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A Review of Next-Generation Sequencing Technologies and Their Impact on Clinical Research: Assessing Clinical Efficacy and Cost-Effectiveness

Pratibha M.S, Saranya Kanukollu, Anand Babu Vangala, Rajani Kanth Vangala & Pramod N. Nair*

Neuome Technologies Private Limited, Helix Biotech Park, Bangalore Bio innovation Centre, Electronics City Phase-I, Bengaluru, Karnataka 560100, India.

*Email: pnnairin@gmail.com

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Abstract

In contrast to microarray methods, sequence-based technologies directly determine the nucleic acid sequence. A number of modern sequencing technologies are referred to collectively as "next-generation sequencing" (NGS), often known as "high-throughput sequencing." Compared to conventional Sanger sequencing, NGS gives orders of magnitude more data at a much lower ongoing cost. These new technologies allow for much faster and more affordable sequencing of DNA and RNA, revolutionizing the study of genomics and molecular biology. Technical improvements in NGS sequencing methods have rapidly increased sequencing volume to several billion nucleotides within a short period and at a reasonable cost. Currently, NGS is developing into a molecular microscope that is permeating almost all areas of biological research. The last ten years have seen the development of NGS platforms and methodologies, and the quality of the sequences has increased to the point where NGS is now utilized in human clinical diagnosis. Due to significant cost reductions and greater community acceptance of NGS, the utilization of NGS techniques in studying clinical trials has significantly increased. NGS is a useful tool for detecting mutations in people with cancer and genetic abnormalities. To ascertain whether NGS can cost-effectively improve patient outcomes, more thorough cost-effectiveness studies of NGS applied to patient care management are required.

Keywords

Next Generation Sequencing; Hereditary neurological disorders; End-stage renal disease; Renal disorders; Heritable connective tissue disorders; Cost-effectiveness

Introduction

DNA sequencing techniques have started around 60 years ago, but in that time, they have advanced amazingly and quickly, and it lead to significant improvements in rate reduction, high throughput, competence, and applications (1,2). The history of DNA sequencing started with the introduction of two techniques such as Sanger sequencing (3) and Maxam and Gilbert's method (4). The first human genome was sequenced in 2001 (5) owing to advancement in polymerase chain reaction, the availability of high-quality enzymes to alter DNA, and fluorescence automated sequencing (6). NGS is a modern technique used for DNA and RNA sequencing as well as variant/mutation detection. In a short amount of time, NGS can sequence tens of thousands of genes or the entire genome. Any genomics question or DNA-based clinical activity can now be investigated using next-generation sequencing techniques that have been developed and put forth. These sequencing techniques have seen a massive revolution in DNA sequencing techniques, chemical processes,

and bioinformatics analysis methodologies (7, 8) (Figure 1). Due to technical advancements in sequencing the researchers can quickly and cheaply sequence to several billion nucleotides in short span of time and that increased the amount of data in genomic analyses (9).

The NGS-detected sequence variation and mutations have been used broadly used for decision making in disease diagnosis, prognosis, and treatment and provide accurate medicine (10). Clinical research needs a patient's genetic profile to analyze quickly and cheaply, and it is easily possible with NGS technology, and that made a revolution. In the future, mostly for all regular disease diagnostic testing may include sequencing analysis (11). NGS combines the benefits of different sequencing chemistries, sequencing matrices, and bioinformatics technology. Therefore, it enables immense analogous sequencing of different lengths of DNA or RNA sequences, or even whole genomes, in a relatively short period of time. NGS sequencing entails several major steps such as fragmenting DNA, preparing libraries, sequencing, bioinformatics study, and gene variants or mutation annotation and interpretation (12). The introduction of these NGS platforms to the market in recent years made a significant impact in the collective characterization and quantification of pools of biological molecules, owing to the massive amount of data obtain with a noteworthy reduction in time and cost (13). Indeed, NGS has been used in a variety of contexts, including genomics, transcriptomics, and epigenomics. (14).

