Medical Genetics – Clinical practise

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Presentation Contents

- Medical genetics - genetic counselling, ethical and legal aspects
- Primary and secondary genetic prevention
- Prenatal diagnosis
- Reproductive genetics – genetic testing of couples with reproductive disorders
- Newborn screening and early postnatal diagnosis

History

Gregor Mendel first publicly presented results of his research on plant crossbreeding in 1865. Audience did not understand his lecture at the time. Most of the listeners had no idea what he was talking about.

1965 Janáček Theatre Brno - Conference to celebrate the 100th anniversary of the publication of Mendel's discoveries
1967 – the field of Medical Genetics was established

Medical Genetics 1967…

- Medical history
- Genealogy
- Karyotype

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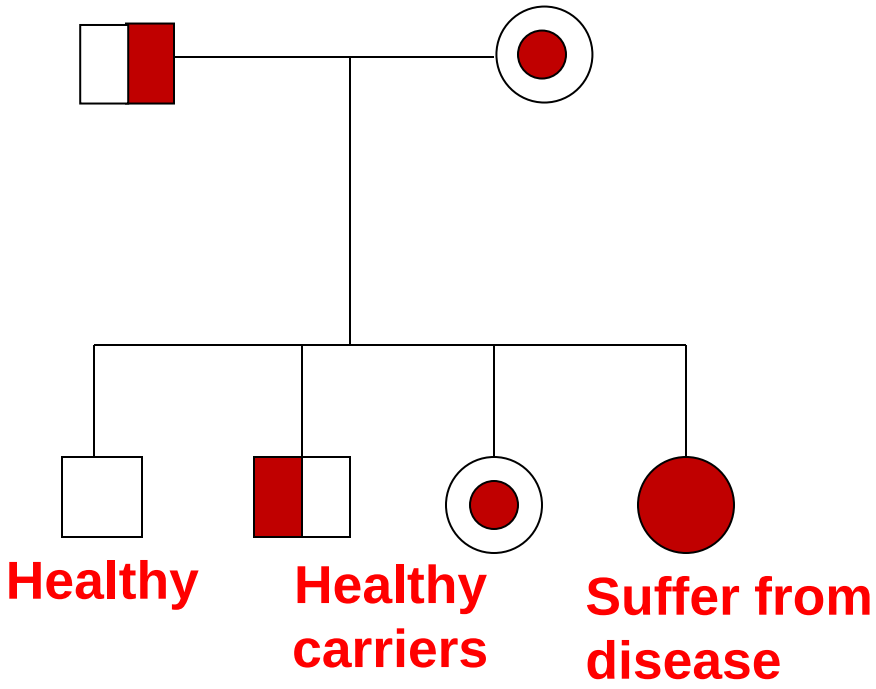
- Genetic prognosis



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Medical Genetics 2020....

- **Medical history**
- **Genealogy**
- **Karyotype**
- **Molecular cytogenetics – FISH, MLPA, array-CGH**
- **DNA analysis- scoring of the most frequent mutations, analysis of hot spot areas, indirect DNA analysis… Sanger sequencing, MLPA, NGS – MPS, WES, Whole-genome sequencing….**

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- **Genetic prognosis**

Medical Genetics

- Medical genetics deals with the diagnosis of hereditary diseases, their therapy and preventive care
- Medical genetics deals not only with medical, but also with social and psychological aspects of hereditary diseases.
- As in all other areas of medicine, it is crucial to make a proper diagnosis and provide appropriate care.
- The care must include not only the affected individual, but also his/her family members who should also understand the nature and consequences of the disease.

Genetic counseling

- Combines patients risk assessment with psychological care and educational activities.
- It has evolved into a new healthcare profession.
- clinical geneticists are dedicated to the care of patients with genetic disorders and their families.
- In addition to direct, human to human, contact with patients, clinical geneticists provide necessary laboratory diagnostics and identify patients/relatives at risk of genetic disorders

Genetic disorders

- Congenital chromosomal aberrations
- Monogenic diseases
- Mitochondrial diseases
- Diseases with complex inheritance, congenital defects, multifactorial or polygenic disorders

Orphan or rare disesases

- A rare disease is defined by a population frequency of less than 5 patients per 10,000 healthy patients. Patients with rare disease and their families often find themselves in a very difficult life situation
- Diagnosis of these diseases requires specialized procedures, and due to the rare occurrence of these diseases, a correct diagnosis can take several months or even years.
- Another serious problem with rare diseases is that there is basicaly no effective cure and if there is an effective cure, it is usually extremely expensive.
- Care for patients with orphan diseases is centralised into specialised centers - European Reference Networks(ERN)
- In the Czech Republic - Czech Association for Rare Diseases (ČAVO) since r.2012

What are the major issues affecting people with rare diseses?

- Late or incorrect diagnosis
- Inaccessible expert health care
- Inaccessibility of so-called orphan drugs (i.e. drugs for rare diseases)
- Failures in the social support and benefits network due to lack of knowledge on the part of assessing doctors, social workers, etc.
- People with similar diseases who lack patient organizations have limited possibilities to share experiences

Orphan or rare disesases

- 1 patient per 2000 births
- There is cca 6000 – 8000 different diseases,
- about 30,000,000 patients with rare diseases,
- hundreds of new diagnoses per year,
- about 80% of rare diseases have a genetic origin.
- In thousands of sick children and adults, not only in the Czech Republic, doctors do not yet know what disease they suffer from, which brings considerable complications, it is not clear how to treat them, include or not include them in the social care system...

