TIBBİ GENETİK DEĞERLENDİRMELERİ

Editör: Doç.Dr. Meryem Şenay ŞENGÜL DEMİRAK

yaz
yayınları

Tıbbi Genetik Değerlendirmeleri

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yaz yayınları

2025

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E_ISBN 978-625-8508-15-4

Ekim 2025 – Afyonkarahisar

Dizgi/Mizanpaj: YAZ Yayınları

Kapak Tasarım: YAZ Yayınları

YAZ Yayınları. Yayıncı Sertifika No: 73086

M.İhtisas OSB Mah. 4A Cad. No:3/3
İscehisar/AFYONKARAHİSAR

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İÇİNDEKİLER

The Role of miR-21 In Cancer Drug Resistance: Mechanisms and Translational Opportunities.....1
Murat KAYA

The Role of Next-Generation Sequencing in the Diagnosis of Rare Genetic Diseases 17
Tuğçe YAŞAR KÜÇÜK, Fümet Duygu ÜSTÜNDAĞ

THE ROLE OF miR-21 IN CANCER DRUG RESISTANCE: MECHANISMS AND TRANSLATIONAL OPPORTUNITIES

Murat KAYA[1]

1. INTRODUCTION

Drugs resistance may be the strongest obstacle in modern oncology, resulting in metastasis, relapse, and ultimately death (Javanmardi et al., 2016). The development of therapeutic resistance is a complex phenomenon involving genetic mutations, epigenetic modifications, and intricate interactions with the tumor microenvironment (Lei et al., 2023). miR-21 is recognized as a crucial oncogenic microRNA (oncomiR) and a key regulator of therapy resistance in cancer (Javanmardi et al., 2016). Its overexpression is strongly associated with intrinsic and acquired resistance to chemotherapy, radiotherapy, targeted therapy, and emerging immunotherapies in a wide range of malignancies, such as lung, stomach, pancreas, hepatocellular, and glioblastoma (Gupta et al., 2024). Mechanistically, miR-21 acts by regulating cell survival through the pan-repression of tumor suppressor PTEN, PDCD4, TIMP3, and SPRY2 to liberate oncogenic pathways such as PI3K/AKT/mTOR and MEK/ERK (Javanmardi et al., 2016). Besides traditional targets, miR-21 is directly involved with metabolic reprogramming and modulation of the immunosuppressive tumor microenvironment. Regulatory complexity is also supplemented by non-coding RNA axes,

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including the LncRNA PTENP1/miR-21/PTEN axis that controls Epithelial-Mesenchymal Transition (EMT) and DNA damage response (Gupta et al., 2024). Notably, intercellular miR-21 transfer by extracellular vesicles (EVs), i.e., within aggressive metastatic niches like cerebrospinal fluid (CSF), provides a mechanism of systemic drug resistance transmission (Im et al., 2024).

One of the most significant non-coding regulators, miR-21, is now targeted because of its high and universal expression in almost all human cancer types. It is referred to as being among the oldest and most important oncomiRs, with its activity maintained in numerous cancers, hence acting as a "hub" for non-discriminatory pro-survival and anti-apoptotic signals (Najjary et al., 2020). Whereas earlier studies focused on its direct targets, recent studies narrate miR-21's involvement in broader cell processes such as metabolic adaptation, autophagy modulation (Gu et al., 2020), and immunomodulation (Zhao et al., 2021). The sophisticated regulatory network is extended to complicated non-coding RNA networks (Gupta et al., 2024) and robust, long-range signal mechanisms by means of extracellular vesicles (Im et al., 2024). This chapter integrates new findings about miR-21 drug resistance relation, and it highlights novel treatment strategies, including aptamer-mediated delivery platforms and combination regimens with immune checkpoint inhibitors, to advance miR-21 blockade to a robust clinical strategy for overcoming drug resistance.