NGS is now a common practice in many clinical laboratories, particularly for detecting germline and somatic mutations. The causative mutations of inherited diseases are investigated using various methods such as targeted gene panel, whole exome sequencing, whole genome sequencing, and mitochondrial DNA (mtDNA) sequencing. To be more precise, targeted gen panel analysis is frequently used to test for genetic conditions such kidney, neurologic, connective tissue, cardiomyopathies, skeletal muscle, immunological deficiencies, blindness, deafness, and other hereditary or non-inherited malignancies. The widespread application of these quick, high-throughput technologies in recent years, along with their improved functionality and surmounting of early technological hurdles, has promoted their transition from fundamental research to clinics, with major advantages for managing normal patient care. Diagnostic pathology, clinical medicine, and biological research have all been substantially altered by NGS technologies. As technology and bioinformatics evolve to overcome present limits, increase the number of clinical applications, and enhance the quality of results, the usage of NGS in clinical laboratories will almost probably increase in the future.

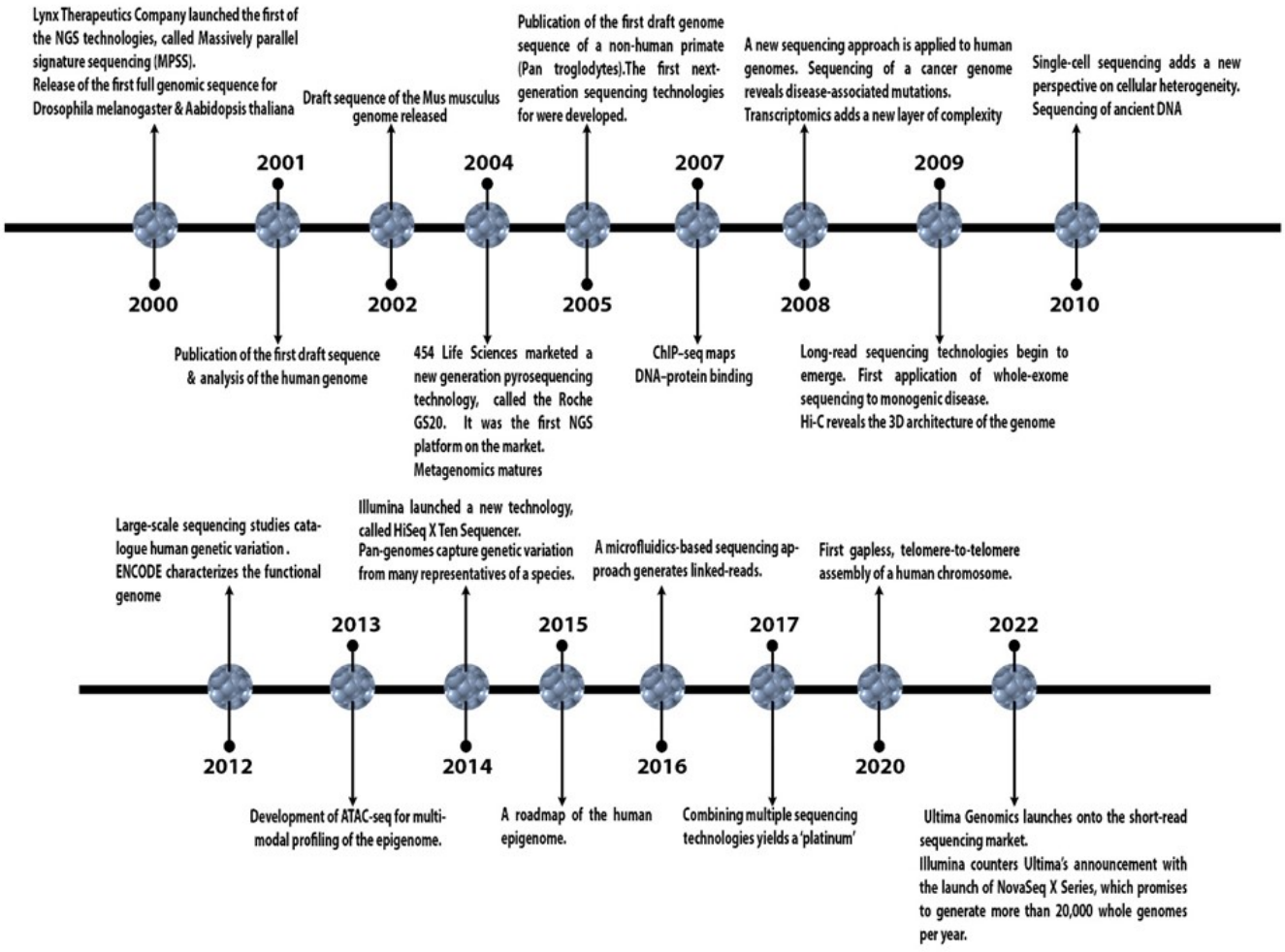

Figure 1. Revolution in DNA sequencing techniques 2000 -2002

2. Next generation sequencing and their impact on diagnosis and therapy

Next-generation sequencing (NGS) represents a revolutionary advancement in molecular diagnostics, allowing for rapid and comprehensive analysis of genetic material. With its ability to detect a wide range of genetic variations with unparalleled precision, NGS plays a crucial role in the diagnosis of various diseases. It enables early detection of genetic disorders, cancer biomarkers, and infectious pathogens, facilitating timely interventions and personalized treatment strategies. Moreover, NGS facilitates targeted therapies by identifying specific genetic mutations, guiding the development and implementation of precision medicine approaches tailored to individual patients. Despite challenges such as data analysis complexity and cost, ongoing technological advancements and efforts to integrate NGS into healthcare systems promise to expand its accessibility and utility in clinical practice.

