UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
"CAROL DAVILA" din BUCUREȘTI

UNIVERSITY OF MEDICINE AND PHARMACY
„CAROL DAVILA", BUCHAREST
DOCTORAL SCHOOL
GENERAL MEDICINE

THE IMPORTANCE OF WHOLE EXOME SEQUENCING (WES) IN PRENATAL DIAGNOSIS IN THE ROMANIAN POPULATION

SUPERVISOR:

Prof. Dr. Hab. Iuliana Ceaușu

DOCTOR OF PHILOSOPHY:

Drd. ILEANA-DELIA SĂBĂU

2025

Contents

Introduction

Medical genetics has grown to be a crucial specialty with a big impact on a global scale, which is related to both individual health and public health systems. Some of the most important advantages include: early detection and intervention through genetic predisposition tests, accurate diagnosis, personalized medicine, meaning a tailored approach to each individual pacient, life management and care for patients with rare disorders, also can offer researchers the possibility to trace back ancient lineages and learn about the evolution of populations. On a broad scale, the impact on public health systems is beginning to show in developed countries that chose to implement genetic tests for screening and/or diagnostic.

1. History

Gregor Mendel was a monk, teacher and biologist, born in present day Czech Republic, at the time the Austrian Empire, who discovered the very basic principles of heredity. He lived between 1822 and 1884 and is considered to be the "father" of modern, or Mendelian genetics. Through his well-known experiments with peas, he postulated the basis of inheritance, and named "factors" what we call now "genes" to explain what drives the formation of the traits in an organism. The three laws of inheritance published in his paper "Experiments in plant hybridization" from 1856, the Law of Segregation, the Law of Independent Assortment and the Law of Dominance and Uniformity remain indisputable up to present day. Mendel's work remained uncovered until 1900, when three European biologists discovered his work simultaneously, Hugo DeVries, Carl Correns and Erich von Tschermak. They raised awareness about his work, making it known to the scientists and to the public.

2. Current picture

In the 21st century we notice the rise of personalized and genomic medicine that led scientists to aim as high as producing gene therapies through gene editing, or CRISPR (clustered regularly interspaced short palindromic repeats) technique.

In the field of gene therapy, there are two primary groups. Through the process of ex vivo gene therapy, cells are extracted from the patient, new genetic material is introduced, and the cells are then returned to the patient after being packaged in a delivery vehicle known as a vector. This approach is being utilized for the treatment of a variety of conditions, including sickle cell disease, adrenoleukodystrophy, chronic granulomatous disease, and others. During in vivo gene therapy, the vector is either injected into a target organ like the eye or infused directly into the circulation through intravenous (IV) administration. The in vivo gene therapy can treat a variety of conditions, including hemophilia and ornithine transcarbamylase deficiency, among others.

The two pioneers who worked on CRISPR are Emmanuelle Charpentier and Jennifer Doudna, who were awarded the Nobel Prize in Chemistry in 2020, “for the development of a method for genome editing”.

CRISPR is a method employed by researchers to selectively edit the DNA of organisms. CRISPR was repurposed for laboratory applications from naturally occurring genome editing mechanisms identified in bacteria.

Gene therapy is a method that utilizes genes to treat, prevent, or cure diseases or medical disorders. Gene therapy often functions by introducing additional copies of a malfunctioning gene or by substituting a defective or absent gene in a patient's cells with a functional version. Gene therapy has been utilized to treat both inherited genetic illnesses (e.g., hemophilia and sickle cell disease) and acquired conditions (e.g., leukemia).

3. General objectives and hypotheses

The coding part of DNA is called the exome. A gene is made up of the letters A, T, C, G, or DNA, which is made up of introns and exons. All cellular processes that occur in the body are regulated by DNA. However, not all types of DNA do this. It is known that only 1-2% of all genes encode or contain instructions for proteins, which are the basic components of cells and underlie all processes in some organisms. Even if researchers thought they were going to discover many more genes in humans, approximately 20,000–25,000 genes are contained within the human genome. One of the most important aspects of human complexity is the manner in which these genes are controlled by the remaining 99% of our DNA, which is referred to as the “unknown” of the genome.

Although sequencing the entire genome of a person is possible, this process is expensive and requires a significant amount of storage space for the bioinformatic data obtained. Whole-exome sequencing is more commonly used and more cost-effective, as it is estimated that 85% of all genetic variants that cause diseases can be found in the exome.

More than about 6% of all problematic pregnancies are diagnosed by the use of molecular karyotyping, whereas the gold standard testing, which is classical karyotyping from amniotic fluid, detects between 8 and 10 percent of all abnormal pregnancies. On the other hand, a significant number of pregnancies remain undiagnosed. According to research conducted on a global scale, prenatal WES is responsible for bringing a diagnosis to light in twenty to eighty percent of cases, and in other instances, even more than ninety percent of cases.

The purpose of this study is to demonstrate the significance of prenatal genetic testing through WES in the Romanian population, specifically in pregnant women who have malformed fetuses or IUGR

on ultrasound. The study aims to determine the diagnosis with absolute certainty and the precise percentage of the population for which the testing makes the diagnosis. This will allow for a more informed decision regarding the pregnancy as well as a significantly improved management of the case, including the possibility of treatment being administered in utero or immediately after delivery in certain circumstances.

The study thus aims to develop a guide for doctors in Romania and beyond, based on evidence, depending on the variants discovered in our population regarding prenatal diagnosis and the stages of its performance, when it is required, in selected patients.

WES is a next-generation DNA sequencing technique that can obtain all the coding regions of the human genome (exons) – over 22,000 genes. This modern and cost-effective technology allows for the rapid and cost-effective identification of single nucleotide polymorphisms/variants (SNP/SNV), copy number variations (CNV) and small insertions or deletions (indels), as well as rare de novo mutations. This library of raw data thus obtained can be reinterpreted and reclassified over time, depending on the development of databases and knowledge in the field, without the need for a new blood or other biological material collection from the patient.

At the international level, there is no strict regulation regarding the recommendation of this personalized testing, as it has only recently been done for diagnosis and not for research.

Various European countries are already using this technology, with a great deal of uncertainty surrounding Solo or Trio testing - that is, testing only the fetus through amniotic fluid analysis, or testing the parents from the first stage, which is called WES Trio. Another question that arises is whether specific panels such as cardio or neurogenetics are more cost-effective than the clinical exome panel.

Motivation of the chosen topic

The existence of an increasing number of pregnancies with malformations or fetal growth restriction not diagnosed by existing methods.

The need to know whether the malformation detected by ultrasound of the fetus is unique, or part of a complex genetic syndrome.

The prevalence of congenital anomalies in the general population is approximately 3%-5%. Since the first report of the use of ultrasound in obstetrics, it has become an important tool for detecting fetal structural defects, detecting their presence in 2-3% of cases. The assessment of nuchal translucency in the first trimester also represents a screening tool for fetal structural anomalies. There is an

association between increased nuchal translucency and chromosomal anomalies, especially trisomy 21 or monosomy X, as well as with the presence of various structural fetal cardiac, gastrointestinal or lymphatic system anomalies. Over 75-80% of fetal anomalies occur in the first 3 months of gestation. Therefore, good visualization of the fetus at this stage allows early detection of structural anomalies. Detection rates by ultrasound examination of major structural anomalies in the first and second trimesters range from 12.0% -44% and from 20% to 87%, respectively.

Research question

How many cases of malformed fetuses with negative molecular karyotype testing have positive results in WES testing in the Romanian population?

Finding out the percentage of cases for which WES makes the diagnosis, compared to molecular karyotype (current prenatal diagnosis) in the population of our country.

