it. An allele is said to be penetrant in an individual known to be heterozygous for the allele, show signs of the disorder, either by clinical symptoms or pedigree analysis and molecular investigation.

When the frequency of expression of a phenotype is below 100 percent - that is, when some of those who have the appropriate genotype completely fail to express it - the gene is said to show **reduced penetrance**. In statistical terms, it is the percentage of people with a particular genotype who are actually affected.

- Complete penetrance: All individuals with the mutation show the phenotype
- Incomplete (reduced) penetrance: Some individuals with the mutation do not express the phenotype

It is influenced by genetic background, environment, and stochastic factors

An example of an autosomal dominant malformation is a type of *ectrodactily* variously knows as split - hand deformity. Review of the literature on split hand deformity suggests that the disorder has about 70 percent penetrance (i.e., only 70 percent of the people who have the gene exhibit the defect). Another example: BRCA1 mutations – not all carriers develop breast cancer.

Expressivity

Definition: Variation in the severity or nature of phenotype among individuals with the same genotype. When the manifestation of a phenotype differs in people who have the same genotype, the phenotype is said to have *variable expressivity*. Multiple phenotypic effects characterize many single-gene disorders, and patients with one of these disorders may differ with respect both to the spectrum of abnormalities present and to the severity of any one manifestation. Can involve differences in age of onset, organ involvement or clinical severity. The examples of autosomal dominant disorders with variable expressivity are: *neurofibromatosis,* Marfan *syndrome,* and many others.

Neurofibromatosis (NF) is a common autosomal dominant disorder of the nervous system, with a complete penetrance and a great variability in its expression. The clinical features consist of “café au lait” spots, peripheral dermal neurofibromas and Lisch nodules; in addition the patients can have several minor disease features depending on the expression of the gene. The severity ranges from mild café-au-lait spots to multiple neurofibromas. Approximately half of cases result from new mutation, their parents being all unaffected. The mutation is proposed to be at a tumor suppressor gene.

Marfan syndrome is a disorder of fibrous connective tissue in which there are characteristic abnormalities of several systems (skeletal, ocular, cardiovascular). Not only is there a wide range of clinical severity in this condition, but individual patients may show no clinical abnormality in at least one of the three systems that are typically affected. Therefore, the diagnosis of this disorder may be complicated by variable expressively.

Onset age

Definition: The age at which symptoms of a genetic disorder first appear.

Not all-genetic disorders are present at birth; many are not expressed until later in life, some at a characteristic age and others at variable ages. Early vs. late onset may influence diagnosis and management. A classic example of a genetic disorder usually of a late onset is ***Huntington disease***, characterized by choreic movements, progressive dementia and onset typically in 5th decade (late onset). In this disorder, the penetrance is age dependent and it is said to show *delayed penetrance.*

Pleiotropy

refers to the phenomenon in which a single gene is responsible for a number of distinct and seemingly unrelated phenotypic effects. For example, the allele causing neurofibromatosis (previously discussed as a dominant autosomal disorder) can produce abnormalities of skin pigmentation, neurofibromas of the peripheral nerves, short stature, skeletal abnormalities and other symptoms. Each one of the pleiotropic effects of an allele can show non-penetrance and variable expressivity.

Pleiotropy can be explained as followed; each gene has only one primary effect; it directs the synthesis of a polypeptide chain. From this primary effect, however, there may be multiple consequences. When a single abnormal gene or gene pair (in a recessive disorder) produces diverse phenotypic effects, its expression is said to be *pleiotropic.* The various characteristics seen in the Marfan syndrome are classical examples of pleiotropy.

Clinical syndromes offer many other examples of pleiotropy, and for most of these, the connection between the various manifestations has not yet been established. In the Marfan syndrome, the underlying defect appears to be in the primary structure of fibrillin, a critical component of connective tissue.

In contrast, in the autosomal recessive disorder known as the *Bardet- Biedl syndrome*, the manifestations of hypogonadism, polydactyly, deafness, obesity, pigmentary retinopathy and mental retardation are together expressed in the phenotype.

Sex influence

Sex influence involves the expression of an autosomal allele that occurs more frequently in one sex than the other. An example in human is *gout*, with males affected more frequently than females, an effect probably mediated by gonosomal alleles and then by hormonal differences.

Another example of an autosomal disorder more common in males, is *hemochromatosis.* In this disorder of iron metabolism, there is enhanced absorption of dietary iron, which leads to iron overload with serious pathological consequences. The lower incidence (one tenth) of the clinical disorder in females is thought to be related to some influence of the hormonal status.

Another example is *congenital adrenal hyperplasia,* an autosomal recessive disease, due to 21-hydroxylase deficiency, in which the abnormalities are recognized at birth (or later) in female infants because they have ambiguous genitalia and sometimes other clinical symptoms, but it may go unrecognized in males; however, without treatment, affected males develop consequences of excessive androgen production.

Environmental factors

Environmental influences can affect the expression of genes. This can involve factors of the internal environment, such as hormones, or the external environment, such as_the effect of certain drugs, diet, and a.o. Environment interacts with genes to modify expression and disease outcome.

Examples:

- Phenylketonuria (PKU): Dietary phenylalanine restriction prevents intellectual disability
- Smoking increases cancer risk in individuals with specific mutations (e.g., TP53)
- Gene-environment interaction is central to multifactorial inheritance
- Important for prevention and personalized treatment strategies

Genetic heterogeneity

Definition: Different genetic mutations cause the same or similar phenotype. Genetic heterogeneity means that any one of several genetic mechanisms can lead to the same or similar phenotype. When a genetic disorder that appears to be a single entity is thoroughly analyzed, it is frequently found to be genetically heterogeneous; that is, it includes a number of phenotypes that are similar but are actually determined by different genotypes (to be seen the table below).

- Types:
  - Allelic heterogeneity: Different mutations in the same gene
  - Locus heterogeneity: Mutations in different genes produce similar phenotypes

An example is *Retinitis pigmentosa*, due to mutations in >60 genes.

Selected single-gene disorders with heterogeneity

Disorder	**Type of heterogeneity**
Cystic fibrosis	**Allelic**
Osteogenesis imperfecta	**allelic and nonallelic**
Hemophilia	**Nonallelic**
Hemoglobinopathies	**allelic and nonallelic**
Glucose-6-phosphate dehydrogenase deficiency	**Allelic**
Duchenne and Becker Muscular dystrophy	**allelic**
Homocystinuria	**allelic and nonallelic**
Mucopolysaccharidoses	**allelic and nonallelic**
Congenital deafness	**allelic and nonallelic**
β - thalassemia	**allelic**
Lysosomal storage diseases (most of them)	**allelic and nonallelic**
Adult polycystic kidney	**allelic and nonallelic**
Alzheimer disease	**allelic and nonallelic**

Heterogeneity may be the phenomenon by which a clinical phenotype results from different genetic defects, representing either mutations at separate locus (*nonallelic heterogeneity=locus level*) or different mutations at a single locus (*allelic heterogeneity*). Heterogeneity may be detected by subtle differences in phenotype or documentation of different patterns of inheritance, but confirmation often relies on biochemical, physiological, or molecular genetic analysis.

Clinical implications of genetic heterogeneity:

- Diagnostic complexity: Similar phenotypes may have distinct genetic causes
- Importance of molecular testing and sequencing technologies
- Personalized medicine: Tailored therapies based on genetic variant
- Genetic counseling: Risk assessment for families

In summary, allelic heterogeneity is an important cause of clinical variation, in which mutations of a single gene cause quite distinct phenotypes. Indeed, many loci can contain more than one mutant allele and therefore, at a given locus there may be several or many mutations, resulting in clinically indistinguishable or closely similar disorders. In other cases, different mutant alleles at the same locus result in very different clinical presentations.

In contrast, nonallelic heterogeneity (locus level) is the situation in which mutations at two or more distinct loci (on different chromosomes) can produce the same or closely similar phenotypes. For example, Alzheimer disease is genetically heterogeneous in which, there are several genetic loci on different chromosomes associated with virtually identical clinical presentations. Heterogeneity refers to the locus level (non-allelic heterogeneity), being involved either mutations on chromosome 21, chromosome 14 or on chromosome 19.

Non-classical patterns of inheritance

Non-Mendelian or 'non-classical' inheritance refers to genetic transmission patterns that do not follow Mendel’s laws.

Nonclassic single gene inheritance patterns include trinucleotide repeat mutations (expansion of repeats causing disorders like Fragile X syndrome, Huntington's disease), mitochondrial inheritance (maternally inherited) and gonadal mosaicism (mutation in a small number of the parent's germ cells), genomic imprinting, uniparental disomy (UPD) and anticipation.

These patterns deviate from the classic autosomal and X-linked patterns because they involve mechanisms other than simply inheriting two alleles, one from each parent.

Trinucleotide repeat mutations

The fragile X syndrome is determined by a mutation with an X-linked dominant pattern of inheritance. The fragile X syndrome is the most common but atypical heritable mental retardation, accounting for about one - third to one - half of all X - liked mental retardation.

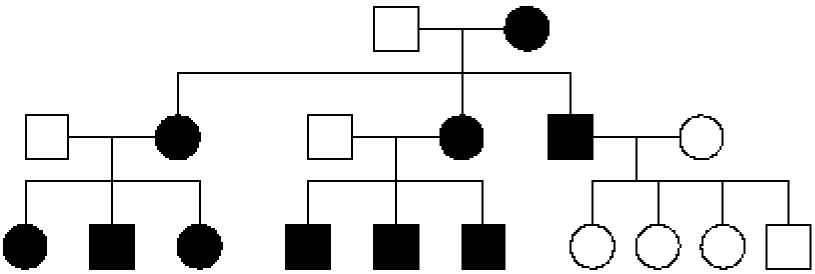
Fragile X syndrome is caused by a mutation in FMR1 gene, in which a nucleotide triplet *CGG* is repeated more than 200 times.

Healthy individuals may have a number of 5 to 40 CGG repeats. Individuals with 55 to 200 CGG repeats are said to have a FMR1 gene premutation. In women, the premutation can expand to more than 200 CGG repeats during oogenesis. Thus, women with the premutation have an increased risk of having an affected descendent, who has the full mutation. By contrast, in men the premutation does not expand during spermatogenesis. Men pass the premutation only to their *daughters*, who will not be affected, but may have affected descendents (daughters receive one X chromosome from their father, the other one from the mother). Their *sons* will not receive the X chromosome, thus, will neither carry the permutation, nor will be affected (the son receives the Y chromosome from the father). The name of the syndrome refers to a cytogenetic marker, a "fragile site" in which the chromatin fails to condense during mitosis, on the X chromosome at Xq27.3.

The fragile X is expressed cytogenetically only in a relatively small proportion of cells: 10% - 40% in most fragile X syndrome at males and in lower percent in fragile X syndrome at females.

Mitochondrial inheritance

It has been recognized for many years that a few pedigrees of inherited diseases cannot be explained by typical Mendelian inheritance of nuclear genes. There is variable expression due to heteroplasmy (mixture of wild-type and mutant mtDNA). A unique feature of these mitochondrial diseases is its *maternal inheritance*, because only mothers transmit mitochondrial DNA to their offspring. Recurrence risk: All children of affected mothers at risk; no transmission by affected fathers.



Pedigree pattern of mitochondrial inheritance

There are few neuromuscular diseases determined by mitochondrial DNA mutations, such as: hereditary optic atrophy, MELAS syndrome.

Mosaicism: Mosaicism for mutations in single genes, in either somatic or germline cells, seems to be a likely explanation for a number of unusual clinical disorders.

Somatic mosaicism is defined as the existence of two genetically distinct cell lines within an individual, derived from a postzygotic mutation. It is a mutation occurring during embryonic development, which may affect morphogenesis, but the patient has healthy parents. These mutations may affect only a part of the body and are not transmitted to the descendents.

Germline mosaicism is represented by a mutation, which has occurred in a germline cell of one of the parents as a new mutation. In this way, healthy parents could have children with a single - gene disorder, such as achondroplasia, osteogenesis imperfecta, Duchenne muscular dystrophy. Germline mosaicism can be seen with any inheritance pattern.

Genomic imprinting:

One assumption of Mendelian inheritance is that the alleles of a given gene from both parents are equally expressed in the offspring, but today, on the basis of molecular investigations, it is known that in some cases the two alleles are expressed in functionally different ways, a phenomenon called as genomic imprinting. In other words, the imprinted allele is inherited in a mendelian manner but its expression is determined by the gender of the transmitting parent. One of the mechanisms involved in this process is a different pattern of methylation of nucleotides in DNA molecules. Differential expression of a gene depends on its parent of origin. It is caused by DNA methylation and epigenetic modification. The imprinted gene → only maternal or paternal copy active. It is the phenomenon whereby a small subset of all the genes in our genome are expressed according to their parent of origin. Some imprinted genes are expressed from a maternally inherited chromosome and silenced on the paternal chromosome; while other imprinted genes show the opposite expression pattern and are only expressed from a paternally inherited chromosome. Contrary to expectation, 'imprints' can act as a silencer or an activator for imprinted genes.

Normally, a healthy child inherits two sets of chromosomes, one from the mother and one from the father. A living child cannot be produced when both sets of chromosomes come from the same parent because imprinted gene expression will be unbalanced. Because of the way imprints work, a fetus that has two maternal sets of chromosomes will have twice the normal level of some imprinted genes, and completely lack expression of other imprinted genes. No naturally occurring cases of parthenogenesis exist in mammals because of imprinted genes.

Recurrence risk: Usually low but depends on imprinting defect or chromosomal abnormality

In a considerable number of genetic disorders, the expression of the disease phenotype depends on whether it has been inherited from the father or from the mother.

Example 1: a **severe early onset** of miotonic dystrophy that occurs when the mutant gene has been inherited maternally, and the relatively early onset **in Huntington** disease when the mutant gene is inherited paternally.

Example 2: increased severity of neurofibromatosis with maternal transmission.

Example 3: Differences in expression that depend on the sex of the transmitting parent has also come from the study of two syndromes: **Prader - Willi syndrome and Angelmam syndrome.**

In many cases there is a microdeletion involving the proximal long arm of chromosome 15 inherited from the patient′s father. Thus, the genomes of these patients have genetic information (corresponding to the gene originated from fathers), only from their mothers. Obesity, short stature, and hypogonadism, small hands and feet and, mental retardation characterize the Prader - Willi syndrome. In contrast, in many patients with the Angelman syndrome, which is phenotypically quite different from Prader - Willi syndrome, there is a deletion of the same chromosomal region on chromosome 15 inherited from the mother. Therefore, the patients have the genetic material only from their fathers. Epilepsy, severe learning difficulties and severe mental retardation characterize Angelman syndrome.

Other conditions, such as Beckwith-Wiedemann syndrome, are associated with abnormalities of imprinted genes on the short arm of chromosome 11.

Uniparental disomy (UPD) occurs when a person receives two copies of a chromosome, or part of a chromosome, from one parent and no copies from the other parent. UPD can occur as a random event during the formation of egg or sperm cells or may happen in early fetal development.

In many cases, UPD likely has no effect on health or development. Because most genes are not imprinted, it doesn’t matter if a person inherits both copies from one parent instead of one copy from each parent. In some cases, however, it does make a difference whether a gene is inherited from a person’s mother or father. A person with UPD may lack any active copies of essential genes that undergo genomic imprinting. This loss of gene function can lead to delayed development, mental retardation, or other medical problems.

Thus, someone who inherits both chromosomes 15 from one parent (called uniparental disomy) has Prader-Willi or Angelman syndrome, depending on which parent they come from.

Prader-Willi syndrome is due to 2 copies of the chromosome 15 being inherited from the mother, and the locus is imprinted, while Angelman syndrome gene is due to 2 copies of the chromosome 15 inherited from the father and the locus is similarly imprinted.

Prader-Willi syndrome is a genetic disorder in which seven genes (or some subset thereof) on chromosome 15 are missing or unexpressed (chromosome 15q partial deletion). Prader-Willi syndrome is characterized by severe hypotonia and feeding difficulties in early infancy, followed in later infancy or early childhood by excessive eating and gradual development of morbid obesity, unless externally controlled. All patients have some degree of mental retardation and distinctive behavioral problems. Hypogonadism is present in both males and females. Short stature is common.

Angelman syndrome is a neurological disorder in which severe learning difficulties are associated with a characteristic facial appearance and behavior.

Anticipation: refers to an earlier onset and increased severity in successive generations. It is caused by expansion of unstable trinucleotide repeats. In affected individuals, these repeats are unstable and prone to errors during cell division. The number of repeats can increase as the gene is passed from parent to child.

A larger number of repeats often results in a more severe form of the disease and an earlier onset of symptoms.

Recurrence risk: Depends on size of expansion; larger expansions leading to higher risk.

Examples:

- Huntington disease (CAG repeat)
- Myotonic dystrophy (CTG repeat)
- Fragile X syndrome (CGG repeat)
- Spinocerebellar ataxia type 1

Chapter III

SINGLE-GENE DISORDERS

Single-gene (monogenic) disorders arise from pathogenic variants in one gene. They affect enzymes, structural proteins, receptors, or transporters. Inheritance can be autosomal dominant, autosomal recessive, or X-linked.

The molecular and biochemical basis of genetic diseases

The molecular basis for a genetic disease is a mutation. Mutations can be either inherited or acquired. Genetic diseases occur when changes in the DNA of essential genes reduce the amount or function (or both) of the gene products to below required levels. Single-gene diseases in which the molecular defect has been identified result from alterations in DNA sequences at control genes or DNA sequences at structural genes that encode the proteins.

The pathogenesis of a genetic disease can be understood completely when the primary biochemical abnormality caused by the mutation is known. A specific protein deficiency has been demonstrated in many single-gene diseases (both autosomal and X-linked) out of more than 8000 presently recognized (https://www.omim.org/).

The study of genetic diseases at its various phenotypic levels (the protein, the cell, the tissue and the whole body) has provided a wealth of important information about specific disorders and has greatly increased understanding of the normal biological processes that are disrupted by mutations.

Pathogenesis of genetic diseases

Archibald E. Garrod established the field of human biochemical genetics. He introduced the term “inborn errors of metabolism” in 1902 to describe lifelong diseases that arise because the genetic deficiency of a specific enzyme causes a block in a normal metabolic pathway.

The classical definition of an inborn error of metabolism is a monogenic disease resulting in deficient activity in a single enzyme in a pathway of intermediary metabolism.

The prognosis of a certain disorder and the management of the affected individual depend on its pathogenesis, among other factors. For example the age of onset, the rapidity of the evolution or the moments of the occurence of complications depend on the pathogenesis of the disease. Molecular genetics has proven to be very helpful.

A mutation can affect the quantity, the quality of a protein or sometimes both. The quantity of a protein is regulated at the levels of transcription and translation. Usually, due to a mutant gene the production of a protein is decreased. The quantity may also be increased. A change in the primary structure of the protein may alter its function. There may be qualitative gain or loss of function mutations. Sometimes a change in the protein′s function may occur.

Based on their function proteins can be classified in three classes:

- proteins that interact with small molecules such as enzymes, receptors, transporters
- proteins that perform regulatory roles
- proteins functioning in complex systems, often associated with other proteins

Some proteins that interact with others to form multimers may be subject to enhancement of a pathologic effect when an allele is mutant. Even if the patient is a heterozygous for a mutation, the defective protein will interact with the normal one encoded by the wild-type gene and will consume the normal protein. The phenotype will be much more severe than would be expected in a heterozygote. This is called the *dominant negative effect.*

Sometimes a *severe* mutation (e.g. one eliminating transcription of the mutant allele) has less effect on the phenotype than has a small, missense mutation that will lead to transcription and translation of an abnormal protein.

Genetic disorders of hemoglobin

Hemoglobin is formed by heme and four globin chains. In adults the combination of two alfa (α) and two beta (β) chains will produce the normal globin.

Hereditary disorders of hemoglobin (Hb) or the **hemoglobinopathies** can be divided into two broad groups:

- Structural variants that alter the globin polypeptide without affecting its rate of synthesis (hemolytic anemia);
- Thalassemias, in which there is decreased synthesis of one or more globin chains, resulting in an imbalance in the amounts of α or β chains.

There are also many mixed forms, in which combined features of the two groups can be found.

Hemoglobinopathies are the most common single-gene disorders. It is estimated that about 7% of the individuals are carriers. Initially they were described in the Mediterranean area and in some regions in Asia and Africa.

Hemolytic anemias

Sickle cell hemoglobin (Hb S) is due to a change in only 1 of the 146 amino acids in β globin chain: a substitution of valine for glutamic acid at the sixth position of the polypeptide. This point mutation, which can be abbreviated as β 6 Glu→Val, results from a single nucleotide change in the codon (point mutation). Homozygosity for this mutation is the cause of sickle cell disease, a serious disorder that in some parts of the world is relatively common (in Mediterranean area, in India, equatorial Africa and other countries).

Sickle cell disease is a severe hemolytic condition characterized by a tendency of the red cells to become grossly abnormal in shape (sickle) under conditions of low oxygen tension. Patients have anemia, failure to thrive, splenomegaly, repeated infections and loss of immune function.

Heterozygotes, who are said to have sickle cell trait, are clinically normal, although their red cells will sickle when subjected to very low oxygen pressure in vitro.

Hemoglobin C is also due to a substitution at sixth position of the β globin chain, the glutamic acid being replaced by lysine (β 6 Glu→Lys). Patients have a hemolytic disorder that is milder than sickle cell anemia.

Lepore hemoglobin. Some patients with moderate to severe β-thalassemia have been found to have an unusual fusion chain (delta-beta) resulting in the formation of Hb Lepore. This variant has arisen by a process of homologous unequal crossing over, which is possible because the two genes are highly contiguous on chromosome 11. Misalignment based on the sequence homology between the delta gene of one chromatid and the beta gene of the corresponding chromatid, occurring during meiosis could happen as an accident. The patients have decreased synthesis of Hb.

Thalassemias. The thalassemias, the most common human single-gene disorders, are a heterogeneous group of diseases of Hb synthesis in which the mutation reduces the level of synthesis of either the α or β globin chain. Two main groups are defined: the *α-thalassemias,* in which α-chain synthesis is reduced or absent, and β-*thalassemias*, in which β-chain synthesis is impaired. Both α- and β-thalassemia have a high frequency in diverse populations.

Genetic disorders of α-globin production affect the formation of both fetal and adult Hb and therefore cause intrauterine as well as postnatal disease. In the absence of α-globin chain, the chains from the β-globin form a tetramer called Hb H, and Hb with gama-globin chain from Hb Bart's. These Hbs are completely ineffective oxygen carriers. The most common forms of α-thalassemia are the result of deletions or duplications due to misaligned homologous pairing

The β-thalassemias share many features with α-thalassemia. Decreased β-globin production causes a hypochromic, microcytic anemia and a damage of the red cell membrane.

As a general rule, individuals with two β-thalassemia alleles (homozygotes) have thalassemia major with severe hemolytic anemia, failure to thrive, hepatosplenomegaly and an expansion of the bone marrow that causes distinctive skeletal changes that are evident, as thalassemic facies.

Carriers of one β-thalassemia allele are said to have thalassemia minor. They have a mild anemia, with hypochromic microcytic red cells.

Many types of mutations affecting β-globin gene, causing β-thalassemia have been identified. Many of the mutations decrease the synthesis of mRNA, such as: promoter mutations, RNA splicing mutations and nonsense or frameshift mutations in the coding regions.

The clinical manifestations of the hemoglobinopathies are very heterogeneous, ranging from mild hypochromic anemia to severe anemia, with the involvement of many organs. Severe forms benefit from stem-cell transplant, which is the preferred treatment. Supportive treatment consists of periodic blood transfusions, combined with iron chelation. More than 90% of patients survive into adulthood.

The inborn errors of amino acid metabolism

The inborn errors of amino acid metabolism are genetic conditions in which an enzyme deficiency typically results in the accumulation of an amino acid (aminoacidopathies).

Phenylketonuria (PKU). Classic PKU, an autosomal recessive disorder of phenylalanine catabolism, results from mutation in phenylalanine hydroxylase, the enzyme that converts phenylalanine to tyrosine. Because patients cannot degrade phenylalanine, it accumulates in body fluids, damaging the developing central nervous system in early childhood and interfering with the function of the mature brain. A small fraction is metabolized producing increased amounts of phenylpyruvic acid and other metabolites, which are excreted in the urine. The neurological damage due to the metabolic block in classic PKU may be largely avoided by dietary that prevents phenylalanine accumulation.

Only four alleles account for the great majority of defects at the phenylalanine hydroxylase (PAH) locus; the most common mutation is the defect in one exon resulting in premature translation (the aberrant mRNA).

When there is some residual phenylalanine hydroxylase activity result a less severe phenotypes = benign hypherphenylalaninemia.

Population screening of newborns for PKU is done widely. PKU is the prototype of genetic diseases for which mass newborn screening is appropriate. A droplet of blood is obtained, dried on filter paper and sent to central laboratories for assay of blood phenylalanine levels.

Homocystinuria results in cystathionine β-synthase deficiency and is an autosomal recessive condition. Patients may be suspected of having homocystinuria on the basis of clinical phenotype, the presence of dislocated lenses in a child, or the occurrence of a thromboembolic phenomenon and mental retardation. Some patients with cystationine β-synthase deficiency respond to vitamin B_6 therapy.

Cystinuria. In classical cystinuria the urinary excretion of lysine, arginine and cystine is greatly increased. Three hereditary patterns of excretion have been described in heterozygotes for classical cystinuria. Homozygote, including heterzygotes between two types, cannot be distinguished.

Hartnup disease is primarily a neutral amino-acid transport defect. Histidine, glutamine and asparagine are also increased in the urine. The disease appears to be inherited as an autosomal recessive pattern. The clinical manifestations of the disease are very variable. The patients may have any combination of cerebellar ataxia, delayed development and severe retardation.

Maple Syrup Urine Disease (MSUD) is a defect in branched-chain α-ketoacid dehydrogenase complex. It leads to accumulation of leucine, isoleucine, valine, and their ketoacids. It manifests as a constellation of clinical symptoms due to the central nervous system, immune system, and skeletal muscle dysfunction. Clinical features: poor feeding, lethargy, seizures, severe CNS defects, maple syrup odor of urine. Diagnosis: elevated branched-chain amino acids; confirmed by gene testing. Treatment: restrict branched-chain amino acids, thiamine supplementation. Maple syrup odor in neonatal urine is diagnostic clue.

Tyrosinemia Type I: Deficiency of fumarylacetoacetate hydrolase causes toxic metabolite accumulation and results in severe liver and kidney dysfunction; risk of hepatocellular carcinoma. Symptoms: failure to thrive, hepatomegaly, Fanconi-like renal syndrome. Diagnosis: succinylacetone in urine; FAH gene variant. Treatment: nitisinone, tyrosine/phenylalanine restriction, liver transplant.

Tyrosinemia Type II: Deficiency of tyrosine aminotransferase. Symptoms: corneal ulcers, palmoplantar hyperkeratosis, mild intellectual disability. Treatment: tyrosine and phenylalanine restriction.

Tyrosinemia Type III: Deficiency of 4-hydroxyphenylpyruvate dioxygenase. Symptoms: intermittent ataxia, seizures, developmental delay. Treatment: tyrosine and phenylalanine restriction.

Defects in purine metabolism

Mutations in the gene that encode enzymes needed for purine metabolism are associated with specific disorders. Clinical manifestations are variable, but often the nervous system is more seriously affected than the other organs.

Lesch-Nyhan syndrome. Between the extremes of phenotype produced by the complete functional deficiency of a protein and mutations that produce no clinical abnormalities, a variety of clinical phenotypes of intermediate severity may be found. Individuals provide a dramatic illustration of such phenotypic variation with deficiency of the X-linked enzyme hypoxanthine guanine phosphoribosyltransferase (HPRT). Patients with no residual HPRT activity have a remarkable phenotype called Lesch-Nyhan syndrome, characterized by movement disorder, spasticity, mental retardation, self-injurious behavior with biting of the buccal mucosa, lips or digits, and uric acid overproduction that causes gout. The neurological abnormalities may result from changes in brain purine levels produced by the disease. Prenatal diagnosis is feasible.

Adenosine deaminase (ADA) deficiency leads to a severe syndrome, severe combined immunodeficiency, with recurrent and often lethal infections during infancy or early childhood. Diagnosis and treatment must be early established. These cases are subject to bone marrow transplant or enzyme replacement.

Lysosomal storage disease

Genetic defects of the hydrolases (hydrolytic enzymes) lead to accumulation the lipids inside the lysosome, resulting in severe cellular dysfunction, eventually cell death.

The gradual accumulation of the substrate is responsible for the one uniform clinical feature of these diseases: their unrelenting progression. In most of these conditions, substrate storage is manifested clinically as an increase in the mass of the affected tissues and organs. When the brain is affected, the picture is one of neurodegeneration.

Another feature of lysosomal storage disease is their clinical and genetic heterogeneity. Different defects in one gene (*allelic heterogeneity*) may cause both severe phenotypes with onset in infancy and disorders with onset in adult life, as well as a range of intermediate presentations.

Alternatively, a single phenotype may result from defects in separately encoded enzymes (*nonallelic heterogeneity*) that act a different step in a catabolic pathway. More than two dozen lysosomal hydrolase deficiencies, all of which are recessive in inheritance have been described.

Tay-Sachs disease is one of a group of heterogeneous lysosomal storage disease, the gangliosidoses that result from the inability to degrade a sphingolipid (ganglioside). Affected infants appear normal until about 3 to 6 months of age but then undergo progressive neurological deterioration.

