GENETICS: Methods of studying heredity in humans

Plan of the lecture:

1. **human genetics - identification and main sections; contemporary state;**
2. **excursion into the history of human genetics;**
3. **human genetics a sabject;**
4. **methods of genetic analysis of people**

Human Genetics - anthropogenetics

THIS section of fundamental genetics and medicine that is:

Studying patterns of inheritance and variability symptoms in humans (including pathological) and factors influencing the distribution of allelic (mutant) genes in human populations ,

Exploring the connection between genes and certain types of Human Pathology (the problem of genetic markers) .

Examining the contribution of genetic and nongenetic factors in processes of individual human development and life rights, including the purely human aspects (intelligence, sociability , work) , health data, ways to improve genodiagnostic , gene therapy and genoprevention

- ***The task of medical genetics is the identification and prevention of hereditary diseases.***

- ***One of the founders of Medical Genetics is an outstanding Soviet neurologist SN Davidenkov (1880-1961), who began his fruitful work in the twenties in the Ukraine. He first applied the ideas of genetics in the clinic, an analysis of a number of hereditary diseases, some of which were described by him for the first time. Important merit of SN Davidenkov is to develop methods of genetic counseling and its first practical application in our country.***

Human genetics (anthropogenetics) - The main directions

general human genetics studies -

* the laws and rules of inheritance and variability symptoms in humans ;

* Structure and function of human genetic apparatus ; gene pools of human populations and the factors determining interpopulation differences - natural and social (demos and isolates , ethnic groups and sects , marriages ,infectious and parasitic agents as factors of fixation or elimination of alleles) , gene (allele) and geographical (regional) pathology ;

genetic polymorphism in humans , genes - markers

genetic certification of the population;

2. *social genetics studies* -

* genetic background of behavioral change

paradigm: with the animals - the "dictatorship" of genes is instincts),with men - freedom of will;

* genetic background and talent genius, high intelligence and high morality, on the one hand, and asocial, on the other hand, the problem of the person;

correlative contribution of genetic and

nongenetic factors in the development of human as an individual (whether valid thesis a "man is born ") and they became human.

GENETIC BACKGROUND of gift, genius, intellect, high morals, social success -

- * ***Gift (TALENT)*** ***- human Availability of original, creative, resourceful,***

mind that is abilities;

- ****GENIUS - the highest degree*** ***of talent (gift); genius applies to all mankind.***

- ***Three components*, *the combination of which is prerequisite for gifted people (talented) human genius*:**

1.potential talent or genius must be born;

2.potential talents and geniuses appear, if there is a desire to work and self-realization (intrinsic motivation)

3. and if there is a suitable environment (extrinsic motivation);

The first two components are almost entirely genetically determined,

Third - creates the necessary conditions for the realization of genetic conditions;

GENETIC BACKGROUND of gift, genius, intellect, high morals, social success

IQ (iQ, intelligence quotient) - is an integrative quantitative index characterizing such genetically modified abilites as the ability to think logically, remember to imagine items in 3 D etc.;

-***general RANGE of iQ is from 0 to 140;***
Distribution:

iQ70-50 – morons

iQ 50-25 - imbeciles (mental age corresponds to 3-7 years)

iQ below 25 - idiots (mental age corresponds to 1-2 years);

iQ 90-100 - norm

MAIN AREAS OF HUMAN GENETICS (anthropogenetics) -

medical genetics studies and develops -

* ***Genetic determinants*** of health and ill-health

* ***Common genetically*** determined patterns of development of various pathological states - invasive and non-invasive (infectious and noninfectious);

* ***General organizational*** principles, approaches and methods of genetic counseling;

* ***The effective use*** of scientific knowledge of classical and modern molecular genetics in the medical and health care;

CLINICAL GENETICS STUDIES–

*** *hereditary diseases* within specific areas and disciplines of clinical medicine: symptoms and diagnosis, ways of correction of phenotype (treatment), prevention; work is carried out in collaboration with physicians**

Genetic counseling;

*** *polymorphism* of clinical manifestations according to a hereditary disease, factors determining the presence of a polymorphism - and genocopies and phenocopies;**

*** *multifactorial disease*: on the way to preventive health care;**

Man as an object of genetic research: adverse circumstances-

- *great genetic diversity of people (assuming that human chromosomes have just one gene, it is onlydue to independent divergence of homologous chromosomes in anaphase of meiosis-there are 2^{23} = 8,388,608 different gametes;*
- *Note- the man during the active reproductive life span forms in 10^{11} sperm);*

* *karyotype is represented by a large number of chromosomes - 46 or 23 pairs;*

* *reproductive maturity comes late;*

* *many factors (primarily social) restricting panmixia (ethnic, racial, religious, educational, etc.);*

* *relatively few children in the family;*

* *significant length of generation (25 years) and the same for the observer (genetic) and observed;*

* *great variety of habitats of different populations of people, ethnic groups and way (way) of life;*

- ***CONCLUSION: Features of Human Genetics***

•impossibility of experimental crossbreeding;

- slow generational change;
- small number of children in each family;
- humans have complex karyotype and a large number of linkage groups

* **due to the inability to apply the method in Hybridological anthropogenetics developed specific methods ingenetic studies (*classical genetics - genealogical, twin, cytogenetic, and dactylo palmoskopy, etc.);***

* ***new techniques arising from the achievements of cell and molecular biology, projects such as the "human genome"***

***cloning DNA site**

***** ***PCR amplification of DNA sites a***

DNA probes method, molecular cytogenetic ,

***** ***DNA sequencing, genome-wide screening ,***

***** ***somatic cell hybridization,***

***** ***technology "knock out" and "knock in" to create genetic models of pathological conditions, etc***

- ***Impossibility*** of experimental crossing of the person is compensated by the fact that the researcher watching extensive human population can take thousands of married couples those needed for genetic analysis.
- A method of somatic cell hybridization, which allows you to study experimentally the localization of genes in the chromosomes, analysis of linkage groups.

