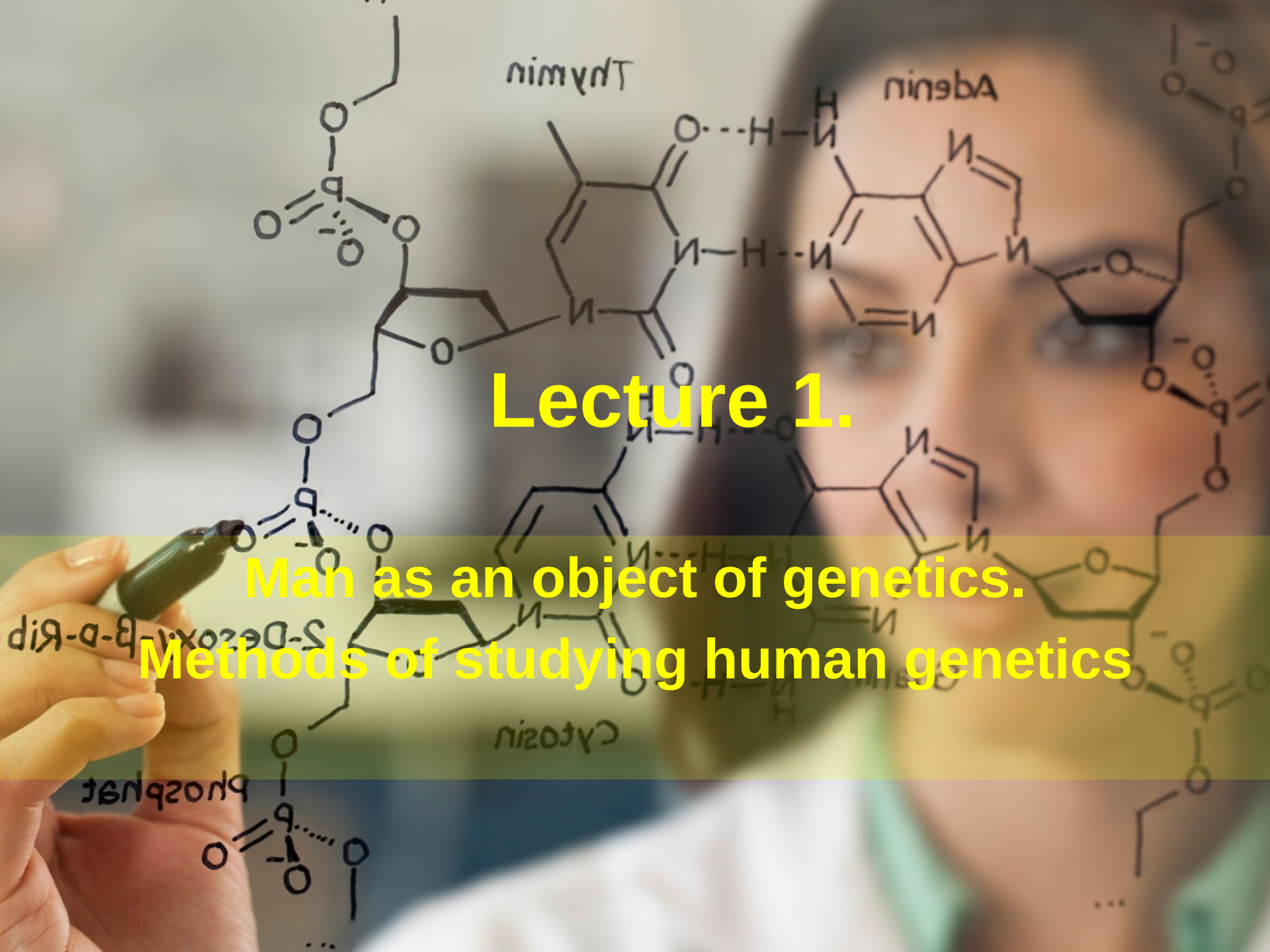


Lecture 1.

Man as an object of genetics.
Methods of studying human genetics

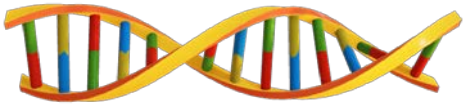
Features of the person as object of genetics

What creates difficulties:

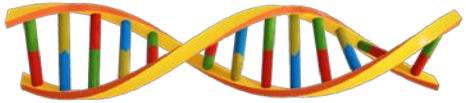
You can not cross at the request of the experimenter

The number of descendants is small

A rare generational change



Many signs A lot of chromosomes



However, the great interest outweighs all the difficulties

The main methods of studying human genetics

- Twin
- Cytogenetic
- Genealogical
- Biochemical
- Population and statistics
- Dermatoglyphic
- Somatic cell genetics
- DNA diagnostics

Twin's method allows to determine:

role of heredity and environment in the expression of some phenotypic traits.

It is necessary to calculate the following coefficient:

Coefficient of pair concordance (K) :

$K = C/(C + D) \cdot 100\%$

Where: C – number of concordant twin pairs; D – number of disconcordant (differ) twin pairs.

The twin method studies the correlative role of genotype and environment by comparing twins

$$H = \frac{C_{MZ} - C_{DZ}}{100 - C_{DZ}} \cdot 100$$

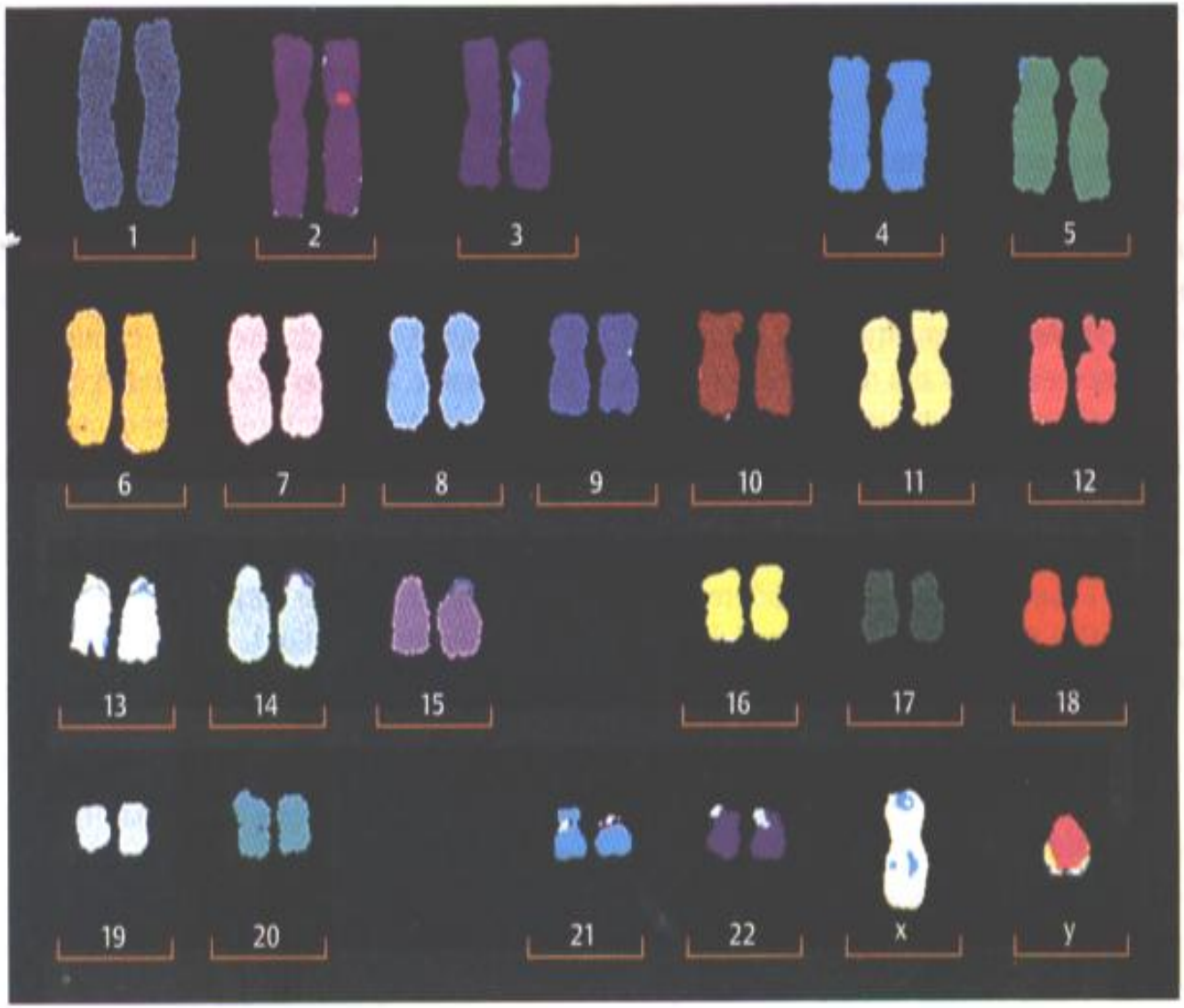
Holzinger formula
For in this formula:
H – heritability coefficient;
C_{MZ} – percentage of concordant pairs in the group of monozygous twins;
C_{DZ} – is the percentage of concordant pairs in the dizygotic twin group.
E – influence of the medium is calculated by the formula
E = 100 – N;
The coefficient of heritability (H) – an indicator of the relative role of heredity and environment in the formation of a sign (disease).
For all monogenic diseases – H = 100%;
Chromosomal – H = 100%.
For multifactorial diseases – 50% < H < 100%
(diabetes H=57%, rheumatism – 36%, schizophrenia – 65%, IQ =80%).

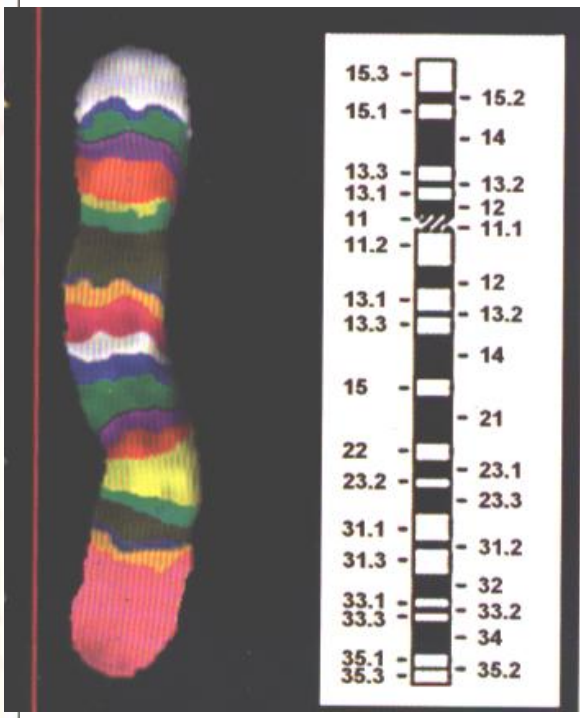
H = 70 – 100% trait is mainly determined by genotype (ex. blood groups).

H = 40 – 60% trait is determined by combined action of genotype and environment (ex. hypertension, diabetes).

H = 0 – 30% trait is determined by environmental factors (ex. infectional diseases: cholera, malaria).