Niemann-Pick disease results from deficiency of acid sphingomyelinase and thus, accumulation of sphingomyelin and other lipids. This storage disease is also inherited as autosomal recessive traits.

Gaucher disease is a lysosomal glycolipid storage disorder characterized by the accumulation of glucocerebroside. Symptoms often occur in childhood, with growth failure, progressive splenomegaly and pancytopenia.

Fabry disease is an inborn error of glycosphingolipid catabolism. It is caused by deficient or absent activity of alpha-galactosidase. The inheritance is X-linked. Symptoms, typically starting during childhood, include pain in the hands and feet, small dark red spots on the skin (angiokeratomas), decreased sweating, and eye problems. More serious complications like kidney failure, heart failure, and stroke can develop later in life.

Mucopolysaccharidoses

The mucopolysaccharidoses are a heterogeneous group of storage diseases in which mucopolysaccharides accumulate in lysosomes as a result of a deficiency of one of the enzymes required for their degradation.

Examples:

- X-linked recessive *Hunter syndrome,* characterized by breathing problems due to thickened tissue and blocked airways, heart disease, joint and bone abnormalities, hernias, seizures, declining brain function.
- more severe autosomal recessive *Hurler syndrome*. Affected children are mentally retarded, intellectual disability that getting worse over time, have skeletal abnormalities, abnormal spine bones, stiffness, short stature and other abnormalities. Other clinical features: inability to fully open the fingers (claw hand), cloudy corneas, deafness, congenital heart defects.

The difference in the pattern of inheritance of the autosomal Hurler and X-linked Hunter syndromes indicates that they are due to defects in different proteins.

Defects in receptor proteins

Familial Hypercholesterolemia is one of several disorders, characterized by elevation of plasma cholesterol. Familial hypercholesterolemia is inherited as an autosomal dominant trait, due to mutations in the structural gene encoding the low-density lipoprotein receptor (LDL). Patients have clinically significant coronary heart disease, hypercholesterolemia accounting for 5% of the cases. The disease presents earlier and more severely in homozygotes than in heterozygotes, these patients suffering myocardial infarction before the age of 20-30 years, reflecting a greater reduction in the number of LDL receptors and a greater elevation of cholesterol in plasma. Heterozygous individuals have myocardial infarctions before the age of 55 years and have cholesterol levels twice that of normal individuals.

Cholesterol is carried by LDL and is taken into cells by the attachment of the LDL-cholesterol complex to the LDL receptors located on the surface of the cells. In affected patients the number of LDL receptors is reduced, resulting in an increase of the levels of cholesterol in plasma. More than 300 mutations in LDL-receptor gene have been identified as typical loss of function mutations. The position of the mutation within the gene alters the receptor protein differently, depending on which of the LDL-receptor protein functions are impaired. The LDL-receptor gene is located on chromosome 19p and is made up of 18 exons corresponding for a protein consisting of more than 800 amino acids. Mutations that occur in the specific exons alter specific functions of the LDL receptor protein and have been classified into five groups. Each of these classes represents a defect in different stages involved in the synthesis or function of the receptor protein. The excess cholesterol is stored as atherosclerotic plaques in arteries and as xanthomas.

Defects in membrane transport

Cystic fibrosis (CF) is an autosomal recessive disorder and classically described as a triad: chronic obstructive pulmonary disease, exocrine pancreatic insufficiency and elevation of sodium, chloride concentration in sweat and failure to thrive due to malabsorption, but cystic fibrosis can have a highly variable phenotype depending on type of allele mutant. Chronic cough, pulmonary infiltrates, reactive airway disease are frequent symptoms. Neonatal intestinal obstruction due to meconium ileus is particularly suggestive of this disorder.

The mutation involved in this disease is in gene transmembrane conductance regulator (CFTR). The most common mutation is the deletion of three-nucleotide (F508) resulting in a deletion of a phenylalanine residue at position 508. Other mutations have been found in the coding region such as: small deletions, insertions that cause frameshift, nonsense mutations and point mutations that affect RNA processing.

Prenatal diagnosis for fetuses at 8-10 weeks with tissue obtained by chorionic villous biopsy is very useful.

Inborn errors of carbohydrate metabolism

These groups include disaccharides deficiencies, disorders of monosaccharide metabolism, glycogen storage diseases, and gluconeogenic disorders.

Galactosemia is transmitted as an autosomal recessive condition. More than 30 mutations have been identified among galactosemia patients. The manifestations include hypotonia, hepatomegaly, susceptibility to infections, cataracts and physical and mental retardation.

Glycogen storage diseases result from deficiencies of various enzymes in the catabolic pathways of glycogen metabolism, resulting into abnormal storage of glycogen in the body. Their incidence is estimated at 1/ 20000-43000 live births. Over 20 types are known, and many subtypes. These disorders can be divided in two groups: those predominantly affecting the liver and those predominantly affecting muscle tissues. Glycogen storage diseases with liver involvement are classified into types 0, I, III, IV, VI, IX and XI, depending on the affected enzyme. Hypoglycemia and hepatomegaly are hallmarks of disease, but muscular and renal tubular involvement, dyslipidemia and osteopenia can also occur. The usual inheritance pattern is autosomal recessive. Eight types have been described until now. The patients (children) usually survive childhood, but growth is slow and progressively there is severe myopathy, cardiomyopathy and neural involvement during adolescence. Treatment approaches: diet, pharmacological treatment, physical therapy, enzyme replacement therapy (ERT) and organ transplant.

Defects in hemostasis

Hemophilias

Hemophilia A is a classic X-linked recessive disorder in which the blood fails to clot normally because of deficiency of factor VIII.

The mutant factor VIII allele causing hemophilia A is noted X_h, and the normal allele X_H. If a hemophiliac mates with a normal female, all the sons receive their father's Y chromosome and a maternal X and are unaffected, but all the daughters receive the paternal X chromosome with its hemophilia allele and are obligate carriers.

Clinical features of hemophilia: blood clotting disorder characterized by prolonged bleeding time, easy bruising and hemorrhage into joints and muscles. The bleeding is painful and leads to long-term inflammation and deterioration of the joint. There are many different mutations reported; the gene location is on X_{q28}.

Hemizygous males have genotype X_h/Y and are affected, but females with genotype X_HX_h are carriers.

Healthy male	X carrier female
X_H/Y	X_H/X_h

	X_H	X_h
X_H	X_H/X_H	X_H/X_h Daughters: ½ healthy, ½ carriers
Y	X_H/Y	X_h/Y Sons:1/2 healthy, 1/2 affected

Hemophilia B is due to deficiency of factor IX. The hereditary nature and pattern of transmission for both hemophilia A and B have been recognized since ancient times.

Management of hemophilia cases includes control of bleeding episodes, factor VIII or IX replacement and medications, rehabilitation of patients and primary and/or secondary prophylaxis.

Factor X deficiency. The gene for factor X is located on chromosome 13. The deficiency is inherited as an autosomal recessive disorder, although a subtle bleeding tendency occurs in heterozygotes. Patients show abnormal bleeding episodes only in connection with major surgery or trauma.

Genetic disorders of fibrinogen. Congenital abnormalities of fibrinogen are rare and include afibrinogenemia, hypofibrinogenemia and dysfibrinogenemia. Afibrinogenemia corresponds to the homozygous state of an autosomal recessive disorder. The cardinal symptom is a lifelong predisposition to easy bruising, mucosal hemorrhage and prolonged bleeding from acute wounds and trauma.

Von Willebrand disease (vWD) is a very common inherited bleeding disorder, frequently confused with hemophilia A. However, vWD is characterized by mild to moderate bleeding which frequently goes undiagnosed.

Inherited disorders of bilirubin metabolism

Three inherited syndromes associated with a deficiency of bilirubin glucuronidation have been described. A near complete deficiency of enzyme for bilirubin metabolism results in *Crigler-Najjar syndromes types I* and *type II.* A mild reduction of this enzyme result in a common benign disorder, *Gilbert syndrome,* in which mild and fluctuating unconjugated hyperbilirubinemia, is the only important clinical sign. The inheritance pattern of Crigler-Najjar syndrome is autosomal recessive. It is considered that Crigler-Najjar syndrome type II would be a homozygous form of Gilbert syndrome. Crigler-Najjar syndrome is due to a mutation in the UGT1A1 gene, which encodes the enzyme bilirubin uridine diphosphate glucuronosyl transferase (bilirubin-UGT) enzyme needed for glucuronidation.

Disorders of iron

Hemochromatosis is a common genetic disease causing tissue iron overload, frequently associated with the HLA-A3 seroantigen. It is also the most common cause of severe iron overload. It is known as primary, idiopathic, juvenile, familial and genetic hemochromatosis, all of which refer to the same recessively inherited disease, associated with HLA-A3. The most common symptoms are hepatomegaly, hyperpigmentation, diabetes mellitus, weakness, abdominal pain arthralgia, weight loss.

Inherited porphyrias

The inherited porphyrias are a diverse group of inborn errors of metabolism, each resulting from the deficient activity of a specific enzyme in the heme biosynthetic pathway. The porphyrias have been classified as hepatic or erythropoietic.

The clinical manifestations include intermittent porphyria, abdominal pain, neuropathy and photosensitivity, but the picture is variable depending on form of the porphyria.

Examples:

Acute intermittent porphyria has autosomal dominant inheritance. Clinical manifestations appear usually after puberty with neurovisceral and circulatory disturbances. Often clinical manifestations begin with abdominal pain and distention. Other manifestations include nausea, vomiting, hypertension, excess sweating, tachycardia. Steroid hormones are the most important precipitating factors for the expression of the disease in phenotype.

Congenital erythropoetic porphyria is inherited as an autosomal recessive trait. Genetic heterogeneity in this disease accounts in part for the considerable variation in the severity of symptoms. Clinical manifestations include severe skin photosensitivity in early infancy, manifestated by increase blistering of the epidermis on the hands and face or other sun-exposed areas. The skin may be thickened, with areas of hypo and hyperpigmantation. Hypertrichosis of the face and extremities is often prominent.

Erythropoetic protoporphyria is inherited as an autosomal dominant trait, with considerable variation in the phenotypic expression. Clinical manifestations include photosensitivity of sun-exposed areas, which usually begins in childhood or in youth. Usually during spring or summer the skin symptoms may be burning, itching, erythema.

Disorders affecting structural proteins

Duchenne and Becker muscular dystrophies

Duchenne muscular dystrophy is a typical X-linked recessive with clinical manifestations in males; females can be carriers of mutation in dystrophin gene. The localization of gene is to Xp21. Patients may occasionally present at birth as hypotonia, or later as failure to thrive or as delay in learning to walk and exhibit muscle weakness. The earliest manifestations of the disease are inability to run. Almost all affected boys have enlarged calf muscles and such pseudohypertrophy may affect various other muscle groups as well. Up to 70% of cases of this disease are associated with deletions or duplications. The remainder is due to a variety of point mutations.

The great majority of carrier females have no clinical manifestations, but about 8 percent of adult female carriers have significant muscle weakness (this is in accordance with random inactivation of the X chromosome, the normal X chromosome being inactivated in a critical proportion of cells in some female heterozygotes).

Becker muscular dystrophy is also due to mutations in the dystrophin gene, but the alleles produce a phenotype that is milder. A high proportion of cases are inherited from the mothers, and few (about 10 percent) represent new mutations (“de novo”).

Facio-scapulo-humeral muscular dystrophy is inherited in an autosomal dominant fashion, with “sporadic” cases in whom there is no clinical abnormality in either parent. The features include onset in the facial or shoulder girdle muscles with weakness or wasting. Asymmetry of muscle involvement is a common finding. In some patients muscle involvement remains restricted to the facial, scapular and proximal upper limb musculature; in others, anterior tibial and peroneal muscles may be involved subsequently.

Osteogenesis imperfecta: heterogeneous mutations in collagen genes

Osteogenesis imperfecta is a group of inherited disorders of type I collagens that predispose a patient to easy fracturing of bones (even without trauma) and to skeletal deformity. Other clinical features are blue sclera, hearing loss. The clinical heterogeneity can be explained by locus ***nonallelic*** and ***allelic heterogeneity***. More than 50 mutations affecting the synthesis or structure of type I collagen have already been found in-patients with osteogenesis imperfecta. Mutations in COL1A1and COL1A2 genes are responsible for more than 90% of the cases. Mutations in the *CRTAP* and *P3H1* genes account for fewere cases, but usually severe ones. 17 types of OI have been described until now.

Most of the mutations that cause the disease are autosomal dominant, but a few are autosomal recessive. Generally, this disease illustrates the genetic complexities that result when mutations alter structural proteins.

The effect of mutation on protein function: By definition, deleterious mutations cause pathology by changing the function of a protein, either by coding for an abnormality in structure or, less often, by altering a regulatory domain to change the number of normal protein molecules produced.

Loss of function mutations

The most common mutation, in either the coding or the regulatory region, is the loss of function mutation leading to the loss of function of the protein.

Apart from any direct effect on function, a structurally abnormal protein may be unstable, so that its cellular concentration is decreased and its function secondarily reduced. Regardless of the specific reason for the loss of function, the clinical phenotype will be the same, as exemplified by the β-thalassemias.

Gain of function mutations

Mutation can also alter the biochemical phenotype by enhancing the function of a protein, either by increasing its amount or by increasing the ability of each molecule to perform a normal function.

Although mutations that cause gain of function are uncommon, they may provide insight into the regulation of the expression of a gene or the molecular mechanism of a protein's function. Few variants of this class have come to clinical attention; they lead to the acquisition of a new function by the mutant protein. For example, subsets of patients with the autosomal dominant form of von Willebrand disease exemplify gain of function mutations that cause disease.

This condition is the most common inherited bleeding disorder, affecting 1 in 125 individuals, if all allelic variants and all degrees of severity are considered.

Novel property mutations

In a few important diseases, some or all of the pathology results from the acquisition of a novel property due to the change in the amino acid sequence, rather than from loss of normal function.

One of the examples of a mutation that confers new properties on a protein is represented by some variants of ***Alpha 1-antitrypsin (α1-AT) deficiency***, an autosomal recessive condition that leads to chronic obstructive lung disease and cirrhosis of the liver. The α1-AT specified by a gene on chromosome 14, inhibits a wide spectrum of proteases, particularly elastase released from neutrophils in the lower respiratory tract. Substantial genetic variability has been found in α1-AT, over 75 genetic variants.

The lung disease is due to the decreased plasma level of α1-AT, which alters the normal balance between elastase and α1-AT and allows progressive degradation of the elastin of alveolar walls. The progression of the emphysema is greatly augmented by smoking and is a powerful example of the effect that environmental factors may have on the phenotype of a genetic disease (an interaction between environmental factors and different human genotypes).

The relationship between the molecular pathology of proteins and the clinical phenotype

Two generalizations can be made about the relationship between the molecular pathology of mutant proteins and the clinical phenotype of the resulting disease:

1. ***Different mutations in a single gene may produce very different clinical phenotypes.*** In other words, genetic heterogeneity at a locus (allelic heterogeneity) is often responsible for clinical heterogeneity. For some proteins, such as hexA (the enzyme deficient in Tay -Sachs disease), the phenotype caused by total loss of function is the same whether the loss is due to absence of the protein or to a defect that cripples catalysis without affecting quantity. For other proteins, it may be important whether the mutation results primarily in normal quantities of an abnormal protein

or reduction in quantity of a normal protein. Thus, thalassemia mutations lead to an underproduction of globin chains, whereas point mutations that do not substantially reduce the amount of a globin chain produce quite different phenotypes (for example, methemoglobinemia). Most clinical phenotypes may result from one of many different mutations that have a similar effect on protein function. In contrast, the only β-globin mutation that has been found to cause sickle cell disease is the β 6 Glu→Val substitution.

T***he biochemical and clinical consequences of a mutation are often unpredictable.***

No one would have foreseen that the sickle mutation would lead to polymerization of deoxygenated hemoglobin S or that the only significant consequence of phenylalanine hydroxylase deficiency would be mental retardation. Equally unexpected was the discovery that point mutations in the visual pigment rhodopsin are responsible for many cases of a slow degenerative disorder of the retina, autosomal dominant retinitis pigmentosa. Patients with this condition have relatively normal vision for many years. How the defect in the mutant allele led to the slow destruction of the photoreceptor cells, with loss of vision in adulthood, is unknown.

Mono-allelic expression

In most cases, both alleles of a pair are transcribed; this is known as bi-allelic expression. Some genes may show mono-allelic expression (only one allele will be expressed). *Monoallelic gene expression is the process in which transcription occurs from only one of two alleles from one pair, in a diploid cell.* This is also called allelic exclusion.

Monoallelic gene expression may occur:

- Depending on the parental origin of the gene = *genomic imprinting*
- Independently, through the random expression of a certain allele, such as: inactivation of an X chromosome in females, a process whereby only one Ig light chain and one heavy chain gene are transcribed in one cell or other mechanisms.

Mitochondrial diseases

Mitochondrial diseases are a group of conditions that affect mitochondrial genes. Mitochondria produce energy in the cells. When mitochondria aren't able to produce enough energy, it affects how the function of different organs, such as brain, nerves, muscles, kidneys, heart, liver, pancreas, eyes.

It is estimated 1 in 5,000 people has a genetic mitochondrial disease. Symptoms of mitochondrial diseases vary based on the type and location of the affected cells. They can range from mild to severe and could include: poor growth, muscle weakness, muscle pain, hypotonia, vision and/or hearing loss, developmental delay, seizures, fainting. Symptoms can be present at birth, but they can occur at any age. Symptoms of the same disease can vary from person to person, even among family members.

Examples:

- Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome.
- Leber hereditary optic neuropathy (LHON).

Chapter IV

MULTIFACTORIAL INHERITANCE. MULTIFACTORIAL DISEASES.

Multifactorial inheritance

There are many common disorders that appear to run in families but are neither of single - gene nor of chromosomal origin. These disorders are said to show *multifactorial inheritance,* indicating that they are caused by multiple factors, both genetic and environmental. Some of these disorders are congenital defects and others appear as common disorders during adult life, such as: hypertension, asthma or diabetes.

Multifactorial inheritance means that many factors are involved in causing a disease. The factors are usually both genetic and environmental, a combination of genes from both parents, in addition to unknown environmental factors, produce the trait or condition. Often one gender (either males or females) is affected more frequently than the other in multifactorial inheritance. There appears to be a different threshold of expression, which means that one gender is more likely to show the problem over the other gender. For example, hip dysplasia is nine times more common in females than males.

Multifactorial traits and diseases do recur in families, because they are partly caused by genes. The chance for a multifactorial trait or condition to happen again depends upon how closely the family member with the trait is related to you. For example, the risk is higher if a brother or sister has the trait or disease, than if a first cousin has the trait or disease. This is due to the fact that family members share a certain percentage of genes in common, depending upon their relationship. The tendency of a trait to run in families is called familial aggregation.

The most important assumptions of the polygenic, multifactorial model are:

- each locus has an additive effect on the phenotype
- the loci are independent of each other

A useful way of comparing the contributions of genetics and environment to phenotypic variation is the concept of **heritability**. This represents a ratio of variance caused by additive genetic influence. The higher the heritability, the more important is the contribution of the genetic factors.

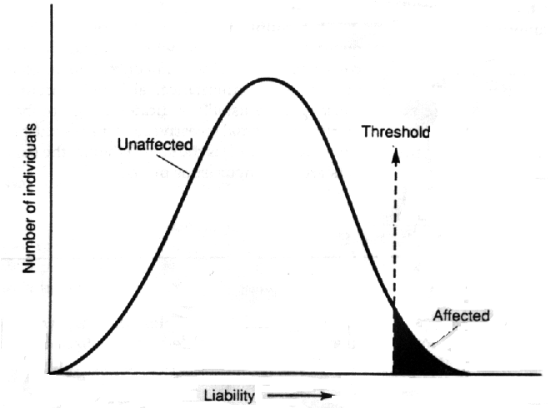
The quantity of genetic factors is termed ″the liability″ of an individual. This can be the result of a polygenic effect together with environment effects.

Recurrence risk in multifactorial inheritance: Empirical risks (approximate):

- 1st-degree relatives: 2–5% (may be higher for some conditions)
- 2nd-degree relatives: <1%
- Recurrence risk doubles if ≥2 relatives affected
- Example: neural tube defect risk = 3–5% after one affected child, 10% after two children

Multifactorial threshold in congenital defects

The basis for a number of common congenital anomalies is a liability to the defect with a threshold marking the point at which the liability is involved in expression of the abnormal phenotype.



Several common congenital malformations with an average frequency in population show a pattern predicted by the multifactorial threshold model. These congenital malformations include:

- *pyloric stenosis,*
- *neural tube defects (anencephaly and spina bifida),*
- *congenital heart defects*
- *cleft lip and palate*
- *positional foot defects*
- *congenital dislocation of hip*
- *hypospadias*

Clinical Implications:

- Requires careful pedigree interpretation
- Empirical risk counseling
- Role of environmental modification and prevention
- Increasing use of polygenic risk scores in precision medicine

Multifactorial disorders

Pyloric stenosis is a defect of the pylorus in which hypertrophy and hyperplasia of the smooth muscle narrows the antrum of the stomach (the antrum becomes almost obstructed), which leads to feeding problems. Pyloric stenosis is five times as common in boys as in girls, but its family pattern is distinctive: in term of the risk, *affected females are much more likely than affected males to have affected children* and among the children of both affected males and affected females, the sons are more likely than daughters to be affected.

The inheritance does not fit any Mendelian pattern. Sons of affected mothers have the highest risk for pyloric stenosis. Liability to the malformation is assumed to be continuously distributed in the population and to be determined by multiple factors, some genetic (the disorder runs in families) and others possibly environmental. If the underlying liability to a trait is continuous but the threshold is lower in males than in females, affected females have, on the average, more extreme liability than do affected males; thus, the offspring of affected females have a higher mean liability to pyloric stenosis than do offspring of affected males. Sons of affected mothers are the highest risk, about 20%.

Neural tube defects

Anencephaly and ***spina bifida*** are neural tube defects that frequently occur together in families and are considered to have common pathogenesis.

Neural tube defects, spina bifida (open spine) and anencephaly (open skull), are seen in 1 to 2 per 1000 live births. During pregnancy, the human brain and spine begin as a flat plate of cells, which rolls into a tube, called the neural tube. If all or part of the neural tube fails to close, leaving an opening, this is known as an open neural tube defect (ONTD). This opening may be left exposed (80% of the time), or covered with bone or skin (20% of the cases). Anencephaly and spina bifida are the most common ONTDs, while encephaloceles are much rarer. Anencephaly occurs when the neural tube fails to close at the base of the skull, whereas spina bifida occurs when the neural tube fails to close somewhere along the spine. Babies with anencephaly are stillborn or usually live for only a few days after delivery. Babies born with spina bifida may have minimal or transient (temporary) problems, or may have permanent, often serious, physical problems. These may include paralysis, lack of bowel and bladder control, club feet, hydrocephaly (a condition marked by an accumulation of spinal fluid in the head) and mental retardation. In most cases, one or more surgeries after birth may be necessary.

In spina bifida, there is a failure of fusion of the arches of the vertebrae, typically in lumbar region, but also possible in other regions. There are varying degrees of severity, ranging from spina bifida occulta, in which the defect is in the bony arch only, to spina bifida aperta, often associated with meningocele (protrusion of meninges) or meningomyelocele (protrusion of neural elements as well as meninges). The incidence of neural tube defects is a little higher in females than in males. The frequency also appears to vary with social factors and season of birth. It has been believed that nutritional factors may account for at least part of the variation. A small proportion of neural tube defects can have other causes: for example, *amniotic bands* (fibrous connections between the amnion and fetus, caused by early rupture of the amnion, which may disrupt structures during their embryological development), *multifactorial,* some *single - gene* *defects with pleiotropic expression*, some *chromosome disorders* and, some *teratogenic factors*. Most neural tube defects are presumed to have multifactorial inheritance.

ONTDs happen to couples without a prior family history of these defects in over 90% of cases. ONTDs are seen five times more often in females than males. Once a child has been born with an ONTD in the family, the chance for an ONTD to happen again is increased to 3 to 5%. It is important to understand that the type of neural tube defect can differ the second time. For example, one child could be born with anencephaly, while the second child could have spina bifida and not anencephaly.

ONTDs can be diagnosed before birth by measuring a protein called alpha-fetoprotein present in the amniotic fluid.

Fetal ultrasound during pregnancy can also give information about the possibility of an ONTD, but is not 100% accurate, since some babies with an ONTD may look the same on ultrasound as those without these defects. Measurement of the AFP, and other biochemical markers from amniotic fluid, is over 95% accurate for detecting ONTDs. Small or closed defects (which do not leak spinal fluid) may not be picked up by this test.

Congenital heart defects

Congenital heart defects are very common, with a frequency of about 8 - 12 cases per 1000 births. They are a heterogeneous group, caused in some cases by single - gene or chromosomal mechanisms and in other cases resulting from teratogenic exposure such as: rubella infection, maternal diabetes, viral infection, anticonvulsivants, etc. In most cases, however, the cause is regarded to be multifactorial.

There are many types of congenital heart defects with different population incidences and risk and having a multifactorial threshold model. Ventricular septal defect has the highest incidence, followed by defect of ductus arterious, atrial septal defect, and aortic stenosis a. o.

Cleft lip and cleft palate

Oro-facial clefts (cleft lip with or without cleft palate) are among most common congenital defects. It is estimated that 1 case occurs in every 500-55o births. They are caused by a failure of fusion of the frontal process with the maxillary process at about 35th day of gestation. About 60-80% of affected cases are males. They can occur separately or together.

The etiology of orofacial clefts is heterogeneous. Most isolated forms are caused by the interaction of genetic and environmental factors, showing multifactorial inheritance. They may also occur in single - gene syndromes, in chromosomal disorders and many cases result from teratogenic exposure (rubella, anticonvulsants, other drugs, maternal smoking). Genetic factors: MSX1, IRF6.

Severity and recurrence risk increase from unilateral to bilateral malformations and from isolated cleft lip to cleft lip associated with cleft palate. The multidisciplinary management involves surgery, speech therapy, dental care.

Congenital dislocation of hip

Congenital dislocation of hip is characterized by the displacement of the femoral head outside the acetabulum, occurring as a congenital subluxation. Also called hip dysplasia, it is nine times more common in females than males. Genetic and environmental factors are thought to have important role in the etiology. One of the environmental influences thought to contribute to hip dysplasia is the baby's response to the mothers' hormones during pregnancy. Once a child has been born with hip dysplasia, the chance for it to happen again in a male or female child is about 6 percent overall. In other words, there is a 94 percent chance that another child would not be born with hip dysplasia. The defect appears to be inherited as a multifactorial trait, as familial aggregation and high concordance rate for monozygotic twins have been noticed.

Club foot

Club foot is a common congenital anomaly that shows familial aggregation. Many types of transmission have been reported: autosomal recessive, X-linked recessive, multifactorial.

Deformities include: adduction of the forefoot, inversion of the heel, plantar flexion, dorsal flexion. About 70% of these malformations occur in males. Surgery is needed in order to correct these defects.

Etiology may be:

- multifactorial
- chromosomal syndromes (especially trisomy 13)
- single-gene syndromes
- teratogenes, such as: rubella virus, anticonvulsivants, etc.

Clinical and genetic counseling aspects

- Risk of recurrence increases with number of affected relatives.
- Importance of family history and risk estimation.
- Counseling emphasizes prevention, early diagnosis, and multidisciplinary care.

Prenatal Diagnosis and Prevention combine screening (ultrasound, maternal serum markers, amniocentesis), preventive measures: folic acid, avoiding teratogens, maternal health optimization and the genetic counseling for at-risk couples.

Complex disorders of adult life

Several common chronic disorders are definitely familial, and in a broad sense they behave as multifactorial threshold traits, but depending on the effect of environment.

Examples of complex disorders of adult life:

- *coronary artery disease*
- *high blood pressure*
- *congenital heart defects*
- *hypertrophic cardiomyopathy*
- *obesity*
- *diabetes mellitus*
- *asthma*
- *mental and behavioral disorders*

Coronary artery disease

Coronary artery disease is a major health problem, particularly in males aged 45 or more. Most cases appear to be multifactorial with an effect of environment. Familial hypercholesterolemia, an AD defect of the low-density lipoprotein receptors accounts for about 5% of survivors of myocardial infarction. Although there are other single-gene causes, the adverse risk factors associated with coronary artery disease include nongenetic as well as genetic factors, most cases of coronary artery disease showing multifactorial inheritance:

Genetic factors	***Nongenetic factors***
male sex	increasing age
family history of coronary disease	smoking
single-gene abnormalities of lipoproteins and lipids	stress
hypertension	physical inactivity
obesity	
diabetes mellitus	

The risk factors for coronary artery disease include several other multifactorial disorders with genetic components: hypertension, obesity, and diabetes mellitus. In this context, the abnormal phenotype associated with these disorders contributes to an environment that enhances the risk of coronary artery disease.

One feature of coronary artery disease that is consistent with multifactorial inheritance is that whereas males are at higher risk of death from myocardial infarction both in the population and within affected families, the recurrence risk in relatives is somewhat greater when the proband (the patient) is female. However, the risk of ischemic heart disease in some families is greater when there is a family history with first-degree relatives having this disorder.

High blood pressure is a major cardiovascular risk factor, which is in part genetically determined. With the notable exception of two AD forms of hypertension related to an abnormality in aldosterone secretion and to an overactivity of the epithelial sodium channel, there is no indication on the number of genetic loci involved in hypertension.