- ***In the study of human genetics, the following methods are applied:***
- ***genealogy***
  - ***twin***
  - ***population-statistical***
  - ***dermatoglyphic***
  - ***biochemical***
  - ***citogenetic***
  - ***somatic cell hybridization***
  - ***modeling***
  - ***prenatal diagnosis of fetal***

genealogical method

This method is based on tracking a normal or pathological feature in several generations specifying kinship between members of the pedigree.

The essence of this method is to find kinship and trace the presence of normal or pathological feature of close and distant relatives in the family.

possibilities of the method

- ***It can help you to solve a number of problems:***

- ***1.For studying character trait: hereditary or not;***
- ***2. For studying penetrance gene type nasledovaniyai;***

- ***3. For studying linkage groups of genes and chromosome mapping;***
- ***4.For studying the intensity of the mutation process;***

- ***5. For deciphering the mechanisms of gene interaction;***

- ***6.For genetic counseling.***

Stages of pedigree formation

- ***1.A collection of information***
- 2. ***Diagram drawing pedigree***
- ***3. Geneological family analisis***
- ***4. Conclusions***

A collection of information starts from the proband.

Proband is a person whose pedigree you want to create.

- *They may be sick or healthy people*

- agents or person who seeks advice from a gentic doctor.

- *Brothers and sisters arecalled proband siblings.*
- *Usually pedigree is compiled on one or more features.*

- ***To compile pedigree you need brief notes about each member of the pedigree with a precise indication of its relationship to the proband - they constitute legend***
- ***Genealogical pedigree method is more informative, the more there is reliable information on the heath of the patients relatives.***
- ***When collecting genetic information and analysis must be borne in mind that the feature can be expressed in varying degrees, sometimes minor – micro features.***

Stage 2

- After collecting information, make a graphic image of pedigree. For this there are standard symbols.

Для составления родословной применяются условные обозначения и делаются графические изображения

Used to produce pedigree symbols and graphics are made

- femail (женщина)
-male (мужчина)
-proband (пробанд)
-miscarriage(невынашиваемость)
-deadbborn (мёртворожденный)
- marriage line (брачная линия)
- clos related (близко-родственный брак)
marriage
-parents(родители)
- children (дети)
-twins(близнецы)
-identical twins (monozigous)(однояйцевые)
- traternal twins (разнояйцевые)
- sick relatives
- agents
-dizygotic(гетерозиготный носитель)
-genetic carrier(носитель гена)
-strapper(здоровый)

After compiling a pedigree begins the third stage - the genealogical analysis, the purpose of which is to establish the genetic patterns:

in the beginning you want to set whether a sign is hereditary :

** if any indication was met in pedigree several times , then you can think about its hereditary nature ;*

** but this may not be the case , for example, any external factors or occupational hazards can cause similar disease in members of the same family*