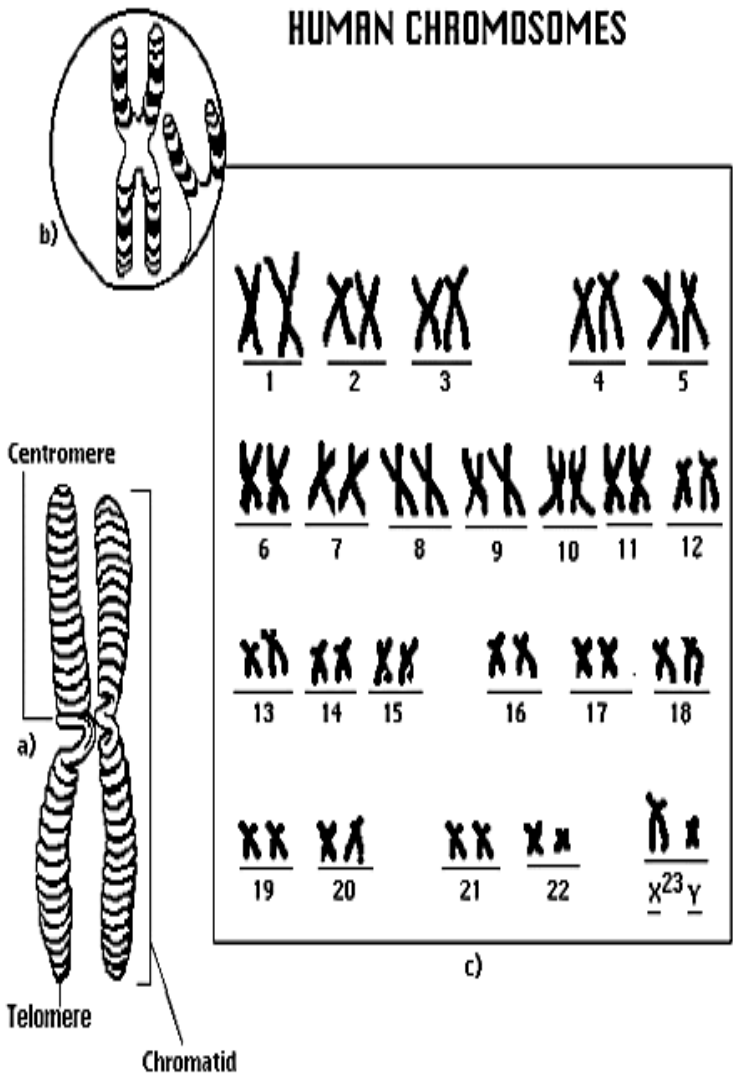
Method cytogenetic studies of chromosomes



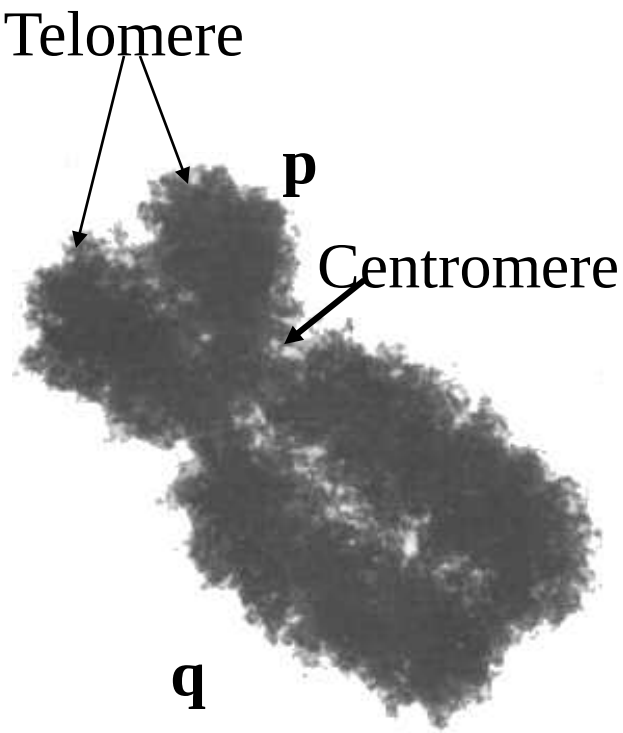


The Normal Human Chromosomes

* Normal human cells contain **23 pairs of homologous chromosomes:**
  - 22 pairs of **autosomes** (numbered as 1-22 in decreasing order of size)
  - 1 pair of **sex chromosomes**.
* **Autosomes** are the same in males and females
* **Sex chromosomes** are:
  - **XX** in females
  - **XY** in males.
* Both **X** are **homologous**. **Y** is much smaller than **X** and has only a few genes.



Chromosome Structure



* At the metaphase stage each chromosome consists of **two chromatids** joined at the **centromere** or primary constriction
- The centromere divides chromosomes into **short (p i.e. petit)** and long **(q e.g. g=grand)** arms. The tip of each chromosome is called **telomere.**
- The exact function of the centromere is not clear, but it is known to be responsible for the **movement of the chromosomes** at cell division.

Classification Of Chromosomes

Chromosomes are classified (analysed) accordig to:

1. Shape and
2. Staining

1. Morphologically (shape)

According to the position of the centromere as:

(i) metacentric,

(ii) sub-metacentric,

(iii) acrocentric,

(iv) telocentric (with centromere at one end.

This occurs in other species, but not in man).

Staining Methods for cytogenetic analysis of chromosomes

There are several staining methods for cytogenetic analysis of chromosomes.

Each stain produces specific banding patterns known as "Chromosome Banding"

- G banding,
- Q banding,
- R banding,
- C banding.

The pattern is specific for each chromosome, and is the characteristics utilized to identify each chromosome.

Staining Methods for Cytogenetic Analysis

G Banding:

Treat with trypsin and then with Geimsa Stain.

R Banding:

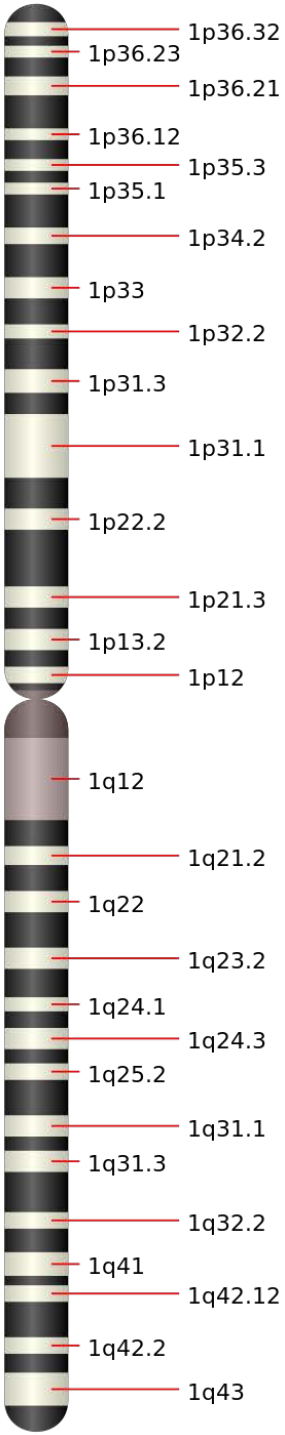
Heat and then treat with Geimsa Stain.

Q Banding:

Treat with Quinicrine dye giving rise to Fluorescent bands.

C Banding:

Staining of the Centromere.



Chromatogram of the first human chromosome and the beginning of articles about it in Wikipedia

Human chromosome 1 is the largest of human chromosomes, one of the 22 autosomes in humans. The chromosome contains more than 248 million base pairs, which is approximately 8% of the total DNA material of a human cell. Currently, it is believed that there are 4505 genes on chromosome 1, which is higher than previously made predictions about the number of genes that were based on chromosome size. According to the cytogenetic classification, chromosome 1 belongs to the chromosomes of group A, which means that this chromosome belongs to the group of the largest human chromosomes and is metacentric, that is, it has arms (p - and q-) of approximately equal length. Chromosome 1 has a large heterochromatic region, which includes two centromeric bands 1 p11 and 1q11, as well as a centromeric band 1q12, together constituting about 5% of the length of chromosome 1.

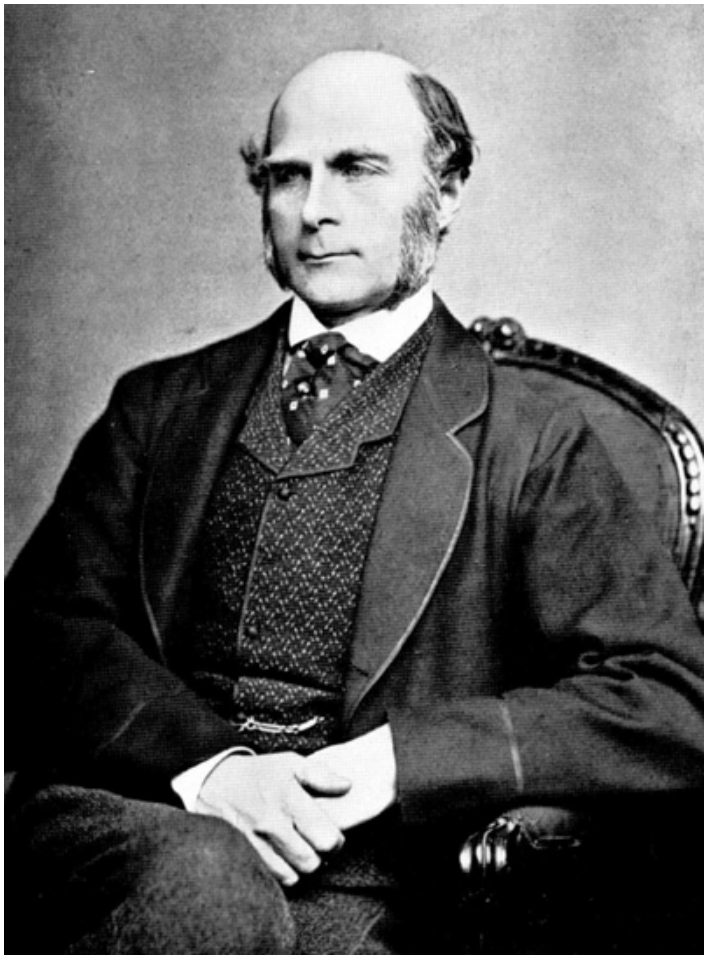
Genealogical method – method of analysis of pedigrees

Genealogical method

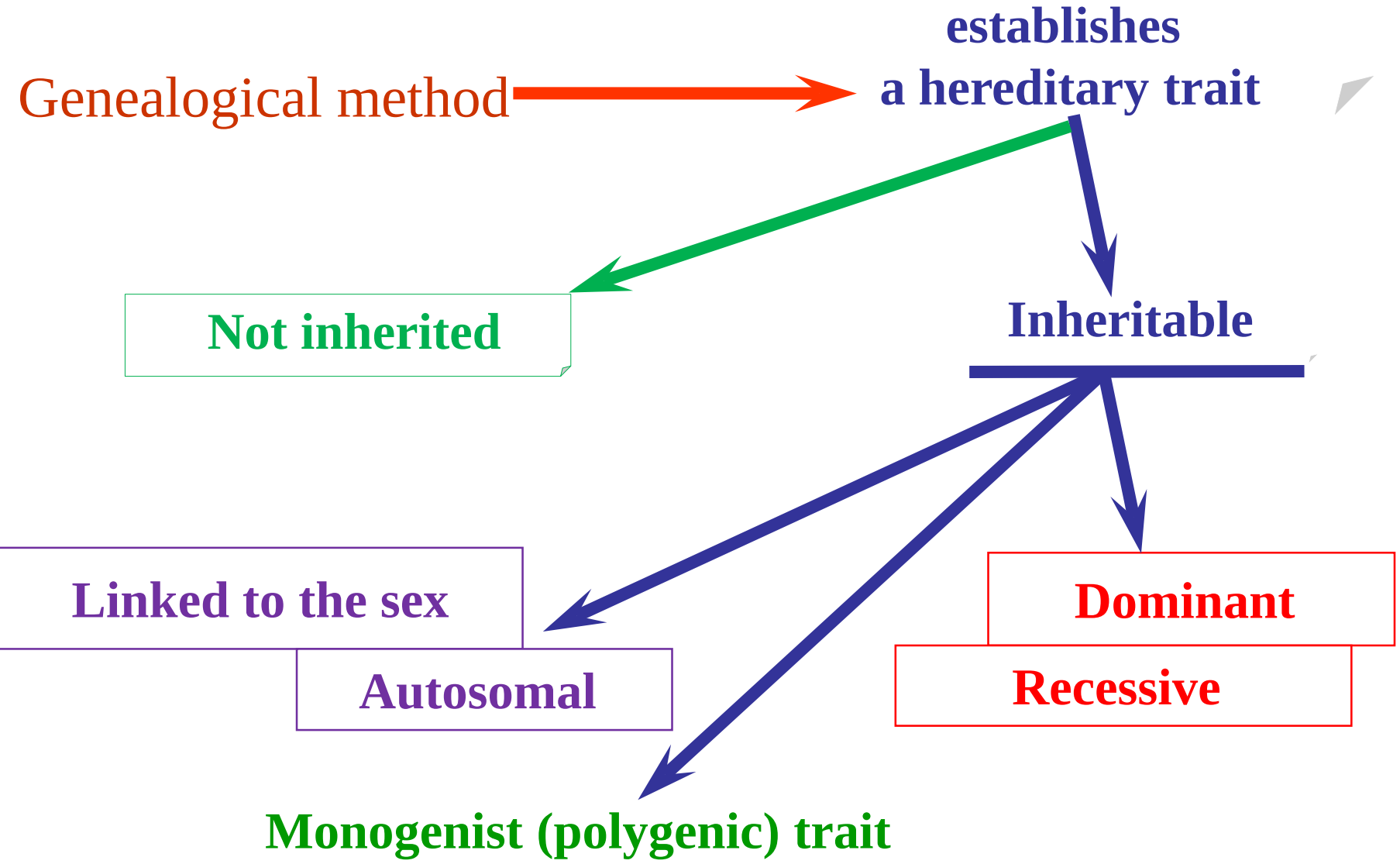
it was proposed in 1883 by F. Galton. The method allows you to set: 1) whether this trait is hereditary (by its manifestation in relatives); 2) the type and nature of inheritance (dominant or recessive, autosomal or sex - linked); 3) zygosity of persons of pedigree (Homo-or heterozygotes); 4) gene penetrance (frequency of its manifestation); 5) the probability of having a child with a hereditary pathology (genetic risk).