Even though the genetic loci controlling blood pressure are unknown, a first approach is to study genes that may contribute to the variance of blood pressure due to their well-known effect on the cardiovascular system. The genes of the renin angiotensin aldosterone system are a good illustration of such a “candidate gene” approach, since this system is well known to be involved in the control of blood pressure and in pathogenesis of several forms of human hypertension.

Environmental influences: diet, smoking, sedentary lifestyle. Preventive strategies: blood pressure control, exercise, diet modification.

Obesity

Large differences in body weight and fat distribution, even among people of the same sex and age are due to genetic factors, although surely that environment also plays a role.

Two recent studies of body weight in monozygotic twins (MZ) demonstrate the twin method of assessing the relative importance of genes and environment in obesity.

The comparison of MZ twins reared together or apart and of dizygotic twins (DZ) reared together or apart is a classical way of measuring heritability of complex traits. Several studies showed thus that the genetic component represented by a high heritability is important in weight gain and also in overfeeding.

Single-gene and syndromic causes of obesity represent a clinically significant subset of severe obesity that is often under recognized in clinical practice. These include monogenic disorders, such as leptin deficiency and melanocortin 4 receptor deficiency, as well as Prader-Willi syndrome and Bardet-Biedl syndrome.

Diabetes mellitus

The genetics of diabetes mellitus is complex; many studies have shown that diabetics have a positive family history- in other words there is a familial aggregation of diabetes. The study of twins (MZ and DZ) leaves no doubt as to the importance of genetic factors in the etiology of diabetes as well as predisposing nongenetic factors. In fact, in diabetes it is known that there is a complex genetic heterogeneity; the clinical genetic studies have suggested that juvenile - and adult - onset diabetes differed genetically.

Diabetes mellitus may be divided into two types: insulin-dependent diabetes mellitus type I (IDDM) and non-insulin-dependent diabetes mellitus type II (NIDDM). They differ in typical age, of onset monozygotic twin concordance, and HLA associations.

IDDM type I is an autoimmune, polygenic disease. The concordance rate (30 to 50 percent) for IDDM in monozygotic twins (Mz) provides strong support for the hypothesis that genetic factors contribute to the predisposition to IDDM. Nevertheless, the fact that the concordance rate is less than 100 percent is usually interpreted as evidence those environmental factors may contribute as well.

Type 1 diabetes is one of the most widely studied complex genetic disorders, and the genes in HLA are reported to account for approximately 40% to 50% of the familial aggregation of T1D. The major genetic determinants of this disease are polymorphisms of class II HLA genes encoding DQ and DR. The DR-DQ haplotypes conferring the highest risk are DR3 and DR4. The risk is much higher for the heterozygote formed by these two haplotypes than for either of the homozygotes DR3/DR3 or DR4/DR4. Some haplotypes confer strong protection from disease, such as DR2.

About 95% of all IDDM patients (compared to about half the healthy population) have HLA-DR3 or HLA-DR4; heterozygotes DR3/DR4 are particularly susceptible to IDDM. This is one of the strongest HLA-disease associations known. Families' studies suggest that the HLA association account for more than half the heritability of IDDM.

Different genetic variants could represent unpreventable causal components of the disease, but their study could improve our understanding of the underlying mechanisms, which could lead to identification of drug targets.

Variable	***IDDM***	***NIDDM***
Onset age	usually<30 years	usually >40 years
MZ twins concordance	30-50%	close to 100%
HLA associations	Strong	none

New insights into the basis of IDDM have come from molecular analysis of the HLA class II genes, which are responsible for immune responsiveness. Thus, in the DR4 haplotype, the presence of aspartic acid (Asp) at position 57 of the DQ β chain is closely associated with resistance to IDDM, whereas other amino acids at this position (alanine, valine or serine) confer susceptibility. About 90 percent of patients with IDDM are homozygous for DQ β genes that do not encode Asp at position 57.

Although there is a strong association of IDDM with HLA genes, there are probably other susceptibility genes outside the major histocompatibility complex and nongenetic factors involved in the etiology of this disease.

Unlike type I, type II diabetes mellitus (NIDDM) is not an autoimmune disease. Available evidence strongly suggests that the susceptibility to type II diabetes mellitus be determined primarily by genetic factors. The concordance of diabetes type II in MZ twins is close to 100%.

Asthma, a reversible obstructive pulmonary disease closely associated with bronchial hyper-responsiveness and airways inflammation is a complex disorder with genetic susceptibility. Although an increased risk to relatives has been reported, the asthmatic phenotype is not inherited in a simple Mendelian fashion. Family studies are complicated because the clinical expression of this disorder is affected by age and gender as well as by exposure to allergens, pollutants and viral respiratory infections.

Studies on monozygotic and dizygotic twins are used as a first step in determining the role of genetics in a multifactorial disorder. Thus, many twin studies showed significant differences of the concordance in MZ twins, 19% compared with 4,8% in DZ twins. These studies provide evidence for a genetic component in the development of asthma. In addition to twin studies, many family studies have also demonstrated a heritable polygenic component to susceptibility to asthma.

Mental and behavioral disorders

Several other common complex genetic polygenic, multifactorial disorders are:

- *schizophrenia*
- *bipolar/unipolar psychosis*
- *autism*
- *dyslexia and specific learning disorders*
- *attention deficit and hyperactivity disorders*
- *addiction*

In ***schizophrenia***, family, twin, and adoption studies suggest that 65-85% of the susceptibility to disease can be attributed to genes. In linkage studies, families with ill members have been tested by genotyping the variable sequences (allele) at DNA marker loci. If a disease gene is close to a marker, then offspring who inherit the disease allele will also tend to inherit nearby marker allele (linkage) because meiotic recombination is unlikely across a short chromosomal distance. Linkage analysis determines whether ill relatives have inherited the same marker allele more of than expected by chance. Thus, linkage studies can focus on the candidate regions for schizophrenia. These regions have been found many chromosomes, eg. 2q, 4q, 9q, 11q, 6p, 8p and 22q; it is obvious that more chromosomal regions contain schizophrenia susceptibility genes, which suggests that the disease has a multifactorial inheritance.

In ***bipolar disorder*** linkage studies have shown that there are marker loci on the long arm of chromosome 18 (18q), but there is compelling evidence from twin, family and adoption studies to suggest that bipolar disorder results from multiple genetic loci acting independently or in concert; some of the genes are probably located on chromosome 8, others on chromosome 21q.

Most of these disorders are heterogeneous and a large part of their analysis will involve dissecting the heterogeneity, finding genes responsible for different genetic forms and establishing risk factors, separate from the genetic background, that increase the probability that the disease will develop in an individual patient. Many methods of genetic linkage analysis show in these disorders multifactorial and polygenic traits.

Autism is a behavioral disorder characterized by abnormalities in language and social communication, stereotype, repetitive movements, tics, abnormalities in reciprocal social interaction. Implication of genetic factors in its etiology was sustained by family studies that revealed familial aggregation, twin studies (higher concordance in monozygotic than in dizygotic twins) and associations with chromosomal abnormalities. All these studies suggest that genetic liability plays an important role, together with social factors, obstetric complications a.o.

Dyslexia and other specific learning disabilities**:** Patients with these disabilities have difficulties in learning, reading and spelling despite normal intelligence and without neurological handicap. Linkage analysis revealed that few loci are involved in the etiology, thus, this trait is called oligogenic.

Attention deficit and hyperactivity disorder is the most common impediment to learning seen in children. Patients have difficulty in attention, impulsivity, hyperactivity. There is evidence for a substantial genetic influence in this disorder. Twin studies, as well as family and adoption studies have shown that few loci together with environmental factors cause the disease.

Addiction: Genetic factors play an important role in different types of addictions, but it is a very complex trait, multiple genes and environment can have additive effects.

Characteristics of multifactorial inheritance

1. Although the disorder is familial, there is no distinctive pattern of inheritance within a single family.

2. The risk to first - degree relatives is evaluated in the following way: the lower the population incidence, the greater the relative increases in risk for first - degree relatives.

3. The risk is lower for second - degree than for first - degree relatives.
4. The recurrence risk is higher when more than one family member is affected.
5. The more severe the malformation, the greater the recurrence risk.
6. If a multifactorial trait is more frequent in one sex than in the other, the risk is higher for relatives of patients of the less susceptible sex.
7. If the concordance rate in DZ twins is less than half the rate in MZ twins, the trait cannot be AD, and if it is less than a quarter of the MZ rate, it cannot be autosomal recessive.
8. An increased recurrence risk when the parents are consanguineous suggests that multiple factors with additive effects may be involved.

Twins and twinning

Twins can be either dizygotic (fraternal) or monozygotic (identical). Dizygotic twins (DZ) are the result of two different ova fertilized by two different sperm. Monozygotic twins (MZ) are the result of one ovum fertilized by one sperm that divides to form two embryos.

Twins and genetic studies

In the past, the only way of differentiating between MZ and DZ twins at birth was their sex, appearance and placentation. Today, cord blood type, HLA antigens and DNA fingerprinting are all used to differentiate between MZ and DZ twin pairs. However, DNA fingerprinting has become the only accurate method.

The importance of twins in genetic studies was first recognized by Galbon, who in 1875 suggested the study of twins as a model for investigating the differences between the environmental and genetic effects on disease. Comparison of concordance rate in MZ and DZ twins is a standard method used in medical genetics for comparison of the effects of genes and environment.

Concordant in human genetics means a twin pair in which both members exhibit a certain trait. ***Discordant*** means a twin pair of which one member shows a certain trait and the other does not. If MZ pairs are not completely concordant for a given trait, genetic factors alone cannot account for the trait.

Comparison of concordance rates in MZ and DZ twins is used to measure the "***heritability***" of complex traits.

There are many types of studies in which twins can provide valuable information. The purpose of all twin studies is to obtain results that are applicable not only to twins but also to the human population.

In studies that compare the differences between MZ and DZ twins with regard to a specific trait and then draw conclusions for the general population, it is necessary to know whether nongenetic factors act differently on MZ and DZ twins, under the assumption that MZ and DZ twins are exposed to the same pre- and postnatal environmental factors.

The incidence of MZ twins is thought to be constant throughout the world. By contrast, the incidence of DZ twins varies from population to population, with a higher prevalence in some areas, such as Africa. In North America, the incidence of twins (the combination of MZ and DZ) is estimated at 1 in every 80 births.

The number of twin conceptions is harder to asses, since studies have shown that at least 70% of twin pregnancies diagnosed by ultrasound examination before the 10th week miscarry or convert to singleton. Several investigators have confirmed that the number of twins at delivery is considerably less than the number of twin conceptions seen on ultrasound examination in early pregnancy. The “disappearance” of the co-twin recognized on ultrasound examination seems to occur during the second half of the first trimester or early in the second trimester.

Dizygotic twins

Dz twins are derived from the fertilization of two ova by two sperm and may be of the same or different sex. Their genetic contribution is different, since it comes from two different ova and two different sperm.

Multiple ovulation, that is, the release of more than one ovum from the ovaries is necessary for DZ twinning. Women who have given birth spontaneously (without the use of fertility drugs) to DZ twins are known to have higher levels of follicle–stimulating hormone (FSH) and luteinizing hormone (LH). This evidence has led to the now established association of increased levels of FSH and LH and DZ twinning. DZ twins produced by the fertilization of multiple ova may be the result of superfecundation.

DZ twins would be expected to have two placentas, two chorions and two amnious. However, placentas in DZ conceptions may fuse and look like one.

There are many reports of familial DZ twining. The female members of these families are thought to have an inherited predisposition to multiple ovulation and a higher number of DZ twin pair when compared to the general population. An established association between higher gonadotropin levels and higher incidence of DZ twins in certain families is thought to be the basis for familial DZ twinning. The use of fertility drugs may artificially duplicate what is a natural occurrence in some families.

Monozygotic twins

MZ, or "identical" twins are the result of the fertilization of one ovum by one sperm. The single fertilized ovum then divides into two embryos; both embryos are thought to have the same genetic contribution; in the past, it have been expected to be genetically identical. However, with new molecular genetic techniques, it has become clear that some MZ twins are not completely identical.

MZ twins may have separate or contiguous placentas and may be monochorionic monoamniotic.

A few families have been reported in which MZ twinning occurs more frequently than expected. Because familial MZ twinning is inherited both maternal and paternal side of the family it has been suggested that it may be caused by a single gene effect. Although, the etiology of MZ twinning in humans is unknown, several mechanisms have been proposed. In 1970, Bulmer suggested that MZ twinning is associated with disturbance of developmental clocks or thresholds and that delayed fertilization or delayed implantation may play a role in MZ twinning. Edwards in 1986 suggested that abnormalities or rupture of the zona pellucida may lead to herniation of the blastocyst and predispose to MZ twinning.

Other investigators have suggested that twinning itself may a type of congenital anomaly of development.

MZ twins may sometimes be discordant for a variety of congenital defects and genetic disorders. In addition to environmental differences and chance variation, the following reasons for discordance are recognized:

- mechanisms of embryological development, such as vascular abnormalities, that can lead to discordance for malformation;
- postzygotic changes such as somatic mutation leading to discordance for cancer, or somatic rearrangement of immunoglobulin genes;
- chromosome abnormalities originating in one zygote after the twinning event;
- uneven X inactivation between female MZ twins with the result that one twin preferentially expresses the paternal X, the other the maternal X.

Studies of MZ and DZ twins reared apart have been helpful in establishing the significance of genetic and environmental factors regarding susceptibility of several diseases with multifactorial inheritance. For example, these studies have been helpful in measuring the extent of genetic contribution compared to environmental contribution in complex psychiatric disorders like schizophrenia and bipolar disorders.

Chapter V

CLINICAL CYTOGENETICS

ANEUPLOIDIES

Cytogenetics is the study of chromosome characteristics, their normal structure and abnormalities associated with chromosomal syndromes.

Indications for cytogenetic analysis include the following:

1. Confirmation or exclusion of the diagnosis for known chromosomal syndromes
2. Unexplained psychomotor retardation with or without dysmorphic features
3. Abnormalities of sexual differentiation and development
4. Recurrent miscarriage or stillbirth
5. Infertility
6. Pregnancies shown to be a risk of aneuploidy
7. Neoplastic condition particularly hematological malignancies for which the identification of specific chromosomal aberrations may be valuable in diagnosis and management.

Chromosome analysis is performed using karyotyping or molecular cytogenetic techniques (FISH, array CGH).

It is known that chromosome disorders form a major category of genetic diseases, accounting for a large proportion of all-reproductive wastage, congenital malformations, and mental retardation, as well as playing an important role in pathogenesis of malignancy.

Abnormalities of chromosomes may be either numerical or structural and may involve one or more autosomes, sex chromosomes, or both simultaneously.

By far the most common type of clinically significant chromosome abnormality is *aneuploidy*, an abnormal chromosome number due to an extra or missing chromosome, which is always associated with physical or mental maldevelopment, or both.

Aneuploidies (Abnormalities of chromosome number)

Aneuploidy is the most common and clinically significant type of human chromosome disorder, occurring in at least 3 to 4 % of all clinically recognized pregnancies.

Although by definition a person is aneuploid if he or she has fewer or more chromosomes than exact multiple of the haploid set, most aneuploid patients have either trisomy (three instead of the normal pair of a particular chromosome) or, less often, monosomy (only one representative of a particular chromosome). Either trisomy or monosomy can have severe phenotypic consequences.

Trisomy can exist for any chromosome of the set, but trisomy for a whole chromosome is rarely compatible with life. By far the most common type of trisomy in liveborn infants is trisomy 21 (Down syndrome); other major autosomal trisomies are trisomy 13 (Patau syndrome) and trisomy 18 (Edwards syndrome).

Polyploidy refers to having one or more complete extra sets of chromosomes (e.g., triploidy, where there are three sets).

The common aneuploidies involving sex chromosomes include 47,XXY and 47,XYY in phenotypic males; aneuploidies in phenotypic females include 47,XXX and 45,X with or without associated mosaicism.

Chromosomal mosaicism means that a single individual may possess two or more cell lines or clones, each differing in chromosomal constitution.

Monosomy for an entire autosomal chromosome is lethal, but monosomy for the X chromosome can be seen at any age in females.

The causes of aneuploidy are not well understood, but is known that whatever the underlying molecular mechanism, the most common chromosomal mechanism is meiotic nondisjunction, the failure of a pair of chromosomes disjoin in the normal way during one of the two meiotic divisions, usually during meiosis I. The consequences of nondisjunction during meiosis I and meiosis II are different.

If the error occurs during meiosis I, the gamete with 24 chromosomes contains both the paternal and the maternal members of the pair. If it occurs during meiosis II, the gamete with the extra chromosome contains both copies of either the paternal or the maternal chromosome (however, recombination almost certainly has taken place in the preceding meiosis I, resulting in some genetic differences between the chromatids and thus, the corresponding daughter chromosomes).

To determine the parental origin of the error and the meiotic stage at which it occurred, as well as to learn whether an association exists between nondisjunction and recombination, both polymorphic DNA probes spanning the long arm of chromosome 21 and cytogenetic heteromorphism of the short arm have been used. In many cases analysis of markers on the chromosomes of the parents and the aneuploid patient (child) reveals the stage and parental origin or error.

More complicated forms of multiple aneuploidy have been reported. A gamete occasionally has an extra representative of more than one chromosome. Nondisjunction can take place at two successive meiotic divisions or by chance in both male and female gametes simultaneously, resulting in zygotes with bizarre chromosome numbers, which are extremely rare except for the sex chromosomes.

Nondisjunction can also occur in a mitotic division after formation of the zygote. If this happens at an early cleavage division, clinically significant *mosaicism* may result. In some malignant cell lines and some cell cultures, mitotic nondisjunction can lead to highly abnormal karyotypes.

Autosomal aneuploidies

The vast majority of chromosomal abnormalities affect autosomes. Most of autosomal abnormalities are trisomies or monosomies. All autosomal monosomies lead to spontaneous abortion. Most all embryos/fetuses with autosomal trisomies are also aborted. Still, some may be born alive, but they will have many congenital defects, developmental delay and mental retardation.

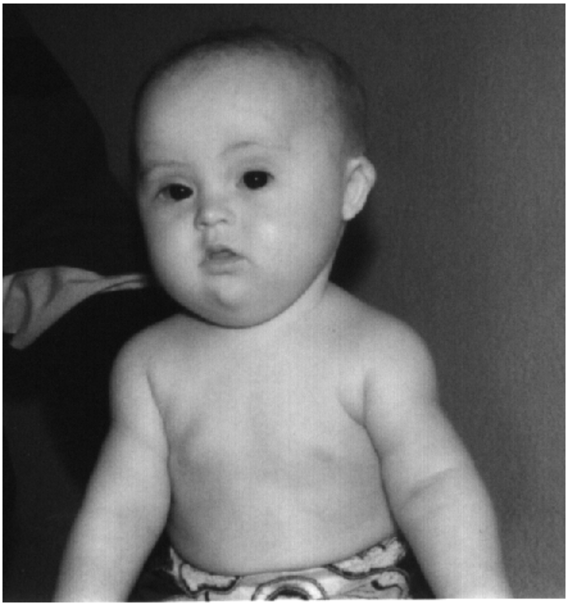
Autosomal trisomies: rates and survival

Although the cumulative birth frequencies of trisomy 21,18 and 13 are considerably less than 1% of all birth, in large series of spontaneously aborted pregnancies that have been karyotyped, autosomal trisomy is present in 20% and is the most common type of chromosomal abnormality. The vast majority of all trisomic conceptions either fail to develop or die during the embryonic period. Most intrauterine deaths occur in early pregnancy.

Trisomy 21 – Down syndrome

Down syndrome can be diagnosed at birth by clinical investigation. Although no single phenotypic feature is pathognomonic, the combination of associated dysmorphisms results in a recognizable and specific appearance (distinctive phenotype).

A plethora of clinical signs have been described, such as brachycephaly, hypertelorism, upwards and outwards orientation of the eyes, epicanthic folds, flattened nasal root, midfacial hypoplasia, low-set ears, protruding tongue. In addition the neck is short, there is hypotonia, hands are short and broad, with simian crease.



Phenotype of an infant with trisomy 21

The major cause for concern in Down syndrome is mental retardation, developmental delay; the delay is usually obvious by the end of the first year. Speech and language develop later and more slowly, compared to healthy children.

Congenital heart disease is present in about one third of the patients. Other medical conditions that may be present are hypothyroidism, gastrointestinal defects and in adults there is an increased risk of developing Alzheimer disease after 40 years of age. Patients with Down syndrome have increased susceptibility to infection due to immunodeficiency and susceptibility for lymphoblastic leukemia.

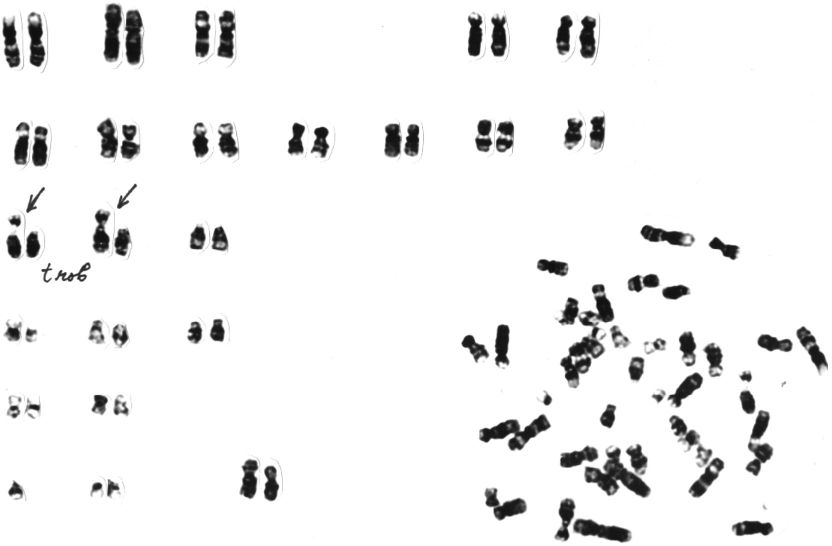
Pattern of karyotype: in about 98% of all patients, Down syndrome involves trisomy for chromosome 21 in all metaphases (complete trisomy); about 1-2% of patients have mosaicism, usually for cell populations with either a trisomy 21 or a normal karyotype.

The trisomy for chromosome 21 results from meiotic nondisjunction of chromosome 21 pair; the risk of having a child with trisomy 21 increases with maternal age, especially after the age of 35 years. The meiotic error responsible for the trisomy usually occurs during maternal meiosis, predominantly in meiosis I, but can also occur in paternal meiosis (about 5% of cases), again usually in meiosis I.

About 4% of Down syndrome patients have trisomy 21 in a *Robertsonian translocation* between chromosome 21q and the long arm of one of the other acrocentric chromosomes (usually chromosome 14, 22 or 21) (figures).

Unlike standard trisomy 21, translocation Down syndrome shows no relation to maternal age but has a high recurrence risk in families when a parent, especially the mother is a carrier of the balanced translocation.

Very rarely, Down syndrome is diagnosed in a patient in whom only a part of the long arm of chromosome 21 is present in triplicate (partial trisomy), that shows mild “downian phenotype”. These patients are of particular interest because they can show what region of chromosome 21 is likely to be responsible for the Down syndrome phenotype.



Trisomy 21 karyotype with G banding. The two arrows show translocations 13q/21q and 14q/21q. (from our collection).

Trisomy 21 with unbalanced t (21; 21) (arrows); G-banded karyotype from our collection

Trisomy 18 (Ewards syndrome)

The features of trisomy 18 always include mental retardation and severe malformation of the heart. Hypertonia is a typical finding. The head has a prominent occiput; the ears are low –set and malformed. The feet are rocker-bottom with prominent calcanei; the fist clenched in a characteristic way. The patterns are distinctive with single creases on the palms.

About 95% of trisomy 18 embryos are spontaneously aborted; postnatal survival is poor.

The trisomy 18 phenotype, like that of trisomy 21, can result from a variety of karyotypes other than complete trisomy. There may be a translocation involving all or most of chromosome 18, which may be either “de novo” or inherited from a balanced carrier parent. The trisomy may also be present in mosaic form, with variable milder expression. As in most other trisomies, late maternal age is a factor.

Trisomy 13 (Patau syndrome)

Trisomy 13 is clinically severe, lethal in almost all cases by the age of six months. Like most other trisomies, it is associated with late maternal age. The extra chromosome usually arises from nondisjunction in maternal meiosis I.

The phenotype of trisomy 13 includes severe central nervous system defects. Growth retardation and severe mental retardation are present. The forehead is sloping; there is ocular hypertelorism, microphtalmia, iris coloboma, or even absence of the eyes. The ears are malformed. Cleft lip and cleft palate are very often present. The hands and feet have polydactyly. The feet are rocker-bottom. The palms often have simian creases. There may be congenital heart defects, urogenital defects, including cryptorchidism in males, hypoplastic uterus and ovaries in females. The most distinctive are the general facial appearance with cleft lip and palate and ocular abnormalities, the polydactyly and the rocker-bottom.

Trisomy 8 mosaicism

Trisomy 8 mosaicism seems to arise frequently from somatic gain of one chromosome 8 in the early development of a zygote.

The phenotype is very difficult for identification because of great variability in clinical expression, but it is clear that mosaic trisomy 8 is always associated with a mild handicap. Typical clinical presentation is in early childhood with developmental delay and dysmorphism such as: bone and joint abnormalities and corneal opacities.

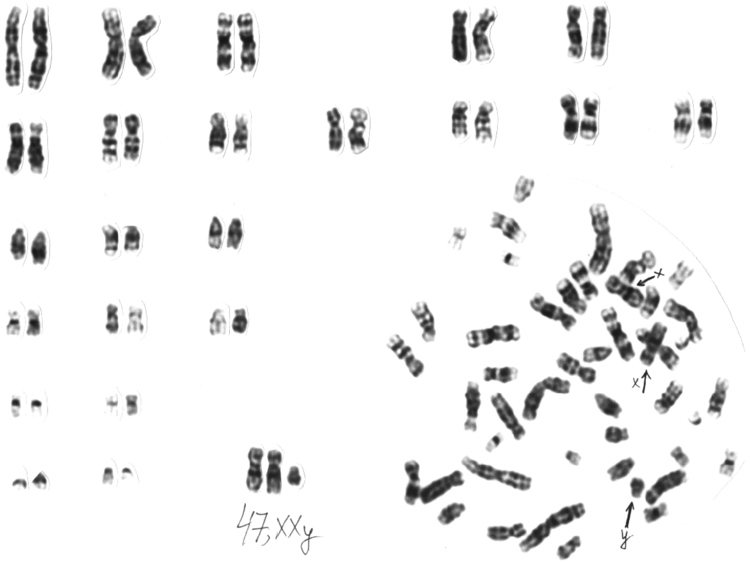
Aneuploidies of the sex chromosomes

Sex chromosome abnormalities, like abnormalities of the autosomes, can be either numerical or structural and can be present in all cells or in mosaic form. X and Y chromosome aneuploidy is relatively common and the most common of all human genetic disorders with an overall frequency of about 1 in 400 birth. The phenotypes associated with these chromosomal defects are, in general, less severe than those associated with autosomal disorders.

The main expressions in phenotype are gonadal dysgenesis, secondary sexual characteristics undeveloped and infertility or sterility.

Klinefelter syndrome (47,XXY)

The patients appear physically normal until puberty, when signs of hypogonadism become obvious. The testes remain small atrophic (sometimes there is cryptorchidism) and the secondary sexual characteristics remain underdeveloped. Klinefelter patients are almost always infertile (with azoospermia). The patients are tall and thin, with relatively long legs. The patients can have learning disabilities, speech delayed and minor neuromotor deficits. Even though the phenotype seems benign in comparison with that in autosomal disorders, half of all 47,XXY conceptions are loss prenatal. Puberty occurs at the normal age but some of the secondary sexual characteristics remain underdeveloped. Gynecomastia emphasized as a typical sign is not always present. Although the disease cannot be cured, early diagnosis is very important, as early initiation of the treatments will help minimize the effects of the presence of one X chromosome in plus. The earlier the treatment is started, the greater the benefits.



G-banded 47,XXY karyotype (Klinefelter syndrome). The arrows show the two chromosomes X and chromosome Y (from our collection)

About 85% of patients have 47,XXY and about 15% have mosaic karyotypes. As a group, such mosaic patients have variable phenotypes. The most common mosaic karyotype is 46,XY/47,XXY, probably as a consequence of loss of one X chromosome in a XXY conceptus during an early postzygotic division. As predicted by the finding that 47,XXY

Klinefelter patients have a Barr body, one of the two X chromosomes is inactivated.

About half the cases result from errors in paternal meiosis I, one-third from errors in maternal meiosis I, and the remainder from errors in meiosis II or from a postzygotic mitotic error leading to mosaicism. Maternal age is increased in the cases associated with maternal meiosis I errors, but not in the other cases.

There are several variants of Klinefelter syndrome, with a karyotype including 48,XXXY and 49,XXXXY. As a rule, the additional X-chromosomes cause a correspondingly more abnormal phenotype with a greater degree of dysmorphism and more severe mental impairment.

47, XYY syndrome

Although the 47, XYY chromosome constitution is not associated with an obviously abnormal phenotype, it became of scientific interest after the observation that the proportion of XYY males was much higher in the population of prisons, especially among the tallest inmates or in mental hospitals than in general population. The origin of the error that leads to the XYY karyotype must be paternal nondisjunction at meiosis II, producing YY sperm. There are no specific signs of the disease. They usually have normal intelligence. Hyperactivity, distractibility, lack of emotional control and increased compulsiveness are present in some of the patients with XYY. Testes are normal developed, but decreased spermatogenesis, subfertility and sterility were reported in a number of patients.