** but in the case of hereditary feature it is necessary to establish the type of inheritance :*

autosomal - dominant ,

autosomal recessive ,

sex-linked : X - linked dominant

X-linked recessive

y -linked

Key features of inheritance, sex-linked

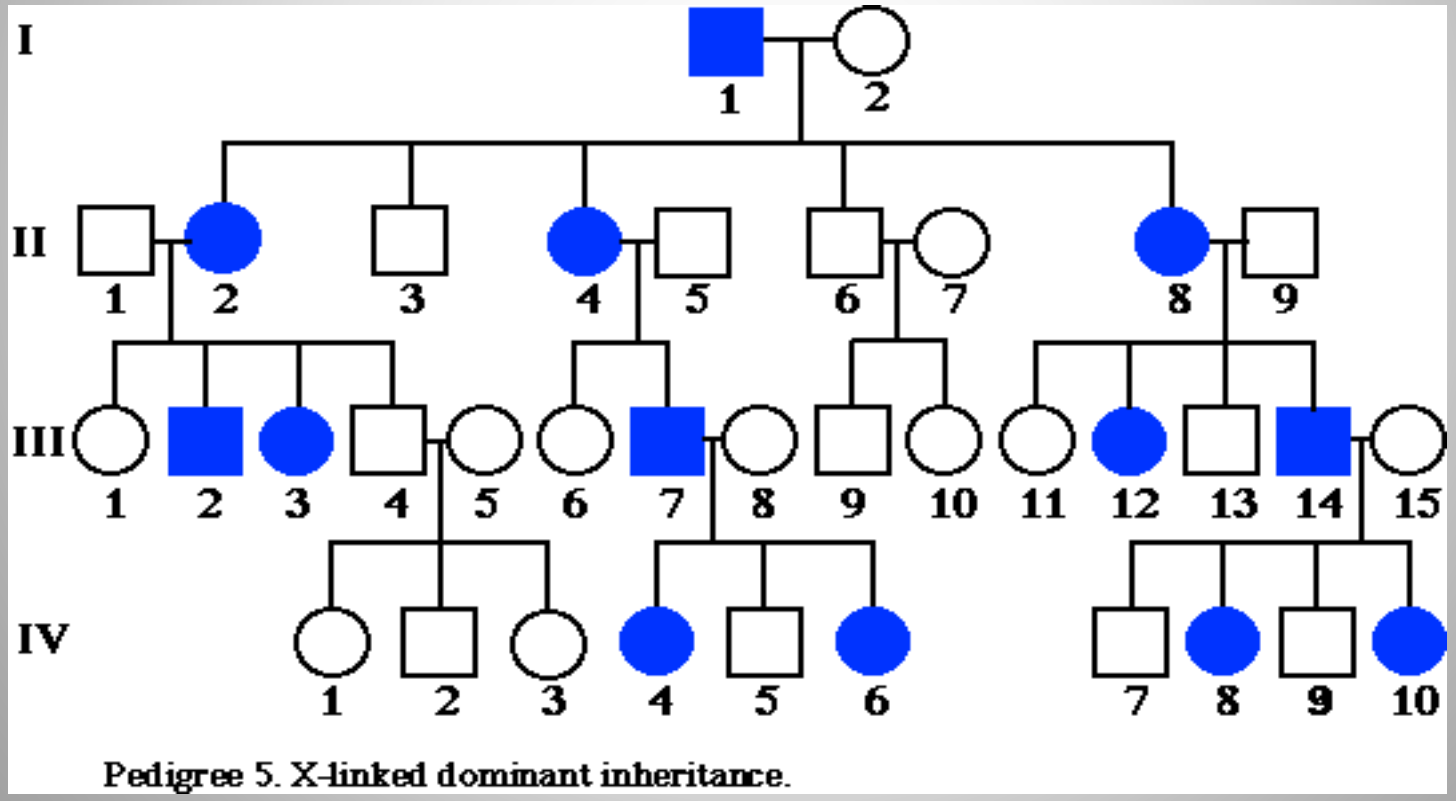
.

* Diseases caused by a gene localized on the X -chromosome can be both dominant and recessive ;

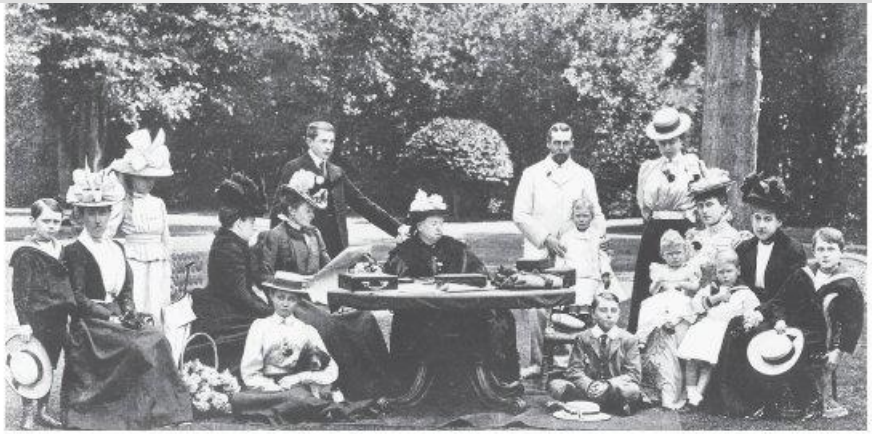
* in X-linked dominant inheritance disease is manifested equally in both men and women and can be transmitted to offspring (in this case, a woman can transmit the gene to half of the daughters and half of sons);

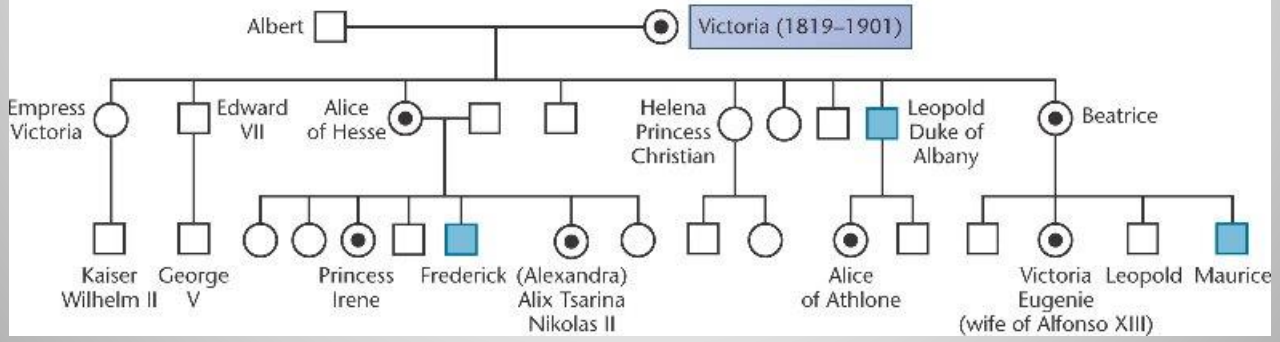
* For recessive inheritance diseases X-linked men tend to suffer (heterozygous carrier - mother - transfers mutant gene to half of sons who are sick and half of daughters who remain phenotypically healthy, as a mother , too, are the agents and recessive gene transfer together with the X chromosome to the next generation) .

