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Evaluating the role of next-generation sequencing and radiological techniques in rare disease diagnosis: Challenges and opportunities

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***Abstract*---**Aim: This article evaluates the utility of next-generation sequencing (NGS) and radiological techniques in the diagnosis of rare diseases, emphasizing the challenges and opportunities presented by these technologies. Methods: A comprehensive review of existing literature on NGS technologies, including first, second, and third-generation sequencing methods, as well as their applications in genomics, transcriptomics, and epigenomics, was conducted alongside radiological imaging techniques such as MRI and CT scans. Results: NGS has revolutionized rare disease diagnosis by enabling high-throughput, cost-effective sequencing, facilitating the identification of pathogenic mutations, and advancing personalized medicine. Radiological techniques provide complementary insights into anatomical abnormalities and disease progression. Despite significant advantages, challenges such as data interpretation, cost, and ethical considerations persist. Conclusion: NGS and radiological imaging offer transformative potential in rare disease diagnosis, enhancing our understanding of genetic and anatomical aspects of disorders and enabling targeted therapeutic approaches. Continued technological advancements and integrative analyses with other omics data and imaging findings will further enhance their diagnostic utility.

***Keywords*---**Next-generation sequencing, rare diseases, genomics, diagnostics, omics integration, radiological imaging.

Introduction

The field of genomics has seen a profound transformation thanks to next-generation sequencing (NGS), which has greatly improved our knowledge of the structure, functions, and dynamics of the genome. This cutting-edge technology has made it possible for scholars to conduct extensive research and explore the complexities of genetic data in previously unheard-of ways. The high-throughput and cost-effectiveness of NGS have made it a valuable tool for researchers in a wide range of disciplines, from basic biology to clinical diagnostics [1]. NGS has advanced transcriptomics, epigenomics, metagenomics, and other omics fields in addition to providing comprehensive genome sequencing [2]. With the ability to sequence millions to billions of DNA segments at once, advanced NGS systems such as those from Pacific Biosciences, Oxford Nanopore, and Illumina have completely changed the field of genomics [3, 4]. This feature has opened up new ways to interpret genetic variety, patterns of gene expression, changes in the epigenome, and diversity of microbes. The identification of pathogenic mutations, the identification of new therapeutic targets, and the clarification of intricate

biological processes including tumor heterogeneity and developmental mechanisms have all been made possible by next-generation sequencing (NGS) technology [3,4,5]. This study provides a comprehensive analysis of NGS technology, highlighting its revolutionary impacts in a number of fields, such as microbiome research, clinical genomics, cancer research, and infectious disease surveillance. We also look at NGS's future potential, considering new technologies, its ability to further genomics research, and its uses in the biological sciences. Next-generation sequencing (NGS) has emerged as a pivotal technology in genomics, significantly transforming our understanding of genetic information. By allowing for rapid and cost-effective sequencing of entire genomes, NGS has enabled researchers to investigate the complexities of genome structure, function, and dynamics at an unprecedented scale. This revolutionary approach has facilitated a wide array of studies, from basic biological research to applied clinical diagnostics, and has become indispensable in various scientific disciplines [1].

The capabilities of NGS extend beyond traditional genome sequencing; they encompass diverse omics fields such as transcriptomics, epigenomics, and metagenomics, providing insights into gene expression, epigenetic modifications, and microbial communities [2]. The advent of advanced sequencing platforms, including Illumina, Pacific Biosciences, and Oxford Nanopore, has further propelled the field by enabling the parallel analysis of millions to billions of DNA fragments, thereby unlocking new opportunities for understanding genetic variation and biological complexity [3,4]. NGS has proven instrumental in identifying disease-associated genetic variants, facilitating the discovery of novel therapeutic targets, and enhancing our comprehension of intricate biological phenomena, such as tumor heterogeneity and developmental processes [3,4,5]. Its impact is evident in various applications, including clinical genomics, cancer research, and infectious disease surveillance, making it a cornerstone of modern biomedical science. This review aims to provide a comprehensive overview of NGS technology, emphasizing its transformative role in advancing genomic research and its multifaceted applications. Additionally, we will explore the future prospects of NGS, highlighting emerging technologies and their potential to further enrich the fields of genomics and biomedical research.