European Reference Networks

- **The ERNs are virtual networks that bring together healthcare providers across Europe to address complex or rare diseases that require highly specialised treatment**
- **The networks are established on the basis of Article 12 of EU directive 2011/24 on the application of patients' rights in international healthcare, which obliges EU Member States to support the European Reference Network, especially in the field of rare diseases.**
- **In March 2017, 24 ERNs were approved, involving over 900 highly specialised healthcare facilities from more than 300 hospitals and 26 Member States.**
- **Health care providers in the Czech Republic have been very actively involved in this networks, with 8 providers participating in 17 networks.**

Czech Association for Rare Diseases

The Czech Association for Rare Diseases (ČAVO) was established in 2012. The mission of ČAVO is to bring together organizations for patients with rare diseases and individual patients, represent their interests and raise awareness of the specific issue of rare diseases among healthcare professionals, representatives of state, international institutions and the public.
http://vzacna-onemocneni.cz/

Medical genetic workplaces

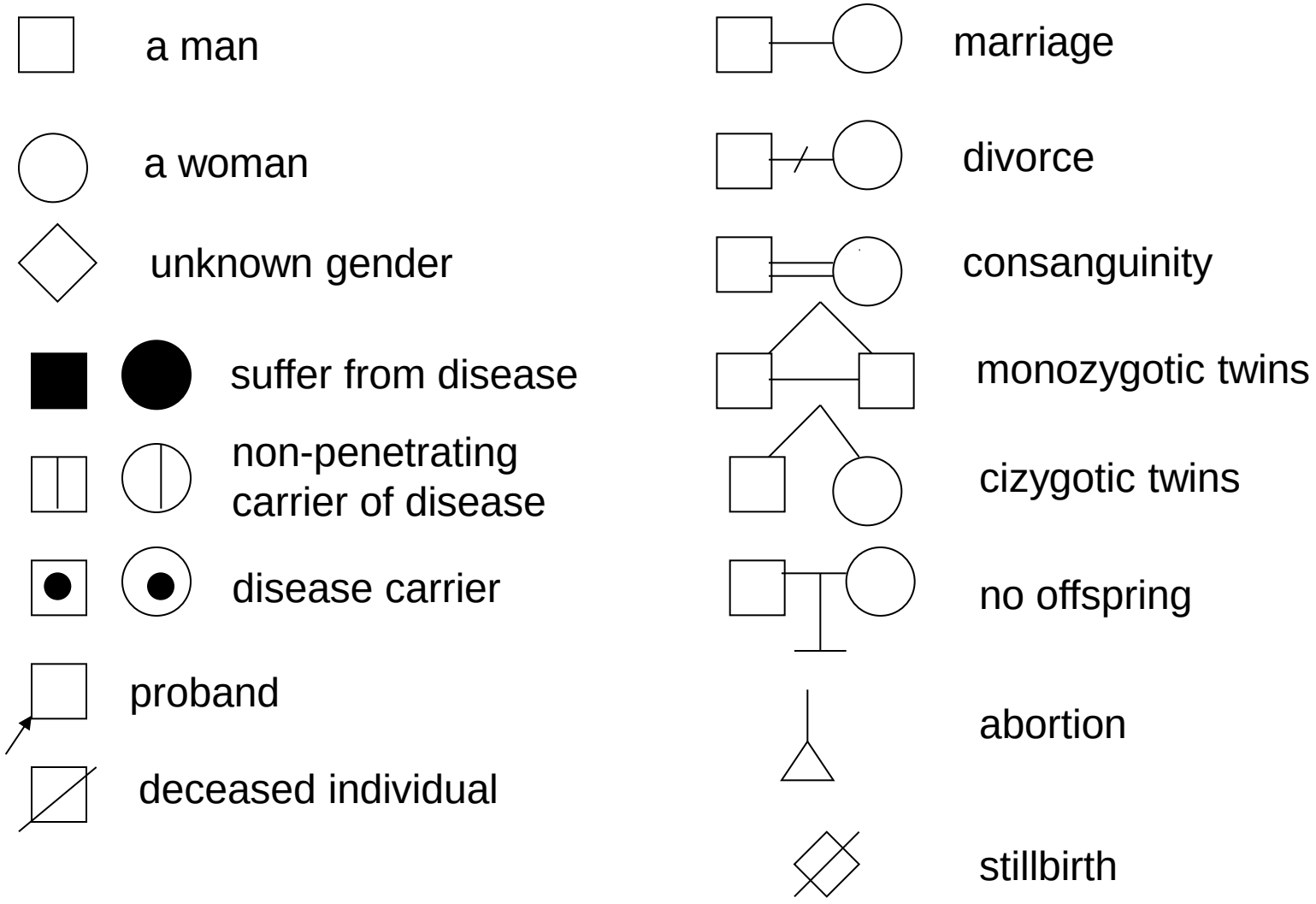
- Outpatient clinic -> Genetic counselling
- Cytogenetic laboratories (prenatal, postnatal, molecular-cytogenetic, oncocytogenetic)
- Laboratories of DNA/RNA diagnostics (monogenic diseases, oncogenetics, identification of individuals..)