X - dominant inheritance



Pedigree 5. X-linked dominant inheritance.




Albert
Victoria (1819–1901)
Empress Victoria
Edward VII
Alice of Hesse
Helena Princess Christian
Leopold Duke of Albany
Beatrice
Kaiser Wilhelm II
George V
Princess Irene
Frederick
(Alexandra) Alix Tsarina Nikolas II
Alice of Athlone
Victoria Eugenie (wife of Alfonso XIII)
Leopold
Maurice

X-linked recessive

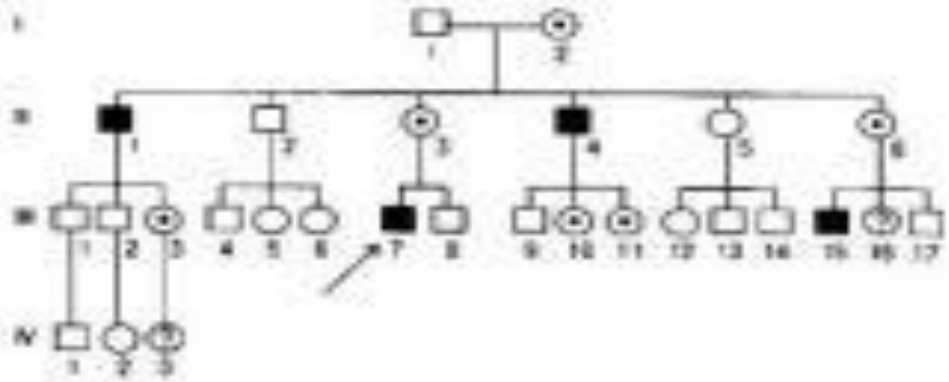
- ***1. men are sick ill more often***
- ***2.mother transmits features to sons and father to daughters***

X-linked recessive mode of inheritance linked males

- ***Homozygous*** male are ill predominantly
- Son never inherit the father's illness
- If the proband is woman, her father is sick
- Heterozygous agent mother passes the mutant gene to half of the sons and daughters

Х – сцепленный рецессивный тип наследования (Х-Р)

- Гемизиготность у мужчин
- Заболевают преимущественно мужчины
- Сын никогда не наследует заболевание отца
- Если пробанд женщина, то ее отец обязательно болен.
- Гетерозиготная носительница – мать передает мутантный ген половине дочерей и сыновей.

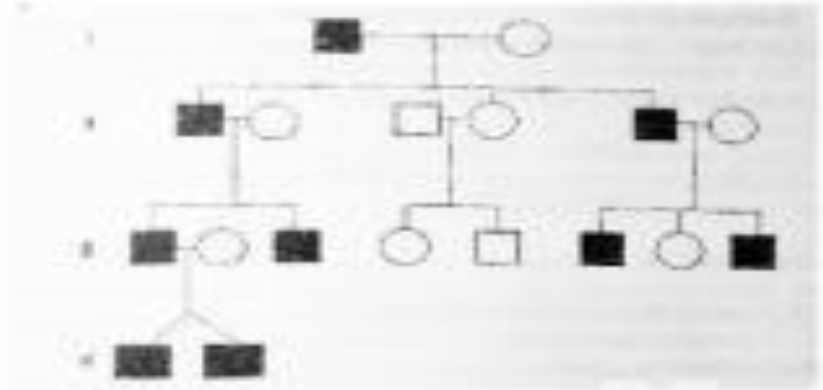


Y-linked pattern of inheritance

- Holandric type of inheritance
- Transmission only through the male line

Y – сцепленный тип наследования

- Голандрический тип наследования
- Передача только по мужской линии



- ***Autosomal – dominant gene***
- ***1.A disease is found in each generation***
- ***2.correlation of girls and boys is the same***
- ***3Probability of birth of a sick child, if one of the parents is sick, is 50%***

The main signs of autosomal dominant inheritance:

- ***manifestation feature*** equally in both sexes availability of patients in all generations (verticaly) with a relatively large number of siblings
- ***availability of patients*** and horizontally (among brothers and sisters of the proband)
- with ***heterozygous parent*** probability of the birth of a sick child (if the other parent is healthy) is 50% Keep in mind that when the dominant type of inheritance may pass from generation to generation due to poorly defined , " erased " forms of the disease (small ekspressiveness of mutant gene) or due to its low penetrance (when the gene carrier has the feature missing).

Autosomal recessive type

- 1. Sick y child is born with clinically healthy parents
- 2. Sick brothers and sisters
- 3.Both sex affected equally
- 4.Often occur blood-related marriages
- 5.If both spouses are sick, all children are sick
- 6.Heterozygous mother passes a feature to half of her children

The main signs of autosomal recessive inheritance

- ***** relatively small number of patients in the pedigree
- * Availability of patients "horizontally" (sick siblings - relatives , cousins)
- * parents of a sick child are often phenotypically healthy,but are heterozygous carriers of the recessive gene
- * probability of the birth of a sick child is 25%
- * Recessive traitis is manifested when in genotype are both recessive alleles
- * In the manifestation of recessive diseases kinship of parents of patients is common. It should be keptin mind that the presence of distant kinship is unknown to family members.You have to take into account indirect considerations , such as the origin of the same sparsely populated points, or belonging to any ethnic isolated or social group.