Sir Francis Galton; February 16, 1822 January 17, 1911)

Cousin Of C. Darwin
He worked on
heredity,
biometrics,
dermatoglyphics,
statistics
and testing;
he was the first to study twins.
Created eugenics.



Genealogical method – method of analysis of pedigrees

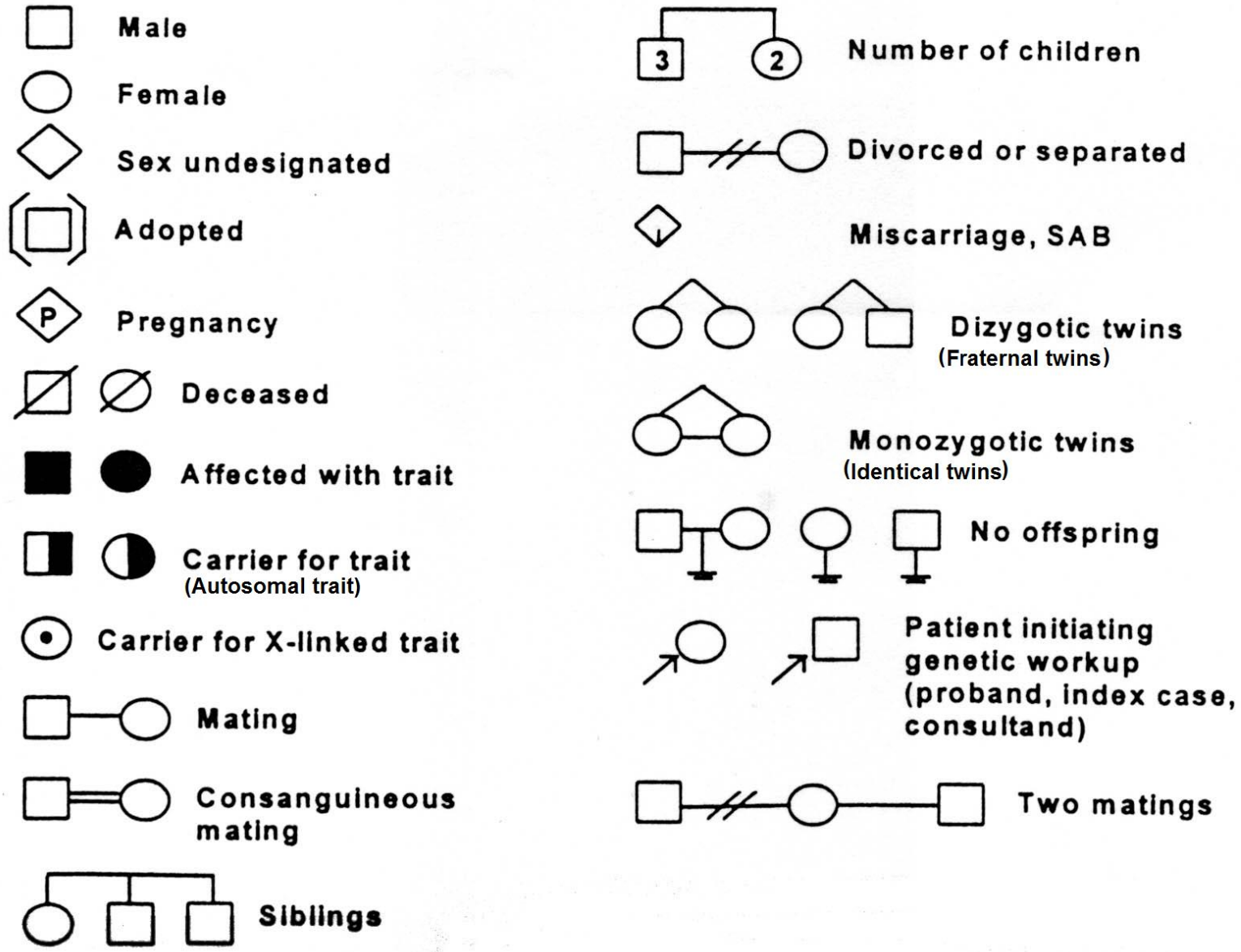


Genealogical method

indicate

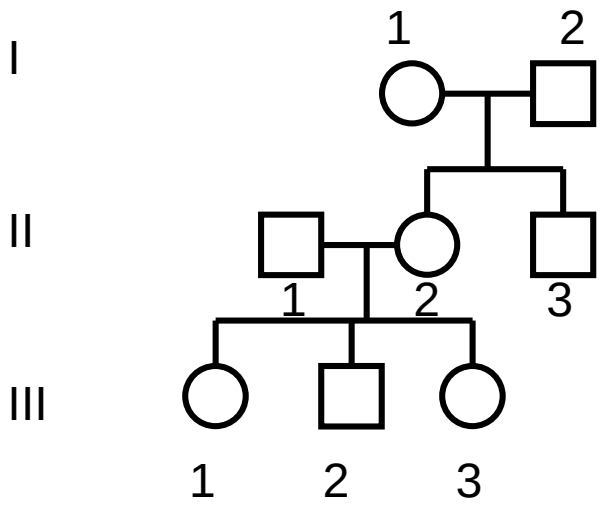
- Homo - or heterozygote individuals pedigree;
- gene penetrance (frequency of its manifestation);
- the probability of having a child with a hereditary pathology (genetic risk).

Symbols used in drawing up pedigrees



Stages of genealogical method

1) genealogy begin to build with probanda-person, which begins the study of the family.
2) each generation is numbered with Roman numerals on the left;
3) individuals of one generation are located on a horizontal line and numbered in Arabic numerals.

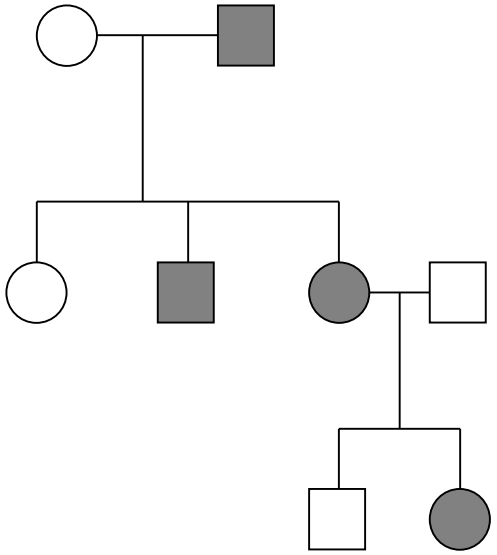


There are 6 main types of inheritance

- AD – autosomal dominant
- AR – autosomal recessive
- XD – x-linked-dominant
- XR – x-linked-recessive
- Y – holandric type of inheritance
- mitochondrial (or cytoplasmic) of inheritance

Autosomal dominant type (AD) inheritance is characterized by the following features:

1) men and women are equally ill;
2) there are patients in every generation - inheritance "vertically".
3) the probability of inheritance is 100% (if at least one parent is homozygous), 75% (if both parents are heterozygous) and 50% (if one parent is heterozygous).



Examples in humans:

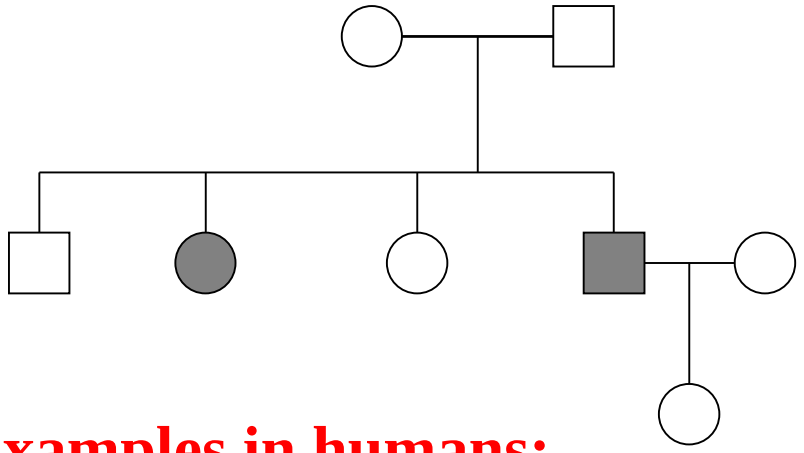
Marfan syndrome

Achondroplasia

Hypercholesterolemia

Autosomal recessive (AR) inheritance type

1. Typical skip generations
2. Men and women alike «Horizontally»
3. The probability in children is 25%, if the parents do not show the sign



Examples in humans:

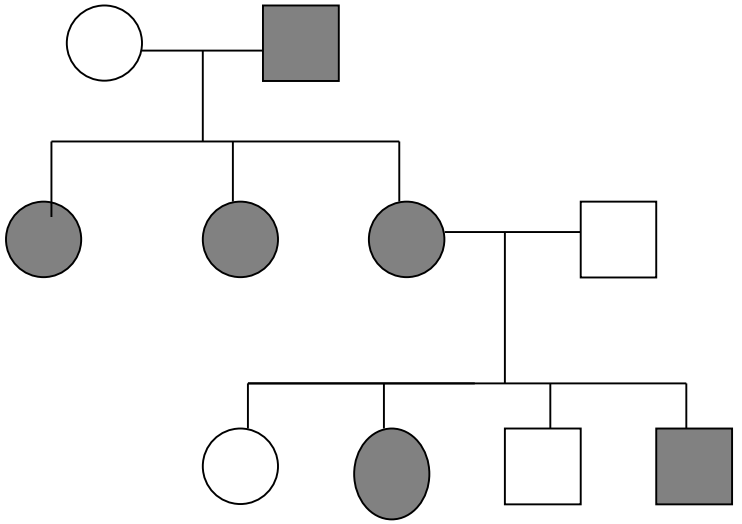
Phenylketonuria

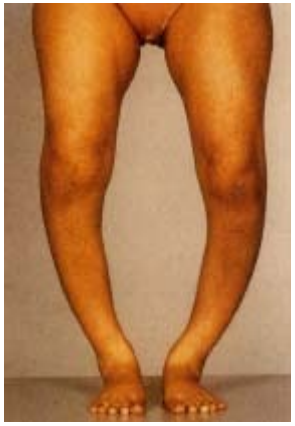
Mucoviscidosis

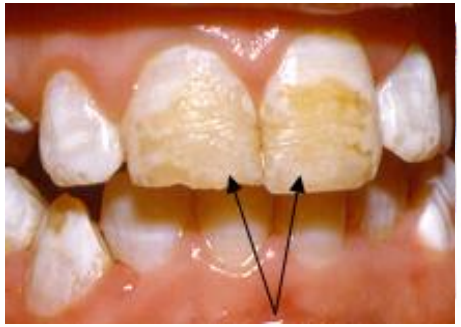
Adrenogenital syndrome

X-linked dominant (XD)

- No missing generations – vertical
- Women are affected 2 times more often
- Passed from father to all daughters; mother-50% of sons and daughters.