Indications for genetic testing

- Families with a history of hereditary disease, chromosomal aberrations, or developmental defects.
- Couples treated for reproductive disorders.
- Pregnant women with an increased risk of fetal abnormalities
- Consanguineous couples
- Persons with an increased risk of induced mutations (environmental influence)
- Gamete donore
- Cancer patients

The course of a genetic consultation → Information gathering

- Personal medical history
- Family medical history
- Genealogical analysis- compilation of at least three-generation family tree with information about health issues of individual family members
- Information about Ethnicity • Consanguinity • (Non)paternity
- Informed consent



Symbols to draw a family tree

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Family tree

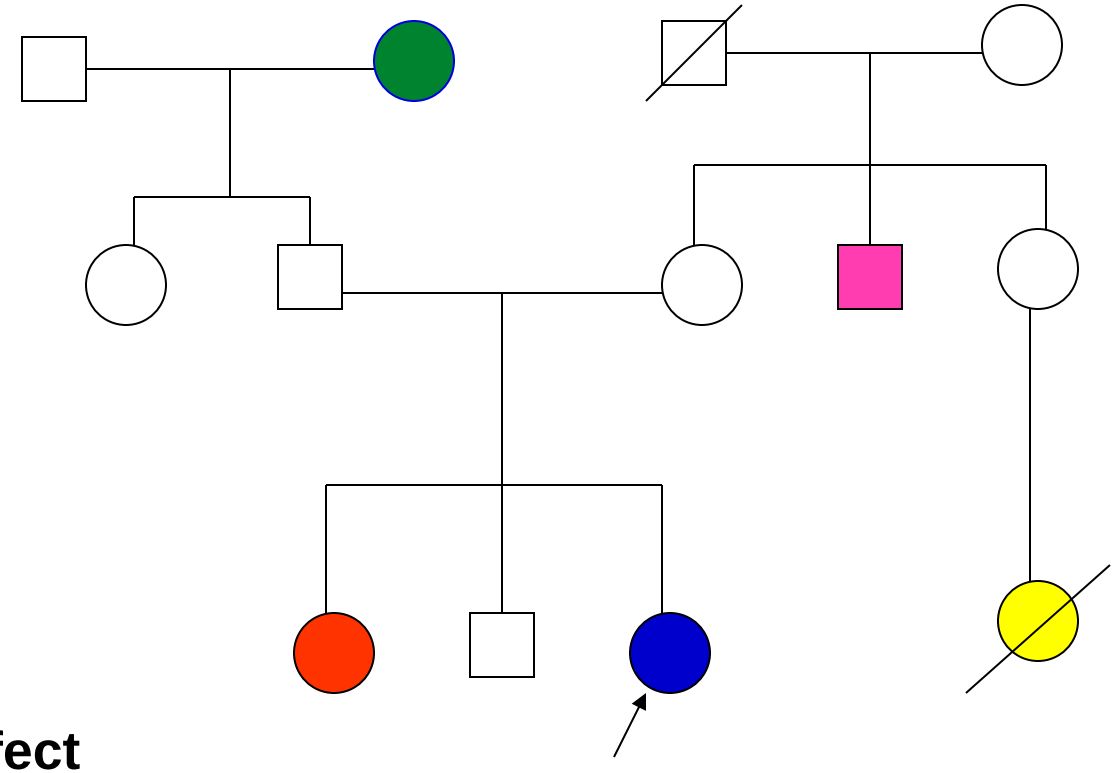
Cleft lip and palate

Death of a newborn

Syndaktilie

Epilepsy

Congenital heart defect



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The course of a genetic consultation → Clinical genetic examination

- Somatic abnormalities – stigmatization – atypical appearance
- Atypical behavior, failure to thrive…
- Congenital developmental disorders
- Psychomotor development
- Intellectual disability
- Dermatoglyphics

The course of a genetic consultation → Indication of laboratory and other specialized examinations

- Cytogenetics
- Molecular cytogenetics
- DNA/RNA analysis
- Specialized complementary examinations – cardiology, neurology,….

Possibilities of genetic testing 1

- Modern methods allow for parallel analysis of a group of patients and dozens or hundreds of genes, or even whole-exome or whole-genome analysis.
- This can be utilized for diagnosing heterogenous diseases or extremely rare diseases in one go. However, the development of molecular biology methods also necessitates a comprehensive approach to interpreting the vast amount of data obtained. The conclusions (classifications) are not always clear-cut and require further "in silico" analyses, functional experimental tests and collaboration with experts in bioinformatics.
- The classification of individual variants may not be permanent and is associated with the current level of knowledge in the field. The clinical application of this data is gradual and much slower and may change over time. This leads to the necessity of repeated analyses of previously addressed diagnoses and their updates in light of new findings, placing additional demands on physicians across all specialties as well as clinical geneticists, molecular biologists, and bioinformaticians.