Objectives and hypotheses

Prenatal WES testing makes the diagnosis in up to 80% of cases selected internationally (malformations or IUGR), helping the clinician and the family to make an informed decision and have an overall vision of the pregnancy.

First objective is to know the percentage for which in Romania the certain diagnosis is found. Other objective is to follow – up the decisions of the informed family and how they choose to continue the pregnancy or not depending on the finding of the definite diagnosis of genetic syndrome.

Pre and post-testing genetic consultation is recommended. The pre-test consultation aims to choose targeted and personalized testing for each individual case and to analyze the utility and possible benefit of specific genetic testing. The post-test consultation is carried out with the aim of interpreting the results and advising the family on the mode of transmission, recurrence and possible complications of the discovered genetic pathology.

Completing the existing gold standard testing algorithm with this new generation testing method should become the new gold standard in selected patients, making the diagnosis certain in up to 80-92% of cases.

The algorithm for testing the fetuses with unspecific malformations is also presented in **Figure 7**. The steps are:

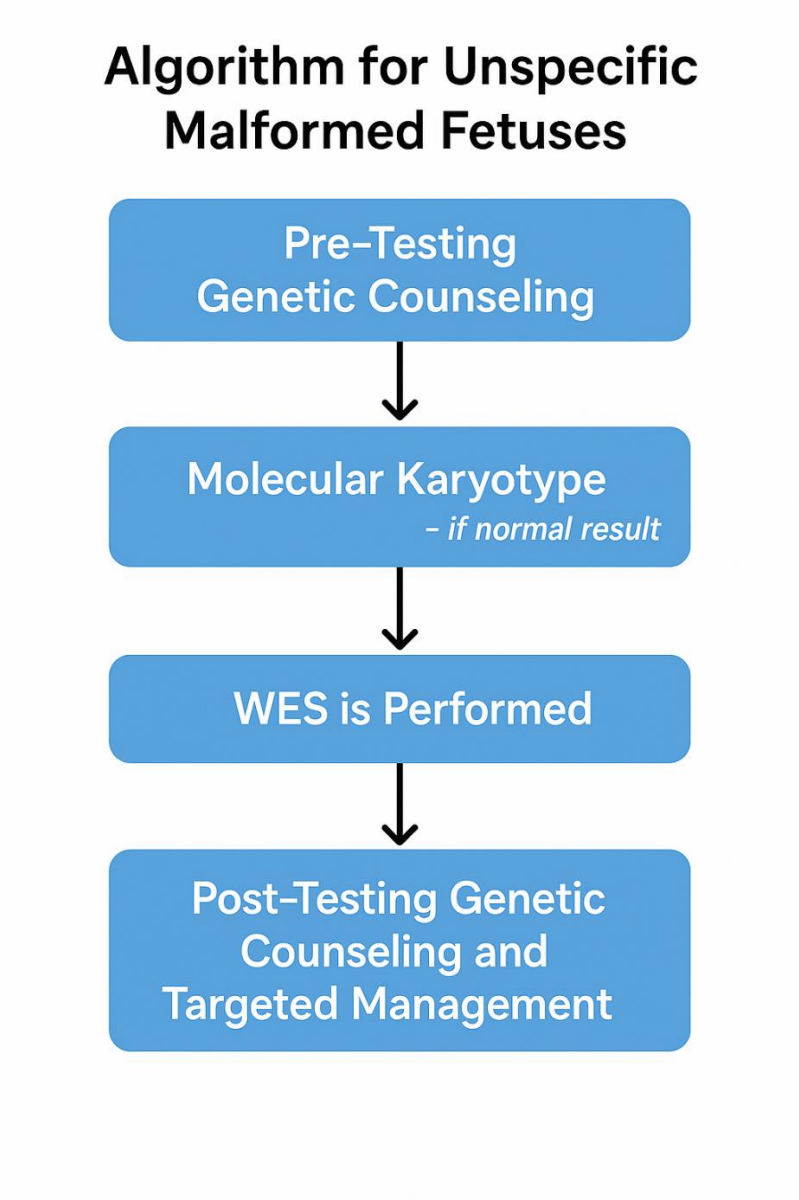
1.pre-testing genetic counseling

2. molecular karyotype –if normal result

3. WES is performed

4. post-testing genetic counseling and targeted management

Figure 7: Testing algorithm for unspecific malformed fetuses



4. The Evolution of Prenatal Whole Exome Sequencing: From Cytogenetics to Precision Medicine

Historically, prenatal genetic testing was limited to identifying chromosomal abnormalities. While groundbreaking, these early methods could not detect more subtle genetic alterations that could profoundly affect fetal development.

In the 1960s, the introduction of karyotyping marked a pivotal advancement in prenatal genetic diagnostics. This technique enabled the visualization of fetal chromosomes, obtained through amniocentesis or chorionic villus sampling (CVS), allowing clinicians to detect major chromosomal abnormalities such as Down syndrome (trisomy 21), trisomy 18, trisomy 13 and sex chromosome aneuploidies. Until the 1960s, the sole method to prevent a genetic sickness was to abstain from marrying an individual from a 'tainted' lineage, and for individuals from such lineages refrain from procreation, a strategy that culminated in eugenics during the twentieth century [12-17].

John William Ballantyne, a Scottish obstetrician, was the first to advocate in the early twentieth century for the treatment of pregnant women to enhance the future health of their children. Ballantyne developed an interest in newborn and fetal deformities, referred to as 'monstrous births.' He concluded that adequate medical care for expecting mothers diminishes the incidence of abnormalities in babies [5,6,7].

By the 1990s, FISH emerged as a powerful advancement in prenatal genetic diagnostics. This technique utilized fluorescently labeled probes that bound to specific DNA sequences on chromosomes, enabling the detection of targeted chromosomal abnormalities with greater precision than traditional karyotyping. However, while FISH improved the diagnosis of specific chromosomal anomalies, it lacked the comprehensive genomic coverage necessary for detecting point mutations or complex structural rearrangements.

Whole exome sequencing (WES) emerged in the early 2010s as a groundbreaking advancement in prenatal genetic diagnostics. Unlike chromosomal microarray analysis (CMA), which primarily detects copy number variations (CNVs), WES sequences the entire protein-coding region of the genome — the exome — where the majority of pathogenic variants associated with genetic diseases are found. Although the exome represents only 1–2% of the genome, it harbors most disease-causing variants, making WES a highly effective tool for detecting point mutations, small insertions and deletions (indels), and certain structural variants linked to monogenic and syndromic conditions. By

offering greater resolution and diagnostic yield compared to previous techniques, WES has significantly expanded the capabilities of prenatal genetic testing, enabling early identification of previously undetectable conditions [19-28].

In the cohort study by Wang et al. from 2024, a total of 60 pregnancies with ultrasound anomalies were included for extended investigation, including karyotype analysis, CMA and WES. The results showed that twelve (20%, 12/60) fetuses were identified with chromosomal abnormalities through karyotyping or CMA. Out of the remaining 48 cases that had undergone WES, P/LP variants were found in 14 cases (29.2%), resulting in an increased diagnostic yield of 23.3% (14/60) of the tested fetuses with congenital anomalies, including a range of fetal conditions such as skeletal abnormalities, heart defects, and other congenital anomalies were found to have genetic causes. The study demonstrated that WES could diagnose a range of congenital anomalies, such as heart defects and skeletal abnormalities, which had been elusive to traditional prenatal diagnostic techniques like karyotyping and chromosomal microarray analysis (CMA). The study also highlighted how WES could identify genetic causes for these conditions, thereby enhancing prenatal diagnostics and influencing clinical decision-making [26].