In addition to its diagnostic applications, NGS is reshaping therapeutic approaches across medical specialties. By guiding the selection of targeted therapies based on individual genetic profiles, NGS contributes to the advancement of precision medicine. Pharmacogenomic studies leveraging NGS data enable the prediction of drug responses, optimizing treatment efficacy and minimizing adverse effects. Furthermore, NGS -based monitoring of treatment response through liquid biopsies offers a non-invasive approach to assess disease progression and therapeutic effectiveness. As research continues and evidence-based practices emerge, the integration of NGS into routine clinical care holds the potential to revolutionize healthcare delivery, paving the way for personalized and optimized treatment regimens tailored to each patient's genetic makeup.

3. Unveiling the Therapeutic Potential and Clinical Effectiveness of NGS

Next-generation sequencing (NGS) stands as a transformative force in clinical efficiency, notably by offering heightened diagnostic accuracy and personalized treatment avenues. Its capacity for detecting genetic variations with unprecedented sensitivity allows for more precise diagnoses of genetic disorders, cancers, and infectious diseases. This comprehensive analysis of genetic profiles enables timely interventions and tailored treatment strategies, optimizing therapeutic outcomes. Next-generation sequencing (NGS) stands as a transformative force in clinical efficiency, notably by offering heightened diagnostic accuracy and personalized treatment avenues. Its capacity for detecting genetic variations with unprecedented sensitivity allows for more precise diagnoses of genetic disorders, cancers, and infectious diseases. This comprehensive analysis of genetic profiles enables timely interventions and tailored treatment strategies, optimizing therapeutic outcomes. The therapeutic applications of NGS and its clinical efficacy are noted below.

3.1. Hereditary neurological disorders

In both research and diagnostics, the use of NGS has promoted a profound change in the ability to detect genetic abnormalities in both rare diseases and heterogeneous diseases NGS can be used to analyse the complete genome or only certain parts of it i.e. genome or exome sequencing, (15) NGS can be used to detect de novo mutations or mosaicism in intermittent patients without a prior hypothesis about the mutated gene (16). Hereditary neurological disorders (HNDs) are a clinically and genetically diverse group of conditions, and these disorders are caused by abnormal electrical impulses that impair the function of the central or peripheral nervous systems. Owing to this clinical heterogeneity, diagnosing these disorders has been difficult for both clinicians and geneticists, and many patients are either misdiagnosed or remain undiagnosed. Using NGS, clinicians have successfully identified disease-causing genomic variants and accurately diagnosed a variety of hereditary neurological disorders. (17).