2. CENTRAL MOLECULAR MECHANISMS OF miR-21-MEDIATED RESISTANCE

2.1. Canonical Target Repression and Signaling Cascade Activation

miR-21 plays a role in the simultaneous repression of many tumor suppressor mRNAs. Downregulation of Phosphatase and Tensin Homolog (PTEN) is one of the best-characterized pathways (Javanmardi et al., 2016). Silencing of PTEN by miR-21 leads to hyperactivation of the pro-survival and anti-apoptotic PI3K/AKT/mTOR pathway, which mediates drug resistance such as cisplatin in lung cancer (Liang et al., 2021). Downregulation of Programmed Cell Death 4 (PDCD4), a tumor suppressor with pro-apoptotic and inhibitory translational activity, by miR-21 results in increased protein synthesis and decreased cell death, underpinning the resistant phenotype (Sun et al., 2020). miR-21-mediated downregulation of TGF-β/SMAD targets is responsible for maintaining stem cell existence and inducing resistance to Cisplatin in gastric cancer (Nie et al., 2024).

2.2. DNA Damage Response and Non-Coding RNA Axes of Regulation

Other non-coding RNAs predominantly regulate miR-21 function as a ceRNA. Long non-coding RNA PTENP1 is an important molecular sponge that sequesters miR-21, thereby re-establishing the expression of PTEN (Gupta et al., 2024). This elaborate process is pivotal in terms of regulating the Epithelial-Mesenchymal Transition (EMT) phenotype along with cellular DNA-damage drug response (Gupta et al., 2024). circRNA-001241 mediates Sorafenib resistance in hepatocellular carcinoma by sequestering miR-21-5p to regulate TIMP3 expression (Yang & Wu, 2022).

2.3. Metabolic Reprogramming

miR-21 regulates metabolic reprogramming that is termed as Warburg effect through HIF-1α nuclear translocation through repression of PTEN (Deng et al., 2024).

2.4. Regulation of Autophagy

In gastric cancer, miR-21 suppresses survival autophagy through the PI3K/Akt/mTOR signaling pathway and potentiates cellular dependence on additional survival mechanisms and modulation of resistance to Cisplatin (Gu et al., 2020).

3. miR-21 AND TUMOR MICROENVIRONMENT

3.1. Intercellular Communication through miR-21

Exosomes play a role in cell-to-cell transfer of miR-21 as a mechanism of horizontal transmission of drug resistance. EVs released by Leptomeningeal Metastasis (LMM) of the Cerebrospinal Fluid (CSF) transduce lung cancer cells to make them methotrexate-resistant through delivering high amounts of miR-21 (Im et al., 2024). Exosomes from CAFs transfer miR-21 to induce STAT3 signaling in monocytic Myeloid-Derived Suppressor Cells (MDSCs) and therefore associate drug resistance to localized immune suppression (Zhao et al., 2021).

3.2. Hypoxia, Stemness, and Immunomodulation

CAFs promote hypoxia-induced stemness and Gemcitabine resistance in PDAC cells through exosomal transfer of HIF-1α/miR-21 axis activators (Deng et al., 2024). MiR-21 inhibits PTEN, PI3K/AKT downstream targets polarizing TAMs towards the immunosuppressive M2 phenotype, which further promotes immune evasion (Najjary et al., 2020).

4. THERAPY RESISTANCE IN CERTAIN CANCERS

The oncogenic potential of miR-21 has been largely confirmed in the oncology field and is not associated with treatment failure through a single mechanism, but rather through highly complex, context-dependent molecular pathways that vary according to the specific cancer type and associated microenvironmental stresses (Figure 1 and Table 1).

4.1. Lung Cancer (NSCLC): Navigating Diverse Resistance Mechanisms

Non-Small Cell Lung Cancer (NSCLC) is a system model in which multi-modal resistance is induced by miR-21 to repress cytotoxic chemotherapy as well as molecularly targeted therapy. Drug resistance to agents such as Cisplatin is caused by a down-regulation of the tumor suppressor PTEN by miR-21, to consequently slow dephosphorylation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Due to this molecular mechanism, sustained activation of survival-promoting PI3K/AKT signaling cascade follows to enhance cell protection from drug-induced DNA damage and apoptosis (Liang et al., 2021). Furthermore, severe hypoxic tumor mass environment stimulates tumor cells to secrete extracellular vesicles packed with miR-21 to paracrinely convey resistance factors to normoxic cells in close proximity and elicit a global, generalizable resistant phenotype to the tumor microenvironment (Dong et al., 2019). Among all common mutations linked to EGFR, resistance to third-generation TKIs like Osimertinib is most often found to be connected to activation of the MEK/ERK/miR-21 regulatory cascade (Huang et al., 2021). Another mode of activation through the Sonic Hedgehog (SHH)/PI3K/AKT pathway has been documented as a leading driver of initial resistance developing against first-generation EGFR-TKIs and so far has made miR-21 an important upstream