5. Comprehensive Prenatal Genetic Analysis: From Non-Invasive Prenatal Testing to Whole-Exome Sequencing in a High-Risk Pregnancy with Gaucher Disease—A Case Report and Literature Review

In this study, we present the case of a 35-year-old Romanian Caucasian patient who underwent non-invasive prenatal testing (NIPT) using the Veragene platform.

NIPT represents a groundbreaking advancement in prenatal screening, leveraging next-generation sequencing (NGS) or other high-throughput technologies to analyze cell-free placental DNA circulating in maternal blood. This approach offers a safer alternative to traditional invasive procedures, such as chorionic villus sampling (CVS) or amniocentesis, as it poses no direct risk to the fetus.

Despite its widespread promotion and commercialization, it is crucial to critically evaluate the benefits, limitations, and risks of NIPT compared to both invasive and other non-invasive testing methods. While NIPT is highly effective for screening common aneuploidies, such as trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), and trisomy 13 (Patau syndrome), its clinical utility is limited by its inability to provide a definitive diagnosis. Additionally, false-positive and

false-negative results may occur due to factors such as placental mosaicism, maternal health conditions, or fetal-specific variables.

The NIPT used was Veragene testing, a validated Laboratory-Developed Test (LDT) from Medicover Genetics Ltd. for prenatal screening. It can test for common chromosomal aneuploidies such as trisomies 13, 21, 18, X, and selected microdeletions through the multiplexed parallel analysis of specific regions of interest. It can also detect 100 monogenic disorders, such as GD.

Veragene is created based on the Target Capture Sequences (TACSs) approach, which consists of long synthetic DNA probes that bind to specific DNA regions of the genome and then utilize NGS technology. This particular methodology is designed to reduce the current described limitations of whole-genome-based NIPT [51-59]. TACS enrichment has been successfully used to screen for fetal risk for monogenic disorders based on parental carrier screening results.

Prenatal WES was performed through NGS technology on Illumina's NovaSeq platform. The bioinformatic pipeline used was Illumina Dragen Bio-IT, and an interpretation of the result was made with the help of VarSeq software version 2.3.0 from GoldenHelix. The data were aligned to Human Reference Genome hg38 (NCBI GRCh38).

The classification and interpretation of variants were performed following the ACMG (American College of Medical Genetics) and Association for Clinical Genomic Science (ACGS) standards [60,61].

This case underscores the necessity of understanding both the advantages and limitations of non-invasive prenatal testing (NIPT) in prenatal care, particularly the importance of confirmatory diagnostic testing when abnormalities are identified. Given these constraints, the medical indications for NIPT remain surprisingly narrow, raising critical questions about what individual pregnant women can genuinely learn from the test and how the results should be interpreted in clinical practice.

6. The importance of Prenatal Whole-Exome Sequencing Testing in the Romanian Population

We hereby describe 10 cases of prenatal WES testing for pregnant mothers from the Romanian population between 2022 and 2023 from a private laboratory in Bucharest, Romania.

Case No.	Phenotype	Gestational Age	Reason for WES Testing Referral	Fetal WES	Pregnancy Outcome	Incidental/Secondary Findings
1	Fetal sinus bradycardia, supraventricular extrasystoles	22 weeks	Negative SNP array Known genes linked to fetal phenotype	Negative	Stillbirth	PALB2:c.93dupA(p.Leu32thrfsTer11)—inherited from the mother
2	Aberrant right subclavicular artery, rocker bottom feet, fetal hydrops, fetal ascites	20 weeks	Negative SNP array	LMX1B:c.718G > A (p.Val240Ile) Focal segmental glomerulosclerosis 10 Nail–patella syndrome	Born, affirmatively without symptoms at the age of 1 y.o.	-
3	Right aortic arch, severe hydronephrosis, caudal regression syndrome, mesoaxial hand polydactyly	18 weeks	Negative SNP array Clinical suspicion of VACTERL syndrome	HOXD13:c.820C > T (p.Arg274Ter) Brachydactyly-syndactyly syndrome Brachydactyly, type D Brachydactyly, type E Syndactyly, type V	Couple decided on termination of pregnancy	-

Case No.	Phenotype	Gestational Age	Reason for WES Testing Referral	Fetal WES	Pregnancy Outcome	Incidental/Secondary Findings
				Synpolydactyly 1		
4	Mega Cisterna Magna, ventriculomegaly	22 weeks	Negative SNP array	ADNP:c.1612G > A (p.Glu538Lys) Helsmoortel–Van der Aa syndrome De novo	Ongoing pregnancy	-
5	Clubfeet, microretrognathia, arachnodactyly, agenesis of the corpus callosum, intrauterine growth restriction	24 weeks	Negative SNP array	TGFBR1:c.734A > C (p.Glu245Ala) Loeys Dietz syndrome	Born, affirmatively without symptoms at the age of 1 y.o.	HBB: c.-151C > T Beta-thalassemia—inherited from the mother
6	Corpus callosum agenesis Oligohydramnios	23 weeks	Consanguinity Negative SNP array	Negative	Born, affirmatively without symptoms at the age of 2 y.o.	BRCA2: c.793 + 1G > A Susceptibility to breast–ovarian cancer, pancreatic cancer, prostate cancer—inherited from mother

Case No.	Phenotype	Gestational Age	Reason for WES Testing Referral	Fetal WES	Pregnancy Outcome	Incidental/Secondary Findings
7	Intracardiac echogenic focus, pyelectasis. Long QT syndrome of the father, family history of sudden cardiac death, arrhythmias due to affected father (without genetic diagnosis)	19 weeks	Negative SNP array	KCNQ1:c.605-28A > G Long QT syndrome 1 Susceptibility to Short QT syndrome 2 Atrial fibrillation, familial, 3 Inherited from the father	Born, affirmatively without symptoms at the age of 1 y.o.	-
8	Right aortic arch	17 weeks	Negative SNP array	Negative	Born, affirmatively without symptoms at the age of 1 y.o.	-
9	Sexual ambiguity on ultrasound morphology.	20 weeks	SNP array: arr [GRCh38]12p13.33p11.22(148769_30138756)x	Negative	Couple decided on termination of pregnancy	-

Case No.	Phenotype	Gestational Age	Reason for WES Testing Referral	Fetal WES	Pregnancy Outcome	Incidental/Secondary Findings
	Intrauterine growth restriction, hypospadias, polyhydramnios.		2 hmz, 12q21.31q24.22 (84757938_117685540)x2 hmz			
10	Borderline bilateral ventriculomegaly, suspicion of hydrocephaly with Sylvian stenosis	19 weeks	Negative SNP array	Negative LAMB1:c.3499C > T (p.Arg1167Ter) Lissencephaly 5 (AR) PTPN23:c.2248C > A (p.Pro750Thr) Neurodevelopmental disorder and structural brain anomalies with or without seizures and spasticity (AR)	Ongoing pregnancy	

The diagnostic yield of WES testing among our patients was 50%, similar to other studies recently published in the literature.

In this article, we present 10 cases that were tested through prenatal WES, showing the great diagnostic yield in comparison to the SNP array in our selected cases. As a closing remark, we wish to highlight the benefits of WES testing in prenatal cases. Not only have we discovered the etiology

of the fetal structural abnormalities in US, but also we discovered some incidental and secondary findings that diagnosed the parents or made the medical multidisciplinary team aware of some high risks for the parents and their families.