Sequencing Technology Generations:

Over the past 20 years, methods for interpreting DNA sequences have rapidly evolved [6,7,8,9,10]. This quick evolution has made it possible for DNA sequencing to progress remarkably, leading to the creation of three different generations of sequencing technology (Figure 1).

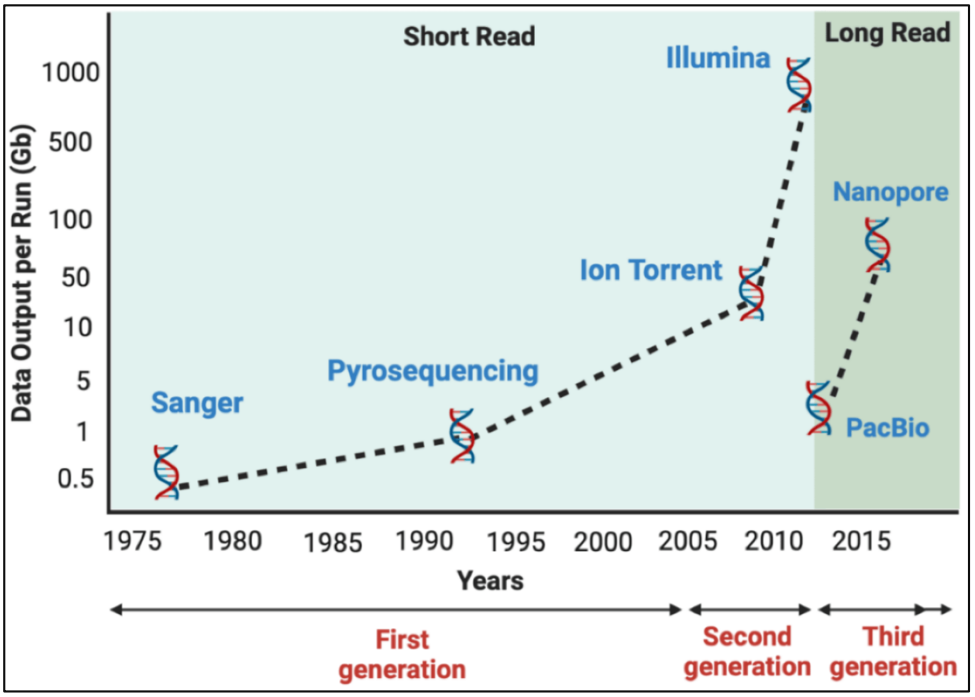

Figure 1: Sequencing Technologies

Sequencing Technology of the First Generation

The first steps in sequencing DNA and RNA involved breaking these molecules down chemically or by cleaving them with an enzyme, which produced pieces that could be examined separately. In 1964, Robert Holley used ribonuclease derived from S. cerevisiae to be the first person to sequence a nucleic acid, namely Alanine tRNA [11]. The full bacteriophage PhiX174 genome may be sequenced concurrently with the introduction of a chemical degradation approach by Walter Gilbert and Allan Maxam [12]. But with Fredrick Sanger's invention of the chain termination sequencing technique, a major advancement was made [13]. Dideoxynucleotides, which prevent DNA strands from lengthening during replication, were used in this method to produce sequence readings that might be several hundred nucleotides long. Sanger's technique was widely adopted and revolutionized molecular biology by making it possible to sequence DNA and RNA quickly [12]. A significant breakthrough was made in 1987 with the introduction of the Applied Biosystems ABI 370, the first automated sequencing equipment sold commercially. This device automated the Sanger sequencing procedure using capillary electrophoresis and fluorescently tagged dideoxynucleotides, greatly increasing the speed and accuracy of DNA sequencing [14, 15]. Following technical advancements, the ABI 370 became the industry standard and was soon followed by higher-throughput sequencers that could process longer reads

[15, 16]. Although more recent and effective sequencing techniques have largely superseded first-generation technologies, they still mark a significant historical turning point in the development of sequencing, having a significant impact on many biological and medical fields and enabling a great number of genetic and molecular discoveries.