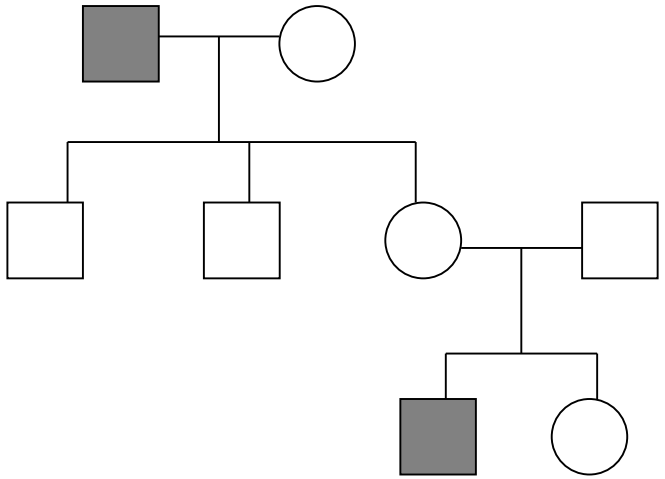
Examples in humans:

Rickets resistant to vitamin D

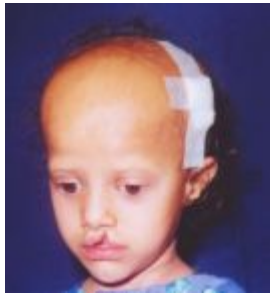
Brown enamel of teeth

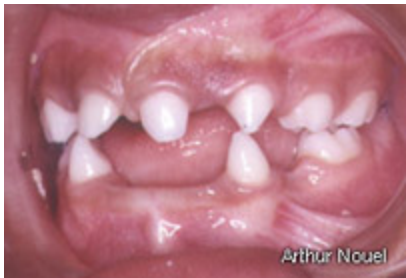
X-linked recessive (XR)

- Transmitted from the grandfather through the mother-carrier to the grandson
- In men, it appears much more often than in women



Examples in humans:
Hemophilia
Daltonism
Muscular dystrophy
Ectodermal dysplasia



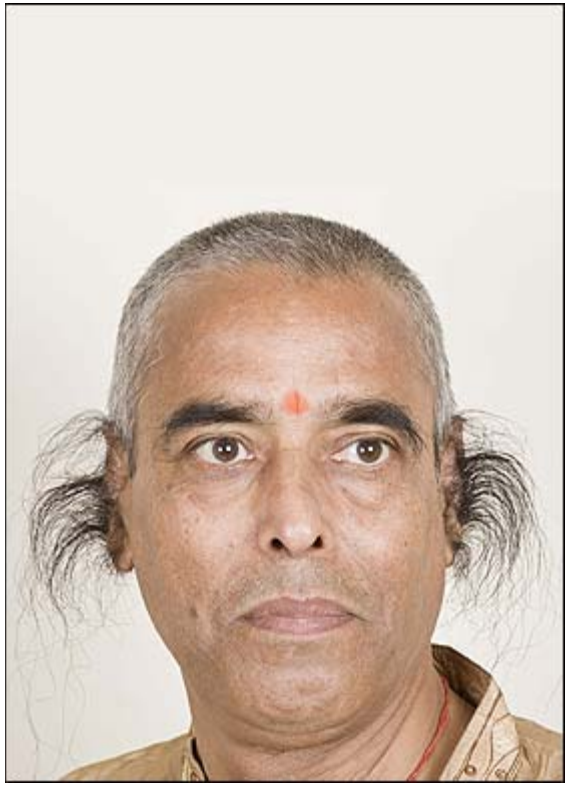


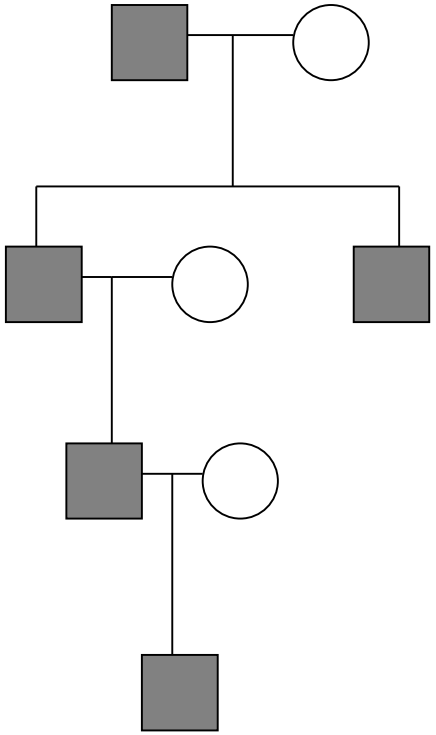
Holandric type (Y) of inheritance

Transmitted through the male line without missing generations

An example in humans:

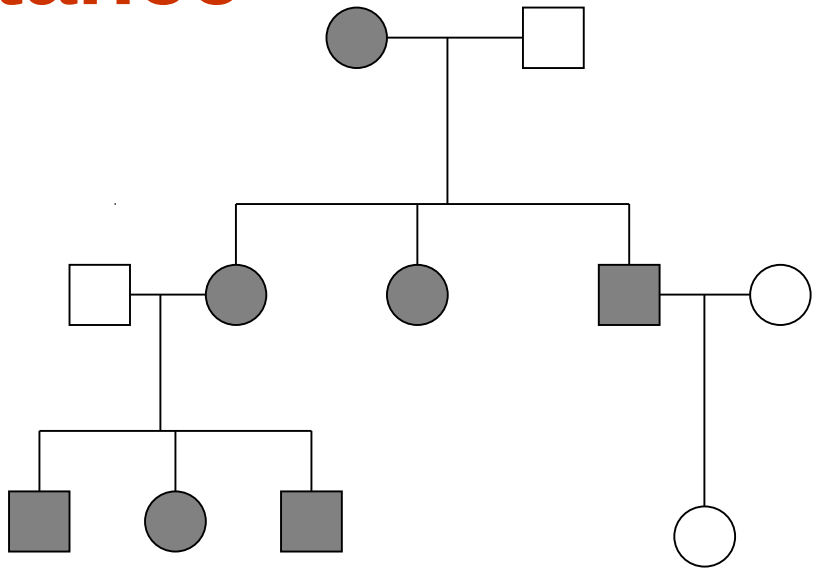
Hypertrichosis of the ear





Mitochondrial (cytoplasmic) inheritance

- Transmitted only through the maternal line
- Men get sick more often and more severely than women
- **example: mitochondrial myopathy**



Plants have also inherited genes of chloroplasts.

Haplotype

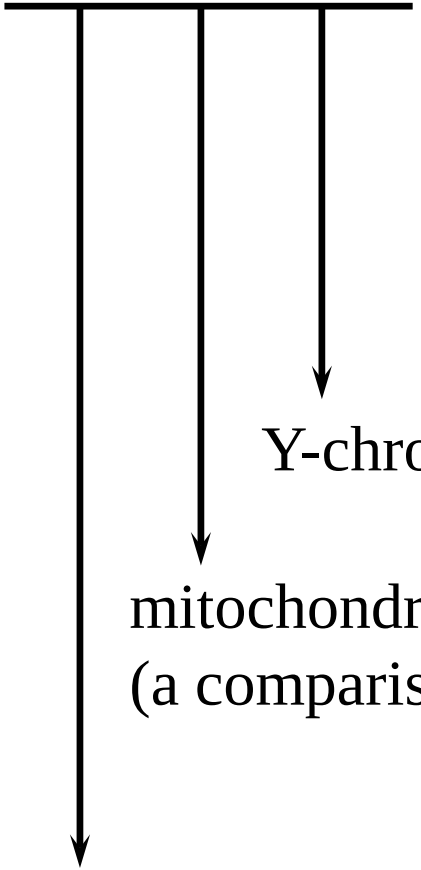
Haplotype (abbr. from "haploid genotype") — a set of alleles at the loci of one chromosome, usually inherited together.

If when crossing the combination of alleles changes (which is very rare), talk about the emergence of a new haplotype.

The number of identical short tandem repeats is investigated (STR, short tandem repeats)

Haplogroup

Гаплогруппа — a group of similar haplotypes having a common ancestor that has a mutation inherited by all descendants (usually a single-nucleotide polymorphism).



Y-chromosome-paternal ancestral line

mitochondrial-maternal ancestral line
(a comparison of the Standard of Cambridge sequences)

centromeric somatic ancestral inheritance

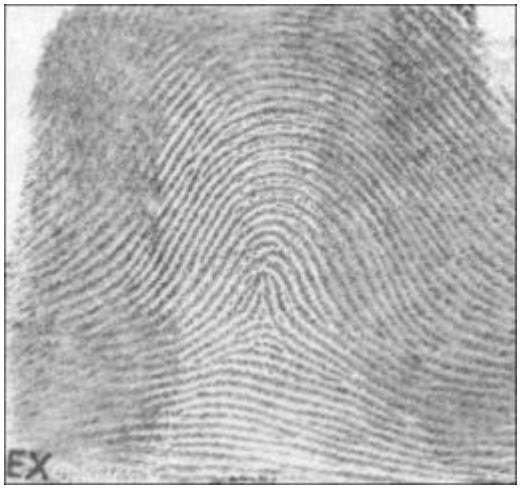
Dermatoglyphic method (also proposed by Galton).
Auxiliary method in the diagnosis of hereditary syndromes

Dermatoglyphic method

Studies the features of scallop skin and the main flexor lines of the palms and soles