The statement by de Jong and de Wert [104] starts a debate about how prenatal testing should be state-insured so that couples have the option to terminate a particular pregnancy where the future child will be born with severe disorders that might lead to the child's death or have the child disabled for life. State health services should remain impartial concerning couples' pre- and post-test choices regarding whether the couple would like to keep or terminate the pregnancy, whilst offering the best support to uphold their wish. For example, if the couple would like to keep a pregnancy where the newborn would have either congenital affections or various other pathologies, the state services could book a consultation prenatally for a particular type of surgeon or organize a multidisciplinary team to better treat and tend to the needs of the patient. On the other side, should the couple choose to terminate the pregnancy, the state services could offer a variety of ob-gyn specialists who could provide them with the best care before and after terminating the pregnancy [91,92].

A crucial step in genetic testing is the requirement of informed consent from the tested patients or the prenatally screened couple's pregnancy. The informed consent form should comprise pre- and post-test options, the lack of legal constraint, a specialized pathology management plan, assuring optimal perinatal care, or, in other cases, as far as palliative care, the limitations of the genetic testing, and the limitations of the current worldwide knowledge.

Diagnostic yields for this technique vary from 8 to 80%, depending on cohort characteristics such as size, family history, ethnicity, and patient selection.

7. Conclusions and personal contributions

Medical genetics is now recognized as a fundamental component modern medicine, facilitating substantial advancements in the diagnosis, treatment, and prevention of diseases by uncovering the genetic determinants of health disorders. Recent breakthroughs in genome sequencing technologies, molecular biology, and analytics enable healthcare practitioners to detect genetic variants and biomarkers linked to many hereditary and acquired illnesses. These breakthroughs have facilitated early identification and enhanced accuracy in the diagnosis of diseases like as cancer, cardiovascular disorders, and uncommon genetic syndromes, hence enhancing patient outcomes and quality of life.

The emergence of pharmacogenomics facilitates personalized medication therapy customized to an individual's genetic profile, improving treatment efficacy and reducing unwanted effects.

Reproductive and prenatal genetic technology facilitate informed family planning and diminish the prevalence of hereditary disorders.

The incorporation of medical genetics into healthcare presents ethical, legal, and societal obstacles that require meticulous consideration, including concerns over privacy, permission, and equitable access to genetic treatments. Consequently, sustained investment in research, education, and healthcare infrastructure is needed to fully actualize the advantages of medical genetics.

Governments, organizations, and healthcare systems are encouraged worldwide to implement rules that advocate for the ethical and appropriate utilization of genetic information, while facilitating collaboration among scientists, clinicians, and public health professionals to guarantee that these advancements benefit different communities worldwide.

Prenatal genetics has transformed reproductive healthcare by offering essential insights into fetal genetic health before to delivery, facilitating informed decision-making and early intervention options. Advancements in genetic screening and diagnostic technologies, including non-invasive prenatal testing (NIPT), chorionic villus sampling (CVS) and amniocentesis, enable healthcare providers to identify chromosomal abnormalities, single-gene disorders, and other hereditary conditions with enhanced precision and safety. These advancements facilitate the early detection of disorders such as Down syndrome, cystic fibrosis, thalassemia, and neural tube abnormalities, frequently prior to the manifestation of clinical symptoms.

The incorporation of genetic counseling into prenatal care guarantees that prospective parents obtain thorough information and assistance to make informed decisions on pregnancy management, reproductive alternatives, and possible therapies.

Furthermore, prenatal genetic medicine facilitates the development of novel therapeutic approaches, such as in utero medicines and gene editing methods that could rectify or alleviate genetic abnormalities before to birth. As these technologies become increasingly available and advanced, they present significant ethical and societal issues, including informed consent, potential discrimination, and the psychological ramifications of genetic discoveries. Consequently, it is imperative that prenatal genetic services are provided with sensitivity, equity, and compliance with ethical norms to guarantee that all persons and families can responsibly and supportively use these medical developments.

To conclude, prenatal WES is the preferred method for an accurate and comprehensive genetic diagnosis, identifying genetic causes of structural anomalies that are not explained by the actual gold standard like karyotyping or microarray. It is particularly helpful in diagnosing rare genetic disorders, and also assessing whether the disease occurred de novo or if it was inherited from a parent. It is

undoubtedly the most efficient and eloquent for families who have history of undiagnosed recurrent pregnancy loss or anomalies in the fetuses. It can help the family and attending physicians about recurrence risk of rare syndromes and help build a personal management plan for the family.

Prenatal WES is the elective diagnostic test when NIPT fails to provide an answer, being uncertain, without result or negative and when karyotype and/or microarray do not explain the ultrasound findings.

Informed decision-making is a crucial element when contemplating prenatal whole exome sequencing. The insights derived from prenatal WES equip parents with essential knowledge to make informed decisions concerning pregnancy care. In places with legal gestational restrictions for termination, prompt results from prenatal WES can be crucial. Furthermore, negative results can offer reassurance and impact decisions on the continuation of the pregnancy.

In addition to facilitating prompt decision-making, prenatal WES outcomes can guide perinatal management methods. Identifying certain genetic abnormalities prenatally enables healthcare personnel to foresee future issues, prepare for requisite interventions at delivery, and organize specialized care teams. This proactive strategy can markedly enhance neonatal outcomes.

Families usually require an exact diagnosis when it comes to malformed fetuses and also the multidisciplinary team attending them have to know if a genetic disorder is the cause of the fetal anomalies. Only by knowing the cause can we provide the correct approach and also counsel the patients to undergo specific in utero procedures or treatment, in utero surgery, or to choose the type of hospital and also the type of delivery methods recommended. In utero gene therapies also can be an option and it will also develop more and more in the coming years. Other possible in utero therapies are stem cell transplantation and enzyme replacement therapy.

It is possible for expecting parents to experience less psychological strain if they are provided with a conclusive diagnosis, which eliminates the uncertainty that is associated with prenatal abnormalities. Because of this clarity, families are able to seek the proper support, connect with networks that are relevant to their situation, and prepare themselves emotionally and practically for years to come.

Knowing the genetic foundations of fetal abnormalities by prenatal WES also enables precise counseling of recurrence risks in subsequent pregnancies. Identifying autosomal recessive disorders informs parents of a 25% recurrence probability, guiding decisions on preimplantation genetic diagnosis (PGD) or early prenatal testing in future pregnancies. De novo arising disorders on the other hand usually don’t have a recurrence rate.

My personal conclusion is the just like similar European countries, Romanian state should also reimburse the testing, since it is more cost-efficient to early diagnose the genetic conditions so that couples might have the opportunity to terminate or keep the pregnancies based on an accurate diagnosis.

In Romania, just like in other European countries we face lack of infrastructure, trained personnel to perform and interpret the results, as well as provide the pre and post-test genetic consultation. In our country, the current situation is that we train a good number of medical geneticists which don't usually find jobs in state hospitals, since the offer is very scarce. They then are offered jobs in the private sector, a place where all technologies and machines are available.

The need for a better public infrastructure that can create the whole work-flow, both with current gold-standard and also more complex tests such as prenatal WES is one statement that I want to underline.

The test, like every other genetic test has its own limitations, that a medical specialist in medical genetics has to know and to also inform the family and the other doctors that are in the multidisciplinary team. Hence, it should only be recommended by a medical geneticist, only after a thorough pre-test genetic counseling. Keeping in mind the limitations and benefits, the attending genetician also has o keep in mind the cost-efficiency, being cheaper and faster than a more comprehensive test like whole genome sequencing.

In conclusion this testing should also be part of a new gold standard panel, alongside with the previous existing tests and to be used only in specific situations, selected cases with malformed fetuses with no genetic diagnosis.