In this regard, not only the patient's attending physicians but also the patient and their family must be informed

Possibilities of genetic testing 2

- The diagnostic process may not be a one-time thing, it can be a long-term process that may yield conflicting results over time.
- A large number of genomic sequence variants need to be processed and correctly interpreted.
- It is necessary to consider the potential impact of information obtained through modern diagnostic methods in medical genetics on the patient, their physician, the clinical geneticist and the molecular biologist.
- Extensive laboratory genetic testing using modern methods creates a large and complex issue of random incidental findings

-> What should be reported? (to the clinical geneticist, the specialist physician, the patient)

Possibilities of genetic testing 3

- **Before laboratory genetic testing, the risc of incidental findings related to genetic predispositions must be discussed with patient during genetic counseling.**
- **These incidental or unexpected findings, which can arise particularly from gene panel analyses and whole-genome sequencing, but also molecular-cytogenetic methods, such as array-CGH, may not be related to the condition for which the patient/family is primarily being tested, but can have significant consequences for the individual carrying the predisposition.**

- **Highlights importance of genetic counseling and informed consent.**

Legislation

- Act No. 372/2011: Act on Health Services and Conditions for Their Provision (Health Services Act)
- Act No. 373/2011: Act on Specific Health Services
- Convention on Human Rights and Biomedicine of 1996 (No. 96/2006 Coll.)
- Recommendations of proffesional associations– in CZ www.slg.cz

Act No. 373/2011 on Specific Health Services, Section 6, §28

- **"Knowledge of basic genetic principles is an indisputable necessity for physicians of any specialization. Collaboration between clinicians from all medical fields and clinicians specializing in medical genetics is a standard practice in modern medicine, as established by Czech legislation:**
- **'If a genetic laboratory test is expected to provide a diagnostic conclusion with a significant medical impact on the health of an embryo, fetus, or the examined individual, including future generations, or on the health of their genetically related individuals, the healthcare provider must always recommend genetic counseling by a physician with specialized qualifications in the field of medical genetics, both before and after the examination.**
- **Genetic counseling, according to the preceding sentence, should be recommended to the parents of the embryo or fetus, the legal guardian or representative of the examined individual, the examined individual, and affected genetically related persons.**
- **If the goal of the genetic laboratory examination is the analysis of somatic (X germinal) changes in the human genome, it is not necessary to conduct genetic counseling by a physician with specialized qualifications in the field of medical genetics.'**

Genetic prognosis

- **Consultation with a Clinical Geneticist:**

  Establishing an accurate clinical diagnosis (in collaboration with other specialists)
  Confirming the clinical diagnosis at the "Molecular level" (cytogenetic analysis, DNA analysis)

- **Genetic Prognosis for the Family:**

  Is there a risk of recurrence of the same disease in the family?
  Which relatives are at risk of recurrence of the same disease?
  Which relatives should be advised to seek genetic counseling and genetic testing?
  Can we reduce the risk of recurrence in the family? How?

- **Genetic Prevention:**

  Non-directive approach – offering the family options for testing
  Provide maximum information
  The family always chooses the course of action; the geneticist informs and helps implement it

Prevention in medical genetics

Primary

&

Secondary

Primary genetic prevention 1

= Preventive measures before (ideally planned) pregnancy

- Genetic counseling
- Reproduction at an optimal age
- Prevention of spontaneous and induced mutations
- Vaccination against rubella, prevention of other infections (e.g., toxoplasmosis)
- Preconception and periconception care
- Vitamin prevention of cleft defects

Primary genetic prevention 2

= Preventive measures before (ideally planned) pregnancy

- Preconception consultation with a primary care physician or specialist
- Evaluation of acquired chromosomal aberrations
- Contraception
- Sterilization
- Adoption
- Gamete donation

Secondary genetic prevention

= Procedures during pregnancy – prenatal diagnosis
= Early postnatal diagnosis

Secondary genetic prevention 2

- Genetic counseling
- Prenatal screening for congenital defects and chromosomal abnormalities
- Targeted invasive and non-invasive prenatal diagnosis
- Preimplantation genetic diagnosis
- Prenatal and perinatal management of pregnancy with identified developmental defects or hereditary diseases
- Prenatal therapy - if possible

Secondary genetic prevention 3

- Termination of pregnancy
- Postnatal screening
- Presymptomatic screening
- Preclinical prevention of clinical manifestation of hereditary disease
- Postnatal examination, testing, care, and therapy
- Retrospective genetic counseling

Prenatal Diagnosis

Set of procedures aimed at identifying statistically significant deviations in structure or function that exceed the boundaries of phenotypic variability.

Prenatal diagnosis of congenital defects and hereditary diseases allows for early detection with possibility to plan optimal perinatal care in advance.

In severe cases, it may lead to the early termination of pregnancy.

Prenatal diagnosis

- Screening tests – for all pregnant women
- x
- Targeted tests – in cases of increased risk of disease/defect in the fetus

- Non-invasive prenatal testing
- x
- Invasive prenatal testing

Non-invasive prenatal testing - methods

Ultrasound examination

Biochemical testing

Testing of cell-free fetal DNA in maternal plasma (NIPT/NIPS)

- Fetal aneuploidy
- Fetal microdeletion syndromes
- Fetal Rh factor
- Determination of fetal sex (XX, XY)
- Certain monogenic diseases in the fetus

Screening

Screening means the process of sorting.