Twin method

Twin method was introduced by F.Gamiltonom, he singled out two groups of twins:

- identical(monozygotic)
- fraternal (dizygotic)

- ***Monozygotic twins*** *with normal embryonic development are always of the same sex.*
- ***Dizygotic twins*** *are born more frequently (2/3 of the total number of twins), they develop from two simultaneously mature and fertilized eggs. These twins can be same-sex and heterosexual. From a genetic point of view they are similar as regular siblings, but they have a big community environmental factors in utero (prenatal) and partly in postnatal periods.*
- *If studied traits are manifested in both twins, they are called* ***concordant.***
- ***Concordance*** *- the percentage of similarity of the studied feature.*
- ***Discordance*** *–the percentage of difference of the studied feature*

Близнецы

- **Symptoms controlled a large number of genes and non-genetic factors independent of frequency (probability) of occurrence similarity % heritability(ON and DB)**

	ON	DB	
Mental retardation	**97**	**37**	**95**
Schizophrenia	**69**	**10**	**66**
Diabetes mellitus	**65**	**18**	**57**
Epilepsy	**67**	**30**	**53**
Average	**≈ 70%**	**≈ 20%**	**≈ 65%**
Crime	**68**	**28**	**56%**

- Twin method is used in human genetics in order to evaluate the effect of heredity and environment on the development of a normal or pathological trait. To assess the role of heredity in the development of a trait, use the formula:

$$H = \frac{\%\ \text{similarity ON} - \text{DB}\%\ \text{similarity}}{100 - \%\ \text{similarity DB}}$$

Where: ***H****- index of heredity,*

ON *- identical twins*

DB *- fraternal twins*

N = 1 feature is fully determined hereditary component

At H = 0 is determined by the sign of the influence of the environment

For H = 0.5 is close to a sign determined approximately equal influence of heredity and environment on formation of characteristic

Biochemical methods

- These methods are used for the diagnosis of metabolic diseases caused by a change in the activity of certain enzymes. Using biochemical methods they discavered about 500 molecular disease manifestations resulting from mutant genes.
- These methods differ intensive by labor, require special equipment and therefore can not be widely used for mass population studies for early detection o patients with hereditary diseases metabolism.

Cytogenetic methods

The method is based on microscopic examination of the chromosomes isolated from human cells.

Material for cytogenetic studies may be: peripheral blood cells, lymphocytes, cells obtained by amniocentesis or chorionic villus sampling. For identification of chromosomes is used quantitative morphometric analisys.

For this purpose, a cell preparation is prepared :

1. blood sampling is taken ;

2. Blood is placed in a special environment fitogemoaglyutinin where there is a rapid growth of white blood cells , ie leukocyte culture is prepared ;

3. culture of cells is grown and is used to prepare " smear cells“

4. smear is fixed and stained , resulting in a preparation with a human chromosome karyotype

5.cell preparation is examined under the microscope and find chromosome karyotype at metophase stage of meiosis

6.preparation is photographed and study the chromosomes

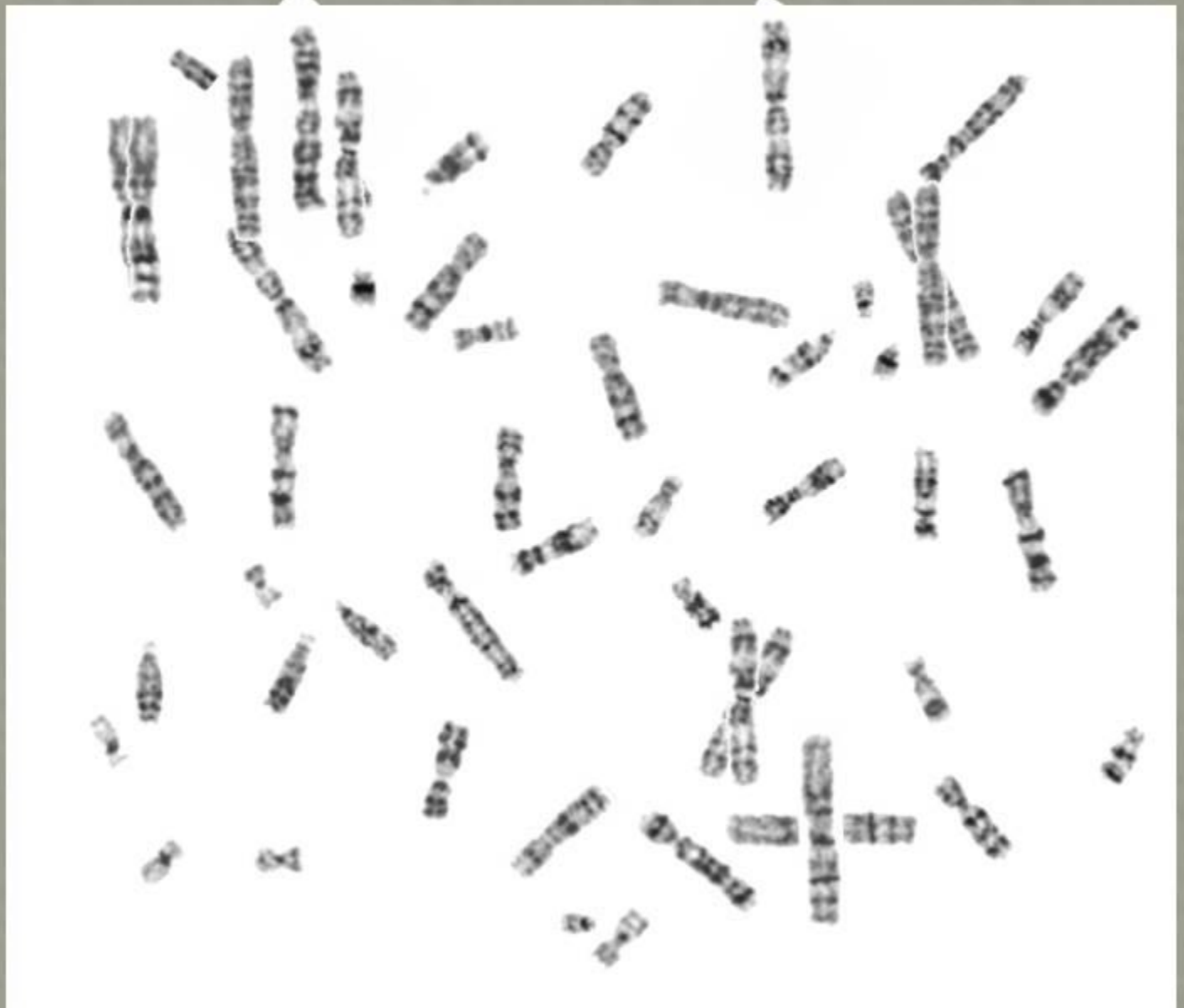
- Measurement of the length of the chromosome in micrometers (microscoping chromosomes is produced in mitosis stage of metophase)