Medical genetics in Romania has experienced significant progress in recent years, driven by increased awareness of genetic disorders, improvements in healthcare infrastructure, and the growing integration of genetic services into clinical practice. Romanian medical institutions, particularly in major cities like Bucharest, Craiova, Iași, Cluj-Napoca, and Timișoara, have developed specialized centers for genetic diagnostics, counseling, and research, offering services such as carrier screening, prenatal diagnostics, and molecular testing for inherited conditions. However, despite these advancements, the field still faces notable challenges, including regional disparities in access to care, limited funding, and a shortage of trained geneticists and genetic counselors, particularly in rural areas.

Efforts to expand education and training programs in genetics are underway, aiming to build a more robust workforce capable of supporting the growing demand for genetic services. Public and professional awareness campaigns have also played a role in reducing the stigma surrounding genetic

diseases and promoting earlier diagnosis and intervention. Furthermore, Romania's participation in international collaborations and research projects, particularly within the European Union, has helped accelerate the adoption of modern genomic technologies and standards.

Nevertheless, ethical concerns, such as patient privacy, informed consent, and equitable access to genetic testing, remain critical areas that require continuous oversight and development. As the country continues to integrate medical genetics into its national healthcare system, sustained investment in infrastructure, education, policy development, and interdisciplinary collaboration will be essential to ensure that the benefits of genetic medicine are accessible to all Romanian citizens.

Bibliografie selectiva

1. Han J., Yang Y.D., He Y., Liu W.J., Zhen L., Pan M., Yang X., Zhang V.W., Liao C., Li D.Z. Rapid prenatal diagnosis of skeletal dysplasia using medical trio exome sequencing: Benefit for prenatal counseling and pregnancy management. Prenat. Diagn. 2020;40:577–584. doi: 10.1002/pd.5653.
2. Guadagnolo D, Mastromoro G, Di Palma F, Pizzuti A, Marchionni E. Prenatal Exome Sequencing: Background, Current Practice and Future Perspectives-A Systematic Review. Diagnostics (Basel). 2021 Feb 2;11(2):224. doi: 10.3390/diagnostics11020224. PMID: 33540854; PMCID: PMC7913004.
3. Wei W, Jiang W, Yang R, Cui W, Zhang L, Li Z. Analyzing the Trends and Causes of Birth Defects - Jinan City, Shandong Province, China, 2005-2022. China CDC Wkly. 2023 Nov 3;5(44):978-983. doi: 10.46234/ccdcw2023.184. PMID: 38023392; PMCID: PMC10652091.
4. Dolk H, Loane M, Garne E. The prevalence of congenital anomalies in Europe. Adv Exp Med Biol. 2010;686:349-64. doi: 10.1007/978-90-481-9485-8_20. PMID: 20824455.
5. Bergman, J. E. H., Perraud, A., Barišić, I., Kinsner-Ovaskainen, A., Morris, J. K., Tucker, D., Wellesley, D., & Garne, E. (2024). Updated EUROCAT guidelines for classification of cases with congenital anomalies. Birth Defects Research, 116(2), e2314. https://doi.org/10.1002/bdr2.2314
6. Passarge, E. Origins of human genetics. A personal perspective. Eur J Hum Genet 29, 1038–1044 (2021). https://doi.org/10.1038/s41431-020-00785-7
7. Steele MW, Breg WR Jr. Chromosome analysis of human amniotic-fluid cells. Lancet. 1966 Feb 19;1(7434):383-5. doi: 10.1016/s0140-6736(66)91387-0. PMID: 4159775.
8. Available online: https://www.genome.gov/human-genome-project/timeline (accessed on 29.04.2025)
9. Hashizume R, Wakita S, Sawada H, Takebayashi SI, Kitabatake Y, Miyagawa Y, Hirokawa YS, Imai H, Kurahashi H. Trisomic rescue via allele-specific multiple chromosome cleavage using CRISPR-Cas9 in trisomy 21 cells. PNAS Nexus. 2025 Feb 18;4(2):pgaf022. doi: 10.1093/pnasnexus/pgaf022. PMID: 39967679; PMCID: PMC11832276.
10. Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. Curr Genomics. 2010 Nov;11(7):537-61. doi: 10.2174/138920210793175895. PMID: 21532838; PMCID: PMC3048316.
11. **Sabau ID**, Bohiltea LC, Varlas V, et al. The evolution of prenatal Whole Exome Sequencing: from cytogenetics to precision medicine. Arch Clin Cases. 2025;12(2):80-89. doi: 10.22551/2025.47.1202.10318
12. Gregory M, Burton V, Shapiro B. Developmental disabilities and metabolic disorders. In: Zigmond MJ, Rowland LP, Coyle JT, editors. Neurobiology of brain disorders. San Diego: Academic Press; 2015

13. Löwy I. How genetics came to the unborn: 1960-2000.Stud Hist Philos Biol Biomed Sci. 2014 Sep;47 Pt A:154-62. PMID: 24968964. doi:10.1016/j.shpsc.2014.05.015.

14. Brock DJ, Sutcliffe RG. Alpha-fetoprotein in the antenatal diagnosis of anencephaly and spina bifida. Lancet. 1972 Jul 29;2(7770):197-9.PMID: 4114207. doi: 10.1016/S0140-6736(72)91634-0.

15. Lorber J, Stewart CR, Ward AM. Alpha-fetoprotein in antenatal diagnosis of anencephaly and spina bifida. Lancet. 1973 May 26;1(7813):1187. PMID: 4123575. doi: 10.1016/S0140-6736(73)91190-2.

16. Press N, Browner CH. Why women say yes to prenatal diagnosis. Soc Sci Med. 1997 Oct;45(7):979-89. PMID: 9257391. doi: 10.1016/S0277-9536(97)00011-7

17. Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. Fertil Steril. 2018 Feb;109(2):201-12. PMID: 29447663; PMCID: PMC5856154. doi: 10.1016/j.fertnstert.2018.01.005.

18. Hillman SC, McMullan DJ, Hall G, et al. Use of prenatal chromosomal microarray: prospective cohort study and systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2013 May;41(6):610-20.doi: 10.1002/uog.12464.

19. Bilgüvar K, Oztürk AK, Louvi A, et al. Whole-exome sequencing identifies recessive WDR62 mutations in severe brain malformations. Nature. 2010 Sep 9;467(7312):207-10. doi: 10.1038/nature09327

20. Goh G, Choi M. Application of whole exome sequencing to identify disease-causing variants in inherited human diseases. Genomics Inform. 2012 Dec;10(4):214-9. PMID: 23346032; PMCID: PMC3543920.doi: 10.5808/GI.2012.10.4.214.

21. Best S, Wou K, Vora N, et al. Promises, pitfalls and practicalities of prenatal whole exome sequencing. Prenat Diagn. 2018 Jan;38(1):10-9.PMID: 28654730; PMCID: PMC5745303. doi: 10.1002/pd.5102

22. Talkowski ME, Rehm HL. Introduction of genomics into prenatal diagnostics. Lancet. 2019 Feb 23;393(10173):719-21. PMID: 26826164;PMCID: PMC4847612. doi: 10.1016/S0140-6736(19)30193-X.

23. Qiao Y, Wen J, Tang F, et al. Whole exome sequencing in recurrent early pregnancy loss. Mol Hum Reprod. 2016 May;22(5):364-72. PMID:9257391. doi: 10.1093/molehr/gaw008.

24. Carss KJ, Hillman SC, Parthiban V, et al. Exome sequencing improves genetic diagnosis of structural fetal abnormalities revealed by ultrasound. Hum Mol Genet. 2014 Jun 15;23(12):3269-77. doi:10.1093/hmg/ddu038.