Yq deletions: Deletions of the long arm of Y can affect testicular function leading to hypogonadism, delayed occurrence of secondary sexual characteristics and short stature.

Trisomy X (47,XXX)

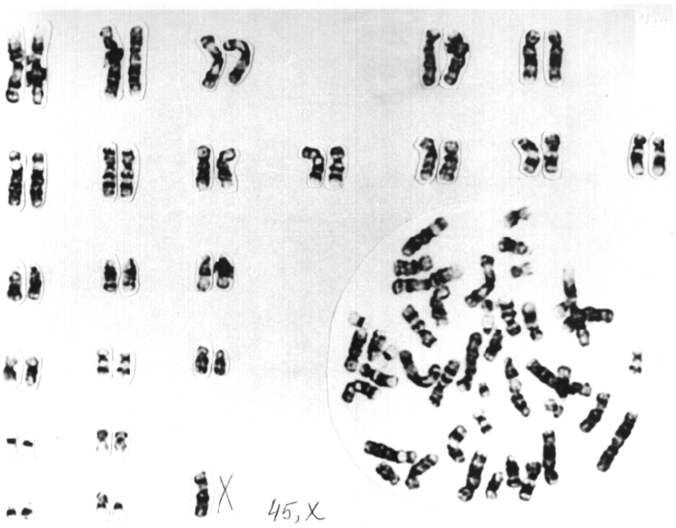
Trisomy X females, though usually above average in stature, are not phenotypically abnormal, but several patients have been identified in infertility clinics and in institutions for the mentally retarded. There is a significant deficit in performance on IQ tests and about 70 percent of the patients have serious learning and educational problems. In 47,XXX cells, two of the X chromosomes are inactivated and late-replicating as suggested originally by the finding of two Barr bodies. Almost all cases result from errors in maternal meiosis I; there is an effect of late maternal age.

The *tetrasomy X syndrome* (48,XXXX) is associated with more serious retardation in both physical and mental development.

Turner syndrome (45,X and Variants)

Unlike patients with other sex chromosome aneuploidies, females with Turner syndrome can often be identified at birth or before puberty by their distinctive phenotypic features. The most frequent chromosome constitution in Turner syndrome is 45,X with no second sex chromosome, either X or Y.

However, about 50 percent of cases have other karyotypes. About one quarter of Turner syndrome cases involve mosaic karyotypes (45,X/46,XX).



G-banded karyotype 45,X (from our collection).

The typical abnormalities in Turner syndrome include short stature, gonadal dysgenesis (usually streak gonads), neck webbing, low posterior hairline, absence of puberty and often renal and cardiovascular anomalies. At birth, infants with this syndrome often have edema of dorsum of the feet and hands, a useful diagnostic sing. Many patients have coarctation of the aorta. Intelligence is usually average or above average, but sometimes patients display a deficiency in perception.

The very high frequency of 45,X in spontaneous abnormality is present in an estimated 1,5 percent of all conceptuses. Usually the single X is maternal in origin; in other words, the meiotic error is usually paternal (the basis of this nondisjunction is unknown). Further more, it is not clear why the 45,X karyotype is usually lethal in utero but is apparently fully compatible with postnatal survival. By the age of puberty the ovaries are replaced by ovarianlike stroma, but no folicle. It follows that in untreated individuals menarch rarely occurs and the patients are sterile. In 45,X/46,XX mosaic patients the clinical features are milder.

Chapter VI

ABNORMALITIES OF CHROMOSOME STRUCTURE

Structural rearrangements result from chromosome breakage, followed by reconstitution in an abnormal combination. Rearrangement can take place in many ways; the most common type, a balanced translocation can be seen frequently.

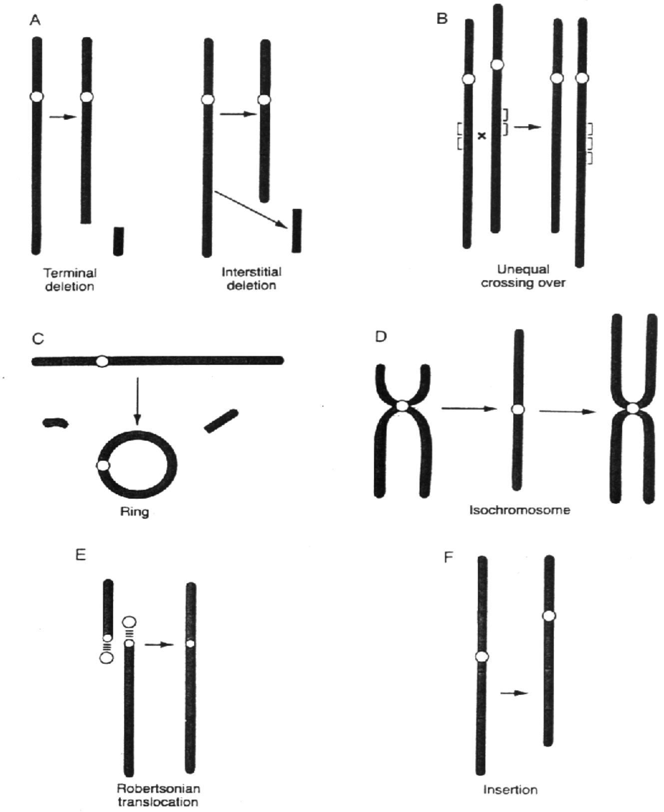
Structural rearrangements are defined as balanced, if the chromosome set has the normal complement of genetic information, or unbalanced if there is additional or missing information. Some rearrangements are stable, capable of passing through cell division unaltered, whereas others are unstable and forming derivate chromosomes. To be stable, a rearranged chromosome must have normal structural elements, including a single functional centromere and two telomeres.

Unbalanced rearrangements

In unbalanced rearrangements, the phenotype is likely to be abnormal because of deletion, duplication, or–in some cases-both. Duplication of a part of a chromosome is comparable with partial trisomy; deletion leads to a partial monosomy. Any change that disturbs the normal balance of functional genes can result in abnormal development.

Deletion

Deletion is loss of a chromosome segment, resulting in chromosome imbalance. A carrier of a chromosomal deletion (with one normal chromosome and one deleted chromosome) is hemizygous for the genetic information on the corresponding segment of the normal homologous chromosome. The clinical consequences depend on the size of the deleted segment and the number and function of the genes that it contains. A deletion may be *terminal* or *interstitial.* Deletions may originate simply by chromosome breakage and loss of the acentric segment. Alternatively, unequal crossing over between misaligned homologous chromosomes or sister chromatids may account for deletion in some cases.



Structural rearrangements of chromosomes

The previous image shows: A: Terminal and interstitial deletions each generating an acentric fragment; B: Unequal crossing over between segments homologous chromosomes or between sister chromatids (duplicated or deleted segment indicated by the brackets); C: Ring chromosome with two acentric fragments; D: Generation of an isochromosome for the long arm a chromosome; E: Robertsonian translocation between two acrocentric chromosomes; F: Insertion of a segment of one chromosome into a nonhomologous chromosome.

High-resolution banding techniques can reveal deletions that are too small to be seen in ordinary metaphase spreads. To be cytogenetically identifiable by high-resolution banding, a deletion must span at least 2000-3000kb, but smaller deletions with phenotypic consequences have been detected by molecular techniques.

Selected examples of deletion syndromes

Disorder	**Deletion**	**Description**
Prader-Willi syndr.	15q11-q13	Dysmorphic syndr. by genomic imprinting
Angelman syndr.	15q11-q13	Dysmorphic syndr. by genomic imprinting
Lejeune syndr.	5p-	Dysmorphic syndr. by genomic imprinting
Hirschorn syndr.	4p-	Dysmorphic syndr. by genomic imprinting
Retinoblastoma	13q14	Embryonic neoplasm of retinal cells (childhood cancer)
DiGeorge syndr.	8q24.11-13	Mental retardation, microcephaly, dysmorphism, etc.

Duplications

Duplications, like deletions, can originate by unequal crossing over or by abnormal segregation from meiosis in a carrier of a translocation or inversion. Because duplication in a gamete results in chromosomal imbalance and because the chromosome breaks that generate it may disrupt genes; duplication often leads to some phenotypic abnormality.

Ring chromosomes

Ring chromosomes are formed when a chromosome undergoes two breaks and the broken ends of the chromosome reunite in a ring structure. If the centromere is within the ring, the two distal fragments, lacking a centromere, are lost.

Ring chromosomes have been detected for every human chromosome, but more frequent can be seen for X- chromosome.

Isochromosomes

An isochromosome is a chromosome in which one arm is missing and the other reduplicated. It results from a division error where a chromosome splits horizontally instead of vertically. An isochromosome forms when a chromosome lines up incorrectly and is pulled apart incorrectly, leading to a division across the centromere. This creates an unbalanced structural abnormality. An individual carrying an isochromosome therefore has a single copy of the genetic material of one arm and three copies of the genetic material of the other arm or, in other words, is partially monosomic and partially trisomic.

The most common isochromosome is an isochromosome of the long arm of the X chromosome i(Xq). However, isochromosome has also been described for the short arm of the X chromosome (fewer cases) and for a number of autosomes, including isochromosomes for the short arm of chromosome 18, i(18p) and for the short arm of chromosome 12, i(12p). Isocromosomes are also frequently seen in karyotypes of both solid tumors and hematological malignancies.

Dicentric chromosomes

A dicentric is a rare type of abnormal chromosome in which two chromosome segments (from different chromosomes or from the two chromatids of a single one), each with a centromere, fuse end to end, with loss of their acentric fragments. Dicentrics, because of their two centromeres, tend to break at anaphase; if the two centromeres are close together, or if one becomes inactivated, a dicentric may be stable. Such chromosomes are sometimes called “pseudodicentrics”. The most common pseudodicentrics involve one or both of the sex chromosomes.

Marker chromosomes

Marker chromosomes are defined as abnormal chromosomes that cannot be fully characterized based on standard cytogenetic analyses because of their modified banding landmarks. These markers are known to be heterogeneous with respect to size and composition.

Balanced rearrangements

Chromosomal rearrangements do not usually have a phenotypic effect if they are balanced, because all the genetic information is present even though it is differently packaged. However, structural rearrangements pose a threat to be subsequent generation, because carriers are likely to produce unbalanced gametes and therefore have an increased risk of having abnormal offspring with unbalanced karyotypes.

There is also a possibility that one of the chromosome breaks will disrupt a gene, leading to mutation. This is well – documented cause of X-linked diseases in female carriers of balanced rearrangements of the X chromosome.

Balanced translocations are rather common in couples who have had two or more spontaneous abortions, in infertility and sterility.

Inversions

An inversion occurs when a single chromosome undergoes two breaks and is reconstituted with the segment between the breaks inverted. Inversions are of two types: *paracentric* (beside the centromere) in which both breaks occur in one arm, and *pericentric* (around the centromere) in which there is a break in each arm.

Because paracentric inversions do not change the arm ratio of the chromosome, only banding can identify them. Pericentric inversions are easier to identify cytogenetically because they wary change the proportion of the chromosome arms as well as the banding pattern. An inversion does not usually cause an abnormal phenotype in carriers, as it is a balanced rearrangement. Its medical significance is for the progeny; a carrier of either type of inversion is at risk of producing abnormal gametes that may lead to unbalanced offspring. This risk is 1 to 10%.

Translocations

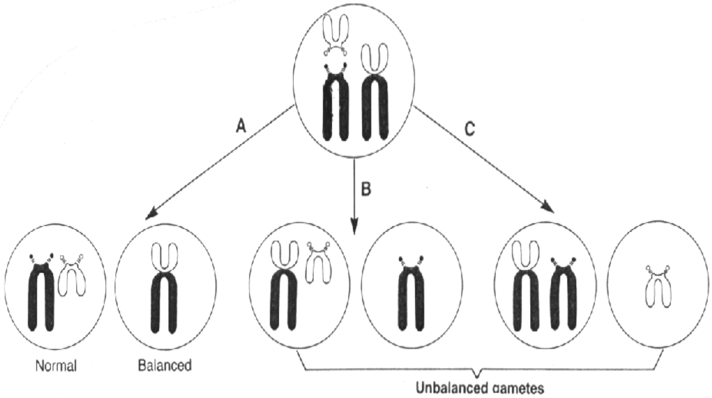
Translocation involves the exchange of chromosome segments between nonhomologous chromosomes. There are two main types: ***reciprocal*** and ***Robertsonian.***

If the transfer of genetic information between the two chromosomes appears to be complete and is not associated with a loss of material, the translocation is reciprocal and balanced.

Robertsonian translocations are a special case, being technically associated with or without a loss of genetic material, but generally not associated with phenotypic abnormalities. They are a specific class of translocation in which two acrocentric chromosomes fuse at their centric ends. In humans, 13, 14, 15, 21 and 22 are acrocentric chromosomes and there are numerous examples of robertsonian translocations involving each of them.

As the short arms of all of the acrocentric chromosomes contain copies of the genes that cod for ribosomal RNA, the loss of the short arms of two acrocentric chromosomes is not deleterious and, individuals with this type of rearrangement are generally phenotypically normal.

Certain robertsonian translocation carriers may, however, be at increased risk for having children with abnormalities due to malsegregation of chromosomes during meiosis. Although a carrier of a Robertsonian translocation is phenotypically normal, there is a risk of unbalanced gametes and therefore of unbalanced offspring (figure). The chief clinical importance of this type of translocation is that carriers of a Robertsonian translocation-involving chromosome 21 are at risk of producing a child with translocation Down syndrome, the most common structural abnormality.



Chromosomes of gametes that can be produced by a carrier of a Robertsonian translocation

Partial monosomy (4p-) Wolf-Hirschorn syndrome

Partial monosomy of the short arm of chromosome 4 causes a distinct syndrome characterized by recognizable facial dysmorphism with marked growth retardation and severe mental retardation. The distinctive face is characterized by 'Greek warrior helmet' aspect, with high forehead, hypertelorism, high-arched eyebrows, protruding eyes, epicanthal folds, strabismus, short philtrum, distinct mouth with downturned corners, and low-set ears a.o.

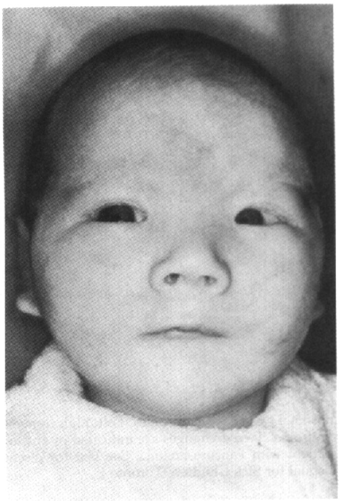
About 15% of patients have micrognathia, closure defects of the mouth, malformations of the teeth. Approximately 85-90% of cases result from "de novo" deletions.

Partial monosomy (5p-) Cri du chat syndrome

Cri-du-chat syndrome was first described by Lejeune et al. being associated with a deletion of part of the short arm of chromosome 5. The deletions can vary in size, from very small and involving only band 5p15.2 to the entire p arm.

Partial deletion of the short arm of chromosome 5 is named "the cri du chat syndrome" because it is associated with a laryngeal defect that causes a distinctive cry. Patients present microcephaly, craniofacial dysmorphism, round face, micrognathia, epicanthal folds, hypertelorism, low-set ears, micrognathia and severe psychomotor and mental retardation. Most patients demonstrate muscular hypotonia, and heart disease. The catlike cry disappears over the first year and development is severely delayed; mental handicap is usually severe. Approximately 85% of cases result from "de novo" deletions.

With advancing age the clinical picture of the syndrome becomes less striking.



An infant with "cri du chat" syndrome (5p-)
(from Genetics in Medicine, Thompson, 1991)

18 q monosomy

The main clinical manifestations are: growth retardation, impaired intellectual development, craniofacial dysmorphism including round face, dysplastic ears, wide mouth, teeth anomalies, abnormalities of the limbs, brain, eyes and heart. Genotype-phenotype correlations showed that patients with deletions in the centromeric region at 18p11.1 had mental retardation, but other cases with distal deletions had normal intelligence or borderline mental retardation, suggesting that a critical region for mental retardation may exist between 18p11.21 and 18p11.1.

18 q monosomy

The breakpoints of the deletions on the long arm of chromosome 18 have been found variable from the telomere to the centromere. 75% of patients have a "de novo" deletion, with the remainder resulting from familial rearrangements. The smaller deletions produce a milder phenotype. The patients have growth deficiency, facial dysmorphia, microcephaly, genitourinary malformations, choreoathetotic movements and spinal muscular atrophy. Mental retardation is generally present, but varies from mild to severe. Usually they have some learning disabilities.

Isochromosome 18p

The marker chromosome for isochromosome 18p is a small, metacentric marker. Patients with isochromosome 18p are characterized by low birth weight, microcephaly, hypotonia and some facial dysmorphism (a round face with small low set ears, short palpebral fissures). Mental handicap in the moderate to severe range is present in all patients.

Cat eye syndrome

The characteristic chromosomal change is the presence of a small, extra-acrocentric marker chromosome, derived from chromosome 22.

One of the features of the syndrome is coloboma of the iris. Other features include heart malformations, imperforate anus, urinary tract anomalies and mild to moderate mental handicap. Some patients have a normal intelligence.

Structural abnormalities of X chromosome

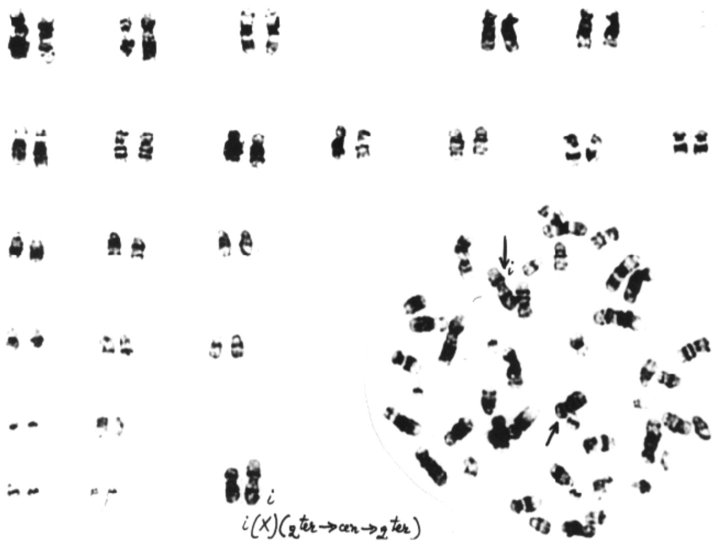
There are some structural abnormalities of X chromosome, which cause other forms of gonadal dysgenesis:

46,Xi(Xq)	isochromosome for the long arm of X
46,Xdel(Xq)	deletion of the long arm of X
46,Xdel(Xp)	deletion of the short arm of X

46,Xr(X)	ring X
46,Xi(Xp)	isochromosome for the short arm of X
46,X,t(X;X) or	X-X translocation
46,X,ter	terminal rearrangement
46,X,t(X; autosom)	X-autosom translocation

An i(Xq) isochromosome consists of two copies of the entire long arm and no short arm. Some isochromosomes have one centromere and are perfectly symmetrical, while others are symmetrical but have two centromeres close to each other. Females with this karyotype resemble those with 45,X but have milder clinical abnormalities. Individuals with 46,X,del(Xp) are often less affected than in which the whole X is missing (they are taller than patients with Turner syndrome and a proportion of them have menstruation). However, deletions in proximal Xp produce primary amenorrhea, while those individuals with telomeric deletions of Xp often have secondary amenorrhea and infertylity. Females with a del(Xq) have primary amenorrhea but, compared with those with 45,X, a greater proportion menstruate. The implication is that normal gonadal development requires the presence of genes on Xq but not the entire Xq. Height is variably affected, depending on how many genes are missing.

Ring chromosomes 46,X,r(X) are special cases of partial monosomy. A ring X chromosome is formed when breaks occur at both ends of chromosome and the proximal ends rejoin. Such patients often display typical features of Turner syndrome.



G-banded karyotipe 46,Xi(Xq), with isochromosome for the long arm of X (our collection).

Syndromes with microdeletions and microduplications

Microcytogenetics studies submicroscopic chromosomal abnormalities, which are chromosomal deletions or duplications that are very small, spanning less than 5Mb, and cannot be detected by light microscopy using conventional cytogenetics methods. Advances in molecular cytogenetics have identified a growing number of diseases caused by changes in some small parts of the genetic material. Newer techniques such as Fluorescence in situ Hybridization (FISH) allow visualization of such submicroscopic chromosomal losses or gains of genetic material.

Depending on the molecular mechanisms that generate submicroscopic chromosomal abnormalities in human pathology there are:

1. syndromes with microdeletion with/or without genomic imprinting
    - Prader-Willi syndrome and Angelman syndrome;
    - Williams syndrome;
    - 22q11.2 deletion syndrome (also known as DiGeorge Syndrome, Velo-Cardio-Facial);
    - Rubinstein Taybi syndrome;
    - Smith-Magenis syndrome;
    - retinoblastoma;
    - nephroblastoma;
2. syndromes with microduplication with/or without genomic imprinting
    - Beckwith-Wiedemann syndrome

Microdeletion syndromes

Microdeletions lead to abnormal phenotypes because they cause haploinssuficiency (the total level of a protein is 50% of the normal level, resulting in an abnormal phenotype).

Prader-Willi syndrome

It is estimated that about 70% of the patients have a deletion of chromosome 15q11-q13 that is inherited from the father (genomic imprinting). The others occur due to other mechanisms, such as maternal uniparental disomy: both chromosomes 15 are inherited from the mother and the locus is imprinted or an imprinting defect.

Clinical features: short stature, developmental delay, hypotonia, obesity, hypogonadism, mild to moderate mental retardation, small hands and feet, almond shaped eyes, arched palate, microdontia.

Angelman syndrome

This syndrome is characterized by severe mental and motor retardation, ataxia, seizures, microcephaly, inappropriate laughter, absence of speech, macrostomia, large jaw, protruding tongue, mandibular prognathism. It is estimated that about 70% of the patients have a deletion of chromosome 15q11-q13 that is inherited from the mother (genomic imprinting). The other cases occur due to other mechanisms, such as paternal uniparental disomy: both chromosomes 15 are inherited from the father and the locus is imprinted, an imprinting defect in one chromosome 15 or point mutations in maternal chromosome 15 critical region.

Willliams syndrome

It is due to a microdeletion 7q11.23. It is usually sporadic, with negative family history. Characteristics: distinct facial dysmorphism with long philtrum, thick, wide lips, open mouth, hypodontia, microdontia; cardiovascular anomalies; growth deficiency; musculoskeletal abnormalities.

Rubinstein Taybi syndrome

The syndrome is characterized by the following phenotype: microcephaly, facial anomalies: antimongoloid orientation of the palpebral fissures, maxillary hypoplasia, ogival palate, beaked nose; broad thumb and first toe; short stature; mental retardation. Genetics of the syndrome: it is due to a microdeletion 16p13.3. It may be familial or sporadic. Transmission is AD with variable expressivity.

Microduplication syndromes

Beckwith-Wiedemann syndrome

It is determined by a microduplicaton of 11p 15.5. Characteristics of the syndrome include: macrosomia, macroglossia, organomegaly, hemihypertrophy, dental anomalies such as: widely spaced teeth likely due to macroglossia, speech problems.

Chapter VII

CARDIOGENETICS

Elements of Cardiogenetics

Cardiogenetics is the field of medicine that focuses on understanding how genetic factors contribute to cardiovascular diseases (CVD).

It merges principles of genetics with cardiology:

- to uncover the hereditary causes of heart diseases,
- identify genetic risk factors,
- improve prevention, diagnosis, and treatment strategies.

Importance of Cardiogenetics:

Cardiovascular disorders represent one of the leading causes of morbidity and mortality worldwide.

Genetics plays a pivotal role in the development of both common and rare heart conditions. Some patients may inherit mutations in genes that predispose them to conditions like arrhythmias, cardiomyopathies, and congenital heart defects. Identifying these genetic predispositions is critical in managing heart disease risk, especially in individuals with a positive family history.

It involves analyzing the genes and genetic variations linked to heart-related disorders eg: coronary artery disease, arrhythmias, cardiomyopathies, familial hypercholesterolemia and congenital heart defects.

By understanding the genetic factors that contribute to these conditions, researchers and health care professionals can develop more targeted and personalized treatments. Identification of the genetic cause in a family enables us to identify individuals who may be at risk for developing these disorders. Early detection, timely preventive measures, and monitoring are as crucial as treatment in managing these conditions, improving outcomes and quality of life.

Genetic Inheritance and Cardiovascular Diseases

Genetic mutations associated with cardiovascular diseases can be inherited in various ways:

- Autosomal Dominant Inheritance: Common cardiovascular conditions associated with this inheritance include familial hypercholesterolemia and hypertrophic cardiomyopathy (HCM). Family members of affected individuals have a 50% chance of inheriting the gene mutation.
- Autosomal Recessive Inheritance: Conditions like familial dilated cardiomyopathy (DCM) or certain forms of arrhythmogenic right ventricular cardiomyopathy (ARVC) can follow this inheritance pattern.
- **X-linked Inheritance**: Some cardiovascular diseases, particularly those involving the muscles of the heart, may follow an X-linked pattern, where males are more commonly affected because they have only one X chromosome. Examples include certain forms of Duchenne muscular dystrophy, which can lead to cardiomyopathy.
- **Mitochondrial Inheritance**: Some conditions related to mitochondrial dysfunction; ex. MELAS syndrome, which can cause

heart failure, follow a mitochondrial inheritance pattern. (MELAS-mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes).

Common Cardiovascular Conditions with Genetic Implications

Familial Hypercholesterolemia (FH)

Hypertrophic Cardiomyopathy: one of the most common inherited cardiac disorders, characterized by thickening of the heart muscle, often leading to obstruction of the left ventricle.

Typically-A.D. pattern. Variants implicated in HCM are predominantly found in genes encoding cardiac-specific isoforms of sarcomere proteins eg. MYH7 (myosin heavy chain) and TNNT2 (troponin T), a.o.

Dilated Cardiomyopathy: is characterized by left ventricular dilation and systolic dysfunction, may result from mutations in a variety of genes, including those for TTN (titin), LMNA (lamin A/C), and DSP (desmoplakin). It can be inherited in both autosomal dominant and recessive patterns.

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC): a disorder of the heart muscle that can lead to arrhythmias and sudden cardiac death. It often affects the right ventricle and is caused by mutations in genes like PKP2 (plakophilin-2), which play a role in the heart muscle's structure. It is characterized by structural and functional abnormalities of the right ventricle caused by the replacement of the myocardium by fatty and fibrous tissue. The inheritance is AD. with variable penetrance and incomplete expression

There are several ECG features in the criteria diagnosis of ARVD:

a) T wave inversions in V1 through V3 (minor diagnostic criterion, but one of most common ECG abnormality present in 85% of patients

b) QRS duration = 110 ms in V1 through V3 (n: 80-100 millisec)

c) Epsilon wave (electric potentials after the end of the QRS complex). It is a major diagnostic criterion found in up to 30% of cases.

Ventricular arrhythmias without structural heart disease mainly include:

- monomorphic ventricular tachycardia classified by location of origin
- polymorphic ventricular tachycardia dominated by primary hereditary arrhythmia syndrome, and ventricular fibrillation, i.e., Brugada syndrome (BrS), congenital long QT syndrome (LQTS), short QT syndrome (SQTS). The clinical presentations vary, including palpitations, vertigo, syncope, seizure-like activity and sudden cardiac death.

Etiology:

- Monogenic factors: mutations in the sodium channel-encoding gene SCN5A, the potassium channel-encoding genes KCNQ1 and KCNH2, and the calcium channel-encoding gene RYR2 cause the majority of ventricular arrhythmias
- Polygenic factors

Their importance is highlighted by the heterogeneity of causative genes across patients and the impact of the accumulation of mutations in multiple genes on the severity of the clinical phenotype

- Epigenetics-examples
  - Non-coding RNA-Circular RNA may serve as a marker for disease progression.
  - DNA methylation usually plays a repressive role in gene transcription. For example, SCN5A (Sodium channel protein type 5 subunit alpha) promoter hypermethylation levels enhance the expression of the gene in cardiac tissue.

Brugada syndrome

- is a rare but potentially life-threatening inherited disease that predisposes patients to fatal cardiac arrhythmias.
- is characterized by the ECG findings of a right bundle branch block and ST-segment elevations in the right precordial leads (V1-V3).

Etiology: loss-of-function mutation in the cardiac voltage-gated sodium channel gene SCN5A-in 15-30% of cases +mutations in calcium and potassium channels genes. Inherited in an AD pattern; but may demonstrate variable expressivity and reduced penetrance. Many environmental and genetic factors may influence the phenotype, eg. temperature, medications, electrolyte abnormalities, and cocaine.

Long QT syndrome

- is characterized by QT prolongation (440 ms in men or greater than 460 ms in women) and T wave abnormalities on EKG. LQTS predisposes individuals to a significant risk of life-threatening arrhythmic events, especially in young individuals.
- Pathogenic variants in KCNH2, KCNQ1, and SCN5A account for 90% of individuals.

