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Exploring The Genetic Basis of Rare Diseases Through Next-Generation Sequencing

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ABSTRACT

An establishment of Next-Generation Sequencing (NGS) as a powerful tool in elucidating the genetic complexity of rare diseases, providing tremendous throughput in the selection of known and/or novel pathogenic variant(s) with exceptional resolution. In this review, we describe the use of technologies such as Whole Exmore Sequencing (WES), Whole Genome Sequencing (WGS), and targeted sequencing in rare disease research by integration with animal models, namely mouse, zebra fish and dogs. They are these important physiological and genetic parallels to human biology that allow functional validation of mutations using gene editing tools like CRISPR-Cas9. To exemplify, while mice are the primary candidates for mutagenesis and studies of neurodevelopment diseases, zebra fish are highly suited for modeling congenital conditions because of transparency and rapid development, and canine models are the best in closely mimicking human neuromuscular diseases to facilitate translational studies. NGS makes possible the discovery of disease mechanisms and drug development; however, there are certain challenges associated with NGS that prevail like the ethical issues, species gene expression variability and poor genomic annotation in non-model species. Nevertheless, further NGS advances including integration with CRISPR-based functional validation, comparative genomics and translational research are taking the field further toward precision personalized medicine. The future directions seek to incorporate multi omics, expand genomic databases, and utilize artificial intelligence to improve variant interpretation and narrow the gap between discovery and clinical use of rare genetic disease treatment.

Key Words:

Next-Generation Sequencing (NGS), Rare Diseases, Animal Models, CRISPR-Cas9, Functional Genomics, Precision Medicine.

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1. INTRODUCTION

Next generation sequencing (NGS) has become a game changing technology in the genetics field that is capable of performing comprehensive and high throughout DNA and RNA analysis. The prevalence of many rare diseases derived from genetic mutations is low, they are clinically heterogeneous, and well characterized inheritance patterns are often lacking due to which these are diagnostically challenging. The traditional diagnostic methods often fail in diagnosing the true genetic causes. Although these NGS technologies – Whole Exome

Sequencing (WES), Whole Genome Sequencing (WGS) and targeted sequencing – have greatly improved our ability to identify known and novel genetic variants with high precision and efficiency [1]. This has allowed for much more precise diagnoses and a better understanding of the mechanisms behind disease states and the development of personalized approaches to treatments.

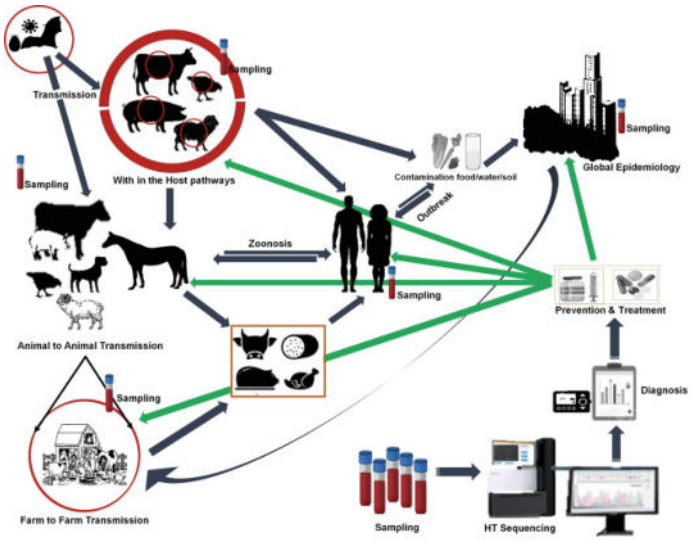

Figure 1: Zoonotic Transmission & HT Sequencing Workflow [2]

The use of animals is important for understanding what NGS finds can mean for us. Because of their genetics and ways they function, mice, zebrafish, and dogs can be used for disease modeling, confirming suspect genes, and testing out new drugs. The models are useful tools for studying genetic variations and, at the same time, can be used for research and development of new treatments. Because of NGS joining forces with technologies like CRISPR-Cas9, it is now easier to test how mutations might influence rare diseases. This paper examines the use of NGS in research on rare diseases, mainly by describing how it is applied in different animal models, and points out the latest technical and methodological breakthroughs supporting progress in this field.