- Also determine the ratio of the short arm length to the length of the entire chromosome (centromere index).

In1968-1970 Caspersson published papers in genetics for the study of chromosomes heapplied ***fluorescent dyes in particular*** , quinacrine mustard and derivatives . Study of chromosomes in the fluorescent microscope showed that ***the chromosomes do not provide uniform*** glow in length. : luminous , revealed several bands that coincide with the localization of structural geterohromtina ***. If chromosome is dyed with Giemsa stain , then they revealed clear differentiation on dark-colored and light stripes - wheels (bends) .***

The sequence of these bends, their drawing is- strictly specific (individual) for each chromosome .

Кариотип человека



- Sex chromatin , chromatin portions defining difference interphase nuclei in individuals of different sexes associated with singularities structure or functioning of the sex chromosomes . Distinguish Y- P. x . (Y- chromatin) and X -P. x . (X - chromatin) . Y- xpomatin - structural Y- chromosome heterochromatin man tapped in the interphase nucleus using fluorochromes in ultraviolet light . X - chromatin , or Barr body - intense coloring basic dyes structure (0.7-1.2 m) , located in the nuclei of different cell types females formed normally one of the two sex chromosomes homogamete sex. This chromosome helical and therefore inactive. In the presence of a larger number of X chromosome inactivation such exposed all but one X chromosome . Therefore, count cells P. x . one less than the number of X- chromosome and is diagnostic sign in determining their quantityt . Such a mechanism of P. x . attaching most mammals

Method of sex chromatin-detects chromosomal diseases caused by changing the number of sex chromosomes

- *To identify the calf sex chromatin body increasingly is used cells of human from buccal mucosa.*
- *To do this they scrape cheek cells and smear is prepared, which is dried and stained with hematoxylin or atsetoarsein or other basic dyes.*
- *Body is detected in interphase cell nuclei of mammals and humans. directly under the nuclear membrane. Normally (XX) in females they reveal one body, and healthy males (XY) have none.*
- *Women with karyotype 46 XXX have 2 will ox-46XO have no bullock sex chromatin;*
- *Males with XXY karyotype-have 1 body; XXXY-2 bodies in cells.*

Methods of somatic cell hybridization

- According to moral and ethical principles of man can not be artificially cross , and therefore the study of man can not be the main method used in genetics - Hybridological . However, in recent years have found a replacement for him . Somatic cells contain the entire amount of genetic information. This makes it possible , using them to explore many issues of human genetics , which can not be investigated on the whole body.
- Thanks methods somatic cell genetics man, as it was one of the pilot sites . Most use connective tissue cells (fibroblasts) and the blood lymphocytes .
- Culturing cells outside the organism allows to obtain sufficient material for studies . it is not always possible to take a person without sacrificing health. ? Held in tissue culture cells can be subjected to a study of different methods : cytological , biochemical , immunological .

- In 1960, the French biologist J. Barsky , growing in tissue culture cells of two strains of mice (outside the body) , has found that some cells in their morphological and biochemical characteristics were intermediate between the original parent cells . ***These cells were hybrid .***
- This spontaneous cell fusion in tissue culture is fairly rare .
- Later it was found that the frequency of somatic cell hybridization increases when administered to a culture of cells containing RNA ***of Sendai parainfluenza virus ,*** which like all viruses alters the properties of cell membranes and makes it possible to cell fusion .
- Under the influence of such a virus in a mixed culture of two types of cells produced cells containing a total cytoplasm both parental cell nucleus - ***heterokaryons .***
- .