- associated with tachyarrhythmias, typically the ventricular tachycardia torsade de pointes (TdP). TdP may cause palpitations, dizziness, or syncope (fainting), the most common symptom in individuals with LQTS. Syncope is typically precipitous and without warning. A careful evaluation of medical history can help distinguish LQTS-associated syncope from common vasovagal and orthostatic forms of syncope in which presyncope and other warning symptoms occur.
-

Hypertrophic and dilated cardiomyopathy

Hypertrophic cardiomyopathy is characterized by a hypertrophied left and/or right ventricle, rapid upstroke pulse, prominent apex beat and a late systolic ejection.

Genetic mutations cause a significant percentage of cardiomyopathies. In hypertrophic cardiomyopathy mutations in genes encoding the heavy chains of myosin and myosin binding protein C (MYH7 and MYBPC3) explain 75% of inherited forms, leading to the observation that this form is a disease of sarcomeres. Many mutations are variants or rare mutations, often occurring in a particular family.

By contrast, dilated cardiomyopathy is more heterogeneous from the genetic point of view, with mutations of cytoskeletal, nucleoskeletal, mitochondrial genes, etc. Over 50 unique gene variants have been identified in this form. It is characterized by left ventricular dilation and systolic dysfunction, may result from mutations in a variety of genes, including those for TTN (titin), LMNA (lamin A/C), and DSP (desmoplakin). It can be inherited in both autosomal dominant and recessive patterns.

Cardiomyopathies with or without arrhythmias are associated with some monogenic diseases (lipidosis).

Congenital heart defects

Congenital heart anomalies are the most common forms of birth defects. They have a complex etiology:

- chromosomal abnormalities, such as trisomy 13, 18 or 21
- single-gene disorders, such as some metabolic diseases
- multifactorial
- teratogenic, due to rubella, maternal drug ingestion, maternal diabetes a.o.

Genetic Testing in Cardiogenetics

Genetic testing plays an important role in cardiogenetics by providing valuable insights into an individual's genetic predisposition to cardiovascular diseases.

It is particularly helpful in:

- **Diagnosis**: Identifying the genetic mutations underlying specific heart conditions can confirm a diagnosis, especially when the clinical presentation is ambiguous.
- **Risk Assessment**: Genetic testing helps in assessing the risk of developing heart disease in individuals with a family history, allowing for earlier intervention and monitoring.
- **Family Screening**: Inherited cardiac conditions often run in families, and genetic testing allows for the identification of at-risk family members who may benefit from early surveillance or preventive measures.
- **Personalized Treatment**: As medicine moves toward precision medicine, genetic information can help tailor treatment plans, from medications to lifestyle modifications.

Gene Panels in Cardiogenetics

Gene panels allow for more comprehensive testing in patients who present with multiple symptoms or who have a family history of heart disease but for whom the specific cause is unclear.

Here are a few examples of commonly used gene panels:

- Cardiomyopathy Panel: This panel tests for mutations in genes associated with both hypertrophic and dilated cardiomyopathies. It often includes genes like MYH7, TNNT2, TPM1, MYBPC3, LMNA, TTN, and others.
- Arrhythmia Panel: This tests for genes related to inherited arrhythmias, including SCN5A (linked to long QT syndrome and other arrhythmic disorders), KCNQ1 (linked to Long QT syndrome), KCNH2, and RYR2 (related to catecholaminergic polymorphic ventricular tachycardia).
- Sudden Cardiac Death (SCD) Panel: This panel may include genes like RYR2 (for arrhythmogenic right ventricular cardiomyopathy), KCNQ1 (for Long QT syndrome), and PKP2 (for arrhythmogenic cardiomyopathy).
-

Key takeaways

- Cardiogenetics is a rapidly evolving field that is transforming the way we understand and treat heart disease.
- By identifying genetic mutations associated with cardiovascular disorders, we can offer better management and counseling for patients and their families.
- Genetic testing plays a vital role in diagnosing inherited cardiovascular diseases, assessing risk, and guiding treatment.

- With ongoing advancements in genetic testing and an increasing focus on personalized medicine, cardiogenetics will play a pivotal role in the future of cardiovascular care, offering hope for improved prevention, diagnosis, and targeted treatment options for patients with inherited heart conditions.

Chapter VIII

NEUROGENETICS

Introduction

Neurogenetics examines the genetic underpinnings of nervous system development, function, and disease. It focuses mainly on the genetic basis of neurodegenerative diseases and mental diseases, as well as on the development of potential methods of preventing these diseases.

By integrating genomics with neurobiology, researchers elucidate mechanisms of neurodegeneration, developmental disorders, and channelopathies.

Key Concepts

- *Repeat Expansion Disorders:* Pathogenic trinucleotide repeats cause diseases like Huntington’s and certain ataxias.
- *Mendelian vs. Multifactorial:* Disorders such as spinal muscular atrophy (SMA) follow simple autosomal-recessive inheritance, while Parkinson’s disease often involves complex interplay of common and rare variants.
- *Somatic vs. Germline Mutations:* Somatic mosaicism can underlie focal cortical dysplasia; germline variants drive hereditary neurodegenerative diseases.

Deciding on the genetic tests to order can be simplified by narrowing the differential diagnosis and defining the patient's phenotype.

Huntington's Disease (HD)

Genetics: A.D., CAG repeat expansion in HTT, with ≥36 repeats leading to disease; larger expansions correlate with earlier onset.

Clinical Features: Chorea, psychiatric disturbances, cognitive decline in mid-adult life.

Genetic Testing: PCR-based sizing of CAG repeats; pre-symptomatic testing with genetic counseling.

Epilepsy

Epilepsy is a disorder of the brain characterized by recurrent uncontrolled attacks (seizures). A partial epilepsy includes benign childhood epilepsy with convulsions that occur from time to time caused by numerous pathological processes such as: head injuries, meningitis, perinatal asfixia, hypoxia, etc. About 20% of the patients with epilepsy have a genetic etiology; thus, any of the genes encoding proteins involved in neuronal excitability can initiate an epileptic seizure. The familial segregation provides evidence for a genetic contribution to etiology in specific human epilepsies.

Spinal muscular atrophy

The term spinal muscular atrophy (SMA) comprises a genetically heterogeneous group of diseases characterized by degeneration and loss of the anterior horn cells in the spinal cord and sometimes also in the brain nuclei, resulting in muscle weakness and atrophy. The spinal muscular atrophies are divided into autosomal recessive and dominant types, but clinically there is no difference between their symptomatology and severity. The clinical signs of the most severe type are evident soon after birth or after 1 month with hypotonia and generalized weakness. Infants are never able to sit unaided, first legs are paralyzed, than he arms also.in other types the onset is during childhood. All patients have severe respiratory insufficiency.

Genetics: Homozygous deletion (or mutation) of SMN1; severity modulated by copy number of paralog SMN2.

Clinical Features: Proximal muscle weakness, absent reflexes; types I–IV distinguished by age of onset and motor milestones.

Genetic Testing: Quantitative PCR or MLPA to determine SMN1 deletion and SMN2 copy number; newborn screening panels increasingly include SMA.

Therapy: **gene therapy** : onasemnogene abeparvovec-xioi (brand name **Zolgensma**®).

- **nusinersen** (brand name **Spinraza**®) and **risdiplam** (brand name **Evrysdia**®) for the treatment of SMA in children and adults.

Amyotrophic lateral sclerosis (ALS)

a neurodegenerative disease; primarily affects motor neurons, leading to progressive muscle weakness and death from respiratory failure within 2–5 years of symptom onset

~ 10% of patients have a + family history

>30 ALS-associated genes have been identified

Pathogenic variants in the most common genes: superoxide dismutase 1 (SOD1), TAR DNA-binding protein (TARDBP), fused in sarcoma (FUS) and chromosome 9 open reading frame 72 (C9orf72), account for ~ 60% of familial cases

~ 10% of sporadic ALS.

- characterized by progressive muscular paralysis reflecting degeneration of motor neurons in the primary motor cortex, corticospinal tracts, brainstem and spinal cord.

Hereditary ataxia

Definition: A heterogeneous group of genetic disorders that involve the degeneration of cerebellar- and extracerebellar-related circuits, leading to the development of progressive abnormal gait, dysarthria (motor speech disorder), and ataxia (lack of muscle coordination).

Prevalence: ~1–2 /100,000 individuals.

Inheritance Patterns: A.D., a.r., and X-linked.

Clinical Features: gait ataxia, dysarthria, dysphagia (difficulty in swallowing), and oculomotor dysfunction.

Classification-based on the mode of inheritance

Autosomal domunant cerebellar ataxias (ADCAs)-spinocerebellar ataxia (SCA). SCA3 (OMIM # 109150) is one of the most common forms. The unstable CAG repeat in ataxin-3 gene characterizes this form. The normal range: up to 44 repeats, while SCA3 patients have: 52- 86 repeats.

Autosomal recessive cerebellar ataxias (ARCAs): Friedreich's ataxia (FA—OMIM #229300) is the most frequent. The onset of clinical symptoms- before 25 (mean age of onset: 10–15 years); disease progression leads to wheelchair dependence within 10 years of disease onset. The classic presentation: progressive gait and limb ataxia, dysarthria, polyneuropathy, and sensory loss caused by a non-coding GAA trinucleotide expansion within the FXN gene encoding frataxin.

Friedreich's Ataxia (FRDA): due to expansion of GAA repeats in the FXN gene. Onset typically between ages 5–15.

Clinical Features: Progressive gait ataxia, dysarthria, and scoliosis. Cardiomyopathy and diabetes are common.

Management: Symptomatic treatment for cardiac and endocrine issues. Omaveloxolone (Skyclarys) approved for treatment.

Advances in Therapeutic Strategies

- Gene Therapy:

AAV-based delivery of FXN gene in FRDA shows promising preclinical results.

- Pharmacological Agents: Omaveloxolone (Skyclarys) for FRDA. EryDex in Phase 3 trials for A-T.
- Symptomatic Treatments: Physical therapy for gait and balance. Speech therapy for dysarthria.
- Cardiac and endocrine management in FRDA.

Charcot–Marie–Tooth Disease (CMT)

Genetics: Over 100 genes; the most common is PMP22 duplication (type 1A, AD.).

Clinical Features: Distal muscle weakness, foot deformities (pes cavus), sensory loss, reduced nerve conduction velocities.

Genetic Testing: Multiplex ligation-dependent probe amplification for PMP22 copy number; NGS panels for rarer subtypes.

Parkinson disease

characterized by an onset age of > 50 years, tremor at rest, gait complaints and falls, bradykinesia, rigidity and painful cramps. The exact etiology is still unknown; mutations in the genes SNCA (4q21.3-q22), LRRK2 (12q12), and VPS35 (16q12) have been implicated in its pathogenesis. Heterozygous mutations in GBA (1p22; glucocerebrosidase) are the most common risk factor. Transmission is AD.

Alzheimer's disease

AD is the most common neurodegenerative disease in the world. It is classified as familial and sporadic. Inheritance: dominant autosomal: 1–5% of the total number of cases. It is categorized as early onset (EOAD; <65 years of age) and presents genetic mutations in presenilin 1 (PSEN1), presenilin 2 (PSEN2), or the Amyloid precursor protein (APP). Sporadic AD represents 95% of the cases, is categorized as late-onset (LOAD), occurring in patients older than 65.

Conclusions

Advances in genomic sequencing and functional modeling have revolutionized our understanding of both ocular and neurological hereditary diseases.

Genetic testing—ranging from targeted panels to WGS—enables precise molecular diagnosis, informs genetic counseling, and paves the way for gene-specific therapies such as antisense oligonucleotides and gene replacement.

As research deepens, integrating multi-omics (epigenomics, transcriptomics) will further elucidate disease mechanisms and accelerate personalized medicine in ophthalmology and neurology.

Chapter IX
OPHTHALMOLOGICAL GENETICS

Introduction in ocular genetics and genomics

Ocular genetics and genomics is the study of how inherited genetic variation contributes to eye development, function, and disease. With advances in sequencing technologies, researchers have uncovered dozens of genes whose mutations lead to vision impairment or blindness.

Genomic approaches enable comprehensive analysis of both coding and noncoding regions, facilitating discovery of novel disease genes and refinement of genotype–phenotype correlations.

Key concepts

- Monogenic vs. Complex Traits: Many early-onset retinal dystrophies are single-gene disorders, whereas common conditions like age-related macular degeneration (AMD) have complex, multifactorial inheritance.
- Allelic Heterogeneity: Different mutations in the same gene can produce variable phenotypes (e.g., missense vs. null variants in the RHO gene causing retinitis pigmentosa).

- Locus Heterogeneity: Similar clinical presentations may arise from mutations in different genes (e.g., >60 genes implicated in retinal dystrophies).
- Modifier Genes: Secondary variants can influence disease severity and age of onset.

Hereditary Eye Disorders-examples

Retinitis Pigmentosa (RP)

Genetics: Over 80 genes (e.g., RHO, USH2A, RPGR) with autosomal-dominant, autosomal-recessive, or X-linked inheritance.

Clinical Features: Night blindness, progressive peripheral vision loss, bone-spicule pigmentation on fundus exam.

Genetic Testing: Comprehensive inherited retinal disease panels (100–300 genes) via NGS; WES for unsolved cases.

Congenital cataract

Genetics: Mutations in crystallin genes (CRYAA, CRYBB2), connexins (GJA3, GJA8), and transcription factors (PITX3).

Clinical Features: Lens opacities apparent in infancy or childhood, leading to vision deprivation and amblyopia if untreated.

Genetic Testing: Targeted gene panels for pediatric cataract; where negative, WES may identify novel or CNVs.

Inherited glaucoma

Genetics: Mutations in CYP1B1 (Cytochrome P450 1B1) (most common autosomal-recessive cause), MYOC (myocilin) (autosomal-dominant juvenile open-angle glaucoma).

Clinical Features: Elevated intraocular pressure, optic nerve cupping, progressive visual field loss; congenital forms present in infancy with buphthalmos (enlargement of the eyeball).

Genetic Testing: CYP1B1 sequencing for congenital cases; glaucoma panels including MYOC, OPTN, TBK1 for adult-onset forms.

Genomic Technologies in Ocular Diseases

- ***Targeted Gene Panels:*** Cost-effective, high coverage for known ocular disease genes.
- ***WES:*** Captures all protein-coding regions, useful for genetically heterogeneous conditions.
- ***WGS:*** Detects noncoding variants, structural rearrangements, and deep intronic mutations.
- ***Copy-Number Variant (CNV) Analysis:*** MLPA or bioinformatic pipelines applied to NGS data to detect deletions/duplications.
- ***Functional Assays:*** eg. CRISPR-Cas9 editing help validate variant pathogenicity.

Chapter X

THE CHROMOSOMAL BASIS FOR SEX DETERMINATION. DISORDERS OF SEXUAL DEVELOPMENT

In humans, the process of sex determination and differentiation is strictly genetically initiated and controlled, mainly by genes on sex chromosomes, but, in different stages, some autosomal genes are also involved. In humans, biological sex is formed through two successive and complex phenomena: genetic determinism of sex and sexual differentiation.

Genetic determinism of sex

Genetic determinism of sex refers to achieving the genetic sex and gonad formation (ovaries or testes). The genetic sex is formed during fertilization of gametes. Chromosomal sex (46, XX or 46, XY) is given by the type of sex chromosome from the spermatozoon involved in fertilization.

Embryology of the reproductive systems

The X and Y chromosomes differ between genders, have their own specific patterns of inheritance and are involved in primary sex determination. It is well known that Y chromosome has a crucial role in normal male development. In the absence of a Y chromosome, the XX embryo will have a female development.

Development of the gonads: By the sixth week of development in both sexes, the primordial germ cells migrate from their extraembryonic location (endoderm of the yolk sac) to the primitive gonadal ridges to form the indifferent gonad. 46,XY and 46,XX gonads are initially indistinguishable and bipotential.

The current concept is that development into an ovary or a testis is determined by the coordinated action of a sequence of genes that leads to ovarian development when no Y chromosome is present or to testicular development if a Y is present. In other words, the primitive gonads develop into testes if the embryo is 46,XY. This process begins about 43 days after conception (7 to 8 weeks after conception).

In the presence of a Y chromosome, the medullary tissue forms typical testes with seminiferous tubules and Leydig cells, which, under the stimulation of gonadotrophin from placenta, become capable of androgen secretion. The spermatogonia, derived from primordial germ cells, form the walls of the seminiferous tubules together with supporting Sertoli cells.

If no Y chromosome is present the gonad forms an ovary; the cortex develops, the medulla regresses and ovogonia begin to develop within follicles. The oogonia are derived from the primitive germ cells. Beginning at about the end of the third month, the oogonia enter meiosis I, but this process is arrested at a stage called dictyotene, in which the cells remain until ovulation occurs at puberty. Many of the oogonia degenerate before birth, and only about 380-400 mature into ova during the 30 years or so of sexual maturity of the female.

Gonadal differentiation are key events that lead to phenotypic sex development, a complex process, regulated by different mechanisms, with the involvement of at least 30 genes located both on the sex chromosomes and on autosomes.

While the primordial germ cells are migrating to the genital ridges, thickenings in the ridges indicate the developing internal genital ducts, the mesonephric ducts (Wolffian ducts) and paramesonephric duct (Müllerian ducts). In the male, the Leydig cells of the fetal testes produce androgen which stimulates the formation of the male genital ducts, and the Sertoli cells produce a hormon that suppresses formation of the Müllerian ducts (female ducts). In the female embryo, the Wolffian ducts regress.

In the early fetus the external genitalia consist of a genital tubercle, paired labioscrotal swellings and paired urethral folds. From this undifferentiated state, male external genitalia develop under the influence of fetal androgenes, or in the absence of a testis, female external genitalia are formed regardless whether an ovary is present.

Neuro-hormonal and neurobehavioral sexual differentiation includes modulation of nervous structures, particularly the hypothalamus, according to the karyotype (XX or XY). They gradually add sex-specific characteristics. In humans, control of sexuality goes from hormone to cortical control.

Pubertal sexual differentiation is the last stage, which establishes secondary sexual characteristics and hence sexual maturity, with the production of gametes capable of fertilization. Age of onset is variable between genders and is influenced by genetic, social, climatic, nutritional or psychological factors.

The X and Y chromosomes

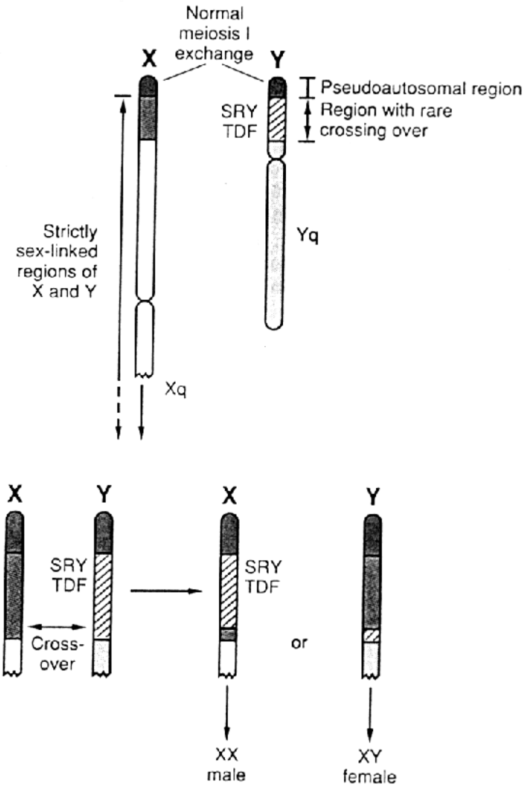
The Y chromosome

The Y chromosome is a small chromosome (~60 Mb) with male-specific region (MSY). It contains SRY and AZF regions, as well as pseudoautosomal regions (PAR1, PAR2) that allow pairing with the X chromosome.

The structures of the Y chromosome and its role in sexual development have been analyzed at the molecular level. In male meiosis, the X and Y chromosomes normally pair by segments at the ends of their short arms and undergo recombination in that region. The pairing segment includes the pseudoautosomal region of the X and Y-chromosomes, so called because the X and Y of this region are homologous to one another. In rare instances, genetic recombination occurs between the X and Y short arms outside of the pseudoautosomal region, and this aberrant exchange mechanism can produce two abnormalities: XX males and XY females.

XX males are phenotypic males with a 46,XX karyotype who usually possess some Y chromosome sequences translocated to the short arm of the X chromosome.

It is estimated that the Y chromosome contains about 70 coding genes, but there are also noncoding genes and pseudogenes. The genes on this chromosome are involved in male sex determination and development.



Schematic of normal (above) and aberrant exchange (below) between X-and Y-linked sequences

SRY gene is located in the male specific region of the short arm "p" of the Y chromosome (Yp11.2), in the immediate vicinity of pseudoautosomal region 1 (PAR1). SRY gene has a dominant role in initiating male development, transformation of undifferentiated bipotential gonad in embryonic testes, a process that begins in weeks 6-7 of intrauterine development. It triggers testis determination by activating SOX9.

KDM5D gene (also known as HYA gene) is on the "q" arm of the Y chromosome. The gene encodes a transcription factor domain, and together with SRY act on the bipotential gonads, resulting in their transformation into testes. A peptide derived from the protein encoded by this gene is a minor histocompatibility antigen which may lead to rejection of a graft from a male donor in a female recipient.

AZF gene (synonyms: AZO, AZF- Azoospermia Factor) has a role in spermatogenesis. Deletions in AZF gene lead to variable phenotypes, from the complete absence of Sertoli cells to blocked spermatogenesis.

GCY gene (Growth Control Y also known as STA, tooth size TS, TSY) is responsible for height development and psycho-behavioral development.

In humans, the presence of the Y chromosome is compulsory for male gonads formation. Male genes on the Y chromosome are highly penetrant, so that the presence of one or more X chromosomes in plus (for example 47, XXY or 48, XXXY) does not alter the gonadic sex, but will disrupt spermatogenesis and phenotypic appearance.

In addition to genes on the Y chromosome, it is clear that testicular differentiation requires loci on X chromosome and from the autosomes. Thus, on the X chromosome there is a gene called gene for androgen receptor (AR), a receptor protein that has the role of forming a complex with testosterone, important for the function of this hormone. Moreover, some of the autosomal genes are also important for testicular differentiation.

The X chromosome

For years, many efforts have been directed toward localizing those regions of the X chromosome important for ovarian development. A locus in region Xp11.2-11.4 and in Xq11.3-Xq27 is clearly important for ovarian development and maintenance. Interestingly, statural determinants must exist in the distal region Xp because individuals with these terminal deletions are short. Similar topography appears to exist on the X long arm (Xq), being involved in ovarian maintenance; their deletions lead to gonadal failure.

The X chromosome carries genes important for ovarian development DAX1 (NR0B1), which antagonizes SRY action.

As mentioned on the "p" arm of the X chromosome is located a gene called AR (androgen receptor), which specifies the androgen receptor. Together with dihydrotestosterone (DHT), the androgen receptors play a role in sexual differentiation of male internal genitalia. Mutations of "AR" gene lead to androgen insensitivity syndrome (complete or partial).

The X chromosome has in the telomeric region of the "p" arms a pseudo-autosomal region, homologous to that of the Y chromosome and crossing-over takes place between these two regions.

Although in female somatic cells one X is partial inactivated, several regions of the short arms contain genes that escape inactivation and continue to be expressed from both chromosomes in females. From the study of structurally abnormal, inactivated X-chromosomes, it has been proposed that there is an X inactivation center, a locus that must be present on an X chromosome for inactivation to occur. Some of the genes escape inactivation; for example, the gene for steroid sulfatase remains fully active. Deficiency of this gene causes the X-linked disorder ichtyosis.

Many other X-linked genes have been mapped to specific regions of the X-chromosome such as: gene for Duchenne or Becker muscular dystrophy, gene for glucose–6-phosphate–dehydrogenase deficiency, gene for hemophilia A (deficiency of factor VIII), gene for hemophilia B (deficiency of factor IX) etc.

Disorders of sexual development

The term “Disorders of sexual development” (DSD) is used today to refer to any problem in which genitalia are atypical in relation to the chromosomes or gonads. The incidence of DSD is estimated at about 1 in 4,500-5,500 births. A former term was intersex, refering to discordances between organic sex criteria. Phenotypic sex is abnormal, sometimes ambiguous, with anomalies that tend to make them resemble those of the opposite chromosomal sex and usually the gender of the newborn cannot be established at birth. Such anomalies may vary from mild forms to very ambiguous genitalia. They may be due to cytogenetic abnormalities or to single-gene defects; fewer may be due to nongenetic causes. Disorders of sexual development are a group of rare diseases that raise questions concerning the diagnosis, treatment and gender assignement.

Classification of ambiguous genitalia is difficult because similar or identical phenotypes may have several different etiologies. The Pediatric Endocrine Society (LWPES) and the European Society for Paediatric Endocrinology (ESPE) consensus group proposed the classification of DSDs into:

1. Sex chromosome DSDs (45,X monosomy and variants, 47,XXY syndrome and variants, 45X/46XY mixed gonadal disgenesis and chromosomal ovotesticular DSD “46XX/46XY chimeric type or mosaic type”
2. 46,XY DSDs (disorders of testicular development or disorders in androgen synthesis/action)
3. 46,XX DSDs (disorders of ovarian development or fetal androgen excess)

1. Sex chromosome DSDs

45,X monosomy (Turner syndrome) and variants, and 47,XXY syndrome (Klinefelter syndrome) and variants were discussed in another chapter (chromosomal disorders).

45,X/46,XY mixed gonadal dysgenesis results from Y-chromosome mosaicism. These individuals manifest a variety of phenotypes, ranging from ambiguous genitalia to a completely male or female phenotype. They have mixed gonadal dysgenesis, with one gonad that is dysgenetic testis and the other a streak gonad. In addition to that, they frequently have müllerian derivates (e.g. uterus). Asymmetry of the external and internal genitalia may also be present. Infants with male sex assignment may have cryptorchidism, partial testicular dysgenesis or hypospadias. Infants with female sex assignment present with varying degrees of virilization and may show features of Turner syndrome. Short stature may be present in both genders. There is an increased risk for gonadoblastomas and dysgerminomas.

Chromosomal ovotesticular DSD “46,XX/46,XY chimeric type or mosaic type” was called hermaphroditism. There is both testicular and ovarian tissue and usually ambiguous genitalia. A testis or an ovotestis is more likely to be present; a uterus is usually present and menstruation is not uncommon, occasionally manifested as hematuria. Breast development usually occurs at puberty, even with predominantly male external genitalia. A small percentage are chimeras, whose tissues are composed of a mixture of XX and XY cells. Unlike a mosaic, in which the cell lines are derived from a single zygote, a chimera is composed of cells derived from different zygotes. Chimerism can occur when dizygotic twins exchange hematopoietic stem cells in utero or, very rarely, when two separate zygotes are fused into one individual. If the original zygotes were of different sexes, chimera may result.

In 46,XY DSDs and in 46,XX DSDs there is a concordance between the karyotype and the gonads, but the external genitalia are ambiguous or characteristic of the opposite chromosomal sex.

2. 46,XY DSDs

The causes of 46,XY DSDs include dysgenesis of the gonads during embryological development, abnormalities of gonadotropins, inborn errors of testosterone biosynthesis and many abnormalities of androgen target cells. These disorders are very heterogeneous both genetically and clinically.

Disorders of testicular development

46,XY complete gonadal dysgenesis (Swyer syndrome) is characterized by anomalies in gonadal development, which occur in the presence of female external and internal genitalia, in the presence of a 46,XY karyotype. Patients have a female phenotype, female external genitalia, but lack of female sexual secondary characteristics at puberty. They have undeveloped streak gonads and an increased risk of malignancy (gonads should be surgically removed). Stature is normal. The etiology is not completely understood, it is considered to be due to failure of testicular development, which occurs as a result of mutations in several genes and disruption of underlying genetic pathways.

Disorders in androgen synthesis/action

Androgen biosynthesis defects may result from deficiencies of some enzymes, such as: 3 β-dehydrogenase, 17 α- hydroxylase, etc. Ambiguous external genitalia may also due to a deficiency of 5α-reductase, the enzyme that converts testosterone to dihydrotestosterone (DHT), encoded by an autosomal recessive gene.

Androgen insensitivity syndrome is due to a mutation of the gene that encodes androgen receptors. It is an X-linked disorder, which may be of two types: complete or incomplete androgen insensitivity. In the complete form affected persons are chromosomal males (karyotype 46,XY) with apparently normal female external genitalia, with a blind vagina and no uterus or uterine tubes. Usually the typical presentation is primary amenorrhea in a teenager girl. Axillary and pubic hair is sparse. As the alternative name “testicular feminization“ indicates, testes are present either within the abdomen or in the inghinal anal. Although the testes secrete androgen normally, there is end organ unresponsiveness to androgens resulting from absence of androgen receptors in the cells. The receptor protein, specified by the normal allele at the X-linked androgen receptor (AR) locus, has the role of forming a complex with dihydrotestosterone. If the complex fails to form, the hormone cannot enter the nucleus and it stops the differentiation in the male direction. In the incomplete androgen insensitivity forms external genitalia are ambiguous, the clinical severity ranging from female external genitalia, ambiguity to male external genitalia in the presence of a 46,XY karyotype.

3. 46,XX DSDs (disorders of ovarian development or fetal androgen excess)

46, XX DSDs due to defects in ovarian development

Disorders of sex development due to defects in ovarian development include ovotesticular 46, XX DSDs and testicular 46, XX DSDs. The ovotesticular form typically presents at birth with ambiguous genitalia and progressive virilization during puberty. In contrast, people with the testicular form, usually have a normal male phenotype and the absence of

Mullerian derivates. About 80-90% of patients will have SRY gene translocated on the X chromosome, which is only rarely detected in the ovotesticular form.