1.1. Background Of the Study

Although individually, rare, when combined, rare diseases affect millions worldwide and many of them arise from genetic mutations. These conditions have low prevalence and clinical variability and traditional diagnostic approaches have often failed to identify the molecular causes. Advances of Next Generation Sequencing (NGS) revolutionized the study of rare diseases by providing rapid and high throughput detailed analysis of genomes and exomes and therefore allowing detection of known and novel pathogenic variants. At the same time, animal models, including mice, zebrafish and dogs, have become critical for validating those genetic findings and understanding their functional impact. Since these models share genetic and physiological properties with humans they provide natural platforms for probing disease mechanisms and testing potential therapeutics [3]. Moreover, the combination of NGS with other gene editing tools that use CRISPR-Cas9 makes it possible to engineer animals with precise genetic alteration, and therefore causes of human disease phenotypes to be replicated and gene function to be tested in controlled environment. In addition to greatly enhancing our biological knowledge of rare diseases, the combination of NGS and animal models spans the

gap from the basic genetic research to the clinical application of the information in the development of the targeted treatment and personalized medicine approach.

1.2. Objectives of the Review

- To highlight the role of Next-Generation Sequencing (NGS) in identifying genetic variants responsible for rare diseases.
- To examine how animal models (mice, zebrafish, dogs) are used with NGS and CRISPR to validate disease genes and study pathogenesis.
- To assess the strengths, limitations, and future directions of using NGS in functional and translational rare disease research.

1.3. Importance of the Topic

The genetic basis of rare diseases is important to understand because, although each of these disorders may be rare, they can affect millions of people in total, and many of them are without effective treatment. The genetic complexity and variability of the diseases are many times more than what can be identified with the traditional diagnostic methods. Integration of Next Generation Sequencing (NGS) with animal models has transformed this field from low resolution genetic analysis to high resolution genetic analysis for functional validation of mutations; and preclinical testing of therapies [4]. But it also helps accelerate gene discovery, and it helps fill the chasm between basic research and clinical application, which could set things in motion for precision medicine and targeted interventions for rare genetic disorders.

2. NGS APPLICATIONS IN ANIMAL MODELS OF RARE DISEASES

Integration into animal models of Next-Generation Sequencing (NGS) has revolutionized rare disease research by allowing identification of genetic variants in high throughput and an in depth understanding of disease mechanisms [5]. The use of mouse models, because of the genetic similarity to humans, has been widespread as mutagenesis screens and gene editing studies to uncover variants responsible for metabolic and neurological disorders. The transparent embryos of the zebrafish along with their rapid developmental rate makes them ideal for studies of development and modeling congenital diseases through CRISPR based gene editing culminating in NGS analysis. An attractive large-animal system to study diseases such as Duchenne muscular dystrophy is provided by canine models, and with Whole Genome Sequencing (WGS) pathogenic mutations directly relevant to human conditions can be identified. Based upon sequencing tools such as Whole-Exome Sequencing (WES), WGS, and targeted sequencing, these diverse models provide researchers the flexibility to twist genetic analysis on the study's goals from broad genome wide discovery to specific mutation validation.

Figure 2: NGS and its Application [6]

However, NGS based research in an animal model has its limitations. Study design may be restricted by ethical concerns of animal welfare and the work may be subject to extensive oversight. Furthermore, animal models do not always work out in humans as they do in animal models because of differences in physiology as well as gene expression between species. Model organisms like mice or zebrafish have annotated genomes and well characterized genetic systems, but non model organisms such as dogs may not have detailed genomic maps, and therefore what is the interpretation of sequencing looks like from a genetics perspective. However, NGS has the strengths of high sensitivity, reproducibility, and compatibility with CRISPR validation, which makes it an essential tool of research in the preclinical space, where there is a gap between genetic discovery and developing therapeutic strategies for rare diseases.

2.1. Use of NGS in Animal Models of Rare Diseases

Next-Generation Sequencing (NGS) offers, in these cases, the possibility to better understand the genetic basis of rare diseases and to simplify patients sample management. Through high throughput, comprehensiveness of the data generated, NGS is a great tool for interrogating disease-causing mutations in [7]. Some of these animals are mice, zebrafish, and dogs whose unique features and genetic relationships with human beings have made them heavily used as animal models for advancing animal experimentation knowledge.

- **Mouse Models**

Humans are similarly genetically and physiologically similar to mice; therefore, mice are by far the most used mammalian models for studying human diseases and the existence of well developed techniques for genetic manipulation. WES has already been used in ENU (N-ethyl-N-nitrosourea) based mutagenesis screens to identify genetic variants associated in a wide variety of pathologies. This consists of metabolic dysfunctions, neurological anomalies and skeletal malformations. For instance, Wang et al. (2020) isolated mutations in the Fbxw7 gene using Whole Genome Sequencing (WGS) this gene regulates cerebellar development and is critical for entire life development. Mice that had it also developed ataxia and shedding light on some possible mechanisms of neurodevelopment disorders in humans [8].