After mitosis and subsequent separation of the cytoplasm of two ***dual-core heterokaryon*** formed mononuclear cells , each of which represents a ***synkaryon - true hybrid cell*** having chromosomes of both parental cells . Depending on the purpose of the analysis was performed ***on study or synkaryon heterokaryons***

Synkaryon can usually be obtained by hybridization within a class . This true hybrid cells , as they merged the two genomes. Application of somatic cell genetics gives you the opportunity to study the mechanisms of the primary action of genes and gene interactions .

Prevention of birth of children with hereditary diseases

In order to prevent the birth of hereditary diseases develope) methods of prenatal diagnosis (prenatal)

- **Methods of prenatal diagnosis can be divided into three groups:**
- **1.Screening methods**
- **2.non - invasive**
- **3. invasive (with subsequent laboratory diagnostics**

Screening methods

- These include methods that identify women at increased risk of having a child with congenital or hereditary disease. The result of this step is the selection of diagnosis of patients at risk (this group is about 10% of the number surveyed). Risk - are women who require prenatal diagnosis

Determination in serum of pregnant women substances, called serum markers of mother:

- The concentration of a-fetoprotein (AFP)
- The level of human chorionic gonadotropin (HCG)
- Unbound levels of estriol
- Pregnancy-associated plasma protein-A
- Serum activin-F
- Identification of cells or fetal DNA from the mother's body

- Change their content than normal may indicate some pathology in the development of the fetus:
- - Such as the concentration of AFP in the blood is reduced
- women carry a fetus with Down syndrome;
- - The level of HCG in Down syndrome increased twice or more;
- - Significantly reduced levels of estriol in Down's syndrome
- All these indicators allow already in utero to 68% reveal pathological pregnancy

noninvasive methods

- These are the methods by which the examination of the fetus is performed without surgical intervention.
- Greatest importance in the prevention of hereditary diseases is a method of fetal ultrasound scanning (USS). The method allows to determine both congenital and functional status of the fetus and its provisional organs (placenta, umbilical cord, meninges). ***Dates U.S. 10 -13 20 -22 -32 and 30 th week of pregnancy.***
- Ultrasound can also be used to identify stunting embryo or fetus as early ***as 6 to 8 weeks of gestation.***

Indications for fetal ultrasound

- 1.Detection of deviations (markers of disease) or fetal malformations during screening ultrasound.
- 2. Delay of sizes fetal gestation.
- 3.Presence in the family child with congenital malformations.
- 4. Presence of disease in women increase the risk of having a child with congenital malformations.
- 5. Action of teratogenic factors (radiation, chemical agents) in the first 10 weeks of pregnancy.
- 6. Presence of congenital malformations in any of the spouses

Range of recognizable defects by this method is great(80-90%)

invasive methods

- ***Chorion and platsentobiopsiya*** used to obtain a small amount of chorionic villi or pieces of placenta from 7 th to 16 th week of pregnancy. Or transabdominal procedure is performed under ultrasound guidance transcervically.

amniocentesis

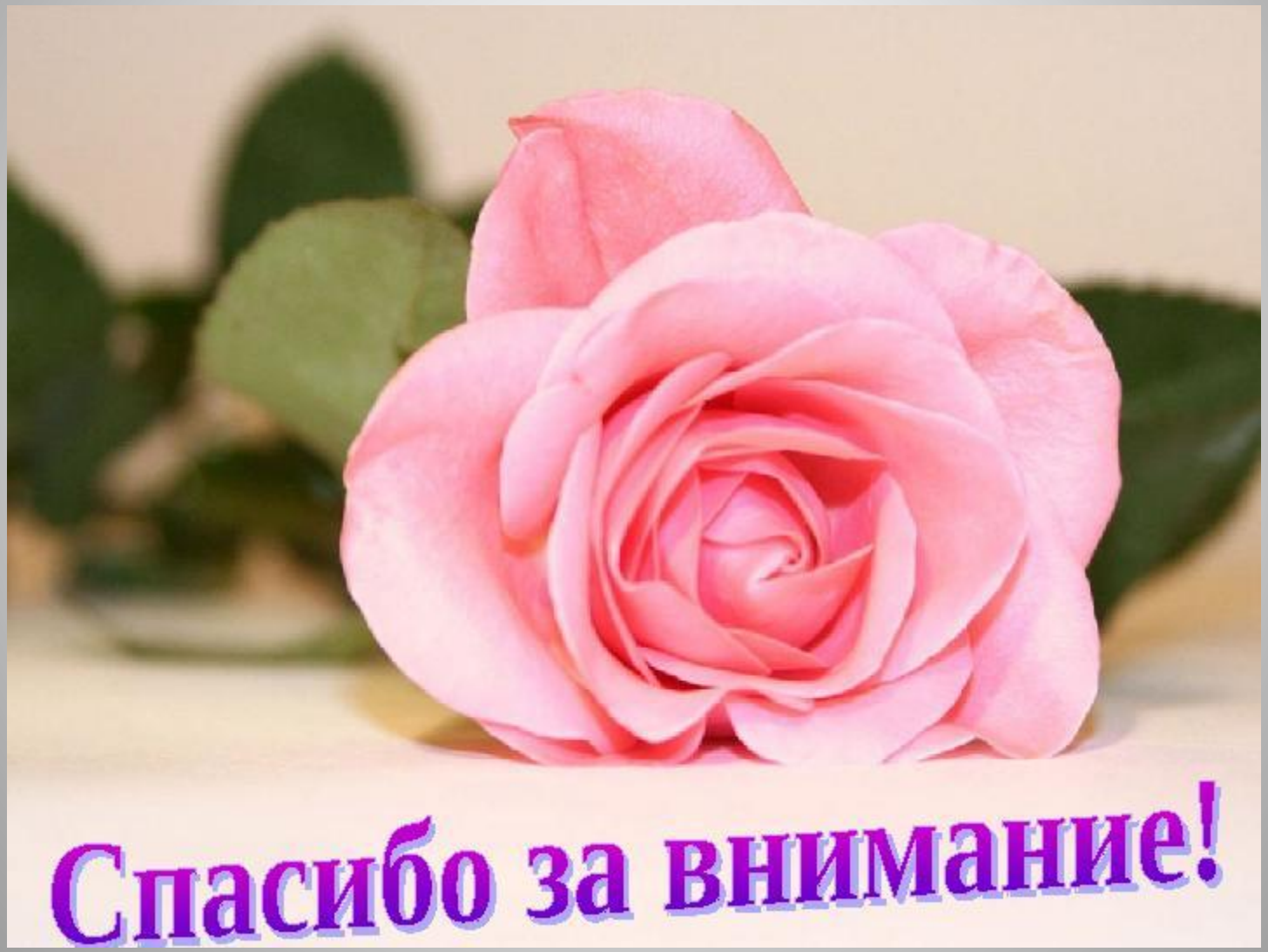
- Amniocentesis or puncture the amniotic sac, in order to obtain amniotic fluid and there are exfoliated cells of the fetus and the amnioninn is used
- for prenatal diagnosis since the early 70s.
- Early amniocentesis was performed on 12-15th week of pregnancy.
- Amniocentesis is made through the abdominal wall of the mother under the control US. From amniotic cavity the take 3-30 fluid.