46, XX DSDs due to androgen excess

Congenital adrenal hyperplasia is a group of autosomal recessive disorders arising from specific defects in the enzyme 21-hydroxylase of the adrenal cortex required for cortisol biosynthesis. Deficiency of 21-hydroxylase blocks the normal biosynthetic pathway, causing overproduction of the precursors, which are then shunted into the pathway of androgen biosynthesis, causing abnormally high androgen levels. Affected male infants have normal external genitalia, but in childhood, androgen excess leads to rapid growth and accelerated skeletal maturation. About 75% have a salt losing type that is clinically more severe and may lead to neonatal death. Female infants homozygous for 21-hydroxylase deficiency are born with ambiguous genitalia, often requiring surgical correction. Females heterozygous for 21-hydroxylase deficiency show a high virilization (hirsutism), oligomenorrhea or amenorrhea and infertility or sterility. This form may be associated with polycystic ovary.

The 21-hydroxylase locus is on the short arm of chromosome 6, within the HLA major histocompatibility complex. Different forms (allelic forms) of congenital adrenal hyperplasia show associations with different HLA haplotypes.

Investigation of a newborn/teenager with a DSD has to be done by a multidisciplinary team. It is necessary for establishing the gender that will be assigned to, anticipate medical problems, explaining etiology and for developing a management plan that will lead to the best results on long term. Gender assignment and surgical timing are sensitive issues.

Chapter XI

ELEMENTS OF TERATOLOGY

Dysmorphology is the study of human congenital anomalies that originate before birth. The term “dysmorphic” (from the Greek terms for abnormal shape) is used to describe people with physical features that are not usually found in other individuals with the same age or ethnic background.

Congenital defects, also known as birth defects, are structural or functional abnormalities that occur during intrauterine life and are present at birth. They may affect different organs or systems and can vary in severity from mild to life-threatening. These anomalies can result from genetic, environmental, or unknown causes.

Prenatal onset of developmental problems

Congenital defect or congenital anomaly refers to structural abnormalities that are present at birth. Some of these abnormalities are single primary defects in development and others are multiple malformations syndromes.

Conceptually, single primary defect in development is an anatomic or morphogenetic designation; in most cases, the defect involves only a single structure. From a practical standpoint, most single primary defects are explained on the basis of multifactorial inheritance.

In contrast to the concept of the single primary defect in development, the designation “multiple malformation syndrome” indicates that the observed structural defects all have the same etiology. The defects themselves usually include a number of errors in morphogenesis. Multiple malformation syndromes are caused by gross chromosomal abnormalities, chromosomal microdeletions, teratogens, and single gene defects usually having Mendelian patterns of inheritance.

Single primary defect in development

Single primary defects can be subcategorized according to the nature of the error in morphogenesis. Thus, single primary defects involve malformation, deformation, disruption or dysplasia of developing structure.

A ***malformation*** implies a primary structural defect arising from a localized error in morphogenesis, usually occurring before 10 weeks of gestation (examples: cleft palate, anencephaly).

A ***deformation*** should be thought of as an alteration usually through compression in shape and/or structure of a part that has differentiated normally.

The term ***dysplasia*** refers to an abnormal organization of cells and the structural consequences. Dysplasia may be localized or generalized.

Disruptive defects occur when there is destruction of a previously normally formed part. These are at least two basic mechanisms known to produce disruption. One involves entanglement followed by renting or amputation of a normally developed structure, usually a digit, arm or leg by “amniotic bands”. The second mechanism through which disruption occurs involves the interruption of blood supply to a developing part.

Sequence of multiple anomalies refers to the pattern of multiples anomalies that occurs when a single primary defect in early morphogenesis produces multiple abnormalities through a cascading process of secondary and tertiary errors in morphogenesis.

When evaluating a child with multiple anomalies it is extremely important from the standpoint of recurrence risk counseling to differentiate between multiple anomalies secondary to a single localized error in morphogenesis (a sequence) and a multiple malformation syndrome. In a sequence, recurrence risk counseling for the multiple anomalies depends entirely upon the recurrence risk for the initiating, localized error.

Multiple malformation syndromes

The category of multiple malformation syndromes includes patients, in whom a primary developmental anomaly of two or more systems has occurred, all of which are thought to be due to a common etiology. Multiple malformation syndromes can be categorized on the basis of etiology.

- Chromosomal disorders (e.g. Patau, Down syndrome)
- Microdeletion syndromes (e.g. Prader-Willi syndrome)
- Single gene disorders (e.g. Apert syndrome)
- Multifactorial disorders (e.g. club foot)
- Environmental causes (teratogenesis) (e.g. rubella, diabetic mother)

Chromosomal abnormalities

The ability to perform chromosomal studies has led to the recognition of a number of multiple malformation syndromes due to chromosomal abnormalities. Since chromosomes are present in all cells of the body, a chromosome aberration may be expected to affect adversely many parts of the body, but some sex-chromosome disorders (e.g., XXY, XXX, XYY) have few, if any, defects recognizable at birth.

The most common disorder associated with a chromosomal abnormality is the Down syndrome (trisomy 21). The principal features of the disorder (flat facies with upward slant to the palpebral fissures, hypotonia and small ears) are usually present at birth.

Other malformations associated with a chromosomal abnormality are well known in trisomy 18 and trisomy 13.

Congenital defects can be classified based on:

1. **Morphological nature**
   - Structural (e.g., cleft lip, heart defects)
   - Functional or metabolic (e.g., enzyme deficiencies)
2. **Etiological basis**
   - Genetic causes
   - Environmental causes
   - Multifactorial inheritance
   - Unknown causes
3. **Timing of developmental insult**
   - Embryonic period (major malformations)
   - Fetal period (functional or minor structural defects)

The etiology of congenital defects is complex and includes:

1. Genetic factors–Chromosomal abnormalities or single-gene mutations.

2. Environmental factors (teratogens) – Drugs, infections, radiation, or chemicals.

3. Multifactorial inheritance – Interaction of genetic predisposition and environmental exposure.

4. Unknown causes – Approximately 40–60% of cases have no identifiable cause.

Teratology

Teratology is the branch of science that studies abnormal development and congenital anomalies. It examines the causes, mechanisms, and manifestations of developmental defects. Teratogens are agents that can disturb the development of an embryo or fetus, leading to birth defects or pregnancy loss.

Disorders caused by teratogens include multiple malformation syndromes due to the effect of specific infections, drugs or chemical agents with which the embryo or fetus has come into contact. These disorders take on special importance because they represent the only group of dysmorphologic conditions in which prevention before conception may be feasible, but it is difficult for a pregnant woman to avoid contact with all infectious agents.

A careful history of drug intake and chemical exposure should be obtained from the parents of all children with multiple malformation syndromes.

Although a specific and easily distinguishable phenotype does not exist for each of the infectious agents that are commonly associated with altered fetal development, intrauterine infection can be suspected based on the overall pattern of malformation. But intrauterine infections can have a wide spectrum, from microphtalmia, cataracts to severely affected newborn infant with multiple malformations, to the child with a significant problems in learning.

Characterization of an environmental teratogen

Teratogens may act by a number of pathogenic processes, which may produce cellular death, alter tissue growth (hypoplasia, or asynchronous growth), or interfere with cellular differentiation. Some agents may also act by disrupting normally developing structures. Environmental factors that alter one of these processes are expected to affect more than one tissue. These general effects may be produced by a number of teratogenic agents, which are playing prominent roles.

Such indicators of teratogenic potential are as follows:

- infertility or fetal wastage
- prenatal onset growth deficiency
- alterations of morphogenesis
- alterations of nervous system performance

The teratogenic activities are common to many agents. Several agents have been found to have even a mutagenic potential (e.g., diethylstilbestrol), although not necessarily produced by the same pathogenic mechanism.

The teratogenic potential of an agent is commonly expressed over a wide spectrum when all exposed individuals are considered.

The effect of teratogenic agents depends on:

- dose of agent
- timing of exposure

The possible drug effects vary according to the gestation phase in which they are administrated:

✓ *Until 10 days* the rule of “all or nothing” is thought to be valid, that is either miscarriage or no effect.

✓ *The days from the 7th to the 57th* are the most risky because organogenesis takes place. The toxic action of the teratogens in this phase leads to defects of the structures that are developing at that moment.

✓ *After the 12th week* the fetal period begins, considered relatively less risky. The toxic agent can cause growth delay, reduction of the organic growth and functional damages.

- host susceptibility - e.g. genetic variation (maternal or fetal)
- interactions with other environmental factors.

The most sensible is the CNS because of the immaturity of the haematoencefalic barrier. In this case timing of exposure is not relevant, as structural or functional problems may occur during the entire pregnancy.

Together, these factors probably account for the vast amount of variation encountered among patients adversely affected by prenatal exposure to environmental hazard. It is clear that genetic variations (either maternal or fetal) may account for substantial differences in susceptibility to many agents.

Categories of teratogens

Teratogenic agents may be conveniently grouped into four major categories: infectious agents, physical agents, drug and chemical agents, and maternal metabolic and genetic factors.

Infectious agents

For many years clinicians have been aware of infections agents that may attack the fetus in utero, including viruses, bacteria and parasites.

Recognized effects on the fetus include intrauterine growth retardation, congenital defects, mental retardation but often fetal death.

The pathogenesis of these abnormalities can generally be ascribed to direct invasion of the fetus, with inflammation of fetal tissues and cellular death. Many of these defects represent the “disruption” pathogenic category.

Direct invasion of the nervous system may result in microcephaly, often associated with cerebral calcifications, mental retardation, disorders of movement and muscle tonicity and central auditory and visual deficits. As the central nervous system controls limb movement, in cases where severe central nervous system damage has occurred, contractures and other fetal limb deformations are more frequently encountered. Other general abnormalities associated with prenatal infections include prematurity, low weight, failure to thrive and congenital heart disease.

Viruses

Infants exposed to rubella virus during the first trimester of pregnancy may display - ocular defects as cataracts, microphtalmia, and cardiovascular anomalies including valvular pulmonary arterial stenosis, atrial and ventricular septal defects, other vascular stenotic. The sensory or functional disturbances are also found, including sensorineural deafness. The adverse effects of rubella infection in utero are related to the stage of gestation in which the infection is acquired.

Cytomegalovirus represents the most common congenital infection cause of mental retardation and other central nervous system disorders, including deafness.

As with rubella, the adverse effects of cytomegalovirus infection in utero are related to the stage of gestation. The classical picture of severe congenital cytomegalovirus infection includes central nervous system manifestations such as microcephaly, diffuse periventricular calcifications reflecting extensive encephalitis. Less frequently, hydrocephalus may develop. These central nervous system abnormalities are associated with functional disturbances, including mental retardation, spasticity and hypotonia. Ocular involvement is common with optic atrophy, microphtalmia and cataracts.

Herpes simplex type I and II, may occasionally have teratogenic effects, including intracranial calcifications, ocular anomalies and others.

Infection with varicella in utero may cause growth deficiency, microcephaly and mental retardation.

Several enteroviruses and viral agents for influenza, mumps and measles have also been suggested to produce fetal abnormalities.

Bacteria

Syphilis and perhaps mycoplasmas are thought to have a significant teratogenic potential. Infants with congenital syphilis have widespread manifestations including visual impairment, late neurological features such as mental retardation, hydrocephalus, convulsions and optic atrophy, craniofacial deformities, malformations of teeth, sensorineural deafness, and many other complications.

Listeriosis has been implicated as a cause of abortion, stillbirth and neonatal death.

Parasites

Toxoplasmosis has been clearly shown to produce congenital anomalies. Congenital toxoplasmosis has been recognized for several decades. The severity of effects on fetus is strongly related to the stage of gestation in which the exposure occurs.

Exposure during the first trimester of pregnancy may lead to spontaneous abortion. Clinically apparent disease is commonly associated with central nervous system problems arising from severe generalized infection, resulting in microcephaly, hydrocephaly, intracranial calcification and the functional concomitants of mental retardation and seizures.

Physical agents

A wide variety of physical agents are potentially teratogenic for the fetus. Among these, the most important include ionizing radiation and mechanical factors.

- Ionizing Radiation: causes DNA damage leading to microcephaly, growth retardation, and mental retardation. Example: Hiroshima and Nagasaki survivors.
- Hyperthermia: High maternal fever or external heat can cause neural tube defects and craniofacial abnormalities.

Concern over possible teratogenic effects of high-energy radiation came from studies with experimental animals and epidemiologic studies of the offspring of survivors of nuclear explosions. Defects that may occur are: microcephaly, spina bifida, cleft palate, blindness. Mechanical factors are represented by intrauterine amniotic fibrous strands, which may disrupt the fetal body or craniofacial complex, resulting in many disruptions.

Drug and chemical agents

Since the thalidomide disaster, increasing attention has been focused on the role of drug and chemical agents in the environment to which pregnant women might be exposed. Far too little is known about the teratogenic potential of most drugs and chemicals.

Some agents with potentially significant teratogenic risk

Alcohol	Methyltestosterone
Aminopterin	Oxytetracycline
Captopril	Penicillamine
Carbamazepine	Phenytoin
Cocaine	Primidone
Danazol	Propylthiouracil
Dicoumarol	Quinine
Diethylstilbestrol	Streptomycine
Doxycicline	Testosterone
Isotretinoin	Tetracycline
Kanamycin	Tobacco
Lithium	Toluene
Mercaptopurine	Trimethadione
Methimazole	Valproic acid
	Warfarin

The pathogenic mechanisms by which most drugs produce birth defects include interference with cellular growth; they also can destroy tissue directly. Some drugs and chemical agents may act by interfering with nervous system function, thereby enhancing the liability to deformation-type fetal anomalies.

Prescription drugs

- Most antineoplastic agents have been found to be teratogenic in various animal species.
- Anticoagulant agents are clearly implicated in the pathogenesis of birth defects. Fetal warfarin syndrome is characterized by delayed growth, defects of the limbs, eyes and CNS.
- Antibiotics (most of them) and antiviral agents have teratogenic potential. For example: *Tetracycline* is known to cause teeth discoloration yellow-tawny colour of the teeth, associated to enamel hypoplasia, skeletal malformations, bony growth delay. Vancomycin and aminoglicosides are oto and nephrotoxic for the fetus.
- Anticonvulsivants may also have a significant teratogenic potential for at least some exposed infants. Fetal hydantoin syndrome is characterized by intrauterine growth retardation, developmental delay, mental retardation, cranio-facial abnormalities.
- Thalidomide – limb reduction defects (phocomelia), ear and cardiac anomalies.
- ACE inhibitors – renal dysgenesis, oligohydramnios, skull ossification defects.

It is now clear that prenatal use of ethanol can result in a wide spectrum of effects on the fetus. The *fetal alcohol syndrome* is characterized by a distinctive facial appearance, prenatal onset growth deficiency, increased frequency of developmental delay and mental retardation, and increased frequency of major congenital anomalies. Children with this syndrome display flat midface with narrow palpebral fissures and low nasal bridge. In addition to developmental delay and mental retardation, such children are often poorly coordinated and sometimes hyperactive in later life.

A wide variety of congenital anomalies have been associated with this syndrome, including cleft palate, congenital cardiac defects, microphtalmia, dermal and skeletal abnormalities.

Significant implications for fetal growth and development are associated with tobacco smoking.

Maternal metabolic factors

The main maternal metabolic disorder with possible teratogenic effects on the developing fetus is *diabetes mellitus*. The juvenile onset insulin-dependent diabetes increases the risk for prenatal wastage and for fetal anomalies. Congenital heart defects and sacral agenesis are more frequent in infants born to insulin-dependent diabetics than in the general population. An increased frequency of other major congenital anomalies, in particular clefts of the lip and palate and neural tube defects has also been reported. Maternal *hypothyroidism* causes growth and mental retardation. *Folate deficiency* causes neural tube defects (spina bifida, anencephaly).

Maternal genetic disorders

Maternal phenylketonuria is always associated with serious risks to the exposed fetus. Pregnancy in an untreated phenylketonuric mother results in exposure of the heterozygous fetus to very high serum levels of phenylalanine, phenylpyruvic acid and other potentially toxic metabolites. In nearly all instances this results in serious damage of the fetus including mental retardation in over 90%, and congenital anomalies in some 25%. Intrauterine growth retardation and microcephaly are the most common features.

Approach to a dysmorphic individual

A genetic etiology of the dysmorphic signs should be suspected in a child with:

- Congenital anomalies
- Growth deficit
- Developmental delay,
- Mental deficit or developmental regression
- Ambiguous genitalia
- Failure to develop secondary sexual characteristics

Family history together with the personal medical history and physical examination help establishing the diagnosis.

Confirmation of diagnosis will be made based on laboratory tests, such as chromosome analysis or molecular studies, and other investigations.

Intervention with treatment of symptoms and cause, if this is known. Genetic counseling and follow-up are important not only for the patient, but also for other family members.

Prevention and Counseling

- Preconception care: folic acid supplementation, control of maternal diseases.
- Avoidance of teratogenic drugs and substances during pregnancy.
- Immunization against rubella before conception.
- Genetic counseling for high-risk families.
- Use of safer drug alternatives when treatment is necessary.

Chapter XII

ONCOGENETICS

Cancer is caused by changes in genes that control:

- cell proliferation,
- mitotic cycle,
- DNA repair,
- apoptosis

All cancers derive from cells that have acquired the characteristics of continually dividing in an unrestrained manner and invading surrounding tissues.

Cancer characteristics:

- Most cancers originate in a single cell. In this regard, a cancerous growth can be considered clonal.
- At the cellular and genetic levels, cancer is usually a multistep process: It begins with a precancerous genetic change (i.e., a benign growth). Following additional genetic changes, it progresses to cancerous cell growth.
- Once a cellular growth has become malignant, the cells are invasive (i.e., they can invade healthy tissues). They are also metastatic (i.e., they can migrate to other parts of the body).

Cancer is a genetic disease. Not all cancers are hereditary. It is estimated that about 5% of all cancers are hereditary. Though environmental factors undoubtedly contribute to cancer, the classic view is that cancers arise through a multistem process driven by mutation of cellular genes, with increasingly aggressive growth properties.

Usually, there have to be≥ 6 mutations, which accumulate over time, before a cell becomes malignant – *driver* mutations: confer a fitness advantage to somatic cells in their microenvironment, thereby driving the cell lineage to cancer. They are defined as changes in the DNA sequence of genes that cause cells to become malignant and grow and spread in the body. *Passenger* mutations are mutations that provide no such proliferative benefit. Mutations either increase cell division rates or inhibit normal controls of cell growth, such as apoptosis or cell cycle arrest.

Molecular biology of cancer

Knudson's '2-hit' hypothesis: the first 'hit' in the development of a familial tumour occurs in the germline in a cancer susceptibility gene and the second 'hit' occurs somatically in the other allele of the same gene.

These mutations occur in three classes of cellular genes: proto-oncogenes, tumor-suppressor genes and DNA repair genes.

The vast majority of mutations are somatic, that is, present only in the tumor cells. A relatively small subset of these mutations may be present in the germline of individuals and predispose them to various cancer types.

Usually, there have to be 6 or more mutations, which accumulate over time, before a cell becomes malignant. Little is yet known about the importance of the order of mutation acquisition for malignant transformation. Newer studies revealed the importance of this order in the clinical presentation, evolution and prognosis of some hematological malignancies.

Evolution, differentiation and vital functions content, all require a precise control of the time and place of cell division. Malignant cells divide and grow uncontrollably. Mutations either increase cell division rates or inhibit normal controls of cell growth, such as apoptosis or cell cycle arrest.

Mutations in cancer cells alter the normal structure and/or the expression pattern of the proto-oncogene and thus generate oncogenic variant forms with altered function. In genetic terms, these oncogenic alleles have "*gain of function*" mutations.

Proto-oncogene is defined as a normal gene, involved in some aspect of cell division or proliferation that if activated by a mutational event, is capable of becoming an oncogene. ***Oncogene*** is defined as a dominantly acting gene involved in unregulated cell growth and proliferation, responsible for tumor development. Mutation, over expression or amplification of oncogenes in somatic cells may lead to neoplastic transformation.

Tumor-suppressor gene is a normal gene involved in the regulation of cell growth. Recessive mutations can lead to tumor development. In contrast to the activating mutations that generate oncogenic alleles from proto-oncogenes, the *loss of tumor suppressor gene function* by mutation contributes to tumor development. Both alleles of the tumor suppressor gene have to lose their function to cause malignancies. This involves two steps, in different times. The first step is loss of functionality of an allele and the next step is inactivation/loss of the other allele. Many tumor suppressor genes have been identified. For most tumors, several mutational events must occur in several genes before malignancies develop.

Similar to the proto-oncogenes, the normal functions of tumor suppressor genes are diverse, and the proteins encoded by these genes are found in many cellular compartments.

Recent studies have established the critical role of mutations involving a third class of genes in the cancer process - the *DNA repair genes*. Like the tumor suppressor genes, loss of function mutations in these genes is the basis for their role in tumorigenesis.

Viral oncogenes

RNA tumor viruses and the identification of proto-oncogenes

It is noteworthy that many (but not all) of the oncogenes identified in human tumors have turned out to be related to viral oncogenes isolated from RNA tumor viruses. The demonstration that genetic information from a virus could change a normal cell into a malignant one established that genes could act as central controllers of malignant conversion. RNA viruses, known as *retroviruses*, have the property of transcribing RNA into DNA, using the enzyme reverse transcriptase. The viral DNA can then be integrated into the DNA of the host and expressed.

RNA tumor viruses can be divided into two classes - acute and chronic transforming viruses. The chronic transforming viruses are capable of producing tumors only in hosts in which the virus can replicate; the tumor arises after relatively long latency period and at specific tissue sites. The acute transforming viruses cause tumors after short latency period.

The chronic transforming viruses transform cells by random integration of a DNA copy of the virus, termed the *provirus*, into the host cell genome. The provirus can alter the genes in the region of the host chromosome, where it integrates. If a proto-oncogene is contained in the region, provirus integration can alter the structure and expression of the proto-oncogene and thus contribute to tumorigenesis (e.g. leukemias, lymphomas and breast cancer).

In contrast to the chronic transforming viruses, the genomes of the acute transforming viruses contain nucleic acid sequences that have been acquired or transduced from the host cell as result of genetic recombination. The host derived sequences are termed *viral oncogenes* (V-oncogenes), which are directly responsible for the rapid transforming activity. A viral oncogene is not a true viral gene but a host gene that had been picked up by an ancestor of the virus through a process termed *transduction*.

DNA tumor viruses

In general, while the RNA tumor viruses promote tumor development by harboring or generating oncogenic alleles, several DNA tumor viruses primarily contribute to tumorigenesis by activating or inhibiting the activities of tumor suppressor proteins. DNA tumor viruses contain double-stranded DNA and use the replication system of the host cell.

Examples of DNA human viruses:

- Epstein Barr virus that causes Burkitt's lymphoma;
- hepatitis B virus, which causes hepatocarcinoma;
- herpes viruses, associated with different cances
- papillomavirus, which causes cervical cancer

Oncogenes identified by study of chromosomal translocation

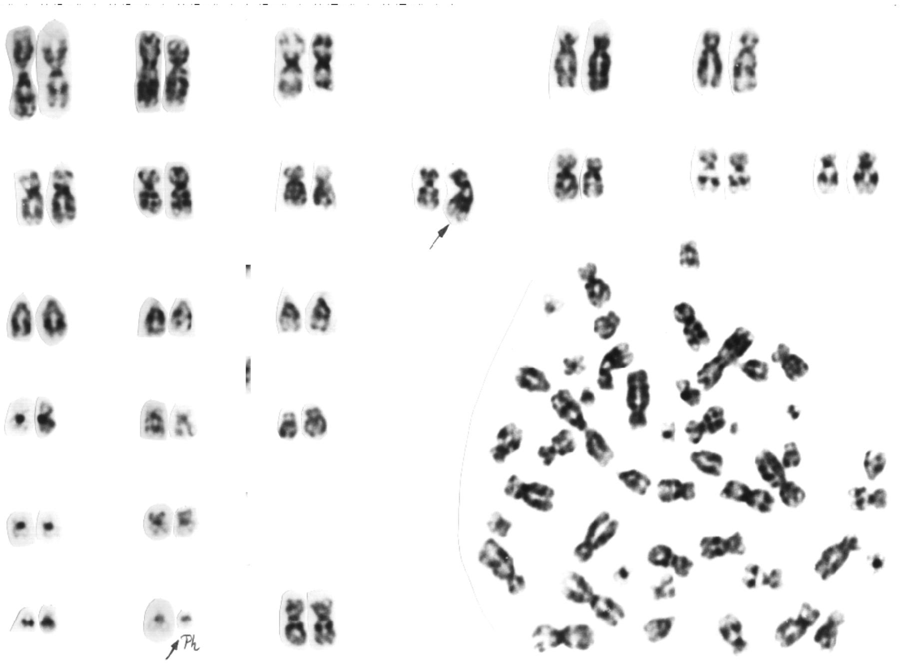
The chromosomal alterations observed in cancer cells have proven particularly valuable for the identification of oncogenic alleles. In many cases, a strong correlation has been established between a particular chromosomal abnormality and the type of the tumor, and even its specific histopathologic subtype. Standard karyotypic analyses have identified an enormous number of specific chromosomal abnormalities in leukemias and lymphomas and in many solid tumors.

Chromosome abnormalities represent a ***genomic signature*** that is linked to cancer prognosis and reaction to chemotherapy and immunotherapy.

Many of the chromosomal abnormalities in leukemias and lymphomas involve translocation between an immunoglobulin (Ig) locus or T-cell receptor locus and a novel gene as a result of a recombination error, which generated the translocation.

A number of other translocation break points have also been characterized in hematopoietic malignancies. Many of these translocations, while deregulating gene expression, also generate chimeric proteins by fusing gene sequences from one side of the break point to gene sequences on the other side of the break point. The first example of such an oncogenic allele encoding a chimeric protein was provided by the elucidation of the molecular basis of the Philadelphia chromosome. The Philadelphia chromosome is generated by a translocation involving chromosomes 9 and 22, and can be identified by cytogenetic studies in the vast majority of patients with chronic myeloid leukemia (CML) and in few cases with lymphoid leukemia.

The translocation fuses chromosome 9q sequences from the ABL gene (a cellular homologue of a V-oncogene) to sequences from chromosome 22q. The novel sequences on chromosome 22q are termed the BCR (for *break point cluster region*) gene. The resultant chimeric BCR - ABL generates a protein kinase with altered structure and function.



G-banded karyotype showing the Philadelphia chromosome translocation t (9q34;22q11) in chronic myeloid leukemia (from our collection)

In Burkitt's lymphoma majority of cases have a chromosomal translocation between chromosome 8 and 14. As a result of this translocation some sequences on chromosome 14 are fused to sequences on chromosomes 8, (oncogene c-myc from chromosome 8 with the gene that encodes the Ig heavy chains from chromosome 14), which alter gene expression.

Gene duplication is common in certain tumors and may be visible like extrachromosomal small fragments or like homogeneous colored regions, in light microscopy. Oncogenes that most often duplicated are members of the following gene families: MYC, RAS, EGFR, FGF, CDK4.

Tumor-supressor genes

Whereas the products of proto-oncogenes promote growth, the products of tumor-suppressor genes normally block abnormal growth and malignant transformation, and contribute to malignancy only when the function of both alleles are lost (mutations in tumor-suppressor genes are recessive).

Tumor-suppressor genes are implicated in several forms of cancer (some with mendelian inheritance) including retinoblastoma (malignant tumor of retina in infants), neurofibromatosis type 1 (a common autosomal dominant disorder that primarily affects the peripheral nervous system and is characterized by neurofibromas), Wilms tumor (an embryonic kidney tumor) and familial polyposis coli (adenomatous polyposis).

Retinoblastoma, the prototype of diseases caused by mutations in tumor-suppressor genes, is a malignant tumor of retina in infants. About 40% of cases are of the heritable form, in which the child inherits one mutant allele at the retinoblastoma locus through the germline, and a somatic mutation (new mutation) which causes loss of function of the remaining normal allele.

The other 60 % of cases of retinoblastoma are nonheritable (they are sporadic); in these cases both retinoblastoma alleles in a single retinal cell have been inactivated by somatic mutation.

The retinoblastoma gene has been mapped to chromosome 13, in band 13q14. The inherited mutation in a few percent of patients is due to deletion or translocation of this portion of chromosome 13.

Wilms tumor, an embryogenic kidney tumor in which is involved a tumor-suppressor gene. It is sometimes associated whit a cytogenetically visible aberration of chromosome 11p13. However, this may not be the only gene predisposing to Wilms tumor, because there are multiple genes expressed in the kidney from this region of chromosome 11 and because in some Wilms tumor families the predisposing locus maps to 11p15 and in others, the map position is still unknown.

Familial polyposis coli, also known as familial adenomatous polyposis is characterized by growing of numerous benign polyps in the colon during the first two decades of life (more than 100 polyps), in heterozygotes persons. In almost all cases, one or more of the polyps become malignant. Surgical removal (colectomy) prevents the development of malignancy.

The responsible gene has been located on chromosome 5q22.2 by demonstration of loss of heterozygozity in colon tumors. The gene has 15 exons and mutations frequently affect exon 15.

Gardner syndrome is determined by a gene that maps to the same region on chromosome 5q and is probably allelic to the gene predispoding to familial poliposis coli. Besides familial poliposis coli, patients have an increased risk of developing other cancers, such as small bowel, stomach, pancreas, thyroid, central nervous system, liver, and/or adrenal gland cancer.