Around 7000 rare diseases have been identified, and 350 million people worldwide are affected by them, with a significant number of them being life-threatening or chronically debilitating. There are three types of gene panels for rare diseases: disease-specific, organ-specific, and all-encompassing panels (18). To begin, gene panel design should consider all genome properties of included genes, such as variant type and GC content. It makes it easier to select gene-capture tools, determine sequencing depth, and interpret data. Second, consider the possibility that a universal panel will not produce a higher diagnostic rate (19). The earlier research proved that the diagnostic rates for various gene panels ranged from 31.3% to 57% and that were unrelated to the number of genes in the panel. Panel-based NGS or targeted sequencing tests are intended to detect causal mutations in genes linked to a specific rare disease (20). Finally, the above research optimized the panel development for clinical diagnosis; promote diagnosis success in rare diseases (**21**). The above success was guaranteed because the NGS gene panel was predesigned, and the ultra-deep, uniform coverage allows for great sensitivity and specific variant calling for uncommon genetic variations. The aforementioned panel-based NGS test has been utilised successfully in various rare diseases with genetic heterogeneity, including allelic heterogeneity, locus heterogeneity, overlapping symptoms, and causative genes participating in common disease-related pathways (22,23). The clinical scenario has been improved and fostered by the high-potential use of NGS-based diagnostics in noninvasive prenatal testing, complicated genetic variations identification, and preventative genetic screening (18).

The development of NGS technology has resulted in significant progress in understanding the causes of Mendelian and complex neurological diseases (24). According to the Online Mendelian Inheritance in Man (OMIM) database, a vast variety of neurogenetic illnesses have been discovered as a result of the advancement of NGS technology. Currently, Pathogenic expansion repeats,

which can span thousands of base pairs (bp), cannot be detected by sequencing technologies due to their read length restriction of roughly 150 bp (25). Meanwhile, third-generation sequencing WGS/NGS has the ability to detect repeat expansion illnesses such as Friedreich ataxia, spinocerebellar ataxias (SCA), Alzheimer's disease, and frontotemporal dementia (FTD) etc. By incorporating recently discovered harmful mutations, NGS has revealed the genetic underpinnings of neurogenetic disorders such Charcot-Marie-Tooth disease (CMT), spinocerebellar ataxias (SCA), epilepsy, and multiple sclerosis (MS) (26). NGS-based studies are being conducted to determine the genetic causes of neurological diseases such as Alzheimer's, Parkinson's, epilepsy, multiple sclerosis, stroke, amyotrophic lateral sclerosis, and spinocerebellar ataxias. NGS has the potential to discover new genes with mutations that cause phenotypic changes (27).

Epilepsy is a neurological disorder with genetic causes in 70-80% of cases. There are hundreds of genes linked to epilepsy syndromes can now be studied using NGS techniques such as targeted gene panels, WES, and whole genome sequencing (WGS) (28). The genetic aetiology of epilepsy may be monogenic, or caused by mutations in just one gene, like the SCN1A mutations in Dravet syndrome. Polygenic epilepsy is thought to be caused by mutations in multiple genes, though the genetic risk factors for this are less well understood (29). Currently, patients without a prior diagnosis have a reported diagnostic rate with NGS that is roughly 25% higher. This is better than the outcomes of other genetic tests of comparably high quality, including kyotyping. Gene panel testing is now the method of choice for epilepsy genetic diagnosis, but NGS will overtake it due to its reduced cost and wider adoption of the technology (28,30,31).