Technologies for Second-Generation Sequencing:

DNA sequencing has been revolutionized by second-generation sequencing techniques, which enable the simultaneous examination of thousands to millions of DNA fragments. These techniques are superior to classic Sanger sequencing in terms of parallel sequencing capability. Leading second-generation platforms include Roche's 454 sequencing, which uses pyrophosphate release detection following nucleotide incorporation into the DNA template to determine the sequence. Another method for determining the sequence is called ion torrent sequencing, which uses the identification of hydrogen ion release during DNA synthesis. Reversible dye terminators are used in the sequencing-by-synthesis method used by the Illumina sequencing platform, which is extensively used in the field. Furthermore, ligation-based technology with reversible terminators is used in SOLiD sequencing (Sequencing by Oligonucleotide Ligation and Detection) to determine DNA sequences. A wide range of applications in genomic research and clinical diagnostics have been made possible by these second-generation technologies, which have significantly boosted the throughput and efficiency of DNA sequencing [17]. Understanding genetic diversity, disease processes, and tailored therapy have advanced significantly as a result of their facilitation of whole-genome sequencing, transcriptome profiling, and targeted sequencing.

Sequencing in the Third Generation:

Third-generation sequencing methods represent the cutting edge of advances in DNA sequencing, providing creative fixes to inadequacies in previous generations. Compared to earlier techniques, these technologies enable the examination of substantially bigger DNA fragments by facilitating long-read sequencing. One notable example is PacBio Sequencing, which uses fluorescently tagged nucleotides in a single-molecule, real-time (SMRT) technique to perform long-read sequencing of DNA segments up to many tens of kilobases in length. Oxford Nanopore Sequencing is another well-known technology that analyzes DNA using nanopore technology. To ascertain the DNA sequence, a single-stranded DNA molecule translocates via a nanopore and changes in electrical current are detected. The long-read capabilities, mobility, and real-time analysis capabilities of Oxford Nanopore sequencing set it apart.

Sequencing for Long-Read and Short-Read:

Short-read sequencing's basic idea is sequencing by synthesis, which is improved by methods like hybridization, amplification, or fragmentation. Long-read sequencing, on the other hand, uses electrical impedance changes or synthesis to identify sequences as a single base passes through a biological membrane hole.

While short-read sequencing usually yields reads of about 600–700 bp, long-read sequencing can provide reads of up to 25–30 kb. Furthermore, because long-read sequencing does not require PCR-based library preparation, it reduces amplification bias and makes it easier to detect base changes like DNA methylation. The accuracy of long-read technologies has increased, and error rates have significantly lowered with the introduction of high-throughput sequencing platforms [29, 31]. Long-read sequencing technologies are superior at providing comprehensive genomic coverage, which makes it easier to identify complex structural variants like large insertions, deletions, inversions, and duplications. Short-read sequencing is useful for determining the abundance of particular sequences, profiling transcript expression, and identifying variants [8,29,31].

Third-Generation Sequencing-Based Omics: Data from several omics disciplines, such as proteomics, transcriptomics, epigenomics, and genomics, must be integrated to fully comprehend complex human disorders. We list a number of omics technologies that are used with the NGS platform below:

Genomics

NGS-based genomic investigations allow for comprehensive DNA analysis using a variety of techniques, such as whole-genome, whole-exome, and targeted sequencing.