Three main types of finger patterns

arc

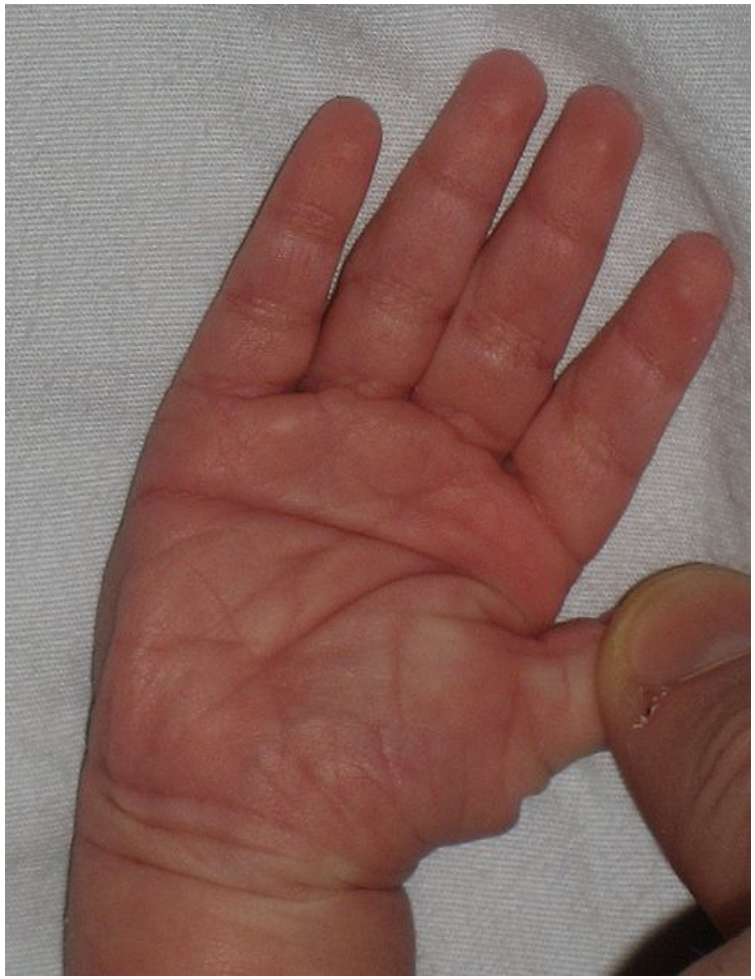


loop

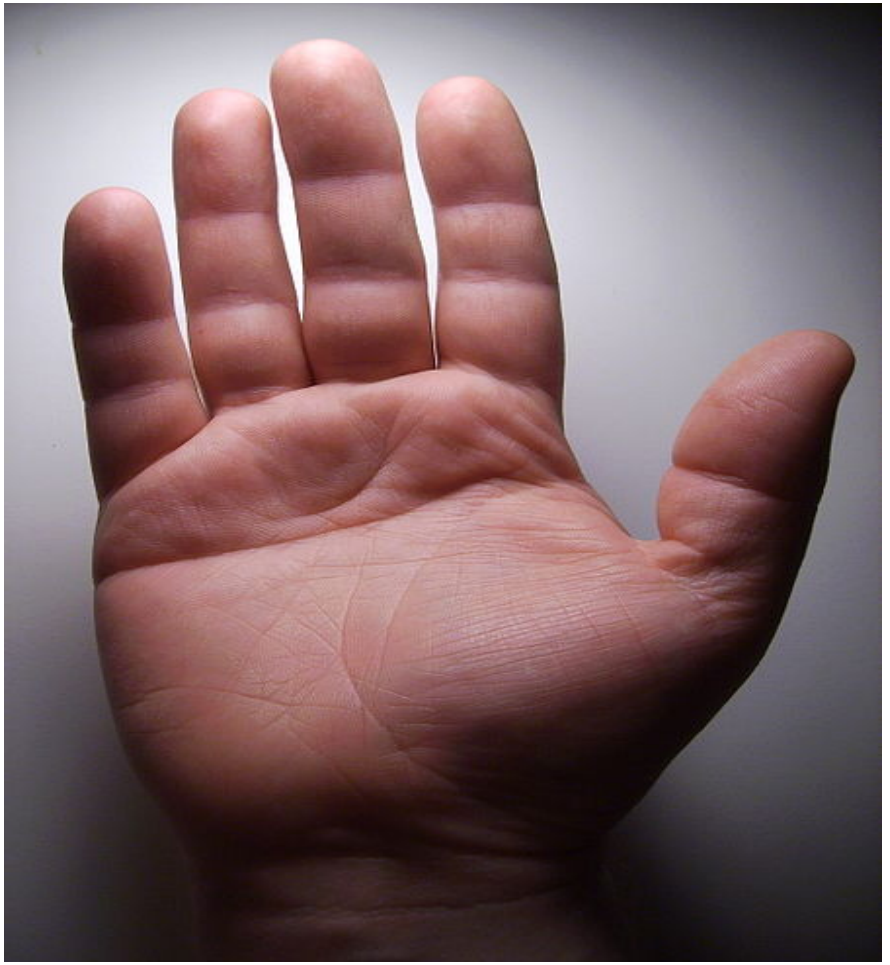
curl

Options flexion creases

normal palm

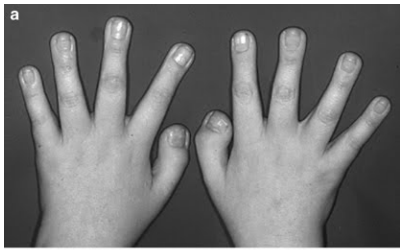


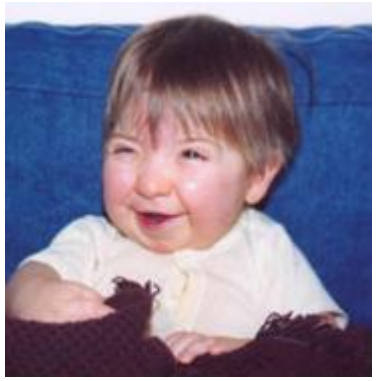
simian crease

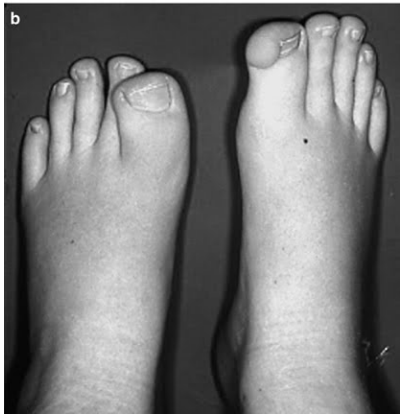


Features of dermatoglyphics in some syndromes

- Edwards syndrome – the arc on all fingers
- Down syndrome is one of the flexion crease (simian)
- Turner syndrome – all curls on the fingers
- Rubinstein-Taby syndrome – a complex pattern on Tenar



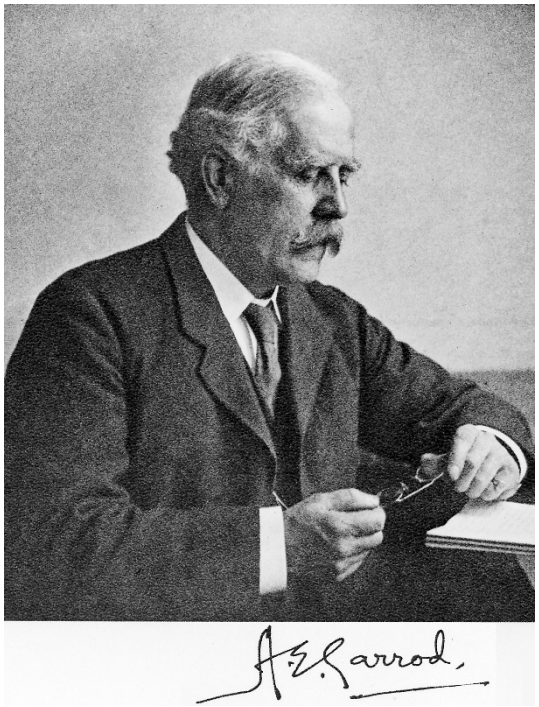


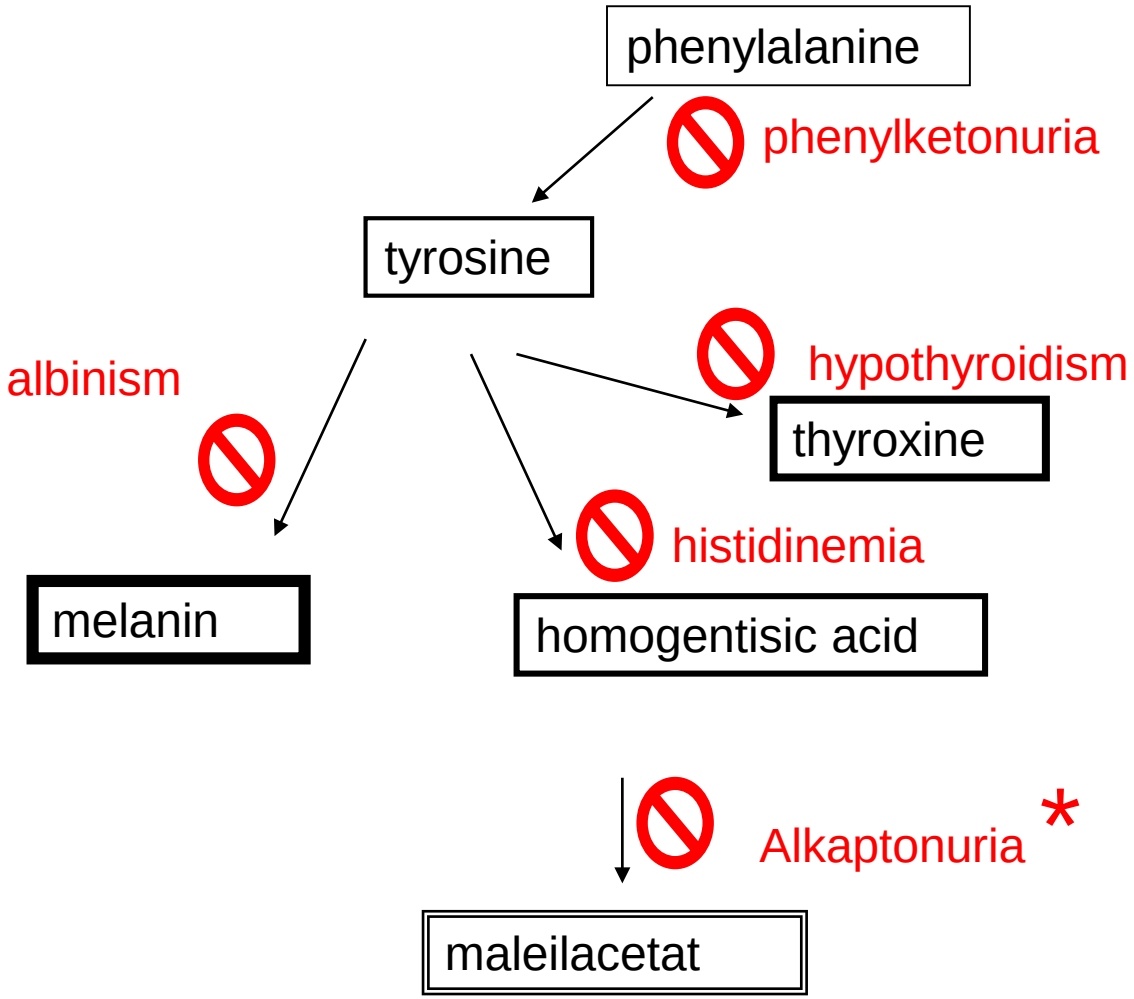


Biochemical method

- Used to study fermentopathy mutations disrupting the work of enzymes.
- In the blood and urine of patients, certain chemical compounds characteristic of this genetic disease are detected.

Examples of the fermentopathy





* The first described hereditary metabolic disorder (Archibald Garrod in the early twentieth century)

and so on

Online Mendelian Inheritance in Man®

Database of gene loci, phenotypes (including hereditary diseases), developed at Johns Hopkins University (USA). Each article in the OMIM classification is assigned a unique six-digit number, the first digit of which indicates the method of inheritance.

1 – (100000 – ...) – autosomal dominant type of inheritance.
2 – (200000 – ...) – autosomal recessive inheritance
3 – (300000 – ...) – loci and phenotypes associated with the X chromosome.
4 – (400000 – ...) – loci and phenotypes associated with the Y chromosome.
5 – (500000 – ...) – loci and phenotypes associated with mitochondrial (cytoplasmic) inheritance.
6 – (600000 – ...) – autosomal inheritance.

Alleles (alleles variants) of a gene are denoted by a six-digit main article number (gene article) followed by a four-digit number denoting the allele. For example, allelic variants (mutations) in the locus encoding the formation of factor IX blood clotting (leading to hemophilia B), are denoted by 306900.0001 to 306900.0101 (306900- the designation of the locus).

An asterisk (*) before the locus or phenotype number indicates that the method of inheritance for a given locus or phenotype has been proven (according to the authors and editors). The absence of an asterisk indicates that the inheritance method is not definitively set.

Symbol # before article means, that this phenotype can be caused by mutation somehow either from two (named) or more genes.

Consider in more detail the exchange of phenylalanine and the development of phenylketonuria (AK) OMIM 261600 and 261630

Phenylketonuria

classical type I

Described By A. Folling, 1934
98% of cases
AR,
12q23.2;
gene PAN phenylalanine-4-mono-oxygenase > 600 mutations
Treated diet

atypical-type II

Described, I. Smith, 1974
2% of cases
AR,
4p15.3;
the QDPR genesynthesis of phenylalanine hydroxylase cofactor-tetrahydrobiopterin
Not treated diet
Treated by L-DOPA with carbidopa, 5-oxitriptofan

Experimental therapy

- substitution phenylalanine lyase (PAL) is a plant enzyme that converts phenylalanine into harmless metabolites,
- gene therapy based on the introduction of a viral vector containing the phenylalanine hydroxylase gene into the body.