Screening in medicine involves examining a pre-defined group of people to detect diseases in their early stages, before the patient has symptoms or problems

Screening during pregnancy

I. Trimester (10-14 weeks gestation):

- NT (Nuchal Translucency): Measurement of the nuchal translucency at the back of the fetus's neck (ultrasound)
- NB (Nasal Bone): Presence of the nasal bone in the fetus (ultrasound)
- PAPP-A (Pregnancy-Associated Plasma Protein A (biochemical test)
- Free β-hCG (Free Beta Subunit of Human Chorionic Gonadotropin): A hormone measured in the blood (biochemical test)
Combined Screening: Combines ultrasound and biochemical tests

II. Trimester (16-18 weeks gestation)

- AFP (Alpha-Fetoprotein)
- Total hCG (Total Human Chorionic Gonadotropin)
- uE3 (Unconjugated Estriol)
Biochemical Screening: Blood test of the pregnant individual

Integrated Screening: Evaluates individual risk based on parameters from both the first and second trimester screenings
- NT + PAPP-A + AFP + Total hCG + uE3

What Screening during pregnancy **good for?**

For screening pregnancies with an increased risk, primarily for Down syndrome, and potentially for Edwards syndrome, Patau syndrome, neural tube defects (NTDs), and Smith-Lemli-Opitz syndrome (SLOS, autosomal recessive) in the fetus.

Comparison of Detection Rates (DR) for different screening methods for trisomy 21 at a 5% false positive rate (Nikolaides).

Screening method	DR in %
Maternal Age (MA)	30
MA and biochemical screening (maternal serum) at 15-18 weeks gestation	50-70
MA and fetal nuchal translucency (NT) measurement at 11-13+6 weeks gestation	70-80
MA and fetal NT measurement along with free β-hCG and PAPP-A in maternal serum at 11-13+6 weeks gestation	85-90
MA and fetal NT + nasal bone (NB) measurement at 11-13+6 weeks gestation	90
MA and fetal NT and NB measurement, along with free β-hCG and PAPP-A in maternal serum at 11-13+6 weeks gestation	95

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Impact of Maternal Age and Gestational Age

(Nikolaides)

- The risk of trisomy increases with maternal age.
- The risk of Turner syndrome and triploidy does not change with maternal age.
- The risk of chromosomal abnormalities is highest in the early stages of gestation.
- The mortality rate for fetuses with trisomy 21 between the 12th week (when NT is performed) and the 40th week of gestation is approximately 30%.
- The mortality rate for fetuses with trisomy 21 between the 16th week (when biochemical serum screening is conducted in the second trimester) and the 40th week is approximately 20%.
- For trisomies 18 and 13 and Turner syndrome, the fetal mortality rate between the 12th and 40th weeks of gestation is approximately 80%.

Ultrasound Screening: Three-Step Process

- 12th + 20th + 33th week of gravidity

- **Detection of detectable developmental abnormalities**
- **Detection of detectable cardiac defects**
- **Detection of indirect signs of chromosomal abnormalities**
- **Monitoring of fetal growth and development**
- **Signs of certain monogenic disorders**:
- **Shortened limb bones**: Small stature, increased risk of conditions such as achondroplasia
- **Increased echogenicity of intestinal loops**: Increased risk of cystic fibrosis in the fetus

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Ultrasound Screening

First Trimester Ultrasound Screening (10-13 weeks gestation):

Information on:

- Number of fetuses
- Size + length of pregnancy
- Nuchal translucency
- Presence of the nasal bone – risk assessment for Down syndrome
- Detection of risk for certain congenital developmental defects

Second Trimester Ultrasound Screening (20 weeks gestation):

Examination focused primarily on:

- Detection of detectable congenital developmental abnormalities
- Indirect signs of congenital chromosomal abnormalities in the fetus

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US Prenatal Cardiology at 20-22th week of gestation

- detection of detectable cardiac defects

- Congenital heart defects are the most common developmental abnormalities in humans and are often associated with additional disorders/defects.
- Prenatal diagnosis of congenital heart defects requires specialized expertise and experience, typically performed by pediatric cardiologists and specialists in prenatal cardiology.
- Identifying a heart defect in the fetus allows for modifying subsequent management based on the severity of the condition, including options such as termination of pregnancy, fetal treatment, monitoring and delivery at a specialized center where cardiological or cardiac surgical treatment for the newborn is available

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NIPT (Non-Invasive Prenatal Testing)

Genetic testing of Free fetal DNA in maternal plasma (**originating from the placenta):**

- Detection of trisomy 21 (Down syndrome), and potentially trisomy 18 and 13, sex chromosome aneuploidies, analysis of triploidy, and possibly microdeletion syndromes
- Performed after the 10th-11th week of pregnancy
- Currently not covered by medical insurance due to relatively high cost
- High reliability of 98-99%
- A pathological finding must be confirmed with invasive testing

Other uses:

- Fetal RhD status determination
- Fetal sex determination (detection of SRY for X-linked diseases)
- Monogenic hereditary diseases in the fetus – currently available for some conditions (e.g., achondroplasia with single mutation scoring)
- Autosomal recessive (AR) hereditary diseases? De novo mutations?