25. Pereira R, Oliveira J, Sousa M. Bioinformatics and computational tools for next-generation sequencing analysis in clinical genetics. J Clin Med. 2020 Jan 3;9(1):132. PMID: 31947757; PMCID:PMC7019349. doi: 10.3390/jcm9010132.

26. Wang Y, Yin F, Chai Y, et al. Prenatal diagnosis of fetuses with ultrasound anomalies by whole-exome sequencing in Luoyang city, China. Front Genet. 2024 Jan 22;14:1301439. PMID: 38318287; PMCID:PMC10838985. doi: 10.3389/fgene.2023.1301439.

27. Qin Y, Yao Y, Liu N, et al. Prenatal whole-exome sequencing for fetal structural anomalies: a retrospective analysis of 145 Chinese cases. BMC Med Genomics. 2023 Oct 25;16(1):262. PMID: 37880672; PMCID:PMC10601195. doi: 10.1186/s12920-023-01697-3.

28. Lei L, Zhou L, Xiong JJ. Whole-exome sequencing increases the diagnostic rate for prenatal fetal structural anomalies. Eur J MedGenet. 2021 Sep;64(9):104288. doi: 10.1016/j.ejmg.2021.104288.

29. Stanley KE, Giordano J, Thorsten V, et al. Causal genetic variants in stillbirth. N Engl J Med. 2020 Sep 17;383(12):1107-16. PMID: 32786180;PMCID: PMC7604888. doi: 10.1056/NEJMoa1908753.

30. Diderich KEM, Klapwijk JE, van der Schoot V, et al. Challenges and pragmatic solutions in pre-test and post-test genetic counseling for prenatal exome sequencing. Appl Clin Genet. 2023 May 15;16:89-97.PMID: 37216148; PMCID: PMC10198275. doi: 10.2147/TACG.S411185.

31. Van Der Merwe N, Ramesar R, De Vries J. Whole exome sequencing in South Africa: stakeholder views on return of individual research results and incidental findings. Front Genet. 2022 Jun 8;13:864822.PMID: 35754817; PMCID: PMC9216214. doi: 10.3389/fgene.2022.864822.

32. Liu S, Yang F, Chang Q, et al. Positive predictive value estimates for noninvasive prenatal testing from data of a prenatal diagnosis laboratory and literature review. Mol Cytogenet. 2022 Jul 6;15(1):29.PMID: 35794576; PMCID: PMC9261060. doi: 10.1186/s13039-022-00607-z.

33. Radoi VE, Bohiltea CL, Bohiltea RE, et al. Cell free fetal DNA testing in maternal blood of Romanian pregnant women. Iran J Reprod Med.2015 Oct;13(10):623-6. PMID: 26644790; PMCID: PMC4668349.

34. Drury S, Hill M, Chitty LS. Cell-free fetal DNA testing for prenatal diagnosis. Adv Clin Chem. 2016;76:1-35. doi: 10.1016/bs.acc.2016.05.004

35. Brand H, Whelan CW, Duyzend M, et al. High-resolution and non-invasive fetal exome screening. N Engl J Med. 2023 Nov 23;389(21):2014-6. PMID: 37991862; PMCID: PMC10711678. doi: 10.1056/NEJMc2216144

36. Setty ST, Scott-Boyer MP, Cuppens T, et al. New developments and possibilities in reanalysis and reinterpretation of whole exome sequencing datasets for unsolved rare diseases using machine learning approaches. IntJMolSci. 2022 Jun 18;23(12):6792. PMID: 35743235; PMCID: PMC9224427. doi: 10.3390/ijms23126792

37. Ji X, Li Q, Qi Y, et al. When NIPT meets WES, prenatal diagnosticians face the dilemma: genetic etiological analysis of 2,328 cases of NT thickening and follow-up of pregnancy outcomes. Front Genet. 2023Aug 2;14:1227724. PMID: 37600658; PMCID: PMC10433188. doi:10.3389/fgene.2023.1227724

38. Qi Q, Jiang Y, Zhou X, et al. Simultaneous detection of CNVs and SNVs improves the diagnostic yield of fetuses with ultrasound anomalies and normal karyotypes. Genes (Basel). 2020 Nov 25;11(12):1397. doi: 10.3390/genes11121397

39. Dempsey E, Haworth A, Ive L, et al. A report on the impact of rapid prenatal exome sequencing on the clinical management of 52 ongoing pregnancies: a retrospective review. BJOG. 2021 May;128(6):1012-9. doi: 10.1111/1471-0528.16546

40. Chen M, Chen J, Wang C, et al. Clinical application of medical exome sequencing for prenatal diagnosis of fetal structural anomalies. Eur J Obstet Gynecol Reprod Biol. 2020 Mar;251:119-24. doi:10.1016/j.ejogrb.2020.04.033

41. Becher N, Andreasen L, Sandager P, et al. Implementation of exome sequencing in fetal diagnostics: data and experiences from a tertiary center in Denmark. Acta Obstet Gynecol Scand. 2020 Jul;99(6):783-90.doi: 10.1111/aogs.13871

42. Petrovski S, Aggarwal V, Giordano JL, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet. 2019 Feb 23;393(10173):758-67. doi: 10.1016/S0140-6736(18)32042-7

43. Lord J, McMullan DJ, Eberhardt RY, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet. 2019 Feb 23;393(10173):747-57. doi:10.1016/S0140-6736(18)31940-8

44. Smith EJ, Hill M, Peter M, et al. Implementation of a national prenatal exome sequencing service in England: cost-effectiveness analysis. BJOG. 2025 Mar;132(4):483-91. PMID: 39572407; PMCID:PMC11794059. doi: 10.1111/1471-0528.18020

45. Deden C, Neveling K, Zafeiropopoulou D, et al. Rapid whole exome sequencing in pregnancies to identify the underlying genetic cause in fetuses with congenital anomalies detected by ultrasound imaging. Prenat Diagn. 2020 Jul;40(8):972-83. PMID: 32333414; PMCID:PMC7497059. doi: 10.1002/pd.5717

46. PFMG 2025–Plan France Médecine Génomique. Accessed online at:https://pfmg2025.fr/ (accessed 14.05.2025)

47. **Săbău, I.-D**.; Bohîlțea, L.-C.; Țurcan, M., Comprehensive Prenatal Genetic Analysis: From Non-Invasive Prenatal Testing to Whole-Exome Sequencing in a High-Risk Pregnancy with Gaucher Disease—A Case Report and Literature Review. *J. Mind Med. Sci.* **2025**, *12*, 25. https://doi.org/10.3390/jmms12010025

48. Dardis, A.; Michelakakis, H.; Rozenfeld, P.; Fumic, K.; Wagner, J.; Pavan, E.; Fuller, M.; Revel-Vilk, S.; Hughes, D.; Cox, T.; et al. Patient centered guidelines for the laboratory diagnosis of Gaucher disease type 1. *Orphanet J. Rare Dis.* **2022**, *17*, 442

49. Available online: **https://www.ncbi.nlm.nih.gov/books/NBK448080/** (accessed on 10 April 2025).

50. Rossi, C.; Ferrante, R.; Valentinuzzi, S.; Zucchelli, M.; Buccolini, C.; Di Rado, S.; Trotta, D.; Stuppia, L.; Federici, L.; Aricò, M. Noninvasive DBS-Based Approaches to Assist Clinical Diagnosis and Treatment Monitoring of Gaucher Disease. *Biomedicines* **2023**, *11*, 2672

51. Koumbaris, G.; Kypri, E.; Tsangaras, K.; Achilleos, A.; Mina, P.; Neofytou, M.; Velissariou, V.; Christopoulou, G.; Kallikas, I.; González-Liñán, A.; et al. Cell-free DNA analysis of targeted genomic regions in maternal plasma for non-invasive prenatal testing of trisomy 21, trisomy 18, trisomy 13, and fetal sex. *Clin. Chem.* **2016**, *62*, 848–855.