- **Zebrafish Models**

The benefits of zebra fish for genetic research are rapid embryonic development, transparency of embryos and high fecundity. These traits, therefore, make these animals ideal to study developmental process and to model congenital disease. A very powerful CRISPR-Cas9 gene editing technology combined with targeted NGS has been extensively used to introduce and study mutations in specific genes. Used sequencing to model genes implicated in congenital

heart disease by CRISPR-Cas9. NGS not only proved a powerful tool in identifying mutations but also to assess off target effects and as such improved the reliability of genotype – phenotype correlations in zebra fish disease models.

- **Canine Models**

Due to the naturally inherited diseases, dogs tend to suffer from conditions that closely resemble how humans suffer from inherited conditions; therefore, dogs are valuable for translational research. There are special reasons, however, for preferring canine models to study neuromuscular disorders like Duchenne muscular dystrophy (DMD). Using WGS, discovered a novel mutation in the dystrophin gene found in Golden Retrievers. The importance of this discovery was underlined by the fact that it helped refine therapeutic strategies, such as gene therapies, through providing a biologically relevant large animal model resembling closely the human form of the disease. The last few years have witnessed the emergence of models that are critical preclinical platforms to test the safety and efficacy of potential treatments [9].

2.2. Methodological Approaches

The development of Next Generation Sequencing (NGS) technologies has permitted researches to investigate genetic variations with high resolution and accuracy [10]. Sequence based approaches used to find disease causing mutations in models of animal disease differ depending on the research goal. WES, WGS and Targeted Sequencing have their own benefits and disadvantages, which affect the type and extent of the genetic information that can be revealed.

- **Whole-Exome Sequencing (WES)**

Whole-Exome sequencing is an approach that only analyzes (captures and sequences) the exonic regions of the genome, which are the regions encoding proteins. Given that the vast majority of disease-associated mutations have thus far been mapped to protein coding sequences WES is therefore especially powerful at identifying pathogenic variants, which account for roughly 85% of all known mutation causing disease associations [11]. As a tool, WES has been successful for screening for mutations in animals ones, like mouse, but these mutations are, often, due to chemical mutagens like ENU (N-ethyl-N-nitrosourea), which are often used in forward genetics studies. WES, a technique that is cost effective and data efficient for variant discovery has a limitation in the sense that it ignores non coding regions (such as enhancers, promoters and other regulatory elements) which also play important role in gene expression and disease.

- **Whole-Genome Sequencing (WGS)**

Whole-Genome Sequencing gives us as complete a picture of the organism's DNA as possible, including both coding and non coding regions. Using this comprehensive approach we are also able to detect not only point mutations, but also more complex structural variants including deletions, duplications, inversions, translocations, etc. Because these types of mutations are often missed by WES, but are vitally important to the understanding of the genetic basis of rare disease. For example, structural change in mouse models of neurological disease, e.g., epilepsy, can be revealed by using WGS. Although WGS has its benefits, it is a lot more data intensive and expensive, needing more computational resources for data analysis and storage.

- **Targeted Sequencing**

This sequencing technology is focused on examining particular genes or parts of the genome that have been selected in advance. This approach gives valuable results when scientists can target genes that are strongly connected to a certain phenotype or disease. It is often employed during genetic studies of phenotype or to confirm crash and CRISPR-Cas9 changes in the genome [12]. They are much more effective and inexpensive than WGS or WES, and they give higher depth of coverage, making it more likely to catch targeted variants. Even so, they are only capable of noticing limited changes and cannot detect different mutations that are not included in the list of predefined genes.

2.3. Evaluation of Strengths and Limitations

Exploring genetics in animals by using NGS has greatly boosted what we know about rare conditions and their underlying causes. Even so, this approach comes with advantages and disadvantages that should always be considered to get the most out of the findings [13].