Sequencing of the whole genome:

The comprehensive process of whole-genome sequencing (WGS) entails figuring out the genome's whole DNA sequence. This approach yields a comprehensive genetic composition map that includes all genes, regulatory components, and non-coding areas. WGS is mostly used in discovery science in fields including population genetics, uncommon genetic disorders, cancer research, eukaryotic and prokaryotic organisms, and plant and animal research [32]. WGS makes it easier to identify genetic variants, ranging from smaller structural changes like insertions, deletions, and rearrangements to bigger variations like single-nucleotide polymorphisms (SNPs), by sequencing an organism's entire genome. The data obtained via WGS has a wide range of uses in several industries [33]. Depending on the size of the genome, two types of whole-genome sequencing (WGS) can be distinguished: (1) large WGS, which analyzes genomes greater than 5 Mb (eukaryotes), and (2) small WGS, which concentrates on genomes smaller than 5 Mb (prokaryotes). Long-read sequencing is used for genome assembly, while short-read sequencing is preferable for mutation detection. Accurate assembly of new genomes without a reference sequence has been demonstrated by the successful fusion of short- and long-read sequencing.

Sequencing of the Whole Exome:

The goal of whole-exome sequencing (WES) is to sequence and capture the exome, or the protein-coding portions of the genome, which makes up 1%–2% of the total genome but is home to most known disease-related variations. Genetic variants inside protein-coding genes, such as single-nucleotide variants (SNVs), insertions, deletions, and copy number variations (CNVs), can be found using WES [34, 35].

When it comes to rare clinical disorders with clusters of symptoms and finding variations related to population and cancer genetics, WES is a more affordable option than WGS [36]. Target-specific amplification or hybrid capture techniques are used to enrich exonic areas, and high-throughput sequencing is then performed. The Illumina NGS platform is interoperable with a range of exome capture kits from vendors including Agilent, NimbleGen, Illumina, Twist, and IDT [37]. The bioinformatics methods used to analyze WES data are similar to those used for WGS because WES is basically a subset of WGS.

Personalized Sequencing:

As the name suggests, targeted sequencing is more focused on certain genomic areas than WGS or WES, which offer more comprehensive exploration capabilities. Many genetic changes, such as SNVs, minor deletions, duplications, insertions, and gene rearrangements connected to disease characteristics, can be found using this technique. Targeted sequencing has several advantages, including cost effectiveness and data management that helps physicians make decisions based on particular, disease-relevant information. It can cover uncommon alleles in genetic illnesses and low-abundance mutant clones arising from tumor heterogeneity or cancer evolution to a depth of up to 5000× [38]. The focused technique, which enables the investigation of both germline and somatic variations, frequently results from WGS/WES population research. These panels usually use amplification with pools of region-specific oligonucleotide primers as part of an enrichment technique, producing libraries of a particular size that are bioinformatically examined and sequenced [39].

The Transcriptomics:

The subject of transcriptomics has seen a significant transformation with the advent of next-generation sequencing (NGS), which has made it possible to examine the transcriptome—the entire collection of RNA molecules contained in an organism or particular cell group. High-throughput and reasonably priced RNA profiling techniques are made possible by NGS technologies, which also produce important insights into a variety of biological processes and diseases as well as alternative splicing, non-coding RNA control, and gene expression [40,41,42, 43]. NGS plays important functions in transcriptomics, such as:

RNA Sequencing: Within transcriptomics, RNA-seq is a widely used NGS application that focuses on the sequencing and measurement of mRNA molecules. With the use of this method, a biological sample's gene expression may be thoroughly analyzed, producing millions of short sequencing reads that can be used to precisely identify and measure the levels of each gene. The investigation of gene expression dynamics across several tissues or developmental stages, the identification of novel transcripts, the assessment of alternative splicing processes, and the detection of differential gene expression across conditions are all made easier by RNA-seq [44, 45].

Analysis of Alternative Splicing: A single gene can produce several mRNA isoforms by alternative splicing, which greatly adds to the transcriptome's complexity. NGS makes it possible to thoroughly investigate different splicing patterns. Researchers can locate splice junctions and alternative splicing events by matching RNA-seq reads to a reference genome. This provides information on isoform diversity, tissue-specific expression, and the functional implications of these differences [46].