52. Stokowski, R.; Wang, E.; White, K.; Batey, A.; Jacobsson, B.; Brar, H.; Balanarasimha, M.; Hollemon, D.; Sparks, A.; Nicolaides, K.; et al. Clinical performance of non-invasive prenatal testing (NIPT) using targeted cell-free DNA analysis in maternal plasma with microarrays or next generation sequencing (NGS) is consistent across multiple controlled clinical studies. *Prenat. Diagn.* **2015**, *35*, 1243–1246

53. Struble, C.A.; Syngelaki, A.; Oliphant, A.; Song, K.; Nicolaides, K.H. Fetal fraction estimate in twin pregnancies using directed cell-free DNA analysis. *Fetal Diagn. Ther*. **2014**, *35*, 199–203

54. Kypri, E.; Ioannides, M.; Touvana, E.; Neophytou, I.; Mina, P.; Velissariou, V.; Vittas, S.; Santana, A.; Alexidis, F.; Tsangaras, K.; et al. Non-invasive prenatal testing of fetal chromosomal aneuploidies: Validation and clinical performance of the veracity test. *Mol. Cytogenet.* **2019**, *12*, 34

55. Koumbaris, G.; Achilleos, A.; Nicolaou, M.; Loizides, C.; Tsangaras, K.; Kypri, E.; Mina, P.; Sismani, C.; Velissariou, V.; Christopoulou, G.; et al. Targeted capture enrichment followed by NGS: Development and validation of a single comprehensive NIPT for chromosomal aneuploidies, microdeletion syndromes and monogenic diseases. *Mol. Cytogenet.* **2019**, *12*, 48

56. Kypri, E.; Ioannides, M.; Achilleos, A.; Koumbaris, G.; Patsalis, P.; Stumm, M. Non-invasive prenatal screening tests—Update 2022. *J. Lab. Med.* **2022**, *46*, 311–320

57. Neofytou, M.C.; Tsangaras, K.; Kypri, E.; Loizides, C.; Ioannides, M.; Achilleos, A.; Mina, P.; Keravnou, A.; Sismani, C.; Koumbaris, G.; et al. Targeted capture enrichment assay for non-invasive prenatal testing of large and small size sub-chromosomal deletions and duplications. *PLoS ONE* **2017**, *12*, e0171319

58. Wang, E.; Batey, A.; Struble, C.; Musci, T.; Song, K.; Oliphant, A. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. *Prenat. Diagn.* **2013**, *33*, 662–666

59. Palomaki, G.E.; Kloza, E.M. Prenatal cell-free DNA Screening Test Failures: A Systematic Review of Failure Rates, Risks of Down Syndrome, and Impact of Repeat Testing. *Anesthesia Analg.* **2018**, *20*,1312–1323. Available online: **http://www.nature.com/doifinder/10.1038/gim.2018.22** (accessed on 27 May 2024).

60. Richards, S.; Aziz, N.; Bale, S.; Bick, D.; Das, S.; Gastier-Foster, J.; Grody, W.W.; Hegde, M.; Lyon, E.; Spector, E.; et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* **2015**, *17*, 405–424

61. Karczewski, K.; Francioli, L.; Tiao, G. ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020. *bioRxiv* **2019**, 531210.

62. Liehr, T.; Lauten, A.; Schneider, U.; Schleussner, E.; Weise, A. Noninvasive Prenatal Testing—When Is It Advantageous to Apply. *Biomed. Hub* **2017**, *2*, 1–11

63. Jayashankar, S.S.; Nasaruddin, M.L.; Hassan, M.F.; Dasrilsyah, R.A.; Shafiee, M.N.; Ismail, N.A.; Alias, E. Non-invasive prenatal testing (NIPT): Reliability, challenges, and future directions. *Diagnostics* **2023**, *13*, 2570

64. Eltabbakh, N.; Mohasin, Y.; Jeddy, R. Advancements of non-invasive prenatal testing: The role of obstetricians. *Front. Med.* **2024**

65. Satam, H.; Joshi, K.; Mangrolia, U.; Waghoo, S.; Zaidi, G.; Rawool, S.; Thakare, R.P.; Banday, S.; Mishra, A.K.; Das, G.; et al. Next-generation sequencing technology: Current trends and advancements. *Biology* **2023**, *12*, 997

66. Gullo, G.; Scaglione, M.; Buzzaccarini, G.; Laganà, A.S.; Basile, G.; Chiantera, V.; Cucinella, G.; Zaami, S. Cell-Free Fetal DNA and Non-Invasive Prenatal Diagnosis of Chromosomopathies and Pediatric Monogenic Diseases: A Critical Appraisal and Medicolegal Remarks. *J. Pers. Med.* **2022**, *13*, 1

67. Spinillo, S.L.; Farina, A.; Sotiriadis, A.; Pozzoni, M.; Giglio, S.; Papale, M.; Candiani, M.; Cavoretto, P.I. Pregnancy outcome of confined placental mosaicism: Meta-analysis of cohort studies. *Am. J. Obstet. Gynecol.* **2022**, *227*, 714–727.e1

68. Grati, F.R.; Bajaj, K.; Zanatta, V.; Malvestiti, F.; Malvestiti, B.; Marcato, L.; Grimi, B.; Maggi, F.; Simoni, G.; Gross, S.J.; et al. Implications of fetoplacental mosaicism on cell-free DNA testing for sex chromosome aneuploidies. *Prenat. Diagn.* **2017**, *37*, 1017–1027

69. Snyder, M.W.; Simmons, L.E.; Kitzman, J.O.; Coe, B.P.; Henson, J.M.; Daza, R.M.; Eichler, E.E.; Shendure, J.; Gammill, H.S. Copy-Number Variation and False Positive Prenatal Aneuploidy Screening Results. *N. Engl. J. Med.* **2015**, *372*, 1639–1645. Available online: **http://www.nejm.org/doi/10.1056/NEJMoa1408408** (accessed on 10 April 2025)

70. Grati, F. Chromosomal Mosaicism in Human Feto-Placental Development: Implications for Prenatal Diagnosis. *J. Clin. Med.* **2014**, *3*, 809–837

71. Gil, M.M.; Quezada, M.S.; Revello, R.; Akolekar, R.; Nicolaides, K.H.; To, C. analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: Updated meta-analysis. *Ultrasound Obstet. Gynecol.* **2015**, *45*, 249–266

72. Brisset, S.; Aboura, A.; Audibert, F.; Costa, J.M.; L'Herminé, A.C.; Gautier, V.; Frydman, R.; Tachdjian, G. Discordant prenatal diagnosis of trisomy 21 due to mosaic structural rearrangements of chromosome 21. *Prenat. Diagn.* **2003**, *23*, 461–469

73. Gregg, A.R.; Skotko, B.G.; Benkendorf, J.L.; Monaghan, K.G.; Bajaj, K.; Best, R.G.; Klugman, S.; Watson, M.S. Noninvasive prenatal Screening for Fetal Aneuploidy, 2016 Update: A Position Statement of the American College of Medical Genetics and Genomics. *Genet. Med.* **2016**, *18*, 1056–1065. Available online: **http://www.nature.com/doifinder/10.1038/gim.2016.97**

74. Guo, Y.; Charoenkwan, P.; Traisrisilp, K.; Piyamongkol, W.; Tongprasert, F. Application of Digital Polymerase Chain Reaction (dPCR) in Non-Invasive Prenatal Testing (NIPT). *Biomolecules* **2025**, *15*, 360.