regulatory node (Xu et al., 2022). Leptomeningeal Metastasis (LMM) is among the most lethal clinical manifestations of NSCLC, whereby resistance to CNS-permeating agents is ubiquitous. Recent studies have confirmed that EVs from LMM patients' CSF are efficient, systemic delivery vehicles of high-dose miR-21 that directly induce methotrexate resistance in recipient cancer cells through a mechanism of transmission of resistance across biological barriers (Im et al., 2024).

4.2. Gastrointestinal Cancers: Leveraging Stemness, Tumor Microenvironment, and Post-Translational Modifications

The intrinsic cisplatin resistance of Gastric carcinoma (GC) in the CD44+ CSC population is exceedingly high. Overexpression of miR-21 in the population maintains the cancer stem-like cell phenotype by controlling the TGF-β2/SMAD pathway, which is a key cell differentiation and stemness regulator, to evade apoptotic signals (Nie et al., 2024). Therapeutic resistance is caused in part by miR-21 by inhibiting the cell survival mechanism of autophagy effectively. This is done through sustained activation of the PI3K/Akt/mTOR signaling pathway that induces a dependence of the GC cells on additional pro-survival mechanisms and lowers the threshold of resistance to Cisplatin (Gu et al., 2020). 5-FU resistance that is central to the FOLFOX chemotherapy regimen against colorectal cancer (CRC) is markedly improved through exosome-based dissemination of miR-21 from tumor as well as stromal cells. This mode of intercellular communication facilitates efficient silencing of the tumor suppressive gene PDCD4 in recipient cells that regulates translation control as well as cell death induction and thereby encourages long-term survival and growth subsequent to chemotherapy (Sun et al., 2020). Resistance to the multikinase inhibitor Sorafenib is highly linked to a number of miR-21-dependent pathways. These include a non-coding RNA-

based regulatory network in which the circular RNA circRNA-001241 is a competitive endogenous RNA (ceRNA) that interacts with miR-21-5p; upon disruption of this interaction, TIMP3 is downregulated and a resistance phenotype is acquired (Yang & Wu, 2022). Additionally, a different post-translational form of regulation is that of miR-21-5p affecting SIRT7 (Sirtuin 7) stability by virtue of its regulation of the deubiquitinase USP24 and consequently pointing to a pivotal role of miR-21 in terms of protein degradation pathway modulation that leads to resistance (Hu et al., 2023). Severe resistance of Pancreatic Ductal Adenocarcinoma (PDAC) to Gemcitabine is mainly due to the dense tumor microenvironment that is highly hypoxic. Under this niche, Cancer-Associated Fibroblasts (CAFs) employ exosomes to convey resistance signals: under hypoxic circumstances, HIF-1α/miR-21 pathway is triggered in CAFs and results in exosome-based delivery of resistance factors driving an improved stem cell phenotype as well as Gemcitabine evading PDAC cells (Deng et al., 2024).

4.3. Central Nervous System Tumors: Glioblastoma (GBM)

Glioblastoma's strong aggressiveness and resistance to the traditional alkylating agent Temozolomide (TMZ) are substantially driven by miR-21. Increased expression of miR-21 directly impairs PTEN, causing dysregulation of cellular DNA damage surveillance machinery. This subsequently activates an uninterrupted signaling of the PI3K/AKT pathway, which effectively bypasses normal apoptotic processes and allows GBM cells to efficiently repair and resist chemotherapy-induced DNA damage and thereby allow their unbridled proliferation (Javanmardi et al., 2016).