3.2. Renal disorders and Connective tissue disorders

End-stage renal disease (ESRD), the majority of whose symptoms first manifested in childhood, was much more common in persons with inherited kidney abnormalities (32). Recent advancements in research have made it possible to explore the molecular mechanisms behind various inherited kidney diseases, revealing certain ailments' previously with unknown genetic causes. Even with genetic sequencing and variant interpretation, there is a good chance that no pathogenic genes or variants will be discovered, and thus no genetic diagnosis will be possible in some cases. In these cases, some novel pathogenic genes may be involved (33). All of these challenges were overcome by NGS technology, but the pathophysiological significance of those novel genes has not been thoroughly addressed, and novel variants in cell lines have been identified (34). Nephronophthisis (NPHP) is a progressive tubulointestinal kidney disease that is inherited in an autosomal recessive pattern. So far, more than 20 different NPHP genes have been identified. A Novel XPNPEP3 Mutation Causing Pediatric Nephronophthisis is discovered by sing Whole Exome Sequencing, and they were able to identify a unique homozygous frameshift mutation in XPNPEP3 (35). A recent NPHP study in China used whole exome sequencing to investigate a faulty candidate gene with complete deletion of the homozygous gene in affected patients. According to the study, making a clinical diagnosis of an unusual NPHP patient can be difficult. WES confirmed that the NPHP1 gene in our case had a complete homozygous deletion. The mutation in this case helps the doctors to understand how the genotype and phenotype of NPHP interact (36).

Renal ultrasound imaging (RUS) is a widely used method for detecting chronic kidney disease (CKD) in children and adolescents. RUS findings were frequently abnormal, and kidney function declines before other symptoms appear, making it difficult for clinicians to provide patient care. A proper diagnosis was difficult, especially in the early stages when there were no accompanying symptoms, so a significant proportion of patients may have received the wrong clinical diagnosis. The recent studies indicate that genetic testing can also be a useful diagnostic tool for both paediatric and adult CKD. NGS comprising gene panels and whole exome sequencing (WES) provides a novel approach to overcoming the aforementioned stages and making an etiologic diagnosis in these situations. NGS has the potential to solve CKD cases with an unknown aetiology in a sizable portion of patients (37, 38).

Heritable connective tissue disorders (HCTDs) are a group of genetic diseases that include Ehlers-Danlos syndrome (EDS), Marfan syndrome, and osteogenesis imperfecta. The clinical presentation and family history are used to guide genetic testing with NGS to identify gene variants in HCTDs. NGS was performed on a group of 100 unrelated patients and most frequently identified ZNF469 and ADAMTSL2 variants in patients. Joint hypermobility was the most prevalent clinical finding, and the aforementioned variants were found in 76% of HCTD patients with different clinical symptoms (39). EDS tissue disorder was distinguished by joint laxity, skin changes, and joint hypermobility, all of which shared clinical and genetic characteristics. This complicates diagnosis and emphasises the importance of molecular diagnostic confirmation, such as NGS, in determining the genetic causes of unsolved EDS types. (40). EDS is a non-inflammatory condition brought on by mutations in the COL5A1 and COL5A2 genes, while the COL1A1 gene is also responsible for the disease. A recent study on genomic DNA in 59 EDS patients was conducted using NGS. Thirty-five genes related to connective tissue were investigated. The pathogenicity of the discovered variants was assessed, and each was identified as having a distinct set of symptoms and inheritance patterns (41). These methods were useful in pregnant women, where complications occur in roughly half of EDS pregnancies. Early genetic identification of variants allows doctors to provide appropriate care and ensures that the majority of these EDS pregnancies have a positive outcome (39). Connective tissue disorders share many characteristics, making it challenging to categorize them into distinct types without comprehensive genetic testing, which is now possible thanks to cutting-edge genomic technology like next-generation DNA sequencing, genomic database searches, and a bioinformatics approach. (39, 40,41).