Analysis of Small RNA and Long Non-Coding RNA (lncRNA): NGS makes it easier to study non-coding RNAs, which are essential for controlling gene expression. Numerous non-coding RNA classes can be identified and characterized by methods like small-RNA and long non-coding RNA sequencing. Regulating RNAs, including as microRNAs, piRNAs, and snoRNAs, are profiled by small-RNA sequencing, which provides insight into their functions in post-transcriptional gene regulation. The identification of lncRNA transcripts linked to many biological processes and illnesses is made possible by long non-coding RNA sequencing [47, 48, 49]. Sequence variants (SNPs) and exon connections within transcribed regions can be found using long RNA-seq reads [50]. Furthermore, novel microRNAs and other small RNAs are found using non-targeted small-RNA sequencing [51]. Our knowledge of the functions of long non-coding RNAs (lncRNAs) and small non-coding RNAs (sncRNAs) in gene regulation during cancer progression has been improved by transcriptomic analyses in conjunction with ChIP-seq studies in cancer biology [52,53,54].

Assembly and Annotation of the Transcriptome: An organism's transcriptome can be reconstructed and annotated using NGS data. Through the use of de novo assembly techniques or RNA-seq read alignment to a reference genome, scientists can find new transcripts, splice variants, untranslated regions, and other transcript properties. This increases reference genome annotations and our understanding of transcriptome complexity, which makes it easier to find genes and regulatory elements that were previously unknown [55].

One-Cell Transcriptomics: Single-cell transcriptomics, made possible by the development of NGS, enables the examination of gene expression profiles at the individual cell level. Transcriptome profiling from individual cells is made easier by single-cell RNA-seq (scRNA-seq) technologies, which also offer insights into cellular heterogeneity, cell type identification, lineage analysis, and gene expression patterns in complicated tissues or developmental processes [56,57].

Transcriptomics with Integration: Integrating transcriptomic NGS data with other omics data—genomics, proteomics, and epigenomics, for example—allows for a comprehensive knowledge of biological processes and gene regulation. The identification of important regulatory mechanisms governing cellular activities and illnesses is aided by integrative techniques, which provide a holistic perspective of molecular interactions [56].

The study of epigenomics

The field of epigenomics is devoted to the study of epigenetic modifications, which are heritable variations in the patterns of gene expression that do not result from

changes to the DNA sequence [58,59]. Histone alterations, RNA methylation (epi-transcriptome), and DNA methylation [60] are examples of common epigenetic modifications. These chemical changes are influenced by environmental factors such nutrition, contaminants, and inflammation [62,63], and they alter DNA accessibility, chromatin remodeling, and nucleosome placement [61]. Whole-genome sequencing in humans, plants, and animals has provided new insights into these epigenetic modifications, especially DNA methylation and hydroxymethylation [64]. The roles of epigenetic modifications in complex disorders, such as memory problems, addiction, cancer, autoimmune diseases, behavioral problems, and neurodegenerative disorders, have attracted attention [65]. As mentioned in [66], a number of platforms and assays have been created to investigate these alterations. NGS has been essential to the study of epigenomics, as will be covered below:

Profiling DNA Methylation: One important epigenetic alteration that profoundly affects cellular functions and gene control is DNA methylation. Single-nucleotide resolution in genome-wide analysis of DNA methylation patterns is made possible by NGS [67]. NGS is used in methods like reduced representation bisulfite sequencing (RRBS) and whole-genome bisulfite sequencing (WGBS) to detect methylated cytosines [68]. Using restriction enzyme digestion, RRBS enriches methylated genomic regions [66,69]. These techniques enable researchers to investigate the dynamics of methylation, identify areas of DNA that are differentially methylated (DMRs) and associated with illnesses, and comprehend how methylation affects gene expression.