4.4. Hematological malignancies: Apoptotic threshold

Resistance to BCL-2 family antagonists like Venetoclax and S63845 that is observed in Multiple Myeloma is to a great extent defined by interactions that occur in the bone marrow microenvironment. Signaling caused by stroma leads to dysregulation of miR-21 that efficiently changes the apoptotic threshold by shifting the stoichiometric ratio of anti-apoptotic proteins, particularly MCL-1 and BCL-2. This adjustment of drug tolerance maintains a barrier against personalized apoptosis (Algarin et al., 2021).

4.5. Immunomodulation and DNA Repair in Breast, Prostate, and Other Solid Tumors

4.5.1. Breast Cancer

Radioresistance and Negative Regulatory Processes: MiR-21 is known to be a critical promoter of radioresistance. Still, studies have revealed that natural regulatory processes make efforts to re-establish susceptibility. For instance, it has been revealed that transcription factor STAT4 suppresses radioresistance by inducing the repressive MALAT1/miR-21-5p/THRB regulatory pathway, which represents an endogenous effort to eliminate the pro-survival effect of miR-21 and re-establish susceptibility to irradiation-induced damage (Guo et al., 2024).

4.5.2. Prostate carcinoma

Multi-Drug Resistance and EMT: High expression of miR-21 directly fosters resistance to compounds like Doxorubicin (Zhao et al., 2021). Additionally, the ubiquitous non-coding RNA pathway of LncRNA PTENP1/miR-21/PTEN is used to facilitate Epithelial-Mesenchymal Transition (EMT), an important entity that imparts multi-drug resistance as well as metastatic potential (Gupta et al., 2024.

4.5.3. Esophageal Squamous Cell Carcinoma

Immune-Mediated Chemotherapy Resistance: Within Esophageal Squamous Cell Carcinoma (ESCC), miR-21 expression extends beyond the malignant cell and into the tumor microenvironment. Cancer-Associated Fibroblasts (CAFs) promote resistance to Cisplatin by inducing intercellular communication of molecules through exosomes that contain miR-21 and consequently stimulate the signaling of STAT3 within monocytic Myeloid-Derived Suppressor Cells (MDSCs). This pathway simultaneously reveals a link between chemotherapy failure and locally generated immunosuppressive microenvironment formation (Zhao et al., 2021).

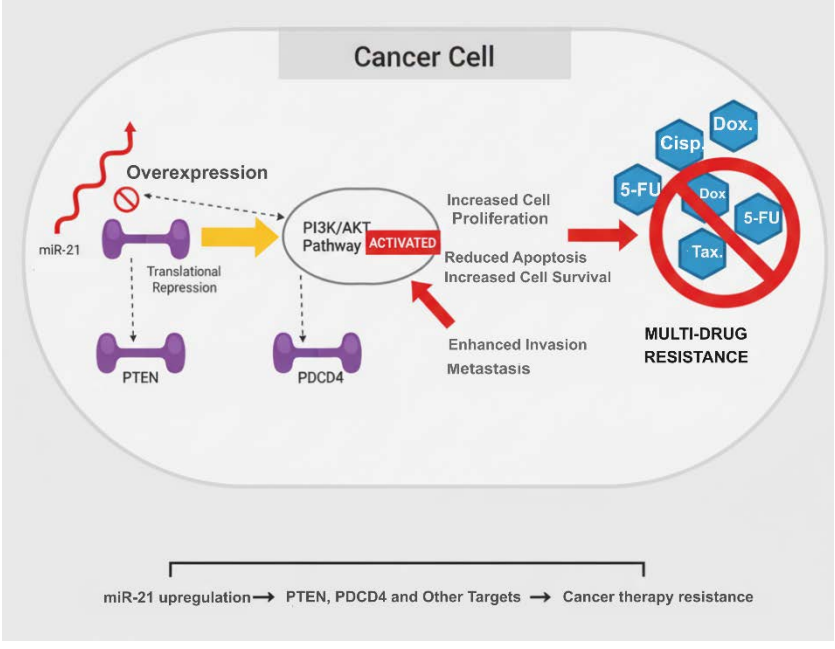

Figure 1. miR-21-Regulated Gene Networks Contributing to Cancer Therapy Resistance

Table 1. Summary of experimental studies on miR-21-mediated drug resistance in human cancers