Phenylketonuria

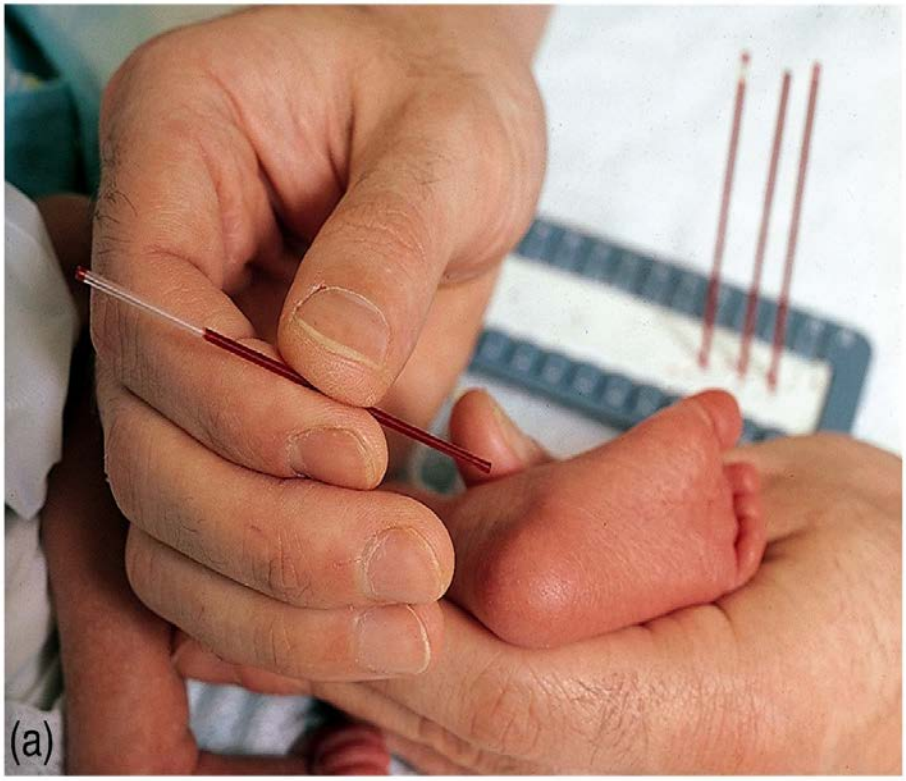
Atypical – type III

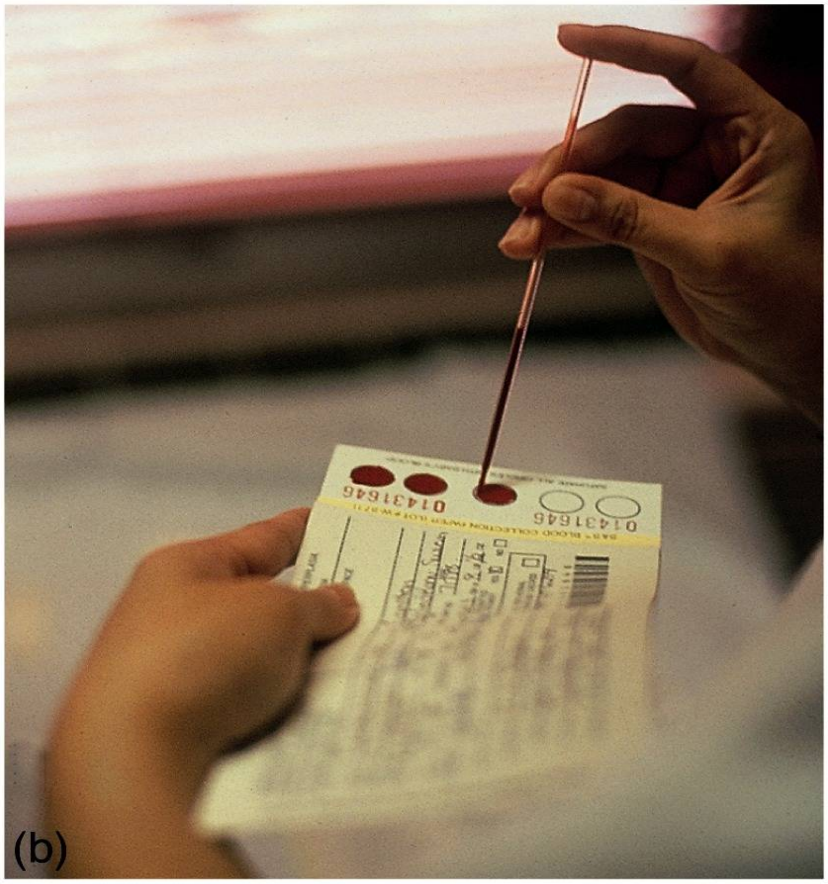
Described, I. Smith, 1974
< 1% of cases
AR
q22.3-23.3
gene *PTPS*
6-pyruvolytetrahydropterinsynthesizing four of dihidrogenofosfat
Not treated diet

All forms are caused by tandem repeats or restriction fragment length polymorphism

Children from birth should follow a special diet with a restriction on phenylalanine

Neonatal screening-screening of all infants for biochemical defects





Currently, children are tested for:
phenylketonuria,
cystic fibrosis,
congenital hypothyroidism,
adrenogenital syndrome
and galactosemia

When choosing diseases for neonatal screening, in accordance with who recommendations, factors such as the severity of disease manifestations, the frequency of these diseases, as well as the simplicity and reliability of the diagnostic methods used, the availability of affordable and effective treatments were taken into account.

Population-statistical method of genetics

- Studies and compares human populations.
- Based on the hardy-Weinberg law

The law of genetic stability of populations

- Formulated in 1908 independently by English mathematician G. hardy and German physician V. Weinberg.
- The law States that if the number of panmictic (free-crossing) population is large, there are no mutations, migration and selection (for the studied gene), the frequency of genotypes AA, Aa and aa in the population remain the same from generation to generation: $\boldsymbol{p}^2(\boldsymbol{AA})$: $\boldsymbol{2pq}$ $(\boldsymbol{Aa})$: $\boldsymbol{q}^2(\boldsymbol{aa})$,

where ***A*** and ***a*** are alleles of an autosomal gene, p-allele frequency ***A***, ***q*** is the frequency of allele ***a***.

Deviations from the hardy-Weinberg equilibrium indicate the effect of one or more factors on the population:

Selection’s

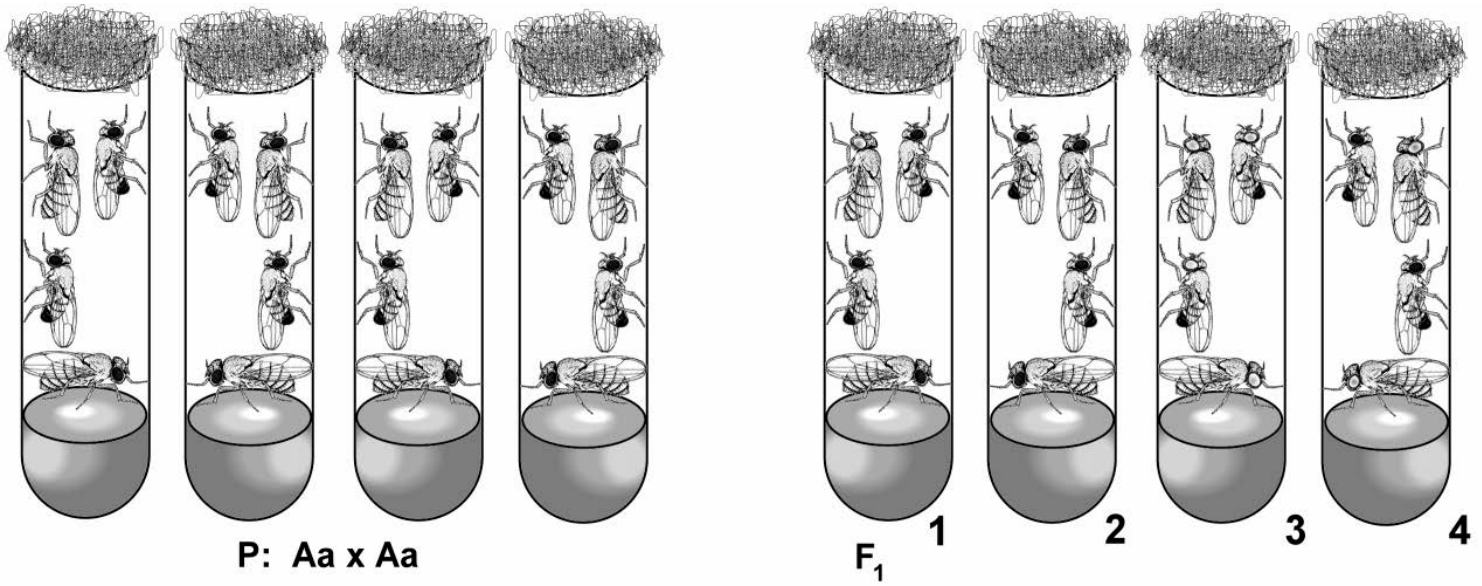
Mutations

Gene drift (non-directional change in the frequency of allelic variants of genes in the population due to random statistical reasons)

Migrations

Insulations

Sewell Wright's experiment was simple: he put two females and two males of Drosophila flies, heterozygous in gene A (genotype AA), into tubes with food. The concentration of normal (A) and mutational (a) alleles was 50 %. After a few generations, it turned out that in some populations all individuals became homozygous for the mutant allele (AA), in other populations it was lost altogether (A A), some populations contained both normal and mutant alleles. Despite the decline in the viability of mutant individuals and, therefore, CONTRARY to NATURAL SELECTION, in some populations, the mutant allele completely replaced the normal one. This is the result of a random process — gene drift.



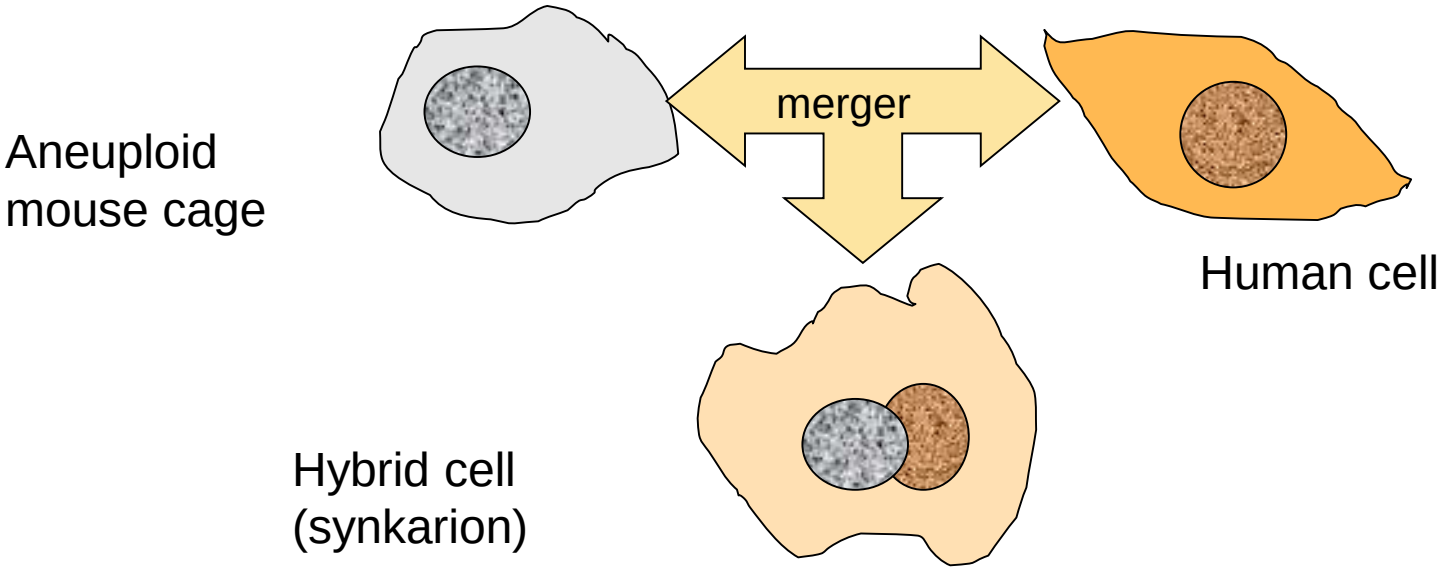
In small populations, the frequency of the mutant allele changes rapidly and randomly