Kordiotsentez is -

- taking blood from umbilical cord. Selection is carried out from 20 weeks pregnancy blood samples are subject to cytogenetic, molecular genetic and biochemical methods for diagnosis of hereditary diseases.

fetoscy

- . This is introduction of the probe and the inspection of the fetus that is visual inspection to detect its congenital malformations It is held on the 18-23-week pregnancy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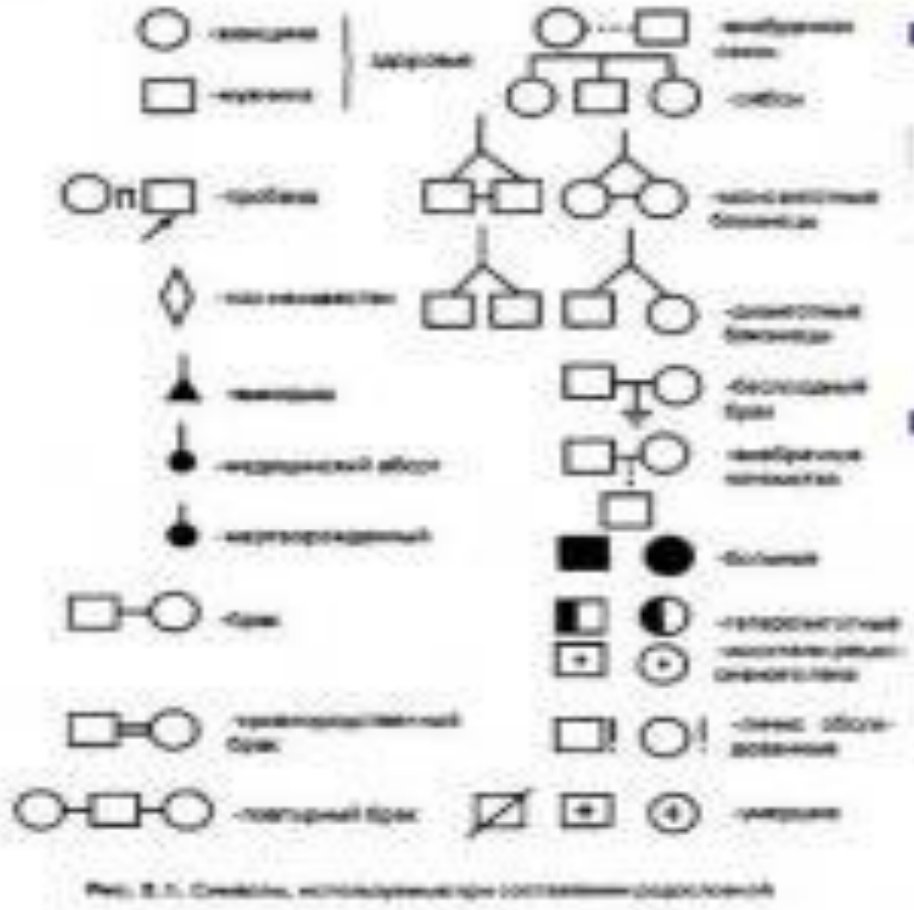

- Сбор данных начинается с **пробанда** - человека, родословную которого нужно составить. Братья и сестра его называются **сибсы.**
- Для составления родословной применяют условные обозначения и делают графические изображения