Cancer Type	Regulation	Drug Resistance	Key Mechanism	PMID
Lung Cancer	Up	Methotrexate	EV-mediated transfer	38542098
Pancreatic Cancer	Up	Gemcitabine	CAF-mediated, Targets PTEN	38197482
Gastric Cancer	Up	Cisplatin	Targets TGF-β2/SMAD	39411053
Hepatocellular Carcinoma	Up	Sorafenib	Targets SIRT7 (via USP24)	37187452
Colon Cancer	Up	General Chemotherapy	Inhibition reverses resistance	31918721
Gastric Cancer	Up	Doxorubicin	Targets PTEN, TIMP3	29393355
Gastric Cancer	Up	Cisplatin	TAM exosome transfer	28407783
Lung Cancer	Up	Gefitinib	EV-mediated transfer	29928355
Prostate Cancer	Up	Doxorubicin	Inhibition reverses resistance	33598946
Hepatocellular Carcinoma	Up	Sorafenib	Sponged by circRNA (Targets TIMP3)	34875312
Gastric Cancer	Up	Cisplatin	Inhibits Autophagy (via PI3K/Akt)	31913198
Lung Cancer	Up	Osimertinib	Activated by MEK/ERK	34885115
Hepatocellular Carcinoma	Up	Sorafenib	Regulates SNHG1, Activates Akt	31053148
Esophageal Cancer	Up	Cisplatin	CAF exosome activates STAT3 (in MDSCs)	34139285
Colon Cancer	Up	General Therapy	Exosomal, Targets PDCD4	32427870
Multiple Myeloma	Up	S63845, Venetoclax	Stroma-mediated, Alters BCL-2 family	33806619
Oral Squamous Cell Carcinoma	Up	Cisplatin	Exosomal, Targets PTEN & PDCD4	28910982
Glioblastoma	Up	Temozolomide	M2 macrophage exosomes	31269723
Tongue Cancer	Up	General Chemotherapy	Targets CADM1	27055844
Glioma	Up	Carmustine	Targets Spry2	29228450
Colorectal Cancer	Up	Topoisomerase Inhibitors	Not specified (in silico insights)	31505885
Breast Cancer	Up	Paclitaxel	Targets PDCD4	31169019
Lung Cancer	Up	Cisplatin	Hypoxic exosome transfer	30881046
Hepatocellular Carcinoma	Up	Doxorubicin (Multidrug)	Inhibition, Hyperthermia reverses resistance	29966415

5. THERAPEUTIC APPROACH AND TRANSLATION OPPORTUNITIES

Owing to its pivotal role, miR-21 is a good prognostic biomarker in addition to a most promising direct drug target (Akhtarkhavari et al., 2022).

5.1. Direct Targeting of miR-21

The easiest approach is to repress activity or expression of miR-21 (Akhtarkhavari et al., 2022).

Anti-miR-21 Oligonucleotides (Antagomirs): These synthetic molecules bind to miR-21, preventing it from binding to its target mRNAs. Delivery challenges are addressed using specialized systems like nanoparticles or targeted exosomes (Akhtarkhavari et al., 2022).

Aptamer-Mediated Targeted Drug Release: RNA or DNA aptamers highly specific to tumor cell receptor binding on the surface are used to decorate carriers having anti-miR-21 to enable highly selective targeting.

Combination Strategies: Anti-miR-21 combinations with traditional treatments have synergistic efficacies as found in gold nanocages encapsulated with anti-miR-21 and hyperthermia against liver cancer (Wang et al., 2018).

5.2. Indirect Modulation and Naturally Derived Compounds

Nature-derived compounds like Astragaloside IV and Salidroside have the potential to regulate the MIR-21/PTEN/PI3K/AKT signaling pathway and subsequently affect insulin resistance and connected inflammatory reactions (Guo et al., 2023; Almohawes et al., 2024).

5.3. Integration with Immunotherapy

Reversing Immune Exclusion: Downregulating miR-21 and re-expression of PTEN could lower the PI3K/AKT signaling, which would facilitate improved cytotoxic T lymphocyte infiltration and improved effectiveness of PD-1/PD-L1 inhibitors.