Strengths

1. **High Sensitivity to Detect Novel Variants**: NGS has unprecedented ability to discover many types of genetic changes, for example, single-nucleotide variants, insertions/deletions, and changes in the structure of genes. This strength matters most in rare disease studies, since new or extremely uncommon gene changes are usually involved in causing the condition. By using NGS, researchers are able to easily find these changes in animals, even when their genes are complicated [14].
2. **Reproducibility in Controlled Environments**: laboratory conditions used in animal studies, less external factors can influence the results and make outcomes easily repeatable. Doing this is important in studying diseases since it separates genetic effects from other outsider factors better than in humans with many genetic variants.
3. **Compatibility with CRISPR-Based Validation**: Both NGS and CRISPR-Cas9 tools are good options for scientist to identify animal genes that need to be further explored. With the information from NGS, researchers use CRISPR to copy or correct the mutation directly, and this lets them confirm cause and effect by watching the organism's behaviors [15]. With these technologies, the understanding of genetic data becomes deeper and tem-ly results in more effective treatment methods.

Limitations

1. **Ethical Considerations in Animal Use**: Conducting research on animals brings up ethical issues, mainly when what they suffer is painful or makes them weaker. According to ethical regulations, animals should be cared for kindly, there should be no unnecessary use of subjects, and the research has to be strongly supported by science. Due to these things, animal studies may be smaller in scale and always need thorough ethical review and permission [16].
2. **Complexity in Translating Findings to Humans**: There are several aspects that make animal models similar to humans, but they create a rough representation only. In some cases, the ways genes are regulated, metabolic procedures function, and how immunity

is formed in animals can become obstacles to applying test results directly to humans [17]. Such challenges are especially noticeable when diseases look different in various organisms or when human lifestyle and environment have a strong impact.

3. **Limited Annotation in Non-Model Species**: Though mice and zebrafish have their genomes well labeled, annotating the genomes of large animals, for example dogs and pigs, is still occurring [18]. This might obstruct the analysis of NGS results because there could be unknown or poorly defined regions of the genome that hide important regulatory parts or irregularities. So, gaps in genomic knowledge may cause researchers to miss vital insights and misread their results.

3. NGS IN FUNCTIONAL AND TRANSLATIONAL RESEARCH

NGS has made a big difference in the research of rare diseases by supporting gene editing, comparative genetics, and the use of animal models for advanced studies. The CRISPR-Cas9 system makes it possible to modify genes accurately to check if different mutations like Scn1a are related to epilepsy [19]. NGS data show that key disease pathways are the same in mice, zebrafish, and dogs, giving more significance to the scientists' works. This information supports the advance of translational research, for example, in dog models of retinal disease, where gene therapy worked and served as a basis for the start of clinical trials in humans.

3.1. Functional Validation and Gene Editing

Functional validation is very important to see if an NGS-identified genetic variant causes the disease. With the help of CRISPR-Cas9, scientists can now intently change, correct, or cut certain genes in animal models. The modifications in genomes can reveal the relationships between mutations and how diseases show up [20].

CRISPR is widely used, for example, to create mice with no function in the Scn1a gene that relates to epilepsy. This kind of genetically engineered mice, which have seizures like humans, proves that Scn1a is linked to the disease [21]. Thanks to this strategy, NGS-found variants can be studied to see if they are relevant and numerous rare diseases can be analyzed for developing new medicines.

3.2. NGS for Comparative Genomics

Comparative genomics makes use of NGS technology to check for similar and different genes in many species. This kind of study helps find typical genes and pathways involved in rare diseases, which improves our view of shared biological mechanisms [22].

Using NGS, researchers can analyze entire genomes or specific bits of DNA in mice, zebra fish, and dogs and see how they resemble the DNA in humans. Such studies found that malfunctions in some genes are common to cardiomyopathies, neurodegenerative disorders, and developmental problems [23]. Such regular findings give scientists more trust in using animals to study human disease mechanisms. As a result, the application of preclinical findings in medicine becomes more possible.

3.3. Applications in Translational Research

NGS findings in animals are commonly used to open the way for translational research by bridging science and medical application [24]. When human disease symptoms appear in

animals, scientists have the chance to try new drugs, follow therapy for genes, and spot signals that indicate disease or successful treatment.

The use of canines with X-linked retinal disease has greatly helped to develop new gene therapy approaches. NGS helped the researchers find the genetic mutation that causes this condition. After the gene therapy, both the genetic change and problems with vision were fixed [25]. The knowledge from animal studies played a big role in setting up clinical trials for humans, proving how NGS-led animal studies led to new treatments.