Invasive prenatal testing

- **CVS (Chorionic Villus Sampling)** – Collection of chorionic villi (11-14 weeks gestation)

- **AMC (Amniocentesis)** – Collection of amniotic fluid (16-20 weeks gestation)

- **Cordocentesis** – Collection of fetal blood from the umbilical cord (after 20 weeks gestation)

- **Placenta Sampling (Placental Biopsy)** – Collection of placental tissue (less commonly used, may be referred to as placentocentesis)

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Indications for Invasive Prenatal Diagnosis

- Pathological result from combined/biochemical/integrated screening
- Pathological ultrasound finding in the fetus
- Carrier status of a balanced chromosomal abnormality in parents
- Congenital chromosomal abnormality in the family or in a previous pregnancy
- Monogenic hereditary disease in the family
- Advanced parental age (?)

  (women over 35 years old, combined age of partners over 70-75 years, men over 45 (?) years old - new mutations like achondroplasia, neurofibromatosis)

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Prenatal Testing for Chromosomal Abnormalities

- **QF-PCR (QuantitativeFluorescent Polymerase Chain Reaction):**

  Tests for the most common chromosomal numerical abnormalities of chromosomes 13, 18, 21, X, and Y (and possibly chromosomes 15, 16, 22, which are common aneuploidies in miscarried fetuses). Results available within 24-48 hours.

- **Karyotyping**:

  Basic genetic test, essential for assessing structural chromosomal abnormalities (e.g., balanced translocations in parents).

- **Array-CGH (Array Comparative Genomic Hybridization):**

  Used when there are pathological ultrasound findings and is increasingly used for all patients.

Prenatal Diagnosis of Monogenic Disorders

- **Based on preconception genetic testing or specific ultrasound findings** (e.g., increased echogenicity of bowel loops suggesting cystic fibrosis, shortening of limb bones indicating achondroplasia).
- **Usually involves scoring of familial** or in population frequent pathogenic variants, rarely NGS panel testing due to time factor
- **Invasive Testing During Pregnancy:**
  - CVS (Chorionic Villus Sampling): Performed after 10 weeks of gestation.
  - Amniocentesis (AMC): Performed after 15 weeks of gestation.
  - Cordocentesis: Performed after 20 weeks of gestation.
- **Material for Testing:**
  - Chorionic villi or amniotic fluid cells. Testing can be done immediately or after sample cells cultivation; rarely, fetal blood is used.
- **Contamination with Maternal Tissue:**
  - Always needs to be excluded, particularly with CVS and amniotic fluid samples without culture.

In families with known risk of a monogenic disorder, it is recommended to consider Preimplantation Genetic Diagnosis (PGD) as part of In Vitro Fertilization (IVF).

Preimplantation Genetic Diagnosis (PGD)

- PGD is an early prenatal diagnostic method that is associated with assisted reproductive techniques.
- PGD is a method that allows the detection of genetic abnormalities in an embryo by examining a few cells taken from the developing embryo. Only embryos without genetic defects can be selected for transfer into the uterus.
- Before performing PGD, we recommend preconception genetic testing and karyotype analysis of the partners, as well as DNA analysis of the parents in cases of monogenetic hereditary diseases.
- This method allows at-risk couples to have an unaffected child without the need to rely on traditional prenatal diagnostics, which may involve making decisions about terminating the pregnancy.

Preimplantation Genetic Diagnosis (PGD)

vs

Preimplantation Genetic Screening (PGS) for Aneuploidies

- **PGD** – Testing for couples with a high genetic risk of disease in the fetus – carriers of translocations or predispositions for monogenic inherited diseases (PGT-Structural, PGT-Monogenic).
- **PGS** – Screening of common aneuploidies, where the risk is increased due to age or an unfavorable reproductive history (PGT-A).

PGS / PGD

- Alternative to prenatal diagnosis
- Prevention of abortions indicated based on results of invasive prenatal diagnosis
- Preventive and targeted diagnosis of specific genetically determined diseases
- Embryo selection for IVF in couples at risk of genetic disease
- Today, testing usually involves 6-8 cells of the embryo on day 5 after IVF (previously one or two blastomeres on day 3)

Prenatal and perinatal management of pregnancy with diagnosed developmental disorders or genetic diseases in the fetus

- **Consultation with specialists who will continue to care for the pregnant woman**: ultrasound specialists, geneticists, gynecologists, psychologist etc.
- **Determination of the most accurate diagnosis and prognosis**, and exclusion of combinations with other conditions, etc.
- **Consultation with specialists who will care for the newborn** with a condition after birth.
- **Planned delivery at a facility with specialized care** (cardiac center, pediatric surgery, cardiology, etc.).

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Genetic indication for interruption

Law of the Czech National Council No. 66/1986 on Induced Termination of Pregnancy, Decree of the Ministry of Health No. 75/86:

Paragraph 2

* After the 12th week of pregnancy, an abortion can only be performed if the woman's life is in danger, or if severe fetal damage is proven, or if the fetus is unviable.
* If there are genetic reasons for an abortion, it can be performed up to the 24th week of pregnancy.

Genetic Reasons:

- Severe hereditary diseases or congenital anomalies diagnosed in the fetus through prenatal diagnostic methods or evidence of their high risk.
- **Risk of serious hereditary disease** or anomaly determined by genetic testing **exceeding 10%**.
- Factors with proven teratogenic or mutagenic effects on the fetus.

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Problems - Conflicts

Prenatal diagnosis does not detect all diseases

Successfully ruling out certain serious conditions through targeted prenatal diagnosis does not guarantee that the baby will be free from other severe diseases. There is always a risk that the child could be born with a different, unrelated serious condition.