Populations differ in the frequency of gene mutations

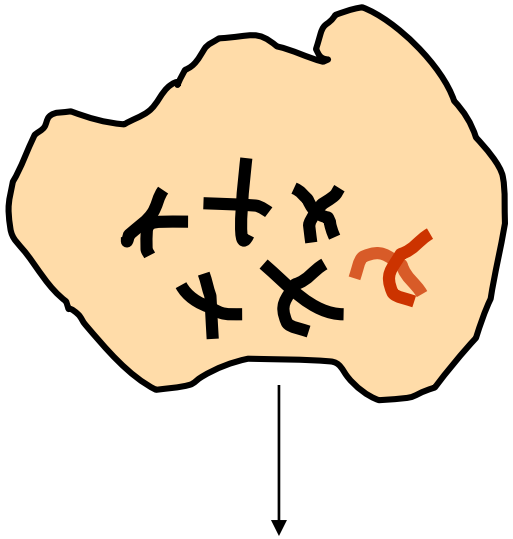
Ethnic group	Disease
The Amish old order (PA)	Chondroectodermal dysplasia; Ellis-van Creveld syndrome; cartilage-hair hypoplasia
Kuhn (San Bias), Indians (Panama) Hopi Indians (Arizona) Pima Indians (southwestern United States) Finns	Albinism Albinism Type 2 diabetes Congenital chloride diarrhea; aspartylglucosaminuria; congenital nephrotic syndrome; dwarfism muliebria
Yupik Eskimos	Congenital adrenal hyperplasia
Afrikaners (South Africa)	Mottled porphyria; lipoid proteinosis; Huntington's chorea; tissue scarring
Ashkenazi Jew	Tay-Sachs disease; Gaucher disease; vegetative dystonia; Canavan disease
The Karaites island Rukan (Japan) Cretans, Sardinians	Werdnig-Hoffman Disease Spinal cord muscle atrophy p-Thalassemia

Somatic cell genetics method

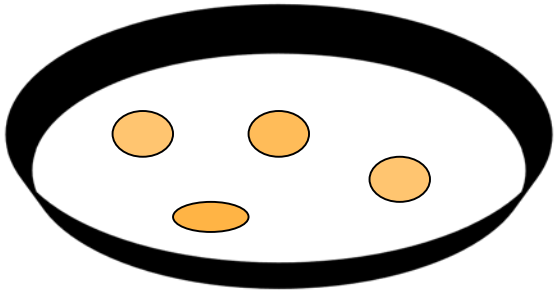
- Cells are grown in culture.
- This method was able to map human genes.
- The method is peculiar:



During cell divisions in a hybrid cell all human chromosomes are lost, except one (for example, № 17)



Seeding into a selective environment where you can only survive if you have a specific human gene (for example, gene A)



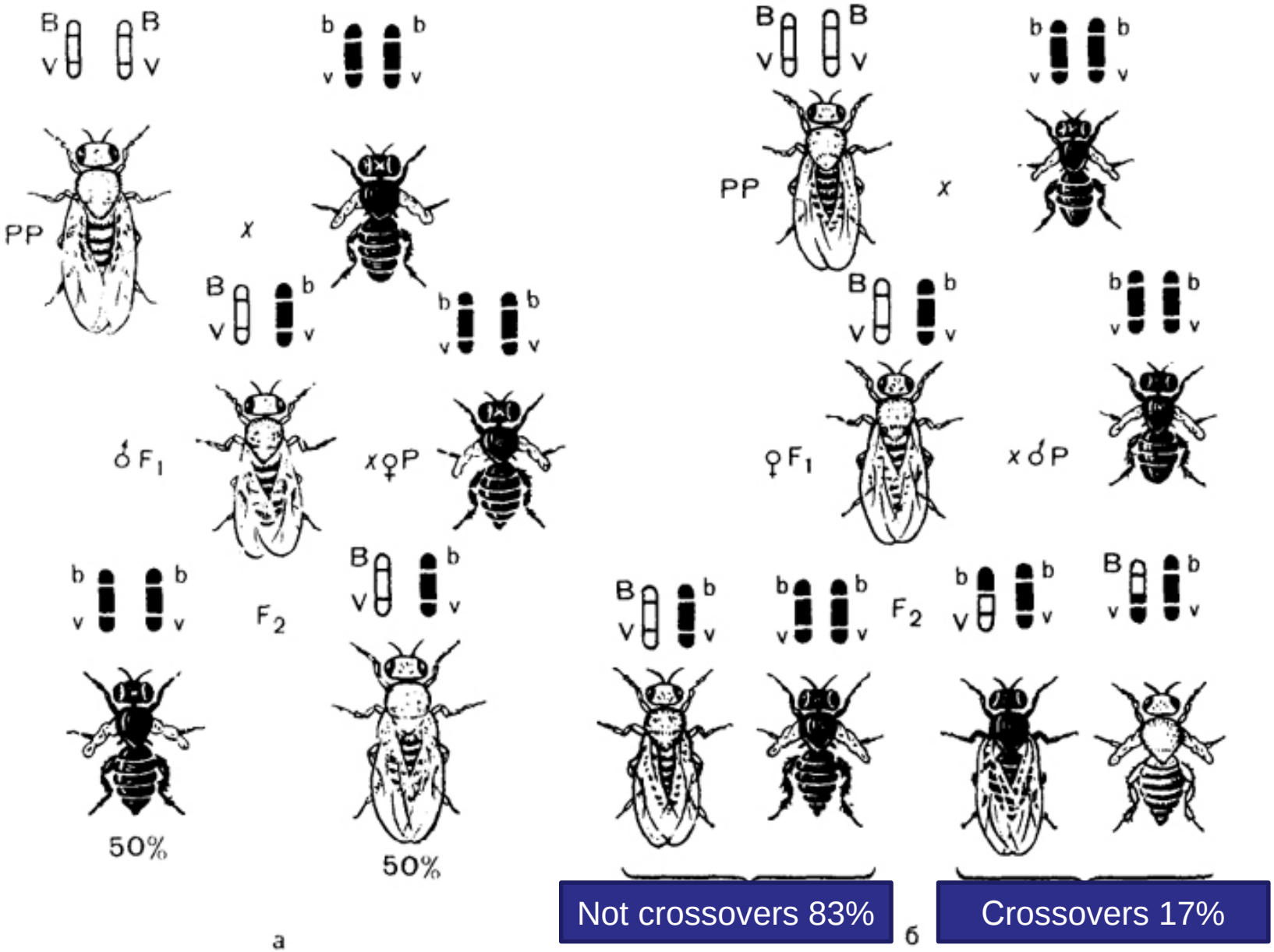
Cells survive, then the gene A lies on chromosome 17

This is one of the methods of gene mapping

Basic methods of genetic (chromosomal) mapping

- On the basis of crossings-not have rights! (hybridological method) - % crossover descendants morganite (centimorgan)
- Based on pedigrees
- Somatic cell genetics methods
- The method of DNA probes (DNA fragments with a known sequence)
- Genome sequencing methods

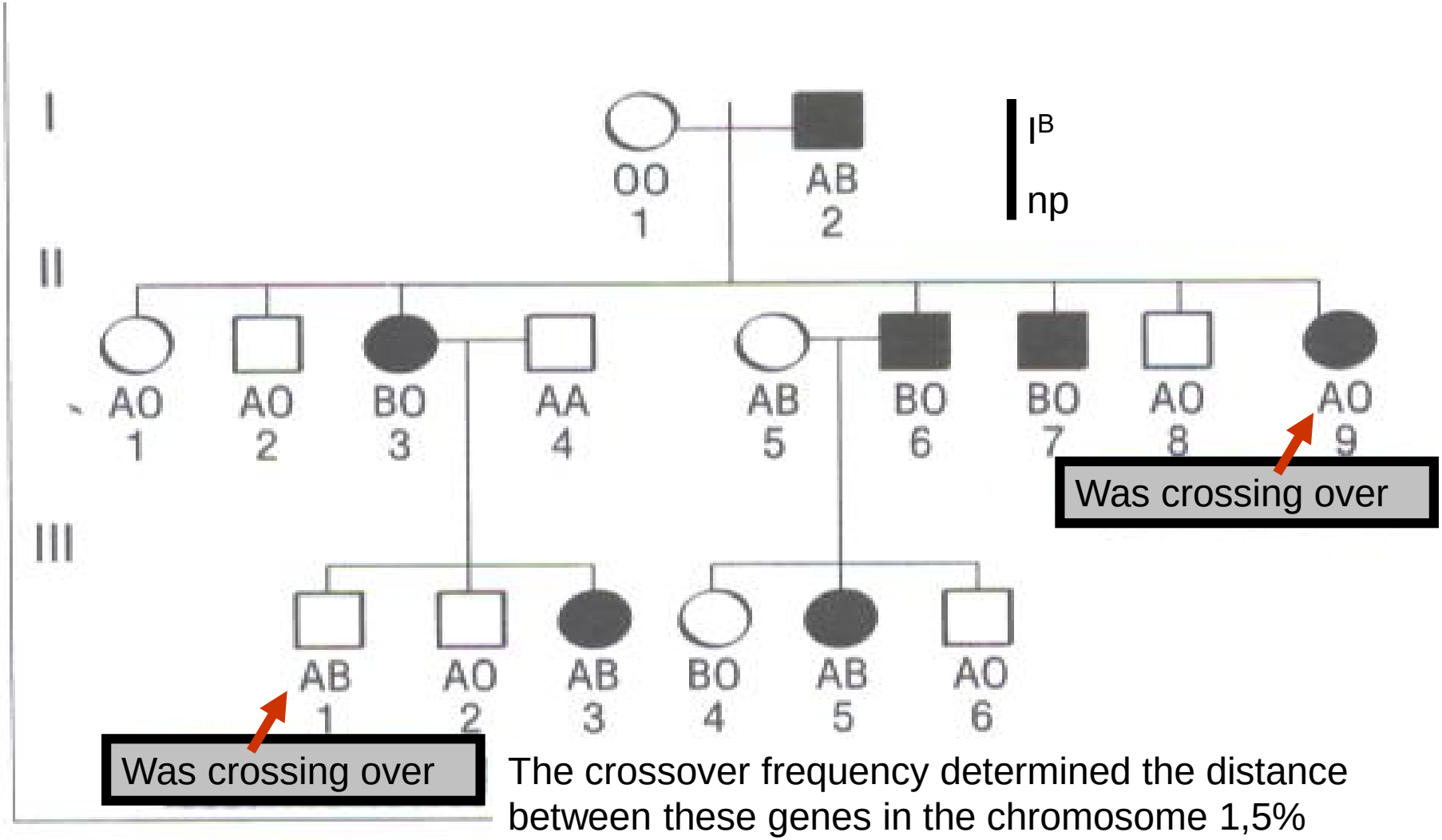
Morgan's experiments on coupling in Drosophila. Distance of genes In V – 17 Morgane



Morgan's Rule

Genes, localized in one chromosome, are inherited, linked, and the strength of the coupling depends on the distance between the genes.

Pedigree showing the coupling of the gene "nail-patella syndrome" np with blood group B (chromosome 9)



The crossover frequency determined the distance between these genes in the chromosome 1,5%

DNA diagnostics identifies gene mutations

- confirming the suspicion of the disease presymptomatic,
- before the onset of the diseasecarriers,
- to identify heterozygous carrierssprenatal-prenatal.

- Fundamentally distinguish direct and indirect DNA diagnosis of monogenic hereditary diseases.