Table 1: Summary of Key Studies on Next-Generation Sequencing (NGS)

Authors Name	Study	Focus Area	Methodology	Key Findings
Marwaha, Knowles, & Ashley (2022) [26]	A guide for the diagnosis of rare and undiagnosed disease: beyond the exome	Rare and undiagnosed diseases	Review of diagnostic tools and sequencing strategies	Emphasized use of genome sequencing, transcriptomics, and multi-omics beyond exome sequencing.
Rajcan-Separovic (2020) [27]	NGS in recurrent pregnancy loss – approaches and outcomes	Recurrent pregnancy loss (RPL)	Application of NGS including targeted panels and exome sequencing	NGS enhanced detection of chromosomal and gene anomalies in RPL cases, aiding genetic counseling.
Robay et al. (2018) [28]	Systematic review on the genetics of male infertility in the era of NGS	Male infertility	Systematic review of studies using NGS	NGS identified novel variants; useful in diagnosing and treating male reproductive disorders.
Russell et al. (2021) [29]	Pharmacogenomics in the era of NGS – from byte to bedside	Pharmacogenomics	Review of pharmacogenomic data and clinical applications	NGS-linked variants improved understanding of drug response; promoted precision medicine.
Satam et al. (2023) [30]	Next-generation	NGS technologies and advancements	Review of sequencing	Highlighted rapid evolution

	sequencing technology: current trends and advancements		platforms and applications	of NGS and its increasing use in clinical and research settings.

4. PRECLINICAL INSIGHTS FROM ANIMAL TRIALS USING NGS IN RARE DISEASE RESEARCH

Animal models are now widely used in preclinical research into rare diseases because of the addition of Next-Generation Sequencing to the field. Such research advantages our knowledge of how differences in genes may result in diseases. Currently, NGS makes it possible for researchers to notice new changes in DNA, understand the causes of diseases, and examine new treatments at a high level of detail. Because mice, zebrafish, and dogs are genetically and physiologically similar to humans, and can be genetically modified, they are very useful [31]. NGS allows researchers to detect mutations that are either natural or result from editing genes and this contributes to better understand how genes are linked to diseases and helps create new treatments.

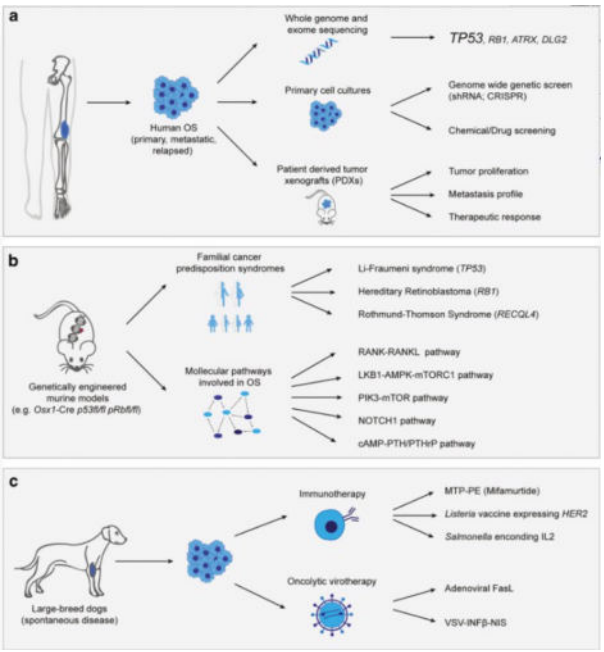

Figure 3: Preclinical Animal Models and NGS Applications in Rare Disease Research [32]

An important progress thanks to NGS in lab animals is confirming how certain genes can lead to diseases. By making use of CRISPR-Cas9, scientists are able to insert the same mutations detected in people into animal genomes [33]. So, creating Scn1a knockout mice showed essential details about the cause of epilepsy and proved that this gene is a proper target for possible treatments. They confirm that the identified variants are dangerous and offer systems for drug testing and measuring effectiveness. Bringing together gene editing and NGS makes it easier to better understand the effects of rare genetic changes.

NGS has played a big role in comparative genomics as well. Studying different genomes and comparing them to areas in human DNA related to a disease allows scientists to find important genes and controls for that medical condition [34]. As an illustration, by studying gene changes shared by mice, zebrafish, and dogs, researchers have figured out various mechanisms linked to cardiomyopathy and neurodegenerative disorders. These results support the use of animals in medical research, because they prove that core features of disease are present in distant species.

Besides, animal testing using NGS has been significant in the progress of gene therapies and advances in medical science. Here, NGS was applied to dog models of X-linked retinal diseases to find the causes of the disorder and watch if the therapy cured the dogs' genes. Thanks to these studies, scientists were able to plan and initiate clinical trials with people. So, animal experiments help scientists learn about rare diseases and also act as important links in getting treatments made and used [35]. The use of NGS in these experiments makes it faster to discover precise treatments for disorders with rare genetic backgrounds.