In order to diagnose cardiomyopathy, NGS was used to sequence an area of interest in the DNA of a single sick patient and compare the results to the reference sequence for the healthy human genome. Testing has primarily leveraged this strategy by providing focused gene sequencing to analyze a small number of disease-related genes in both cardiovascular and non-cardiovascular disorders. Since cardiomyopathies are a genetically diverse collection of illnesses, clinical practice lacks access to thorough and reasonably priced genetic testing. (42, 43). Mendelian illness is diagnosed using NGS in cardiovascular care i.e. disease caused by a single variant in a single gene. Several syndromes, including Marfan syndrome, long QT syndrome, familial hypercholesterolemia, hypertrophic and dilated cardiomyopathies, and hypertrophic cardiomyopathies, are associated with Mendelian cardiovascular disorders (44). The high-throughput mutation screening in disease genes for hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) using microarray-based target enrichment followed by NGS, allowing the detection of cardiomyopathy-causing mutations with high accuracy in a quick and cost-effective manner, making it suitable for use in daily clinical genetic testing (45).

3.3. Skeletal Muscle Disorders and Immunodeficiency Disorders

NGS applications enable the first-tier test for the molecular processes underlying skeletal muscle diseases, facilitating the development of novel therapeutic strategies. (46). A broad range of incapacitating diseases known as skeletal muscle channelopathies include non-dystrophic myotonias and periodic paralysisses. Musculoskeletal illnesses can affect the muscles, bones, and joints, may due to environmental or heridetary reasons, have a role in the aetiology. As a result, finding susceptibility genes and underlying genetic variations may shed light on musculoskeletal illnesses can affect the muscles, bones, and joints and may be caused by environmental or hereditary factors. Genetic analysis of patients is essential for the prognosis and therapeutic goals of the aforementioned disorders and numerous causative genes are involved. NGS is capable of sequencing underlying genetic variations and genome functions, allowing it to be used in a diagnostic setting to provide treatment and care to patients (47).

The study of the genetic and molecular basis of hereditary illnesses has been revolutionized by NGS techniques, which has dramatically increased the finding of new genes linked to immunodeficiencies. Primary immunodeficiencies (PIDs) are a group of inborn immune deficiencies caused by germline abnormalities that manifest as a variety of symptoms such as recurrent infections, autoimmunity, autoinflammation, allergies, and malignancies etc. PIDs have been much more prevalent in recent years, and over 400 different disorders in PID patients have been linked to defects in 430 different genes. Among all known PID genes, the possible mechanism of NGS already accounts for 45% of new genes, which enables doctors to treat immunodeficiencies effectively (48). In order to evaluate the current diagnostic yield of this common diagnostic strategy, the study used next-generation sequencing tools to analyze patient cohorts with PIDs. According to research, the average diagnostic yield for primary immunodeficiencies is 29% (range 10-79%), while the yield for WES or NGS is 38% (range 15-70%) (49). Galo et al. stated in the research study that they discovered 47 variants using NGS and classified them into four types of genetic variations. The first variation is associated with a well-defined PID, the second with the features of a well-defined PID, the third with the immunological features of the PID, and the fourth with a non-diagnostic genotype. In 7/45 cases i.e.16%, a clear genetic diagnosis with NGS was made and the researchers found 31 variations in 10 patients with complicated phenotypes, none of which individually caused the illness (50). Dutch genome diagnostic centers (GDC) conducted an external quality assessment (EQA) to assess the consistency of variant interpretation and reporting in PID diagnosis using NGS. In the Dutch-speaking nations, all clinical laboratory geneticists have started to conduct their business in accordance with best practices for NGS-based diagnostics. The clinical laboratory geneticists were unable to connect the genotype to the phenotypic and immunological information, but after using NGS-based diagnostics, a clear description of the clinical phenotype and immune system test findings were achieved. This study was important since it identified a number of disease-specific characteristics and because new treatments were initiated using data taken from the NGS based evaluation report (51). The vast majority of PID variants classified as pathogenic (P) or potentially pathogenic (LP) were found and accurately reported. These variants produced a clinical diagnosis and were recorded in the findings of a diagnostic research. There were some issues with the processing and filtering of variants, but the researchers were able to work through these issues, and the results show consistency in the data interpretation of variations in NGS-based PID diagnosis (51, 52).