There are several other genetic forms of colon cancer. Some are associated with mutations in MUTYH gene (1p34.1), causing the autosomal recessive familial adenomatous polyposis. This gene encodes a protein needed to correct the errors that occur during DNA replication (DNA repair gene). Mutations of the gene will lead to colon polyps and risk of colon cancer.

Hereditary breast cancer

Breast cancer has long been recognized to have a strong genetic component. A woman’s risk of developing breast cancer is increased up to the threefold if one first-degree relative is affected and up tenfold if more than one first-degree relative is affected. Although as much as 20 % of all breast cancer cases may have a significant genetic component as part of a polygenic or multifactorial mode of inheritance, a small proportion of cases appear to be due to a dominantly inherited Mendelian predisposition to breast cancer. Genetic linkage studies in families with early-onset familial breast cancer have localized a gene (BRCA gene) for increased susceptibility to the long arm of chromosome 17q21.1. The gene has 22 exons, of which large exon11 harbors about 55% of all gene mutations. The other mutations affect the other exons, making their search more difficult. A recent study has suggested that the function of the BRCA protein may be altered in the majority of breast and ovarian cancers.

Another gene with breast cancer susceptibility was recently mapped to chromosome 13q13.1. These findings suggest that the mutations of two genes (or more) may play an important role in many sporadic as well familial breast and ovarian cancers.

Carriers of BRCA1 mutation have a lifetime risk of 65–80% of developing breast cancer and 37–62% of developing ovarian cancer, while BRCA2 mutation carriers have a lifetime risk of 45–85% for breast cancer and 11–23% for ovarian cancer. The frequency and spectrum of mutations are different in different populations.

Neurofibromatosis

Neurofibromatosis type 1 (NF1, also known as von Recklinghausen disease) is a multisystem genetic disorder that is characterized by large clinical variability. Clinical manifestations are café-au-lait spots and axillary freckling (in over 90% of the patients), skeletal dysplasias, multiple neurofibromas and malignant nervous system tumors. The gene is called NF1, is one of the largest human genes and is located on chromosome 17q12. The disease is thus monogenic, autosomal dominant, with 50% risk of recurrence. Besides the familial cases, it is estimated that 50% of the cases occur due to new mutations.

Neurofibromatosis type 2 is a neurological genetic disorder characterized by a predisposition to develop tumors of the central and peripheral nervous systems, frequently associated with acoustic neuromas. Solitary benign tumors may also occur on intracranial nerves (meningiomas, astrocytomas) or the spinal nerves.

Neurofibromatosis type 2 is an autosomal dominant single-gene disease. It is due to a mutation in NF2 tumor suppressor gene, located on chromosome 22q12.

NF type 2 is rarer than NF1, but often much more severe. Morbidity and mortality are largely due to bilateral vestibular schwannomas presenting with hearing loss, tinnitus or vertigo and also due to the other CNS tumors.

Features of hereditary cancer include:

In the individual patient:

- Multiple primary tumors in the same organ.
- Multiple primary tumors in different organs.
- Bilateral primary tumors in paired organs.

- Multifocality within a single organ (e.g., multiple tumors in the same breast, all of which have risen from one original tumor).
- Younger-than-usual age at tumor diagnosis.
- Tumors with rare histology.
- Tumors occurring in the sex not usually affected (e.g., breast cancer in men).
- Tumors associated with other genetic traits.
- Tumors associated with congenital defects.
- Tumors associated with an inherited precursor lesion.
- Tumors associated with another rare disease.
- Tumors associated with cutaneous lesions known to be related to cancer susceptibility disorders (e.g., the genodermatoses).

In the patient's family:

- One first-degree relative with the same or a related tumor and one of the individual features listed.
- Two or more first-degree relatives with tumors of the same site.
- Two or more first-degree relatives with tumor types belonging to a known familial cancer syndrome.
- Two or more first-degree relatives with rare tumors.
- Three or more relatives in two generations with tumors of the same site or etiologically related sites.

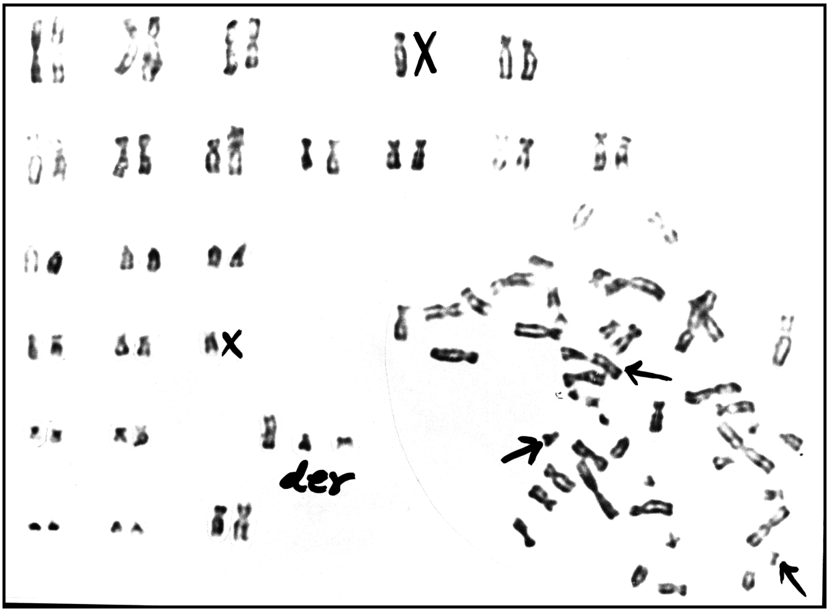
Chromosomal breakage syndromes

A special group of disorders is represented by several rare conditions characterized by excessive chromosome instability, defective DNA repair or genomic instability and increased susceptibility to malignancy. These suggest the existence of a relationship between the genic mutation, the chromosomal mutation and oncogenesis. The inheritance is autosomal recessive for all the following disorders. Examples are: Fanconi anemia, Bloom syndrome, ataxia-telangiectasia, Xeroderma pigmentosum. Some of these disorders are also characterized by immunodeficiency and will be described in another chapter.

Fanconi anemia is characterized by pancitopenia (anemia, leukopenia and thrombocytopenia). Many of the patients have hypoplastic or missing thumbs, sometimes more extensive arm defects such as radial aplasia. Other clinical features are: impairment of growth, brownish pigmentation of the skin. There is an increased tendency to malignancies, especially leukemia and lymphomas.

Mutations in at least 15 genes involved in Fanconi anemia patheway may cause the disease.

Cytogenetic: excess of chromosomal breakage in cultured cells, deletions, and translocations occurring in different chromosomes, not only in the peripheral blood, but sometimes also in the bone marrow.



Chromosomal rearrangements and missing chromosomes in Fanconi anemia (arrows) (our collection)

Xeroderma pigmentosum is characterized by:

- Photosensitivity
- Telangiectasia (sometimes)
- Erythema after sunlight exposure (especially on the exposed areas: face and dorsa of the hands)
- Photophobia
- Very high risk for skin cancers

There is a decreased DNA repair, because of the deficient endonuclease activity. More than 10 genes were associated with the disease. Many of these genes are involved in “nucleotide excision repair” mechanism.

Cytogenetic: chromosomal changes after exposure to ultraviolet light. Breaks, gaps or sister chromatid exchange may be seen in cultured cells, taken from the patients.

Chapter XIII

GENETICS OF HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX. IMMUNOGENETICS

The Major Histocompatibility Complex (MHC) is a group of genes essential for immune recognition. MHC molecules present antigens to T cells, triggering adaptive immune responses. In humans, MHC is known as the Human Leukocyte Antigen (HLA) system.

Classification of HLA genes

The human major histocompatibility complex spanning about 4 million base pairs of DNA is composed of a large cluster of genes located on the short arm of chromosome 6 has a central role in immune response (reaction) and in genetic susceptibility to numerous clinical disorders. These include autoimmune, infectious, neoplastic and metabolic diseases and touch on almost every clinical discipline.

Contains >200 genes, many with immune function.

The major histocompatibility complex (MHC) genes have been divided into three regions on the basis of structural and functional differences, each of which is highly complex and polymorphic.

Two of three classes class I and class II, correspond to the human leukocyte antigen (HLA) genes.

The class I genes (HLA-A, HLA-B, and HLA-C) encode antigens that are integral part of the plasma membrane of nucleated cells. These antigens are involved in transplant rejection (in antigen recognition), in immunocompetence, lymphocyte interactions and the development of self-tolerance. A class I antigen consists of two polypeptide subunits, a heavy chain encoded within the MHC and a nonpolymorphic polypeptide (microglobulin), which is encoded by a gene outside the MHC, mapping to chromosome 15.

The class II locus is composed of several subregions that encode **the HLA-DP, HLA-DQ and HLA-DR antigens.** These molecules are expressed primarily on B-lymphocytes, macrophages and T lymphocytes, but under certain conditions other cell types may express them as well. Like the class I antigens, they are integral to the cell membrane and to immune cellular interactions and function.

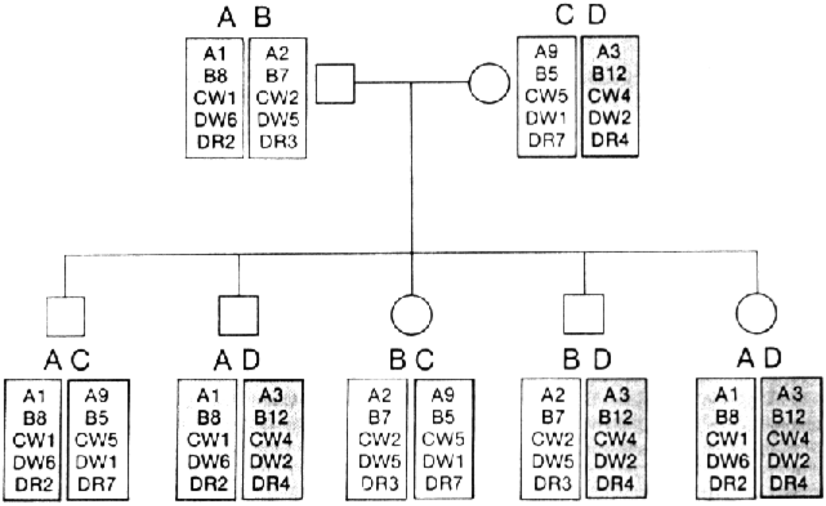
The HLA class I and class II antigens play a critical role in the initiation of an immune response.

The class III genes include genes for proteins such as properdin factors B, C_2, C_4, which are part of the complement system, a series of polymorphic serum proteins and membrane receptors closely involved in immune function. Also in this region are genes that when defective cause single - gene diseases, such as the gene for 21-hydroxylase (deficiency of 21-hydroxylase is associated with one form of congenital adrenal hyperplasia). A number of other gene loci within the MHC are genetically linked to the HLA genes but are functionally unrelated to them; these include the genes for tumor necrosis factor, and lymphotoxin, as well as other genes.

There is a striking similarity between HLA class I, class II genes and the immunoglobulin and T -cell receptors genes; this similarity among these genes has led to define them as "***the immunoglobulin gene super-family***".

The HLA system is highly polymorphic. Numerous distinct antigenic variants have already been recognized at each of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, and HLA-DR. They are encoded by numerous alleles, very different among individuals (among general population).

The HLA alleles on a given chromosome are closely linked that they are transmitted together as a ***haplotype:***



The inheritance of HLA haplotypes

The alleles are codominant; each parent has two haplotypes, expresses both and transmits one or the other to each child. As a result, parent and child share only one haplotype.

Because of millions combinations of haplotypes, it is very hard to find a suitable tissue (organ) for transplant to a patient.

HLA and disease association

The MHC genes have an important influence in genetic susceptibility to a numerous clinical disorders, such as: autoimmune, infectious, neoplastic and metabolic diseases and touch on almost every clinical groups of disorders.

Because the main role played by the products of the MHC genes in the immune system, it is not surprising that genes have an important influence on immune disorders. These disorders can be divided in two types:

- autosomal recessive immune deficiencies caused by genetic defects that abrogate the synthesis of MHC gene products. These include deficiencies of complements C_2, C_4 caused by homozygosity for alleles at these loci and result in compromised humoral immunity, leading to recurrent bacterial and viral infections.
- genetic susceptibility to a large number of disorders that involve MHC alleles, which are also present in the general population. MHC genes encode predisposition to these diseases, probably being the result of individual variations in immune responsiveness against antigens, due to varying abilities of different alleles. This variation in the population provides different degrees of protection against infections, pathogens or tumors. In autoimmune diseases, the etiology and the implication of MHC molecules is represented by a failure of the immune system to tolerate the body's own antigens; that leads to autoimmune disease with an immunologic self-reactivity against organ-specific.

The strong linkage disequilibrium of the MHC genes with several other diseases has been reported, being now known many genes as candidate disease susceptibility loci.

The first HLA haplotype association with inflammatory disease was discovered in 1972 correlating HLA-B27 with ankylosing spondylitis. This remains one of the strongest known associations of a disease with HLA-B27. Since then, more than 100 disease associations have been made, including many ocular diseases and systemic diseases.

Examples of association between HLA genes and disease

Disease	*HLA allele*	*Comments*
Diabetes type I	**DR3; DR4; B8; B15**	**most strong association**
Systemic lupus erythematosus	**DR2; DR3**	**autoimmune disease with hematological and renal involvement; photosensibility, etc.**
Arthrites	**DR4; B27; B28**	**rheumatoid disease**
Thyroidites	**DR3**	**autoimmune form**
Idiopathic hemochromatosis	**A3**	**disease of iron metabolism**
Congenital adrenal hyperplasia	**A3; BW47; C4**	**21-hydroxylase deficiency**
Multiple sclerosis	**DR2**	**demyelination in CNS**
Hodgkin disease	**DPW2**	**Lymphoma**
Hypertrophic cardiomyopathy	**candidate genes on chromosome 6, HLA-DR**	**heart disease**

An HLA disease association is defined as a statistically increased frequency of the HLA haplotype in individuals with the disease compared to the frequency in individuals without the disease. This is expressed as a relative risk. For example, HLA-B27 appears in 80-90% of patients with ankylosing spondylitis. Expressed as a relative risk, an HLA-B27 positive individual is approximately 87 times more susceptible to developing ankylosing spondylitis compared to the general population

A very strong association was established between **diabetus mellitus type 1** and HLA B8, B15, DR3, DR4.

Systemic lupus erythematosus is an autoimmune disorder also associated with a certain HLA haplotype: DR2, DR3. The disease is characterized by hyperproduction of autoantibodies, anemia, pulmonary hypertension, lesions of the skin, photosensitivity.

Multiple sclerosis is a chronic, slowly progressive neurological disorder characterized by demyelinization in CNS and increased permeability of small vessels.

Hemochromatosis is a single gene disorder of iron metabolism, leading to iron overloading; the most common symptoms are weakness, abdominal pain, hepatomegaly, arthropathy with arthralgia.

Congenital adrenal hyperplasia is a single gene disorder, autosomal recessive arising in specific defects in enzyme of adrenal cortex required for cortisol byosynthesis. The enzyme is called 21-hydroxylase and it′s locus is on the short arm of chromosome 6 near the loci for the HLA system. The symptoms of the disease consist of ambiguous genitalia in females.

Cardiomyopathy, a disease of heart muscles, characterized by disfunction of myocard and dysrithmia has also a strong association with HLA haplotypes.

Genetic histocompatibility testing for transplant

Several terms are used to define the source of donor organs.

An *autologous* graft is the tissue from oneself. In certain cases of leukemia or solid tumors, bone marrow from the patient will be harvested and frozen for rescue after aggressive chemotherapy.

A *syngeneic* graft is one from a genetically identical donor, that is the monozygotic twins.

An *allogeneic* graft is from a genetically different donor of the same species. With few exceptions, the donated organs are from unrelated cadaveric donors.

All patients waiting for transplant will be typed for HLA antigens for a possible matching between donor and receiver. The cumulative data clearly show that organ survival is increased by HLA matching between donor and recipients. The HLA matching is the single most important factor to improve the time of graft survival rates.

There are many types of transplants:

- Kidney transplants
- Pancreas –kidney transplants
- Liver transplants
- Bone marrow transplants
- Heart transplants

HLA matching is critical for successful organ and bone marrow transplant. Mismatched HLA antigens can lead to graft rejection.

HLA typing and crossmatching are standard pre-transplant procedures.

Genetics of the immune system

Immunogenetics studies the genetic control of immune responses. It focuses on variability in immune genes affecting susceptibility to infection, autoimmunity, and allergy.

Includes genes for:

- Immunoglobulins (Ig genes)
- T-cell receptor genes (TCR)
- Cytokines and their receptors
- MHC genes.

Genetic factors play a key role in the generation of the normal immune response and in the development of aberrant immune reactions and consequent immune-mediated diseases.

Much of human immunological uniqueness depends on the expression of the genes of the major histocompatibility complex (MHC), as well as two others within the same superfamily that encode additional components of the immune response: immunoglobulins (Igs) and T-Cell antigen receptors (TCRs).

There is a striking similarity, both in organization and in DNA sequence, between the HLA genes (class I and class II) and the immunoglobulin and T-cell receptor genes, which has led to their classification into a gene family designated the ***immunoglobulin gene superfamily***.

Immunoglobulins exist in two forms: a membrane- bound form, on B lymphocytes, and a soluble form produced by plasma cells, which are derived from B lymphocytes by proliferation and maturation. A primary significance of immunoglobulins from the perspective of genetics is that they exhibit a unique property, ***somatic rearrangement,*** by which genes of the germline are rearranged in somatic cells to generating diversity of specific antibodies, capable of promptly responding to any antigen.

The genes encoding the light (L) chains of the Ig are located in three unlinked regions on chromosome 2 and the genes encoding the heavy (H) chains are on the chromosome 14. The structure of the heavy chain allows classification of immunoglobulins into 5 major classes: Ig G, IgM, Ig A, IgE and Ig D.

During B-cell differentiation, the DNA at immunoglobulin loci undergoes somatic rearrangement. There are 40 genes for the κ chain variable region and 5 for the joining region, 30 genes for the λ chain variable region and 4 for its joining region. Regarding the heavy chain genes there are 40 genes for the variable region, 25 for diversity and 6 for the joining region.

Germ-line DNA from *Progenitor B cells* provides H and L chain locus and direct synthesis of heavy and light chains. They have Variable, Diversity and Joining segments in un-rearranged form.

- H chain (chromosome 14) locus has 3 regions – V, D, and J
- L chain (chromosome 2 for κ chain and 22 for λ chain) locus has 2 regions – V and J.

VDJ rearrangement occurs during the maturation of B cells. Initially a V gene together with one D and a J one will combine in *Pro-B cells* and form Heavy chain. VJ rearrangement on 'L' chain occurs in *Precursor B cells* to produce Light chain.

The other member of immunoglobulin gene superfamily, the T-cell receptor (TCR) shares the mechanism of generation of diversity characteristic of the immunoglobulines. The TCR represents the T-cell analog of membrane-bound immunoglobulin on B cells. The TCR genes are encoded in three separate regions on chromosomes 14 and 7.

Immunodeficiency disorders

Genetic disorders of the immune system are collectively known as Primary Immunodeficiency Diseases (PIDs) or Inborn Errors of Immunity (IEI). They result from inherited mutations affecting immune development or function. Over 500 genetic causes have been identified, affecting innate and adaptive immunity.

Classification by affected immune component: B cells, T cells, phagocytes, complement, and regulatory pathways.

The immunodeficiency diseases are part of a spectrum of conditions involving defects in defense against infections.

Examples of single - gene disorders of the immune system

DISEASE	INHERITANCE
Adenosine deaminase (ADA) deficiency	autosomal recessive
Agammaglobulinemia	X-linked recessive
Ataxia-telangiectazia	autosomal recessive
Severe combined immunodeficiency disease	autosomal recessive X-linked recessive

Combined cellular and humoral immune defects

Severe combined immunodeficiency is characterized by profound defects of both cellular and humoral immunity and is the most rapidly progressive and devastating of the primary immunodeficiency syndromes. They may be autosomal or X-linked recessive. Patients present within the first months of life with recurrent, persistent infections due to bacterial, viral and fungal pathogens. Candida infection is almost universal and chronic pneumonitis is extremely common.

X-linked combined immunodeficiency is common in males being inherited as an X-linked recessive disorder. These patients have low numbers of T cells, variable number of B cells, low Ig, and no specific antibodies. Female carriers are clinically unaffected. The gene was mapped to Xq13.1.

Adenosine deaminase deficiency is an autosomal recessive disorder due to the absence of the enzyme adenosine deaminase (ADA).

Approximately 80% of ADA deficient patients are clinically indistinguishable. In 15-20% of the cases, onset of disease may occur in childhood later than 3-6 months. The disease is progressive. While genetic heterogeneity contributes to the phenotypic diversity, some degree of differences in clinical manifestations has been found between sibs, probably indicating influence of environmental factors.

The pathological effects of ADA deficiency result entirely from abnormalities in lymphocytes, accumulating massive amounts of toxic purine metabolites, which inhibit DNA synthesis. Deoxyadenosine accumulation in lymphocytes has numerous adverse effects that impair DNA replication and cell division. The result is a profound failure of both cell-mediated (T cell) and humoral (B cell) immunity.

Characterized by profound defects in both cellular and humoral immunity.

- Genetic causes:
  - IL2RG mutation (X-linked SCID).
  - ADA deficiency (adenosine deaminase deficiency).
  - JAK3, RAG1/2, Artemis (DCLRE1C) mutations.

Primary B-cell deficiencies

Disorders of humoral immunity or antibody deficiency disorders can be infantile or have late onset, involve absence of all classes, or only specific classes of immunoglobulins. The primary clinical manifestation in all of these disorders is recurrent, severe infection with common pyogenic bacteria such as Haemophilus influenze and Streptococcus pneumoniae.

Infantile agammaglobulinemia is an X-linked example of B-cell defect. The clinical course of these patients reveals that they are usually healthy during the first months of life, probably reflecting the presence of adequate amounts of placental transferred maternal Ig G. Then they begin to suffer from multiple recurrent infections most prominent of the upper and lower respiratory tract and skin. These children are more susceptible to infection with hepatitis virus and enteroviruses.

Almost all cases of agammaglobulinemia are X-linked. Infantile agammaglobulinemia must be differentiated from transient hypogammaglobulinemia of infancy, in which there is a prolongation of the normal physiologic decline in Ig G.

Although the classical inheritance is X-linked, about one third of patients should reflect new mutations. The X-linked locus has been mapped to chromosome Xq21.3-22.

Immunodeficiencies with associated abnormalities

Thymic hypoplasia (Di George syndrome) is usually a congenital sporadic disorder in which there are many abnormalities including those of the thymus and parathyroid.

Patients have a characteristic facies with micrognathia, cleft palate and associated abnormalities of the aortic arch and a very low cellular immune function.

In the past years, many cases of Di George syndrome have been reported to show a deletion of 22q11.2, often familial.

Diagnostic Approaches

Clinical evaluation and family history.

- Laboratory tests: immunoglobulin levels, lymphocyte subsets, complement assays.
- Functional assays: oxidative burst, cytokine responses.
- Genetic testing: targeted panels, WES, WGS for variant identification.
- Genetic counseling and newborn screening for severe disorders like SCID.

Treatment strategies

- Immunoglobulin replacement therapy.
- Prophylactic antibiotics and antifungals.
- Hematopoietic stem cell transplant (HSCT) for severe forms.
- Gene therapy trials for ADA-SCID, CGD
- Biologic therapies: IL-1, IL-6, or TNF inhibitors for autoinflammatory syndromes.

Chapter XIV

NUTRIGENETICS

Introduction

Nutrigenetics and nutrigenomics are related but distinct disciplines that investigate how genetic variation and gene–nutrient interactions influence human health, disease risk, and response to dietary interventions. Nutrigenetics typically focuses on how individual genetic variation (single nucleotide polymorphisms, copy number variants) modifies response to nutrients, whereas nutrigenomics uses high-throughput 'omics' approaches (transcriptomics, epigenomics, proteomics, metabolomics) to understand how nutrients and dietary patterns influence gene expression and molecular pathways. Together, these fields aim to provide the biological underpinning for personalized nutrition and precision dietary recommendations in clinical practice and public health.

Definitions and Scope

Nutrigenetics is the study of how inherited genetic differences alter physiological responses to dietary components, affecting metabolism, bioavailability, and disease susceptibility. Common examples include polymorphisms in genes affecting lipid metabolism (e.g., APOE, FTO), folate metabolism (MTHFR), and caffeine metabolism (CYP1A2). Nutrigenetics studies an individual's genes that interact with nutrients to affect health. It focuses on how genetic variations influence the body's response to different foods and nutrients, with the goal of providing personalized dietary advice to improve health and prevent disease. This field helps explain why people may have different reactions to the same foods and can be used to develop personalized diets.

Nutrigenomics: A systems-level approach that assesses how nutrients modulate gene expression and cellular pathways via transcript-tional regulation, epigenetic modifications (DNA methylation, histone modifications), and downstream effects on proteome and metabolome. Nutrigenomics examines both acute responses to dietary components and longer-term changes associated with diet-related disease states.

Key aspects of nutrigenetics

- Personalized nutrition: Nutrigenetics moves away from a "one-size-fits-all" approach to diet by accounting for individual genetic differences.
- Genetic variations: It analyzes specific genetic variations, such as SNPs (single nucleotide polymorphisms), that can affect nutrient absorption, metabolism, and utilization.
- Health outcomes: This information can be used to predict how an individual's body might respond to certain foods and nutrients, helping to address issues related to weight, allergies, intolerances, and chronic diseases.

Methods and Technologies

Both fields leverage modern molecular technologies. Nutrigenetic studies commonly use targeted genotyping or genome-wide association study (GWAS) approaches to link genetic variants with dietary responses. Nutrigenomics relies on high-throughput assays: RNA-sequencing for transcriptomics; chromatin immunoprecipitation sequencing (ChIP-seq) and ATAC-seq for chromatin state; whole-genome sequencing or array-based methods for DNA methylation; mass spectrometry and nuclear magnetic resonance (NMR) for metabolomics and proteomics platforms for protein-level changes. Integrated multi-omics analyses increasingly use bioinformatics pipelines, network analysis, and machine learning to mine complex interactions among diet, genes, microbiome, and phenotype.

Key Biological Mechanisms

Gene–nutrient interactions occur through multiple mechanisms:

1. Variants in nutrient-processing enzymes or transporters alter nutrient handling (e.g., SNPs in MTHFR affecting folate metabolism).
2. Nutrients act as ligands for nuclear receptors (e.g., PPARs, LXRs) and transcription factors, leading to changes in gene expression.
3. Dietary components influence epigenetic marks; methyl-donor nutrients (folate, choline, betaine) modulate DNA methylation patterns with downstream effects on gene regulation.
4. Nutrients and metabolites alter redox status and signal transduction cascades, with consequences for gene expression and cellular homeostasis.

Clinical Applications and Evidence

Personalized nutrition based on genetic information holds promise in areas including obesity management, lipid disorders, diabetes prevention, and micronutrient supplementation strategies. Several randomized controlled trials and pilot implementation studies have tested whether delivering genotype-based dietary advice improves behavioral outcomes or clinical endpoints. Overall, the evidence is mixed: some nutrigenetic-guided interventions show modest improvements in dietary adherence or intermediate biomarkers, but robust, consistent clinical benefit (for endpoints such as sustained weight loss or reduced cardiovascular events) is not yet broadly demonstrated. The clinical utility depends on the strength of gene–diet interactions, effect sizes, and the quality of evidence evaluated through frameworks such as GRADE or other evidence-evaluation tools.

Selected Gene–Diet Examples

APOE genotype modifies lipid responses to dietary saturated fat and omega-3 fatty acids, with APOE4 carriers often demonstrating greater LDL-C sensitivity to saturated fat. Variants in FTO and other obesity-associated loci have been examined for interactions with macronutrient composition and physical activity, with variable replication across populations. MTHFR polymorphisms (e.g., C677T) influence folate requirements and homocysteine metabolism, which has implications for folate supplementation and neural-tube defect prevention strategies in certain populations.

Integration with Microbiome and Metabolomics

Microbiome composition and function are key modifiers of nutrient metabolism and gene–diet interactions. Short-chain fatty acids, bile acid metabolites, and microbial-derived vitamins modulate host epigenetic programming and immune responses; consequently, effective precision nutrition strategies increasingly triangulate host genomics, metagenomics, and metabolomics. Multi-omics integration may improve prediction of individual responses and identify mechanistic biomarkers for targeted dietary interventions.

The rapid growth of direct-to-consumer nutrigenetic testing raises concerns: variable test quality, limited clinical validation for many marketed gene–diet claims, potential for misinterpretation by consumers, and privacy considerations related to genetic data sharing. Integration into clinical care requires validation, clinician education, and regulatory oversight to ensure tests are evidence-based and actionable.

Challenges and Limitations

Major challenges include small effect sizes for many gene–diet interactions, population stratification and ancestry-specific effects, heterogeneity in dietary assessment methods, and limited replication of findings. Implementation barriers include data integration complexities, cost, equitable access, and the need for prospective trials powered for clinical endpoints.

Examples:

Lactose intolerance: A genetic variation in the LCT gene can affect the production of the enzyme lactase, making it difficult to digest lactose from dairy products.

Celiac disease: Most people with celiac disease have specific genetic variants (HLA-DQ2 or HLA-DQ8) that make them sensitive to gluten.