5. DISCUSSION

The discussion shows that using NGS plus animal models and CRISPR helps in finding genes related to rare diseases and discovering potential treatments for them. The study also touches on ethical matters and differences in species, suggesting that the next step should be connecting genomics, proteomics, and DNA methylation with AI technology [36].

5.1. Interpretation and Analysis of Findings

The development of Next-Generation Sequencing (NGS) has made a big impact on researching genetic causes of rare diseases [37]. Due to the use of animals including mice, zebrafish, and dogs in NGS, scientists can identify and study known as well as novel genetic variations that lead to disease. By analyzing DNA this way, researchers have been able to link seldom observed mutations found in Scn1a, Fbxw7, and dystrophin to the problems seen in kids. CRISPR-Cas9 has greatly assisted in checking these discoveries and showing that mutations directly lead to specific health conditions by closely testing them.

5.2. Implications and Significance

This shows that including NGS, gene-editing, and animal models increases progress in studying rare diseases. Making diseases identical to human illnesses in animals supports progress in drug creation and testing their effectiveness. Canine animal models have given important discoveries because they are similar to humans with diseases such as Duchenne muscular dystrophy and retinal disorders as noted in various studies [38]. Using comparative genomics has shown that disease-linked genes are shared among many animals, confirming animal models' role in human disease study and making molecular targets easier to test across different species. All in all, new findings are making personalized medicine and clinical trials more effective.

5.3. Identified Gaps and Challenges

There are still certain obstacles despite the major advances. Worries about the ethics of using animals in genetic studies still affect many types of experiments and require more supervision.

Furthermore, it is sometimes hard to apply research findings from animal models because of species-based differences in genes and body functions. There is a problem in understanding DNA sequencing data for dogs and pigs because their genomes are under-annotated [39].

5.4. Future Research Directions

To amplify the influence of NGS on this field, both the genomes of more species and the models of complex diseases in animals could be improved in the future. Using transcriptomics, proteomics, and epigenomics together with NGS may help us understand more about how diseases develop [40]. Widening databases of genomes beyond a few species will make it easier to study similarities and differences between species. In the end, bringing artificial intelligence and machine learning into NGS data reading could speed up classifying variants, guessing diseases, and finding suitable drugs for treatment, closing the gap between research and healthcare use.

6. CONCLUSION

Next Generation Sequencing (NGS) and animal models have led to major advances within the field of rare disease research, allowing a fantastic level of genetic detail to be achieved that is not otherwise possible. Both known and novel pathogenic variants can be discovered using NGS technologies, which coupled with animal models (including mouse, zebra fish, and dogs) and the advanced gene editing tool CRISPR-Cas9 make it possible to create human disease phenotypes in a dish in a controlled setting. In combination, this enables the study of disease mechanisms at a molecular level and creates a platform of great value for exploring the potential of new therapies. These models are essential in bridging genetic research in the 'lab and clinical application, by validating genetic findings and investigating genotype-phenotype correlations. Finally, the integration of genomic resources with multi–omics, i.e., transcriptomics, proteomics and epigenomics promises to further extend our understanding of complex diseases. In addition, the application of AI and machine learning to NGS data interpretation promises to hasten the identification of disease variants, prognostication and the pinpointing of therapeutic targets. These innovations, however, are facing ethical concerns and are challenged by interspecies genetic differences yet contribute to the advancement of the methods of rare genetic disorders treatment, and more generally to the birth of the era of precision medicine.

REFERENCES

1. Álvarez-Mora, M. I., Sánchez, A., Rodríguez-Revenga, L., Corominas, J., Rabionet, R., Puig, S., & Madrigal, I. (2022). Diagnostic yield of next-generation sequencing in 87 families with neurodevelopmental disorders. Orphanet Journal of Rare Diseases, 17(1), 60.
2. Baldridge, D., Wangler, M. F., Bowman, A. N., Yamamoto, S., Schedl, T., Pak, S. C., ... & Westerfield, M. (2021). Model organisms contribute to diagnosis and discovery in the undiagnosed diseases network: current state and a future vision. Orphanet Journal of Rare Diseases, 16(1), 206.