3.4. Retinal and Hearing Disorders

Retinitis pigmentosa (RP) is a genetic condition that impairs vision and significantly atrophys the retina in young children and adolescents. By employing a successful NGS strategy, substantial genetic heterogeneity in these hereditary retinal dystrophies has been identified (53). Targeted NGS was used in a molecular diagnosis research study on genetic analysis of Ischemic Optic Neuropathies (ION) in known and affected patients. 16 genes were revealed to have pathogenic mutations; the majority of dominant IONs are caused by OPA1 variants, while ACO2 and WFS1 variants are also frequently involved in both dominant and recessive ION. The discovery of all harmful mutations in genes encoding proteins involved in mitochondrial activity highlights the significance of mitochondria in retinal ganglion cell survival. This discovery was novel to science and was only made possible by NGS (54). Inherited monogenic illnesses of the retina and vitreous, which afflict about 1 in 2000 people,

were the subject of investigation by Tiwari *et al.* High genetic heterogeneity and clinical variability, including mutations in about 250 genes and more than 20 clinical symptoms, were used by the researchers to differentiate variability. Retinal dystrophies present clinically as conditions ranging from minor retinal dysfunctions to severe congenital forms of blindness (RDs). Based on a thorough clinical diagnosis, disease-causing mutations were found using a dependable and effective high-throughput study employing WES. According to the research data, this approach was suitable for genetically diagnosing approximately 64% of the patients and discovered 20 novel and 26 recurrent variants in genes associated with retinal dystrophies. (55). Congenital stationary night blindness (CSNB) is a retinal disorder that is clinically and genetically diverse. The gene responsible for CSNB was not identified until NGS discovered compound SLC24A1, which is characterised by heterozygous deletions and a homozygous missense variant that causes CSNB. NGS techniques aid in the proper diagnosis of patients whose clinical characteristics are unclear (56).

Hearing loss is common inherited birth defects which leads approximately 60% of infant deafness. Hearing loss is genetically occurring as a result of mutations in an estimated 500 genes. Due to the large number of those genes and presumably low mutation frequencies, conventional sequencing was not successful. NGS is a paradigm-shifting technology that makes it possible to simultaneously screen mutations in a large number of genes and is a highly effective method for finding novel causal genes and mutations involved in heritable disease (57, 58). Usher syndrome is a rare genetic disease that affects both hearing and vision and is caused by an accumulation of unfavorable gene variants. It is a non-syndromic hearing loss, and NGS has identified approximately 145 loci that cause this genetic disease that are unsuitable for traditional linkage analysis (59). Shang et al. targeted 180 deafness-associated genes in patients with autosomal recessive nonsyndromic hearing loss in Iran using a custom capture panel (MiamiO to Genes) (60). Using NGS technology, the researchers discovered one previously reported and six novel mutations in five different deafness autosomal recessive (DFNB) genes (TRIOBP, LHFPL5, CDH23, PCDH15, and MYO7A) in these five families. Non-syndromic sensorineural hearing loss (NSHL) is the most prevalent form of genetic hearing impairment, an interesting study is reported from China. Both genetically and phenotypically, NSHL exhibits significant heterogeneity, with inheritance patterns varying from autosomal dominant to autosomal recessive to X-linked. NGS was used to identify the novel homozygous loss-of-function ILDR1 gene that causes autosomal recessive NSHL. Hereditary hearing impairment induced by the ILDR1 gene is very rare.This is the first report of a loss-of-function mutation in the ILDR1 gene linked to hereditary hearing impairment in the Chinese population. (61).