6. CONCLUSION

MiR-21 is indisputably a central regulator of cancer therapy resistance, promoting therapeutic failure through well-characterized signaling mechanisms, complex systems of non-coding RNAs, metabolic process changes, and crucial tumor microenvironment interactions. Widespread prevalence of its overexpression combined with its heterogeneity of downstream effects cements its status as a potential core target of prospective personalized oncology therapies. While challenges of clinical application remain an impediment, the unification of nanomedicine and selective molecular therapies holds promising methods of clinical application of anti-miR-21 agents that foretell a new epoch of combined and resistance-manipulative cancer therapies.

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THE ROLE OF NEXT-GENERATION SEQUENCING IN THE DIAGNOSIS OF RARE GENETIC DISEASES

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Fümet Duygu ÜSTÜNDAĞ[2]

1. INTRODUCTION

Rare diseases are not common among individuals, but they are gaining attention as a public health issue that greatly affects society. According to the European Union, diseases that affect fewer than 1 in 2,000 people are defined as “rare diseases.” Today, around 7,000 to 8,000 different rare diseases have been identified. About 80% of these diseases are genetic (Boycott et al., 2023). Most of these diseases start in childhood. They often involve serious, long-lasting, worsening, or physically and/or mentally disabling conditions (Ferreira, 2022). This situation shows how important it is to diagnose conditions early and correctly for effective clinical management. However, the variety in traits, similarities in clinical presentation, and genetic differences in rare genetic diseases make the diagnostic process more complicated. Traditional diagnostic methods like Sanger sequencing, karyotyping, or FISH can be slow and have a narrow focus. These methods are not enough, especially in clinical cases where the genetic cause is unknown or where several candidate genes might be involved. At this point, next-generation

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sequencing (NGS) technologies have emerged as a significant development in clinical genetics over the past decade. NGS has greatly improved diagnostic rates by allowing the analysis of several genes at the same time, quickly and accurately (Stark et al., 2023). NGS plays a key role in diagnosing monogenic diseases, familial syndromes, and studying de novo mutations. This technology is used for diagnosis and in many clinical processes, including genetic counseling, treatment planning, and prenatal screening. This section will discuss the role of NGS technology in diagnosing rare genetic diseases. It will cover its advantages, the challenges faced, and its possible future uses, backed by the literature.

2. THE MOLECULAR BASIS OF RARE GENETIC DISEASES

2.1. Monogenic (Mendelian) Inherited Diseases

Monogenic rare diseases occur when there is a harmful change in one gene. These diseases are usually passed down through autosomal dominant, autosomal recessive, X-linked, or mitochondrial inheritance patterns. For example;

- **Autosomal recessive:** Cystic fibrosis (CFTR mutation),
- **Autosomal dominant:** Marfan syndrome (FBN1 mutation),
- **Affiliated with X:** Hemophilia A (F8 mutation),
- **Mitochondrial inheritance:** MELAS syndrome

NGS technologies have greatly improved the chances of diagnosis, especially in cases with several genes that show similar traits. They allow for the quick and broad detection of these single-gene mutations (Kingsmore et al., 2022).

2.2. Genetic Heterogeneity

The fact that multiple genes can cause the same phenotype, which is known as locus heterogeneity, complicates the diagnosis of rare diseases. For instance, retinitis pigmentosa may be linked to mutations in over 100 different genes. In these situations, using traditional genetic tests for diagnosis is almost impossible. NGS-based gene panels and exome sequencing have become effective diagnostic tools.

2.3. Copy Number Variants (CNV)

In addition to classic mutations, some rare diseases stem from changes in copy number, like deletions, duplications, or microdeletions. For example:

- 22q11.2 deletion syndrome
- Charcot-Marie-Tooth type 1A (PMP22 duplication)

CNV analyses can be done on next-generation sequencing data. This allows for the detection of variants at both the single nucleotide and structural levels (Riggs et al., 2022).

2.4. De Novo Mutations

Some rare diseases, especially neurodevelopmental disorders, epilepsies, and skeletal dysplasias, happen because of new mutations. These mutations are not found in the parents; they occur spontaneously in the child. Detecting these types of mutations requires examining not just the patient, but also their family members.