3. Baltrušis, P., Halvarsson, P., & Höglund, J. (2018). Exploring benzimidazole resistance in Haemonchuscontortus by next generation sequencing and droplet digital PCR. International Journal for Parasitology: Drugs and Drug Resistance, 8(3), 411-419.
4. Bruel, A. L., Vitobello, A., Tran Mau-Them, F., Nambot, S., Sorlin, A., Denommé-Pichon, A. S., ... & Thauvin-Robinet, C. (2020). Next-generation sequencing approaches and challenges in the diagnosis of developmental anomalies and intellectual disability. Clinical genetics, 98(5), 433-444.
5. Bulun, S. E., Yildiz, S., Adli, M., & Wei, J. J. (2021). Adenomyosis pathogenesis: insights from next-generation sequencing. Human Reproduction Update, 27(6), 1086-1097.
6. Cooper, C., Keatley, S., Northover, A., Gofton, A. W., Brigg, F., Lymbery, A. J., ... & Thompson, R. A. (2018). Next generation sequencing reveals widespread trypanosome diversity and polyparasitism in marsupials from Western Australia. International Journal for Parasitology: Parasites and Wildlife, 7(1), 58-67.
7. Den Hoed, J., & Fisher, S. E. (2020). Genetic pathways involved in human speech disorders. Current Opinion in Genetics & Development, 65, 103-111.
8. Domrazek, K., & Jurka, P. (2024). Application of next-generation sequencing (NGS) techniques for selected companion animals. Animals, 14(11), 1578.
9. Donlin, L. T., Park, S. H., Giannopoulou, E., Ivovic, A., Park-Min, K. H., Siegel, R. M., &Ivashkiv, L. B. (2019). Insights into rheumatic diseases from next-generation sequencing. Nature Reviews Rheumatology, 15(6), 327-339.
10. Ea, V., Bergougnoux, A., Philibert, P., Servant-Fauconnet, N., Faure, A., Breaud, J., ... & Kalfa, N. (2021). How far should we explore hypospadias? Next-generation sequencing applied to a large cohort of hypospadiac patients. European urology, 79(4), 507-515.
11. Ergin, S., Kherad, N., & Alagoz, M. (2022). RNA sequencing and its applications in cancer and rare diseases. Molecular Biology Reports, 49(3), 2325-2333.
12. Fernandez-Marmiesse, A., Gouveia, S., & Couce, M. L. (2018). NGS technologies as a turning point in rare disease research, diagnosis and treatment. Current medicinal chemistry, 25(3), 404-432.
13. Firdaus, Z., & Li, X. (2024). Unraveling the genetic landscape of neurological disorders: insights into pathogenesis, techniques for variant identification, and therapeutic approaches. International journal of molecular sciences, 25(4), 2320.
14. Genovesi, M. L. (2019). Next generation sequencing approaches in rare diseases: the study of four different families.
15. Gonzalez-Quereda, L., Rodriguez, M. J., Diaz-Manera, J., Alonso-Perez, J., Gallardo, E., Nascimento, A., ... & Gallano, P. (2020). Targeted next-generation sequencing in a large cohort of genetically undiagnosed patients with neuromuscular disorders in Spain. Genes, 11(5), 539.
16. Groisberg, R., Hong, D. S., Roszik, J., Janku, F., Tsimberidou, A. M., Javle, M., ... & Subbiah, V. (2018). Clinical next-generation sequencing for precision oncology in rare cancers. Molecular cancer therapeutics, 17(7), 1595-1601.
17. Gupta, A. K., & Gupta, U. D. (2020). Next generation sequencing and its applications. In Animal biotechnology (pp. 395-421). Academic Press.
18. Hartley, T., Lemire, G., Kernohan, K. D., Howley, H. E., Adams, D. R., & Boycott, K. M. (2020). New diagnostic approaches for undiagnosed rare genetic diseases. Annual review of genomics and human genetics, 21(1), 351-372.

19. Higuera, A., Herrera, G., Jimenez, P., García-Corredor, D., Pulido-Medellín, M., Bulla-Castañeda, D. M., ... & Ramírez, J. D. (2021). Identification of multiple Blastocystis subtypes in domestic animals from Colombia using amplicon-based next generation sequencing. Frontiers in Veterinary Science, 8, 732129.

20. Hu, T., Chitnis, N., Monos, D., & Dinh, A. (2021). Next-generation sequencing technologies: An overview. Human immunology, 82(11), 801-811.

21. Jensen, T. L., Gøtzsche, C. R., & Woldbye, D. P. (2021). Current and future prospects for gene therapy for rare genetic diseases affecting the brain and spinal cord. Frontiers in molecular neuroscience, 14, 695937.

22. Khan, A. (2024). Biotechnological Approaches to Rare Genetic Disorders: Innovations and Challenges. Frontiers in Biotechnology and Genetics, 1(2), 88-106.