Disease risk: Nutrigenetics may help explain why certain dietary components affect the risk of diseases like obesity or cancer differently in different people.

Genetic testing: A nutrigenetic test analyzes your DNA for specific genetic variants related to metabolism, nutrient processing, and health risks.

Personalized recommendations: Based on the test results, you can receive tailored advice on diet, exercise, and other lifestyle choices to optimize your health and manage potential risks.

Weight is strongly influenced by genetic factors. Obesity is a result of excess body fat accumulation. The heritability of obesity has been estimated at 40% -77%. Besides genetic factors, the variation observed in human body weight is also due to environmental causes.

The genome has not changed very much in the last 40 years, suggesting that environmental factors have important contributions to weight gain.

The prevalence of childhood obesity has tripled in the US and has increased significantly worldwide, and the explanation may be represented by changes of external factors affecting the energy balance in children.

Genetic factors

Parental obesity is considered to be the strongest risk factor for childhood obesity. The risk is even higher if both parents are obese. Inheritance of obesity does not usually follow classic Mendelian inheritance. A combination of genetic mutations, single nucleotide polymorphisms, can predispose to obesity. Each genetic change has only a small contribution to the phenotype, but collectively, inherited genetic variations play a role in determining body mass and in maintaining a balance between physical activity and nutrition.

Classification:

- Single-gene obesity is caused by a single gene mutation with severe obesity as the main symptom.
- Syndromic obesity has several characteristics, obesity is a symptom.

Single-gene obesity:

It is associated with a mutation in a single gene (eg leptin). Phenotypes, usually extremely severe, are characterized by obesity with early childhood onset, often associated with behavioral, developmental or additional endocrine disorders, such as hyperphagia and hypogonadism, significant developmental delay. However, this type has a low frequency. The prevalence of monogenic obesity in children with severe obesity is up to 5%.

Syndromic obesity:

It includes some diseases with Mendelian transmission. Patients have obesity, associated with intellectual disability, dysmorphisms and congenital defects. Occurs in ~ 20 rare syndromes caused by chromosomal abnormalities or gene defects, both autosomal and X-linked. Most of these obesity syndromes associate with intellectual disability.

Multifactorial obesity:

It is the most common type of obesity, which occurs when a genetically susceptible individual is exposed to an "obesogenic" environment. It represents the final result of the interaction between life style, environment, and genetics that can influence individual responses to diet and physical activity. The recent rapid increase in childhood obesity prevalence suggests that environmental factors have a greater impact on body weight in patients with common obesity, although individual responses to these environmental factors are influenced by genetic factors - "susceptibility genes".

Conclusion

Nutrigenetics and nutrigenomics provide a biologically plausible path toward personalized nutrition. Clinical evidence demonstrating improved long-term health outcomes from genomics-guided dietary interventions remains limited. Carefully designed trials, transparent validation of tests and integrated multi-omics approaches will be central to realizing the promise of precision nutrition in medicine.

Chapter XV

PHARMACOGENETICS

Pharmacogenetics studies how genetic variation affects individual responses to drugs. It bridges pharmacology and genetics to personalize therapy and reduce adverse drug reactions. The main focus areas: drug metabolism, transport, and target interactions. It represents the foundation of precision medicine and individualized pharmacotherapy.

Pharmacogenetics is the special area of biochemical genetics that deals with variation in drug response and the contribution of genetics to such variation. What happens when a patient is given a drug medication may be determined by his or her genetic constitution. Clear-cut examples are seen in single-gene phenomena in the fields of both drug metabolism and pharmacological effects. Interethnic variability in this context is a feature that has a genetic basis and that is being increasingly recognized to be of clinical significance in many instances.

Genetic polymorphisms influence drug absorption, distribution, metabolism, and excretion (ADME). Variants may alter enzyme activity, transporter function, or receptor sensitivity.

Pharmacogenomics extends pharmacogenetics to genome-wide analysis of drug response. Pharmacogenomics studies interindividual differences in the genome of the implications for the overall effect of the drug. Pharmacogenomics analyzes the variations in the entire human genome in a given population, and allows certain correlations with the observed pharmacological effects or with the susceptibility of developing various diseases. This approach finds its applicability in drugs designs and can be used for identifying new therapeutic targets. Many genetic polymorphisms affecting genes that encode enzymes, transporters and receptors have been described, as were their consequences on bioavailability and their effects on many drugs.

Polymorphisms are defined as variants that occur in more than 1% of the individuals. The most abundant type is the SNP. Other types are deletions, insertions, tandem repeats.

Pharmacogenetics has focused on the role of genetic variation in pharmacokinetics (e.g., the absorption, distribution, metabolism, excretion of drugs) and pharmacodynamics (e.g., drug-response proteins, such as receptors, channels, and transporters. In the present pharmacogenomics and individualized drug therapy are increasingly influencing medicine and biomedical research.

The ability of predicting the response to treatment according to genetic variation, aims at optimizing therapy in *personalized medicine*, by prescribing the most effective drug in the right dose and the lowest risk of adverse effects.

When a drug is biotransformed by means of more than one way, it may be subject to the influence of more than one genetic polymorphism. Example: an individual who has a low metabolic capacity, will likely develop an adverse reaction or toxicity.

In some instances, it has been clearly shown that a substantial proportion of the total phenotypic variance of drug plasma concentrations is due to genetic factors. This may be established even though the biochemical mechanisms controlling the plasma concentration may not be understood and the effects of single alleles may not be recognized. In practice, this type of variation can be managed by attention to clinical end points and in some cases by plasma level estimations. By these means, it may be possible to avoid adverse reactions caused by high plasma concentrations and lack of efficacy caused by low concentrations.

Cytochrome P450 (CYP) Enzyme System

CYP enzymes metabolize ~75% of all drugs. Key isoenzymes: CYP2D6, CYP2C9, CYP2C19, CYP3A4, CYP1A2.

• Examples:

- CYP2D6: affects metabolism of codeine, antidepressants, beta-blockers.
- CYP2C19: affects clopidogrel activation and proton pump inhibitors.
- CYP2C9: influences warfarin clearance.

Clinical implication: dose adjustment based on metabolizer phenotype

Genetic Variation in Drug Transporters

- ABCB1 (MDR1): encodes P-glycoprotein; affects digoxin, cyclosporine, antiepileptic distribution.
- SLCO1B1: hepatic uptake transporter; influences statin pharmacokinetics.
- SLCO1B1*5 variant → simvastatin-induced myopathy.
- OCT1, OATP, and BCRP variants affect antidiabetic and anticancer drug levels.

Pharmacodynamic Genetic Variability

Genetic differences in drug targets (receptors, enzymes) alter drug efficacy and toxicity.

Examples:

- VKORC1 polymorphisms: sensitivity to warfarin anticoagulation.
- ADRB1/ADRB2 variants: differential response to beta-blockers.
- HLA-B*57:01: hypersensitivity to abacavir (HIV therapy).
- HLA-B*15:02: carbamazepine-induced Stevens–Johnson syndrome (Southeast Asian populations).

Clinical Applications of Pharmacogenetics

- Dose optimization based on genotype (e.g., warfarin, thiopurines).
- Prevention of severe adverse drug reactions through preemptive testing.
- Companion diagnostics in oncology: EGFR, ALK, KRAS, and BRAF testing for targeted therapy.
- Integration into clinical decision support systems for safer prescribing.

Key Pharmacogenetic Examples

- Warfarin: CYP2C9 and VKORC1 genotypes determine dose.
- Clopidogrel: CYP2C19 poor metabolizers show reduced antiplatelet response.
- Codeine: CYP2D6 ultra-rapid metabolizers risk opioid toxicity.
- 6-Mercaptopurine: TPMT and NUDT15 genotyping prevent myelosuppression.
- Irinotecan: UGT1A1*28 variant predicts neutropenia risk.

Pharmacogenomics in Oncology

Somatic and germline variants influence cancer drug response.

Examples:

- EGFR mutations → sensitivity to tyrosine kinase inhibitors in lung cancer.
- KRAS/NRAS mutations → resistance to anti-EGFR therapy in colorectal cancer.
- DPYD variants → fluoropyrimidine (5-FU) toxicity.
- TPMT/NUDT15 → thiopurine toxicity in leukemia treatment.

Pharmacogenetic disorders

Pharmacogenetic disorders are clinical conditions in which genetic variation leads to abnormal drug response. They result from mutations or polymorphisms in genes encoding drug-metabolizing enzymes, transporters, or receptors. Manifestations: toxicity, therapeutic failure, hypersensitivity, or idiosyncratic reactions. Their study aims to identify individuals at risk and guide personalized therapy.

Classification of pharmacogenetic disorders:

1. Disorders due to enzyme deficiency or abnormal activity: e.g., pseudocholinesterase deficiency, G6PD deficiency.

2. Disorders due to receptor or transporter mutations: e.g., malignant hyperthermia (RYR1), statin-induced myopathy (SLCO1B1).

3. Disorders due to immune-related genetic predisposition: e.g., HLA-associated drug hypersensitivity.

4. Multifactorial disorders influenced by multiple genes.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, caused by the mutation of an X-linked gene, is the most common disease due to an enzyme defect in humans, estimated to affect 400 million people worldwide. With over 300 variants described, G6PD deficiency appears to be the most genetically heterogeneous disorder.

In G6PD deficiency, oxidant drugs deplete the cell of reduced glutathione and that leads to hemolysis. Drugs that lead to hemolysis in G6PD deficiency, include sulfonamides, antibiotics, sulfones, primaquine and few others. Numerous other factors can also cause hemolysis in G6PD deficiency such as: hepatitis, salmonellosis, pneumonia and virtually any type of infection.

Several hundred different variants of G6PD have been described:

- class I: severe deficiency with chronic hemolytic anemia
- class II: severe deficiency with intermittent hemolysis
- class III: moderate deficiency with intermittent hemolysis
- class IV: no deficiency or hemolysis

The advances in molecular biology have enhanced the understanding of the abnormality of G6PD deficiency; the gene has been cloned and sequenced. At least 60 different mutations or mutation combinations have been identified in this gene. There are mostly missense point mutations and deletions; however, large deletions or frameshift mutations have not been identified, suggesting that complete absence of G6PD might be lethal.

The clinical observation that the severity of hemolysis due to G6PD deficiency is worse in males compared to females is consistent with the fact that the gene for G6PD is located on the X chromosome. According to the Lyon hypothesis, only one X chromosome is active in any given somatic cell. Depending on the degree of lyonization, the mean red blood cell enzyme activity in females who carry a gene for G6PD deficiency may be normal, moderately reduced, or grossly deficient. The diagnosis of G6PD deficiency is suggested by the presence of episodic hemolytic anemia in association with drug administration or infections.

Cholinesterase deficiency

Soon after the introduction of muscle relaxant in 1951, an occasional atypical patient was encountered. This patient suffered prolonged muscle paralysis and consequently apnea.

It was seen that after drug administration, atypical plasma cholinesterase can prolong the plasma half-time of the anesthetic, thus, leading to toxicity. Such atypical patients showed low activity of the enzyme plasma cholinesterase.

The BCHE gene locus is on chromosomes 3q26.1. The enzyme normally hydrolyzes succinylcholine and mivacurium. Its deficiency causes prolonged apnea and paralysis after anesthesia. Inheritance: autosomal recessive. Clinical management: avoid succinylcholine; use non-depolarizing neuromuscular blockers.

Malignant hypertermia is an uncommon complication of general anesthesia. Genes: RYR1 (most common), CACNA1S. It is triggered by volatile anesthetics (halothane, sevoflurane) and succinylcholine. The inheritance is autosomal dominant. The clinical features of malignant hyperthermia are muscular rigidity, tachycardia, cyanosis and respiratory acidosis. Treatment: dantrolene, supportive cooling, avoidance of triggers.

By gathering large families in which malignant hyperthermia had occurred, the disorder was shown to be usually inherited as a mendelian autosomal dominant trait, with reduced penetrance, but about 20% of cases are sporadic. More than 30 mutations account for the disease. Mutations affect several genes. Half of the mutations affect *RYR1 gene, which* is located on chromosome 19q13.2. Other malignant hyperthermia loci have been proposed on chromosomes 17 and 7. Thus, malignant hyperthermia is a heterogeneous condition.

Statin-Induced Myopathy

Gene: SLCO1B1 is on chromosome 12p12 and encodes OATP1B1 transporter for hepatic statin uptake.

SLCO1B1*5 (c.521T>C) variant → increased plasma statin levels → myopathy risk. Particularly affects simvastatin users. Management: genotype-guided statin selection (pravastatin or rosuvastatin preferred).

The acetylation polymorphism

This important pharmacogenetic polymorphism was first discovered during the treatment of tuberculosis with the drug called isoniazid; its metabolic fate in humans was investigated and large consistent inter individual differences were found. Family studies showed that the metabolism of isoniazid was controlled by a genetic polymorphism. Slow inactivation was an autosomal recessive, while rapid inactivation was an autosomal dominant trait. Two types of experiments established that the enzymatic basis of the polymorphism affect the enzyme N-acetyl-transferase.

The investigations showed that after a test dose, the rate of disappearance of isoniazid from plasma has a bimodal distribution in the population, allowing the identification of individuals as rapid or slow acetylators. Slow inactivators are homozygous for a recessive gene, and rapid inactivators are normal homozygotes or heterozygotes. It is now clear that the slow and rapid inactivation phenotypes are due to two major alleles of the enzyme hepatic N-acetyltransferase, the product of a gene that maps to chromosome 8. The frequencies of the two alleles have marked ethnic differences.

Future perspectives in pharmacogenetics

Pharmacogenomic research areas are:

- identifying disease genes that can be manipulated by drugs;
- identification of genetic polymorphisms involved in drug metabolism;
- stratification of prescription drugs based on SNPs, ie. individualizing drug therapy based on individual genetic profile to avoid adverse reactions;
- population genetic stratification by scanning the human genome to identify genes involved in the response to drugs;
- determining the types of gene expression in target tissues, eg. the interaction between drug and target receptor by analyzing the transcriptional program of target tissue, related to the response to drug administration.

Chapter XVI

STRATEGIES FOR THE TREATMENT OF GENETIC DISORDERS

Genetic disorders comprise a wide spectrum of diseases caused by single-gene mutations, copy-number changes, chromosomal abnormalities, or polygenic risk architectures. Historically, their management was largely supportive and symptomatic. Treatment aims to correct, compensate, or mitigate genetic defects. Approaches include pharmacologic, molecular, cellular, and gene-based therapies. During the past decades, major advances have been made in the elucidation of the molecular pathologies of many genetic diseases and gene therapies have been developed for the treatment of inherited diseases. Molecular genetic techniques have rapidly developed and are applicable in the treatment of various diseases, because of the possibility of synthesis of proteins or vaccines by genetic engineering. Advances in molecular biology, gene-delivery vehicles, genome editing, RNA therapeutics, and cell therapy have produced a new generation of disease-modifying and potentially curative interventions

Treatment in multifactorial diseases

In multifactorial diseases, genetic together with environmental factors cause the pathological phenotype. If *environmental harmful factors* are known, they can be *avoided*. Thus, for example, individuals with alpha-1 antitrypsin deficiency decrease the risk of respiratory disorder and liver complications if they avoid cigarette smoke.

In some multifactorial congenital defects such as congenital heart defects, cleft palate, cleft lip, pyloric stenosis, *surgery* is needed; in 50% of cases, a single surgical intervention will correct the defect.

In other multifactorial diseases such as: hypertension, coronary artery disease, diabetes mellitus, schizophrenia, psychosis there are therapies that serve to ameliorate these disorders.

Treatment in single gene diseases

Treatment objectives:

- Identifying the mutation to perform etiological treatment
- Identifying deficient proteins and mechanisms that determine the onset of clinical symptoms.
- treatment shoul be on long-term, even permanently
- therapy should be differentiated in relation to the genetic heterogeneity of the disease.
- follow-up of patients
- preventing disease onset through early diagnosis and early initiation of therapy

Therapeutic means:

Diet: restrictive diet is recommended in many metabolic disorders. Sometimes it is difficult to establish a complete restrictive diet, but compliance with rigorous diet will have beneficial effects. In phenylketonuria (classic form), if established immediately after birth, diet prevents the installation of neurological complications. In other disorders, it may be necessary to administer a particular product: for example, administration of cholesterol in Opitz Syndrome Smith-Lemli prevents demyelination.

Enzyme Replacement Therapy and Small-Molecule Approaches: Enzyme replacement therapy (ERT) supplies recombinant enzyme to patients with loss-of-function mutations that cause enzyme deficiency (classically lysosomal storage disorders such as Gaucher, Fabry, and Pompe disease). ERT can reduce substrate accumulation, ameliorate organomegaly, and improve biochemical markers; however, it frequently shows limited penetration into the central nervous system (CNS), can provoke immune responses, and usually requires lifelong intravenous administration with substantial cost and logistical burden. Improvements in formulation, dosing, and home-administration programs have improved patient convenience and quality of life, but CNS disease remains a major gap for many ERTs.

Small molecules offer alternative or adjunctive strategies: pharmacological chaperones stabilize misfolded proteins to enhance residual activity; substrate-reduction therapies decrease the production of toxic metabolites; and targeted inhibitors or activators can compensate for dysfunctional pathways. Small molecules are attractive for oral delivery and easier manufacturing, but they often depend on the presence of residual protein function and can be limited by off-target effects.

In haemophilia, the administration of factors VIII or IX (depending on the type of hemophilia), reduce bleeding state.

Administration of vitamins can improve the prognosis of some patients. Thus, in homocystinuria (deficiency of cystathione synthetase), administration of vitamin B6 leads to decreasing plasmatic homocysteine concentration. Vitamin E is beneficial in some neurological disorders, such as, pseudo-Friedrich's ataxia.

Medical rehabilitation: is used in many diseases in order to increase mobility, reduce hypotonia / hypertonia in the muscles and / or reduce pain.

Gene therapy

Germline versus somatic cell gene therapy

Genes can be transferred into the germline or into somatic cells. Germline gene involves the insertion of a normal gene into germ cells such that it will correct the genetic defect and be transmitted in a Mendelian fashion from generation to generation. By contrast, somatic gene therapy is the insertion of the therapeutic gene into somatic cells. In this way only the somatic cells of the patient will be modified, without the possibility of germline transmission.

Germline gene therapy in mice. Although germline gene therapy in humans has been prohibited for moral, scientific and practical reasons, such experiments have been permitted in mice and demonstrate the feasibility of inserting functional genes into the germline with cure of the murine disease.

The technique for insertion of a foreign gene or DNA segment (i.e., a transgene) into the mouse germline is now commonly performed. One - cell mouse embryos are collected immediately following fertilization. The male pronucleus is visible at this stage and foreign DNA can be inserted into the nucleus by microinjection. Following injection, the embryos are surgically placed into the uterus of a pseudopregnant female mouse. After the pups are born, the presence of the transgene can be determined by Southern analysis or polymerase chain reaction (PCR). Because these mice can express the foreign genes, they are called ***transgenic*** mice.

The ability to insert foreign genes into gene germline of mice has provided the opportunity to test the feasibility of gene therapy. Normal genes have been injected into mice with specific genetic defects, and their incorporation into the mouse genome has effectively cured the murine disease. For example, transfer of the human β-globin gene into mice with β-thalassemia (due to a murine β-globin gene deletion) resulted in the regulated expression of β-globin molecules in these mice and correction of the hematopoietic disease.

Somatic cell gene therapy involves the insertion of a normal gene into somatic cells such that sufficient quantities of the therapeutic protein will be expressed to correct the metabolic defect. In contrast to germline gene therapy, only certain somatic cells of an affected individual are target and there is no transfer of the new genetic material to the treated patients' offspring.

Two approaches can be used to deliver genes to somatic cells. "**Ex vivo**" gene therapy involves removal of cells from the patient, introduction of the therapeutic genes using viral or nonviral vectors and the autologous transplant of the gene- corrected cells into the patient. "**In vivo**" gene therapy refers to the direct delivery of the therapeutic gene to the target sites of pathology of the diseased individual (e.g., intravenous infusion) without the need for explanting and culturing cells for gene insertion in the laboratory and then performing transplant procedures.

To date, most gene therapy has relied on "ex vivo" gene transfer using retroviral vectors. Such vectors have been used to transfer cDNA into a variety of cell types, including hematopoietic cells, skin fibroblasts, hepatocytes and endothelial cells.

Hematopoietic cells have been a main target of retrovirus-mediated gene transfer studies, since bone marrow transplant in patients with certain genetic disorders has proved curative.

The major viral and nonviral vector systems used in gene therapy are listed below:

Viral vectors

- Retrovirus
- Adenovirus
- Adeno-associated virus
- Herpes simplex virus

Nonviral vectors

- Plasmid DNA
- Liposome- entrapped DNA
- Protein DNA conjugates

Viral gene delivery systems

Retroviral vectors have been the first and most extensively used delivery system for gene therapy. Retroviruses are uniquely suited to somatic gene transfer, as integration of the retroviral genome into the host cell chromosome is an obligatory part of their life cycle. In general, integration of the virus into the host chromosome and expression of its genes from the integrated provirus has no deleterious effects on the viability and function of the infected host cell.

The virus binds to a cell membrane receptor releasing the viral RNA genome into the cytoplasm. Reverse transcriptase, converts the single-strand viral RNA genome into a double strand DNA copy that forms an “integration complex” with the host cell proteins. This integration complex is transported through the nuclear membrane and directs integration of the viral genome into the host genome as a ***provirus.*** The provirus is able to transcribe and translate the viral genome in the host cell and to produce copies of the viral proteins. These vectors have been successfully used to introduce genes into many different cell types, but the disadvantage is the risk of insertional mutagenesis.

Adenoviral vectors are double-stranded DNA viruses that infect a large range of cell types. The virus does not integrate into human chromosomes, replicating as an episome in the nucleus; thus, there is no risk of insertional mutagenesis or oncogene activation associated with their use. The usual method consists of making a recombinant adenovirus vector. Early experiments demonstrating adenovirus vector- mediated gene transfer to lung cells showed efficient, but short term expression of the gene.

Several other viruses are also being developed for gene therapy, including herpes virus, polio or influenza. However, there are disadvantages of these gene delivery systems, perhaps most significant is the fact that repeated administration for therapeutic effectiveness is needed. Many investigators have turned toward nonviral gene delivery strategies.

Nonviral gene delivery systems

Lyposomes are vesicles composed of continuous lipid bilayers surrounding an aqueous volume, in size of about tens micrometers in diameter. Liposomes binding DNA have an advantage over viral vectors for in vivo gene therapy because they are relatively nontoxic and elicit fewer immunologic reactions. But, despite the promise of this technology, several obstacles must be overcome before liposomes can be widely used in gene therapy. For example, after liposomes were introduced into animals, they often become unstable and just a small amount gained access to the nucleus for expression. In addition, liposomes are difficult to target to specific cell types in vivo, and once the DNA encoding gene is introduced into the cell, most of it is rapidly degraded in lysosomes. To address the issues of cell targeting and lysosomal degradation modified liposomes have been developed, termed “immuno-liposomes", which contained conjugated antibodies targeted to specific cell antigens. This improvement offered an advantage for genetic diseases in which it is desirable to target genes to the reticuloendothelial system (e.g., in lipid storage diseases such as, Gaucher and Niemann-Pick disease). Liposomes offer significant promise for gene therapy and provide an important alternative to viral gene delivery.

Plasmid and conjugate vectors

Several strategies have recently been developed to inject or infuse plasmid vectors directly into cells without encapsidation in a viral particle or entrapment in a liposome. Recent efforts have been directed to use receptor-mediated endocytosis pathways for in vivo targeting of plasmid vectors by conjugated receptor ligands the DNA constructs for efficient delivery to specific sites of pathology (plasmid = independently replicating, extrachromosomal circular DNA molecules in bacteria, used in molecular biology as vectors for cloned segments of DNA).

Conjugate vectors have been used to achieve gene transfer to hepatocytes and to respiratory epithelial cells.

Genome editing technologies correct the DNA mutations in its native location. Programmable nucleases are used and they are able to induce breaks in some specific double-stranded DNA fragments. Subsequently endogenous repair machinery will mediate genome editing. Such technologies were applied in cystic fibrosis, hemophilia B, Duchenne muscular dystrophy, a.o., with promising results.

Genome editing enables precise changes to the DNA sequence. Traditional *CRISPR-Cas9* creates double-strand breaks (DSBs) that are repaired by cellular mechanisms to achieve gene disruption or correction. Newer modalities avoid DSBs: base editors catalyze single-base conversions (e.g., C→T, A→G) and prime editors can create a wider range of precise edits without donor templates or DSBs. Major concerns include off-target editing, delivery efficiency to target tissues, immune responses to editing components, and ethical/regulatory oversight—especially to prevent germline transmission of edits.

RNA-targeting therapeutics constitute a versatile and rapidly expanding class: Antisense oligonucleotides (ASOs) bind target RNAs to modulate splicing, degrade mRNAs via RNase H, or block translation. ASOs have produced approved therapies (e.g., nusinersen for spinal muscular atrophy) and are now used for personalized splice-modulating therapies in ultra-rare disorders. They offer specificity, but can require intrathecal delivery for CNS targets and may have safety or tolerability limitations depending on chemistry and dosing.

Small interfering RNAs (siRNA) induce RNA interference and have been successfully applied for liver disorders leveraging conjugates or lipid nanoparticle delivery; they provide durable knockdown with infrequent dosing schedules.

mRNA therapeutics enable transient expression of proteins and have been propelled to the clinic by successful vaccine platforms; mRNA may be used to express missing enzymes, to transiently deliver genome-editing machinery, or to produce therapeutic proteins without genomic integration.

Epigenome editing, which is aimed to specifically change the gene regulation of the chromatin structure creates new ways to manipulate the genome for gene therapy and cellular therapies.

Disease candidates and cell targets

The initial diseases considered candidates for somatic gene therapy were those in which bone marrow transplant had proven therapeutic. In these disorders such as, hemoglobinopathies, immunodeficiencies and other blood cell defects, the symptoms result from deficient function or lack of a bone marrow gene product. Successful bone marrow transplant results in the cellular replacement of normal blood elements that can produce the normal gene product and correct the metabolic disease. Among such disorders, prime candidates for gene therapy trials are those diseases in which bone marrow transplant is therapeutic, such as immune deficiency due to deficient adenosine deaminase (ADA) and Gaucher disease.

To date, most investigators have concentrated on retroviral gene transfer into pluripotent hematopoietic stem cells. These cells are accessible, can be grown in cultures and provide the opportunity to generate a variety of blood elements or pulmonary macrophages, expressing the transferred gene. Retroviral infected hematopoietic stem cells may be reintroduced into the patient by autologous transplant. Transplanted stem cells will then migrate from the site of injection and will express the foreign gene product.

Other experiments have developed liver gene therapy. The disease candidates for liver gene therapy are those in which the primary metabolic defect resides in the liver (hypercholesterolemia and phenylketonuria). Hepatocytes can be obtained by partial hepatectomy, grown in culture for several weeks, genetically modified using viral or nonviral vectors and then transplanted into animals. For example, a fraction of retroviral marked hepatocytes introduced into mice by intra-splenic injection migrated to the liver parenchyma and continued to express liver specific gene products for up to 1 year.

Many gene-therapy trials have been carried out. Most of them were small-scale studies, but helped understanding of what vectors can do or cannot do. For instance, in cystic fibrosis this procedure involved the use of adenovirus vectors to introduce a specific DNA directly into respiratory epithelial cells, with promising results.

Examples of genetic disorder therapies

Luxturna (voretigene neparvovec-rzyl): A gene therapy for inherited retinal diseases, such as Leber congenital amaurosis, caused by mutations in the RPE65 gene.

Zynteglo (betibeglogene autotemcel): A gene therapy for beta-thalassemia, which involves modifying a patient's own blood stem cells to produce functional hemoglobin.

Lyfgenia (lovotibeglogene autotemcel): Another gene therapy for sickle cell disease that works by using a viral vector to deliver a functional copy of the gene for hemoglobin.

Casgevy (exagamglogene autotemcel): A CRISPR-based gene editing therapy used to treat sickle cell disease and beta-thalassemia.

Zolgensma (onasemnogene abeparvovec): A gene therapy used to treat spinal muscular atrophy (SMA) in young children.

The **genetic basis of personalized therapy** lies in analyzing an individual's unique genetic profile to tailor medical decisions. This includes using genetic markers to predict disease risk, identify how a person will respond to certain drugs (pharmacogenomics), and select the most effective treatment and dosage to minimize side effects and maximize efficacy. The Human Genome Project provided the foundational map to enable this approach, allowing for more precise and individualized approaches to prevention, diagnosis, and treatment.

Genetics enables personalized therapy by:

- Predicting disease risk: An individual's genetic makeup can indicate a predisposition to certain diseases.
- Personalized drug selection: Genetic analysis can predict how a patient's body will metabolize a drug and how the drug will interact with their body, allowing doctors to choose the most effective medication and dosage.
- Optimizing treatment: By considering an individual's genetic profile, doctors can maximize a treatment's effectiveness while minimizing potential adverse side effects.
- Improving diagnosis: Genetic information can help in more accurate diagnosis and can be used to predict disease progression

Conclusion

The therapeutic landscape for genetic disorders has shifted from palliation to targeted, potentially curative options. Each strategy—ERT, small molecules, gene therapy, genome editing, RNA therapeutics, and cell therapy—has unique strengths and limitations. Integration of molecular diagnosis, delivery technology, safety assessment, and policy frameworks is necessary to translate these scientific advances into broad clinical benefit.

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