2.5. Mosaic

In some cases, the harmful variant is not found in every cell of a person, but only in a specific cell line. This condition is called mosaicism. These mutations, which are hard to find with standard methods, can be detected using high-depth NGS analyses.

3. OVERVIEW OF NEW GENERATION SEQUENCING (NGS) TECHNOLOGY

Next-Generation Sequencing (NGS) is a technology that allows for the high-throughput, parallel, and rapid analysis of DNA or RNA sequences. While traditional methods like Sanger sequencing can analyze just one gene, NGS can simultaneously study multiple genes, the whole exome (WES), or the entire genome (WGS). This ability offers a major benefit, especially in diagnosing rare diseases where genetic variation plays a role (Goodwin et al., 2023).

3.1. Fundamental Principles of NGS Technology

At the center of NGS is the process of breaking down DNA samples into small pieces. It involves adding adapters, cloning and increasing these pieces, and then sequencing billions of bases at the same time. The platforms that are often used today include:

- Illumina (short-read)
- Thermo Fisher (Ion Torrent)
- Pacific Biosciences (long-read)
- Oxford Nanopore Technologies such systems are included.

3.2. NGS Approaches

NGS technologies can be used with different strategies. The most common methods in clinical diagnosis are as follows:

a) Targeted Genomic Panel Sequencing

Genes linked to a specific disease group are studied. For instance, a panel of over 100 genes can be developed for neuromuscular diseases. The benefits include lower costs and simpler data analysis.

b) Whole Exome Sequencing (WES)

Only protein-coding areas, or exomes, which make up 1-2% of the genome, are analyzed. Since over 85% of rare diseases come from mutations in these areas, WES is a strong tool for both cost and efficiency (Clark et al., 2022).

c) Whole Genome Sequencing (WGS)

All genomic regions, both coding and non-coding, are analyzed. This approach is great for finding intronic and regulatory region variants, structural changes, and copy number variations (CNVs). However, whole genome sequencing (WGS) has not been fully adopted for regular use because of costs, analysis time, and difficulties in interpretation.

d) RNA Sequencing (Transcriptomic)

RNA-Seq is used to study gene expression and splice variants. It can help diagnose some types of rare diseases, such as neuromuscular diseases.

4. CLINICAL DIAGNOSTIC APPLICATIONS SUPPORTED BY NGS

Next-Generation Sequencing (NGS) is now the main method for diagnosing rare genetic diseases. It has replaced traditional methods in many clinical situations. NGS offers extensive coverage, quick analysis, high sensitivity, and the capacity to screen multiple genes. These features provide important benefits, especially for diseases with varied symptoms, multiple inherited genes, and difficult diagnoses.

4.1. Gen Panel-Based Diagnostic Approaches

Targeted gene panels designed for specific clinical phenotypes may be the best option in the diagnostic process. Panel-based analyses are especially helpful for certain disease groups, including neuromuscular diseases, metabolic disorders, and congenital syndromes.

- Lower cost
- Easier to interpret
- More directly related to the clinic

are preferred because of their features (Wright et al., 2021).

4.2. Increased Diagnostic Rates with Whole Exome Sequencing (WES)

WES is preferred, especially in syndromic conditions where the phenotype is unclear or where many different genes may be involved. The literature reports that WES can lead to a diagnosis in 25 to 40% of cases where conventional methods cannot provide a diagnosis (Monies et al., 2022).

Diagnostic success with WES is especially high in these groups:

- Neurodevelopmental disorders
- Multiple congenital anomaly syndromes
- Suspected mitochondrial disease
- Autoinflammatory disorders

4.3. Whole Genome Sequencing (WGS) and Diagnosis of Complex Variants

WGS has benefits in finding pathogenic variants, structural changes, intronic mutations, and copy number variations (CNVs) in non-coding areas outside the exome. Also,

WGS has higher diagnostic rates compared to WES, but the interpretation process is more complicated (Gilissen et al., 2022).

Specifically

- Cases where WES was done before but no diagnosis was reached
- In cases where mutations in regulatory regions are suspected
- Whole-genome sequencing is suggested for cases of repeated pregnancy loss or fetal abnormality.