23. Koriath, C., Kenny, J., Adamson, G., Druyeh, R., Taylor, W., Beck, J., ... & Mead, S. (2020). Predictors for a dementia gene mutation based on gene-panel next-generation sequencing of a large dementia referral series. Molecular psychiatry, 25(12), 3399-3412.

24. Kristan, A., Pajič, T., Maver, A., Režen, T., Kunej, T., Količ, R., ... & Debeljak, N. (2021). Identification of variants associated with rare hematological disorder erythrocytosis using targeted next-generation sequencing analysis. Frontiers in Genetics, 12, 689868.

25. Liu, X., Zheng, J., Ding, J., Wu, J., Zuo, F., & Zhang, G. (2024). When livestock genomes meet third-generation sequencing technology: From opportunities to applications. Genes, 15(2), 245.

26. Marwaha, S., Knowles, J. W., & Ashley, E. A. (2022). A guide for the diagnosis of rare and undiagnosed disease: beyond the exome. Genome medicine, 14(1), 23.

27. Rajcan-Separovic, E. (2020). Next generation sequencing in recurrent pregnancy loss-approaches and outcomes. European journal of medical genetics, 63(2), 103644.

28. Robay, A., Abbasi, S., Akil, A., El-Bardisi, H., Arafa, M., Crystal, R. G., & Fakhro, K. A. (2018). A systematic review on the genetics of male infertility in the era of next-generation sequencing. Arab journal of urology, 16(1), 53-64.

29. Russell, L. E., Zhou, Y., Almousa, A. A., Sodhi, J. K., Nwabufo, C. K., &Lauschke, V. M. (2021). Pharmacogenomics in the era of next generation sequencing–from byte to bedside. Drug Metabolism Reviews, 53(2), 253-278.

30. Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., ... & Malonia, S. K. (2023). Next-generation sequencing technology: current trends and advancements. Biology, 12(7), 997.

31. Schubert Baldo, M., & Vilarinho, L. (2020). Molecular basis of Leigh syndrome: a current look. Orphanet journal of rare diseases, 15(1), 31.

32. Shi, X., Huang, T., Wang, J., Liang, Y., Gu, C., Xu, Y., ... & Yu, Y. (2018). Next-generation sequencing identifies novel genes with rare variants in total anomalous pulmonary venous connection. EBioMedicine, 38, 217-227.

33. Suminda, G. G. D., Bhandari, S., Won, Y., Goutam, U., Pulicherla, K. K., Son, Y. O., & Ghosh, M. (2022). High-throughput sequencing technologies in the detection of livestock pathogens, diagnosis, and zoonotic surveillance. Computational and structural biotechnology journal, 20, 5378-5392.

34. Thompson, K., Collier, J. J., Glasgow, R. I., Robertson, F. M., Pyle, A., Blakely, E. L., ... & Taylor, R. W. (2020). Recent advances in understanding the molecular genetic basis of mitochondrial disease. Journal of inherited metabolic disease, 43(1), 36-50.

35. Thompson, R., Spendiff, S., Roos, A., Bourque, P. R., Chardon, J. W., Kirschner, J., ... &Lochmüller, H. (2020). Advances in the diagnosis of inherited neuromuscular diseases and implications for therapy development. The Lancet Neurology, 19(6), 522-532.

36. Vora, N. L., & Hui, L. (2018). Next-generation sequencing and prenatal'omics: advanced diagnostics and new insights into human development. Genetics in Medicine, 20(8), 791-799.

37. Vorsteveld, E. E., Hoischen, A., & van der Made, C. I. (2021). Next-generation sequencing in the field of primary immunodeficiencies: current yield, challenges, and future perspectives. Clinical reviews in allergy & immunology, 61(2), 212-225.

38. Wang, H., Zhang, L., Wang, N., Zhu, H., Han, B., Sun, F., ... & Qiao, J. (2018). Next-generation sequencing reveals genetic landscape in 46, XY disorders of sexual development patients with variable phenotypes. Human genetics, 137, 265-277.

39. Wu, F., Sun, H., Lu, S., Gou, X., Yan, D., Xu, Z., ... & Pan, Y. (2020). Genetic diversity and selection signatures within Diannan small-ear pigs revealed by next-generation sequencing. Frontiers in genetics, 11, 733.

40. Yamamoto, S., Kanca, O., Wangler, M. F., & Bellen, H. J. (2024). Integrating non-mammalian model organisms in the diagnosis of rare genetic diseases in humans. Nature Reviews Genetics, 25(1), 46-60.