75. Yang, Y.; Muzny, D.M.; Xia, F.; Niu, Z.; Person, R.; Ding, Y.; Ward, P.; Braxton, A.; Wang, M.; Buhay, C.; et al. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA* **2014**, *312*, 1870–1879.

76. Rabinowitz, T.; Shomron, N. Genome-wide noninvasive prenatal diagnosis of monogenic disorders: Current and future trends. *Comput. Struct. Biotechnol. J.* **2020**, *18*, 2463–2470

77. Snyder, M.W.; Simmons, L.E.; Kitzman, J.O.; Santillan, D.A.; Santillan, M.K.; Gammill, H.S.; Shendure, J. Noninvasive fetal genome sequencing: A primer. *Prenat. Diagn.* **2013**, *33*, 547–554.

78. ACOG. Cell-Free DNA Screening for Fetal Aneuploidy. *Obstet. Gynecol.* **2015**, *126*, e31–e37.

79. Schmitz, D.; Henn, W. The fetus in the age of the genome. *Hum. Genet.* **2022**, *141*, 1017–1026

80. Palanki, R.; Peranteau, W.H.; Mitchell, M.J. Delivery technologies for in utero gene therapy. *Adv. Drug Deliv. Rev.* **2021**, *169*, 51–62

81. **Săbău, I.-D**.; Bohîltea, L.-C.; Rădoi, V.E.; The Importance of Prenatal Whole-Exome Sequencing Testing in the Romanian Population. *J. Mind Med. Sci.* **2025**, *12*, 7. https://doi.org/10.3390/jmms12010007

82. Syngelaki, A.; Hammami, A.; Bower, S.; Zidere, V.; Akolekar, R.; Nicolaides, K.H. Diagnosis of fetal non-chromosomal abnormalities on routine ultrasound examination at 11–13 weeks' gestation. Ultrasound Obstet. Gynecol. 2019, 54, 468–476

83. Wapner, R.J.; Martin, C.L.; Levy, B.; Ballif, B.C.; Eng, C.M.; Zachary, J.M.; Savage, M.; Platt, L.D.; Saltzman, D.; Grobman, W.A.; et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N. Engl. J. Med. 2012, 367, 2175–2184

84. Callaway, J.L.A.; Shaffer, L.G.; Chitty, L.S.; Rosenfeld, J.A.; Crolla, J.A. The clinical utility of microarray technologies applied to prenatal cytogenetics in the presence of a normal conventional karyotype: A review of the literature. Prenat. Diagn. 2013, 33, 1119–1123

85. Srivastava, S.; Love-Nichols, J.A.; Dies, K.A.; Ledbetter, D.H.; Martin, C.L.; Chung, W.K.; Firth, H.V.; Frazier, T.; Hansen, R.L.; Prock, L. Meta-analysis and multidisciplinary consensus statement: Exome sequencing is a first- tier clinical diagnostic test for individuals with neurodevelopmental disorders. Genet. Med. 2019, 21, 2413–2421

86. Clark, M.M.; Stark, Z.; Farnaes, L.; Tan, T.Y.; White, S.M.; Dimmock, D.; Kingsmore, S.F. Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. NPJ Genom. Med. 2018, 3, 16

87. Chandler, N.; Best, S.; Hayward, J.; Faravelli, F.; Mansour, S.; Kivuva, E.; Tapon, D.; Male, A.; DeVile, C.; Chitty, L.S. Rapid prenatal diagnosis using targeted exome sequencing: A cohort study to assess feasibility and potential impact on prenatal counseling and pregnancy management. Genet. Med. 2018, 20, 1430–1437

88. Normand, E.A.; Braxton, A.; Nassef, S.; Ward, P.A.; Vetrini, F.; He, W.; Patel, V.; Qu, C.; Westerfield, L.E.; Stover, S.; et al. Clinical exome sequencing for fetuses with ultrasound abnormalities and a suspected Mendelian disorder. Genome Med ,10, 74.

89. Mone, F.; McMullan, D.J.; Williams, D.; Chitty, L.S.; Maher, E.R.; Kilby, M.D. Fetal Genomics Steering Group of the British Society for Genetic Medicine and Royal College of Obstetricians and Gynaecologists. Evidence to support the clinical utility of prenatal exome sequencing in evaluation of the fetus with congenital anomalies. BJOG Int. J. Obstet. Gynaecol. 2021, 128, e39–e50

90. ISPD; SMFM; PQF. Joint Position Statement from the International Society of Prenatal Diagnosis (ISPD), the Society of Maternal Fetal Medicine (SMFM) and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. Prenat. Diagn. 2018, 38, 6–9

91. Monaghan, K.G.; Leach, N.T.; Pekarek, D.; Prasad, P.; Rose, N.C. ACMG Professional Practice and Guidelines Committee The use of fetal exome sequencing in prenatal diagnosis: A points to consider document of the American College of Medical Genetics and Genomics (ACMG). Genet. Med. 2020, 22, 675–680

92. AlDubayan, S.H.; Conway, J.R.; Camp, S.Y.; Witkowski, L.; Kofman, E.; Reardon, B.; Han, S.; Moore, N.; Elmarakeby, H.; Salari, K.; et al. Detection of Pathogenic Variants With Germline Genetic Testing Using Deep Learning vs Standard Methods in Patients With Prostate Cancer and Melanoma. JAMA 2020, 324, 1957–1969

93. Mellis, R.; Oprych, K.; Scotchman, E.; Hill, M.; Chitty, L.S. Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: A systematic review and meta-analysis. Prenat. Diagn. 2022, 42, 662–685

94. Miceikaite, I.; Fagerberg, C.; Brasch-Andersen, C.; Torring, P.M.; Kristiansen, B.S.; Hao, Q.; Sperling, L.; Ibsen, M.H.; Löser, K.; Bendsen, E.A.; et al. Comprehensive prenatal diagnostics: Exome versus genome sequencing. Prenat. Diagn. 2023, 43, 1132–1141.

Lista lucrarilor stiintifice publicate

1. **Sabau ID**, Bohiltea LC, Varlas V, et al. The evolution of prenatal Whole Exome Sequencing: from cytogenetics to precision medicine. Arch Clin Cases. 2025;12(2):80-89. doi: 10.22551/2025.47.1202.10318

   https://www.clinicalcases.eu/index.php/acc/article/view/1175/352

   This article is found in Chapter 4 (pages 30-60)

2. **Săbău, I.-D**.; Bohîlțea, L.-C.; Țurcan, M., Comprehensive Prenatal Genetic Analysis: From Non-Invasive Prenatal Testing to Whole-Exome Sequencing in a High-Risk Pregnancy with Gaucher Disease—A Case Report and Literature Review. *J. Mind Med. Sci.* **2025**, *12*, 25. https://doi.org/10.3390/jmms12010025

   https://www.mdpi.com/2392-7674/12/1/25

   This article is found in Chapter 5 (pages 61-77)

3. **Săbău, I.-D**.; Bohîltea, L.-C.; Rădoi, V.E.; The Importance of Prenatal Whole-Exome Sequencing Testing in the Romanian Population. *J. Mind Med. Sci.* **2025**, *12*, 7. https://doi.org/10.3390/jmms12010007

   https://www.mdpi.com/2392-7674/12/1/7

   This article is found in Chapter 6 (pages 78-96)