4.4. Trio-Based Analysis: Mother-Father-Child Sequencing

Trio sequencing, which involves the proband and their parents, is very effective for finding new mutations and evaluating whether variants are harmful. This method improves the chances of getting a diagnosis, especially for neurological disorders and developmental delays (Fitzgerald et al., 2022).

4.5. The Effects of Diagnosis on Clinical Management

The diagnosis from NGS identifies the disease and also:

- Allows changes to the treatment plan, such as choosing biological agents
- It allows for genetic counseling.
- Prepares the way for prenatal and preimplantation genetic diagnosis
- Enables screening for family members

5. INTERPRETATION OF DIAGNOSTIC DATA AND CHALLENGES

Next-Generation Sequencing (NGS) technology is a strong tool for finding genetic variants. However, interpreting these variants in a clinical setting and classifying them correctly is one of the toughest parts of the diagnostic process. This is mainly because many of the detected variants are novel variants, whose potential to cause disease is unknown, or variants of uncertain significance (VUS).

5.1. Classification of Variants

In clinical genomic analysis, variants are classified based on criteria set by the American College of Medical Genetics and Genomics (ACMG) as follows:

- Pathogenic
- Potentially pathogenic
- Meaning uncertain (VUS - Variant of Uncertain Significance)
- Potentially benign
- Benign

This classification relies on the type of genetic variant, such as missense, nonsense, or indel. It also considers the population frequency, conservation analysis, predictions from computer tools, and evidence from the literature (Richards et al., 2015).

5.2. Variants of Undetermined Significance (VUS)

A major issue often faced after NGS is the difficulty in clearly identifying the link between the variant found and the disease. In this context, the variant is called a "VUS." VUSs cannot be used directly in clinical decisions and:

- Segregation analysis can be done by testing family members.
- Additional functional studies may show the effect of the variant.
- It may be reclassified over time with new literature and databases.

It has been reported that about 40% of variants are first classified as VUS, especially in exome sequencing applications (Manickam et al., 2021).

5.3. Genotype–Phenotype Correlation

Establishing the relationship between a variant and its phenotype is often difficult. The reasons for this include:

- The same gene variant can cause different traits; this is known as pleiotropy
- The same phenotype can be linked to different genes, which is known as genetic heterogeneity
- The patient's clinical findings do not completely match the classic phenotype.

Therefore, clinical geneticists must work closely with bioinformaticians to ensure correct interpretation (Lindstrand et al., 2023).

5.4. Bioinformatics Infrastructure and Data Analysis Issues

Powerful bioinformatics tools are needed for the proper analysis of data obtained with NGS. These processes include:

- Quality control (QC)
- Variant filtering, for example, selecting variants that have a frequency of less than 1%

- Clinical annotation (matching with databases such as OMIM, ClinVar, gnomAD)
- Automated interpretation systems, such as Franklin and VarSome

However, in data analysis:

- Insufficient annotation
- Inconsistent database records
- Conflicting results from different algorithms

Such problems can make diagnosing more difficult.

5.5. Uncertainties in the Ethical and Clinical Decision-Making Process

Some variants may be important not only for the individual but also for family members. This raises ethical issues like reporting secondary findings and deciding on family screening. Therefore:

- VUS reports should be shared carefully with the family,
- Genetic counseling should be offered to clarify any uncertainties,
- When needed, a management plan should be created based on a decision from a multidisciplinary committee.

6. CONCLUSION

Next-Generation Sequencing (NGS) technologies have changed the way we diagnose rare diseases. Their high efficiency, ability to analyze data thoroughly, and precision boost diagnosis rates. This is especially true for diseases that are complex in their traits or have a lot of genetic variation. However, we expect these technologies to become more integrated, easier to access, and

tailored to individual needs in the future.Although rare diseases have often made diagnosis difficult, next-generation sequencing technologies have provided hopeful solutions. As NGS becomes more available, we can make early, exact, and focused diagnoses. This change improves patients' quality of life and eases the pressure on healthcare systems. In the future, genetics-based personalized medicine is likely to take the lead in everything, from diagnosis to treatment.

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