Direct when a gene and its mutations are well known

Indirect - by closely linked marker - lying next to the site of DNA

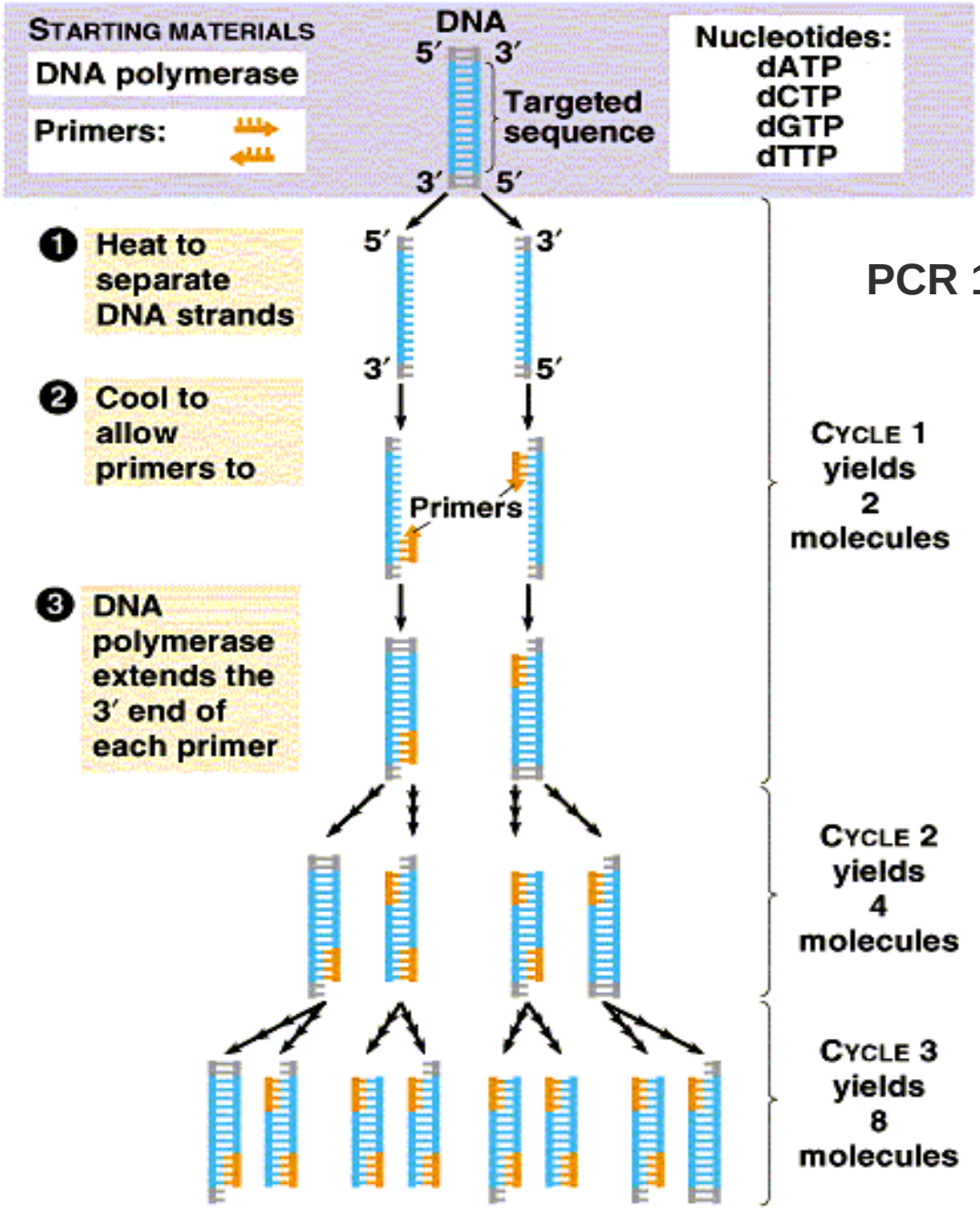
Some terms used in DNA analysis

Cloning-isolation of a gene and its reproduction in the chromosome of a bacterium, phage or plasmid

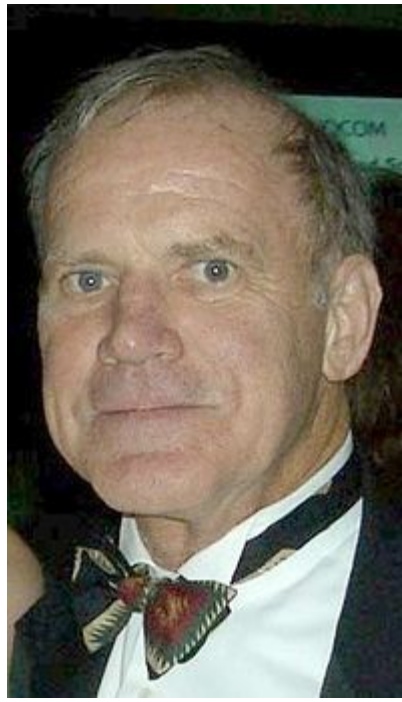
Sequencing - determining the sequence of a DNA site

Polymerase chain reaction, a PCR method for obtaining a large number of copies of a DNA site

Gene fingerprinting-detection of small variations in the structure of DNA



PCR 1983 Carey Mullis



Prenatal diagnostics

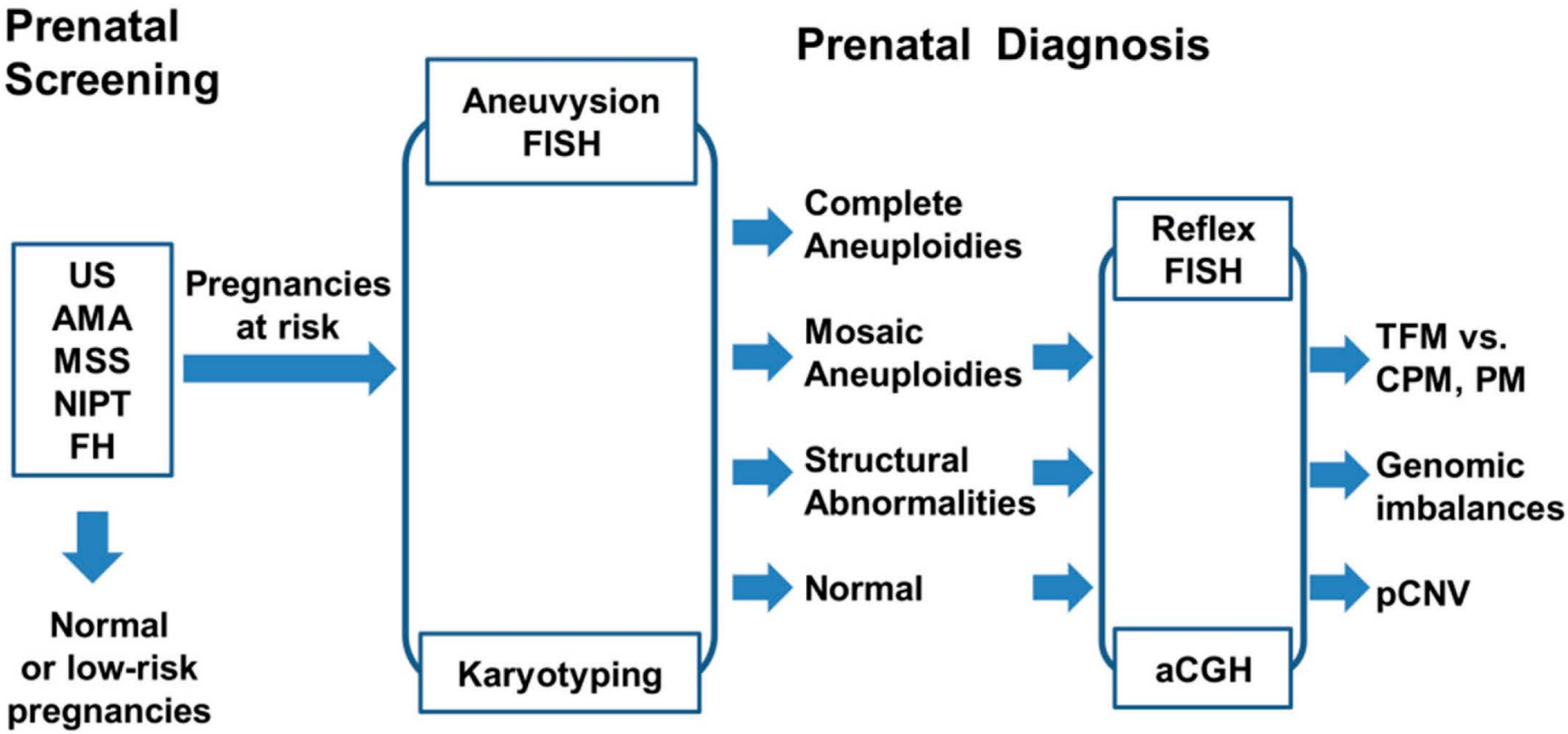
Noninvasive – Ultrasound, mother's blood

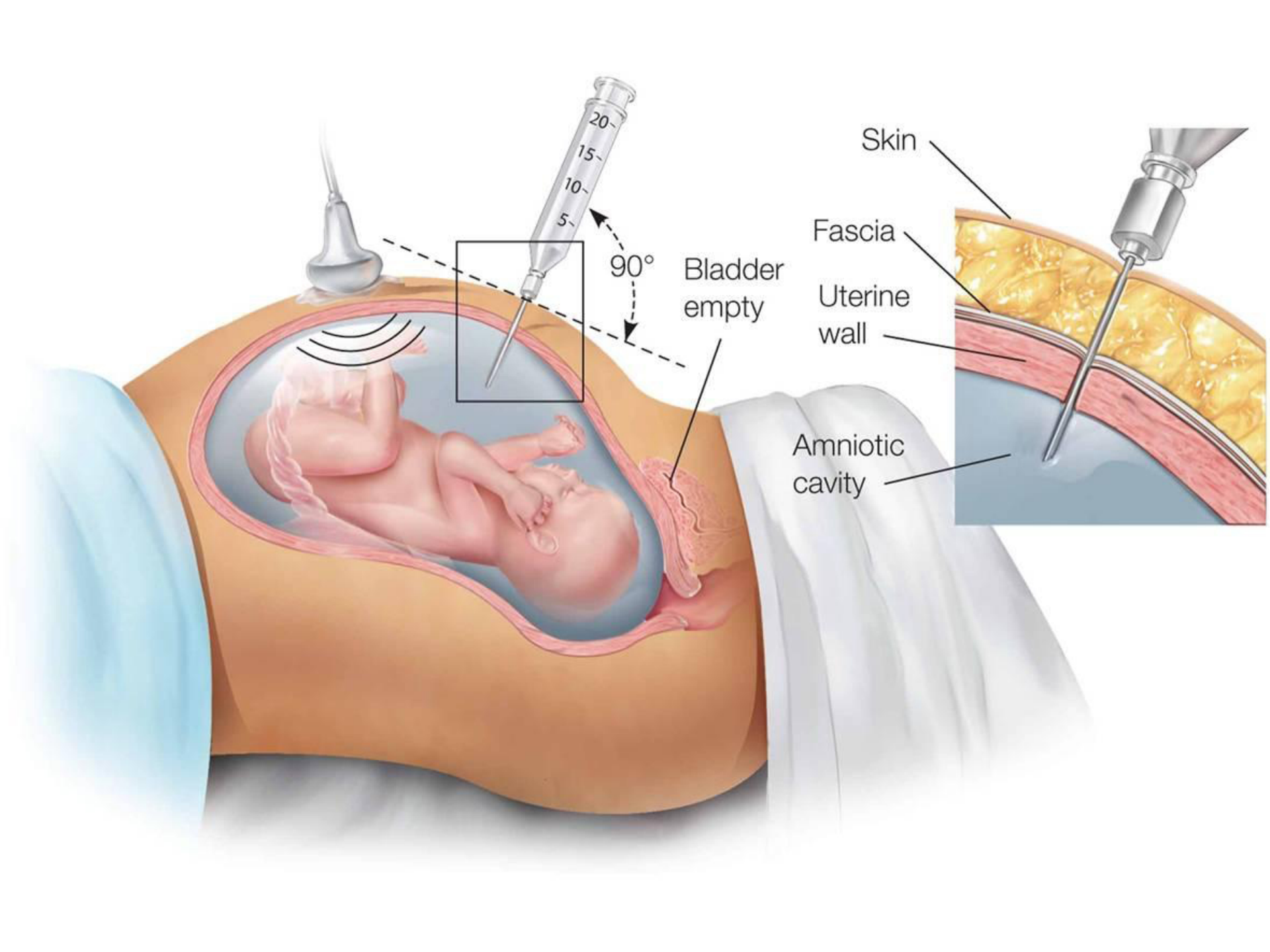
Invasive -

- It is used for the study of fetal tissue or embryonic membranes;
- Uses cytogenetic, biochemical, DNA methods;

Distinguish:

- Preimplantation diagnosis;
- The villus sampling (taking of the chorionic villi);
- Cordocentesis (taking umbilical cord blood);
- Amniocentesis (taking amniotic fluid);
- Placentitis (placental tissue);
- Biopsy of fetal tissue (e.g. skin)


Prenatal Screening
Prenatal Diagnosis
US
AMA
MSS
NIPT
FH
Pregnancies at risk
Normal or low-risk pregnancies
Aneuvysion FISH
Karyotyping
Complete Aneuploidies
Mosaic Aneuploidies
Structural Abnormalities
Normal
Reflex FISH
aCGH
TFM vs. CPM, PM
Genomic imbalances
pCNV


20
15
10
5
90°
Bladder empty
Skin
Fascia
Uterine wall
Amniotic cavity