Ethical aspects

- Equal access to prenatal diagnostics, fair distribution
- Voluntariness
- Offering prenatal diagnostics not conditional on terminating the pregnancy
- Only severe health indications
- Psychological indications are not a priority

Recommendations

- Genetic counseling precedes
- Communication of all relevant information
- Family decision is protected and respected
- Ensuring safe termination of pregnancy
- Supportive counseling after pregnancy termination

Genetic counseling and genetic testing in reproductive disorders

- Is fertility disorder a result of a genetic disorder that could be passed on to the next generation?
- Can fertility correction increase the risk of malformations, diseases, and chromosomal abnormalities in offspring?
- Can genetic testing and prenatal diagnostics reduce this risk?

Couples with Reproductive Disorders - Indications for Genetic Testing

- More than 1 year of unsuccessful attempts to conceive with regular intercourse

- 2 or more spontaneous miscarriages

Genetic Causes of Reproductive Disorders

- Congenital chromosomal abnormalities
- Monogenic hereditary diseases
- Congenital malformations, multifactorial hereditary diseases
- Increased tendency to spontaneous abortions due to thrombophilia
- Disorders of spermatogenesis based on genetic disorders

Genetic Testing for Patients with Reproductive Disorders

- **Genetic Counseling - Genealogy, Medical History**

- **Cytogenetic Examinations:**
  - Karyotyping (for all patients with reproductive disorders)
  - Acquired chromosomal abnormalities (e.g., due to risky work environments, cytostatic treatments in medical history, etc.)

- **Molecular Genetic Testing**
- **CFTR Gene** – family burden (e.g., unexplained child deaths, repeated lung or middle ear infections), prevention in cases of recurrent miscarriages or multiple unsuccessful IVF cycles, men with abnormal sperm analysis.
- **Thrombophilic Mutations** (e.g., Leiden mutation - factor V, Prothrombin factor II - G20210A) - women with recurrent spontaneous abortions and fetal losses.
- **Yp AZF Regions a, b, c** – men with severe oligospermia and azoospermia (sperm count ≤ 5 million/ml).

Congenital chromosomal abnormalities occur with a population frequency of 0.6%.

In individuals being evaluated for reproductive disorders, the risk is reported to be approximately 10 times higher (6-7%).

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Thrombophilic mutations

- Increase congenital risk for deep vein thrombosis, sudden ischemic vascular events, and embolisms even at a young age, as well as an increased risk of recurrent fetal losses, intrauterine growth restriction (IUGR), placental infarcts, HELLP syndrome, and stillbirths – Factors V and II

Leiden mutation G1691A (Factor V)

- Frequency in the white European population is approximately 5-9%.
- Autosomal dominant inheritance.
- Increases the risk of thromboembolism: 50-100 times for homozygotes, 5-10 times for heterozygotes.
- Association with the risk of early fetal losses is not confirmed.
- Increases the risk of fetal losses from the end of the first trimester and in the second and third trimesters.

G20210A variant in Prothrombin (Factor II)

- The mutation is found in about 2-3% of the population in a heterozygous state.
- Increases the risk of thromboembolism.
- Carriers are associated with an increased risk of fetal losses, placental abruption, preeclampsia, and intrauterine growth restriction (IUGR).
- The risk of early spontaneous abortions (SAs) is not confirmed.

Male Infertility

- **Oligoastoenozoospermia – Azoospermia**
- **Chromosomal Abnormalities**
- **Microdeletions Yq11.23 – DAZ Gene – AZF Region**: DAZ (Deleted in Azoospermia) gene is deleted in cases of azoospermia.
- **CFTR Gene**: Mutations, including the 5T allele in the non-coding region of intron 9, are associated with Congenital Bilateral Absence of the Vas Deferens (CBAVD).

CFTR gene

- Carriers of mutations and certain polymorphisms experience sperm production disorders (5T – CBAVD).
- Cystic fibrosis can be a cause of male sterility in patients
- Carrier Frequency: In the general population, 1 in 27 individuals is carrier; among men with abnormal sperm analyses (SPG), the carrier rate is 1 in 19 (possibly symptomatic heterozygotes?).
- Most common monogenic disorder: Preventive testing is recommended before IVF, after recurrent miscarriages (SA), or following unsuccessful IVF attempts.

Microdeletions in AZF regions a, b, c of the DAZ gene:

- Approximately 4-5% in infertile men and about 15-18% in cases of azoospermia.

- When using IVF and micromanipulation techniques, there is a risk of passing the reproductive disorder to sons.