Ability to Access Chromatin Genome-wide profiling of chromatin accessibility is made possible by mapping NGS techniques like DNase-seq and the assay for transposase-accessible chromatin utilizing sequencing (ATAC-seq). By identifying genomic areas that are accessible to transcription factors and DNA-binding proteins, these techniques provide information about enhancers, promoters, and gene regulatory elements. Functional genomic components can be clarified by combining chromatin accessibility data with information on other epigenetic changes and gene expression [70,71].

Analysis of Histone Modification: Acetylation and methylation are two essential epigenetic indicators that control the shape of chromatin and the expression of genes. Histone modifications can be profiled across the genome using chromatin immunoprecipitation sequencing (ChIP-seq), which involves removing changed histones with antibodies and then sequencing the corresponding DNA. This method can be used to analyze histone alterations and find transcription factor binding sites, among other research applications. ChIP-seq offers information on the identification of enhancers and regulatory elements as well as the epigenetic control of gene expression [72,73,74, 75].

Analysis of Chromatin Conformation: NGS techniques that look into 3D chromatin architecture and interactions include Hi-C and 4C-seq. By capturing long-range chromatin interactions, these methods make it possible to create maps of chromatin interactions [76,77]. Researchers can learn more about the spatial architecture of the genome and how it affects gene regulation by combining 3D

chromatin conformation data with information on epigenetic changes and gene expression.

Combining Epigenomic Information: The integration of transcriptomic and NGS data from epigenomics can facilitate the investigation of the connections between gene expression and epigenetic changes. For example, combining RNA-seq data with DNA methylation patterns facilitates the identification of DMRs linked to alterations in gene expression. Similarly, the discovery of epigenetic regulatory mechanisms and the identification of regulatory elements connected to certain gene expression patterns are made possible by the combination of RNA-seq data with information on chromatin accessibility and histone modification.

Main Role of Radiologist

Radiologists play an essential role in the multidisciplinary approach to diagnosing rare diseases. Utilizing advanced imaging techniques such as MRI and CT scans, radiologists provide critical insights into structural and functional abnormalities that complement genetic data from NGS. Their expertise in interpreting complex imaging findings aids in accurate diagnosis, disease monitoring, and treatment planning. Radiologists' ability to detect subtle anatomical changes enhances the overall diagnostic accuracy, making their collaboration with genetic researchers and clinicians indispensable in the effective management of rare diseases.

Conclusion

Next-generation sequencing (NGS) and radiological techniques have fundamentally altered the landscape of rare disease diagnosis, offering unparalleled insights into genetic variation and disease mechanisms. NGS's ability to sequence entire genomes rapidly and cost-effectively has facilitated the discovery of pathogenic variants that were previously elusive, thereby enhancing our understanding of complex genetic disorders. Moreover, NGS's integration with other omics disciplines—such as transcriptomics and epigenomics—provides a more holistic view of disease etiology, enabling personalized therapeutic interventions tailored to individual patient profiles. Radiological imaging, including MRI and CT scans, adds critical information about anatomical abnormalities and disease progression, complementing the genetic data from NGS. While the benefits of NGS and radiological techniques are substantial, several challenges remain. The vast amount of data generated by NGS necessitates advanced bioinformatics tools and skilled personnel for accurate interpretation. Similarly, radiological imaging requires expert analysis to interpret findings accurately. Additionally, the high initial costs and logistical hurdles associated with implementing NGS and advanced imaging in clinical settings may hinder their widespread adoption, particularly in resource-limited environments. Ethical considerations, including patient consent and data privacy, must also be rigorously addressed as these technologies advance. Looking forward, the potential for NGS and radiological imaging to inform clinical practice is immense, particularly as emerging technologies continue to refine sequencing accuracy, imaging resolution, and reduce costs. By overcoming existing barriers and fostering collaborations between genomic researchers, radiologists, and healthcare providers, these technologies could transform rare disease diagnosis

and management, ultimately improving patient outcomes and paving the way for novel therapeutic discoveries. As we continue to unravel the complexities of the genome and anatomical structures, NGS and radiological techniques will undoubtedly play central roles in shaping the future of precision medicine.

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