Genetic Testing in Couples with Reproductive Issues

- Genetic counseling provides partners with information on options of genetic testing and the implications of the results for them and their offspring
- Targeted Basic Genetic Testing:
  - Karyotyping both partners, possibly performing Array-CGH
  - Testing for thrombophilia in women with recurrent miscarriages.
  - Testing for CFTR gene mutations and microdeletions in AZF regions a, b, c of the DAZ gene, and other relevant genes in men with abnormal sperm parameters.
- Extended preventive couples testing
  - screening for carrier status of autosomal recessive (AR) and X-linked (XR) disorders - „Carrier test“
- Recommendations based on abnormal/pathological findings
  - targeted prenatal or preimplantation genetic diagnosis
  - preventive testing of relatives at risk

Preventive Genetic Testing for Gamete Donors

- **Donors medical history**
- **Three-Generation Family Pedigree:** Identification of genetic family history (developmental disorders, hereditary diseases, reproductive issues).
- **Karyotyping:** Determination of chromosomal structure and identification of abnormalities.
- **CFTR Gene Carrier Screening**: Analysis for carriers of common mutations associated with cystic fibrosis.
- **SMA Carrier Screening**: Analysis for carriers of mutations associated with spinal muscular atrophy.
- **Carrier Screening for Non-Syndromic AR Hearing Loss**: Analysis for carriers of mutations in the GJB2 gene, associated with autosomal recessive hearing loss.
- **Extended DNA Analysis**: Panel testing for common recessive hereditary disorders to identify potential genetic risks.

Neonatal screening

- **Neonatal screening is active, nationwide detection of diseases in their preclinical stage in newborns.**
- Analysis of a dried blood spot on filter paper, collected in the standard way from the heel of the newborn.
- Founder: Prof. Robert Guthrie (1916-1995).
- Phenylketonuria screening in the USA since 1963.

Neonatal screening until 10/2009

- Phenylketonuria
- Congenital hypothyroidism
- Congenital adrenal hyperplasia

- Orthopedic examination for hip dislocation
- Screening for congenital cataracts
- Screening for hearing impairment

Neonatal screening

- **Bulletin of the Ministry of Health of the Czech Republic No. 6/2009**

  Expanded newborn screening from 3 to 13 diseases starting from 10-12/2009

- **Bulletin of the Ministry of Health of the Czech Republic No. 6/2016**

Methodological guide for ensuring newborn screening and follow-up care (pages 2-11),

Issued on May 31, 2016, effective from June 1, 2016

Expansion to include 5 additional diseases

Diseases Screened Since October 2009

- **Endocrine diseases** – Congenital hypothyroidism, Congenital adrenal hyperplasia (CAH)
- **Inherited metabolic disorders** – Phenylketonuria (PKU, HPA), Leucinosis, MCAD, LCHAD, VLCAD, Deficiency of carnitine palmitoyltransferase I and II, Deficiency of carnitine-acylcarnitine translocase, Glutaric aciduria, Isovaleric aciduria…
- **Others** – Cystic fibrosis

Diseases Screened Since October 6/2016

- **Endocrine diseases** – Congenital hypothyroidism, Congenital adrenal hyperplasia (CAH)
- **Inherited metabolic disorders (IMD)**: Argininemia (ARG), Citrullinemia Type I (CIT), Medium-chain acyl-CoA dehydrogenase deficiency (MCAD), Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD), Biotinidase deficiency (BTD), Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD), Carnitine palmitoyltransferase I deficiency (CPT I), Carnitine palmitoyltransferase II deficiency (CPT II), Carnitine-acylcarnitine translocase deficiency (CACT), Phenylketonuria (PKU) and hyperphenylalaninemia (HPA), Glutaric aciduria Type I (GA I), Homocystinuria due to cystathionine beta-synthase deficiency (CBS), Homocystinuria due to methylenetetrahydrofolate reductase deficiency (MTHFR), Isovaleric aciduria (IVA), Maple syrup urine disease (MSUD)
- **Other** – Cystic fibrosis
- **Cumulative risk of all screened diseases**: 1 in 1200
- www.novorozeneckyscreening.cz

Samples for Newborn Screening

- Blood sampling for newborn screening can only be performed with the consent of the newborn’s legal guardian.
- An overview of information for legal guardians and a sample informed consent form for newborn screening are provided in Appendix 1 of the Methodological Guideline.
- If the informed consent is in written form, the healthcare provider must store it in the medical documentation of the newborn.
- If the legal guardian of the newborn refuses to have the newborn screening performed, this refusal must be documented in writing in the medical documentation of the newborn.
- The recommended form for recording the refusal of newborn screening is provided in Appendix 2 of the Methodological Guideline.

Newborn screening - Conditions for the disposal of cards/samples of DNA

- **Disposal of Screening Cards**

For blood collection, double self-copying screening cards are used (referred to as screening cards). Providers who have performed the laboratory screening tests retain these cards for five years, with shredding conducted under S5 conditions.

- **Disposal of DNA Samples for Newborn Screening**

In the case of analyzing hereditary changes in the CFTR gene, DNA isolated from the screening card is disposed of within 2 months after the test is completed.

Postnatal Genetic Testing

- Early diagnosis

-> Monitoring

-> Specialized care

-> Interdisciplinary collaboration

Presymptomatic Testing

- diseases with late onset of symptoms
- hereditary cancer disorders

Preventive programs

- Consultation with a clinical geneticist, creation of a three-generation family tree based on medical history
- Karyotype analysis
- Analysis of acquired chromosomal abnormalities
- Testing for cystic fibrosis carrier status (carriers in the Czech Republic approximately 1/30-40)
- Testing for spinal muscular atrophy carrier status (carriers in the Czech Republic approximately 1/60-80)
- Testing for the most common cause of autosomal recessive non-syndromic hearing loss (GJB2 gene)
- Testing for carrier status of other common autosomal recessive and X-linked inherited diseases

If you have any questions, you can contact me by email at:

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