3.5. Cancer Diagnosis and therapy

In the field of medical research, NGS is well known for its contribution to cancer detection. Cancer is thought to be a hereditary disease in some circles, so clinicians look for specific mutations in tumour biopsies, surgically removed tissues, and blood samples from patients. Oncologists frequently use search results because they can be a valuable target for specific treatments (62). The number of possible target mutations that can be found varies depending on the type of cancer. According to recent research, looking for mutations required doing either a single test to check for a single unique mutation or possibly numerous separate tests to hunt for various specific targets. An abundant number of target mutations for cancers have emerged as a result of personalized medicine research. This knowledge of research has shown that the accumulation of molecular alterations is the main driver of carcinogenesis, which regulates the emergence of the malignant phenotype. (63). There are two types of genes involved in cancer oncogenesis. Tumor transformation is induced by oncogene activation, while cellular proliferation is encouraged by oncosuppressor inactivation. Oncogene mutations can be acquired and caused by errors in DNA replication and/or exposure to carcinogens, although they are more frequently acquired mutations that are passed down genetically (germline) (64). NGS can be performed on it, and the likelihood of finding a significant germline mutation varies greatly between cancer types.

Lynch syndrome and the Hereditary Breast and Ovarian Cancer (HBOC) syndrome are thoroughly researched. The identification of the moderate-risk genes for those syndromes and the susceptibility genes for HBOC was aided by genetic investigations based on linkage and positional cloning. (65). Numerous studies have looked into and found additional genetic risk variations as a result of the high penetrance of colorectal and endometrial cancer in Lynch syndrome, which is caused by four mismatch repair genes. Furthermore, several cancer syndromes confer a high risk for one type of cancer while having low penetrance and low- to moderate-risks for tumour development at other sites. (66,67). Despite the identification of several moderate-risk variants and the existence of clinical guidelines for some of these variants for genetic counselling, no additional high-risk casual genetic factors were discovered. Due to these considerations, genetic testing based only on one or a few genes is currently inefficient for detecting inherited disorders. The genetic complexity and underlying causes of cancer syndromes are beginning to be uncovered through NGS, which examines the genomes of families NGS, which examines the genomes of families, is beginning to reveal the genetic complexity and underlying causes of cancer syndromes. (68,69).

Intestinal-type sinonasal adenocarcinoma (ITAC), a rare tumour, has a grim prognosis and necessitates novel therapeutic strategies. A study on ITAC discovered clinically beneficial gene mutations that will help in the future to target specific medications for this rare tumour.

As a result, the researchers investigated the value of sequencing a specific panel of genes for this purpose and made a significant discovery by sequencing the relevant germline DNA to customise treatment with certain inhibitor medications. In general, the researchers concluded that NGS testing could benefit all newly diagnosed ITAC patients (70,71). Liquid biopsy is the collection of samples from cancer patients' blood, urine, and body fluids that can be used to monitor the progression of cancer non-invasively and in real time at all stages of cancer diagnosis and treatment. NGS has been used in the field of liquid biopsy to sequence circulating tumour DNA (ctDNA), which can provide a molecular profile of cancer because it is made up of DNA fragments produced by tumour cells (72). It is challenging to separate ctDNA from blood because the fragments range in length from 100 to 10,000 bp and are extremely fragmented. The ctDNA sequencing techniques are sensitive enough to identify cancer at an early stage with extremely low levels of mutation frequency. Numerous options exist for NGS-based protocols to increase the sensitivity of ctDNA assays, and ctDNA has demonstrated numerous promising outcomes for cancer categorization, monitoring, prognosis, and treatment choice (73).

A group of researchers conducted NGS-based studies to present a complete molecular characterization of breast cancer in tumour biopsies collected from breast cancer patients. They used sequencing to recognize novel genetic alterations involved in oncogenesis, cancer progression, metastasis, tumor complexity, heterogeneity, and evolution. They also investigated the relationship between the variable clinical features of oestrogen-receptor-positive breast cancer and somatic alterations. As a result, they have discovered eighteen significantly mutated genes such as RUNX1, CBFB, MYH9, MLL3, and SF3B1. In oestrogen-receptor-positive breast cancer, various phenotypes are connected to particular somatic mutational patterns that map into cellular pathways involved in tumour biology, was a complete characterization with NGS (74). Another type of cancer is high-grade serous ovarian carcinoma (HGSOC), and circulating tumour DNA (ctDNA) in the patients' plasma has been suggested as a useful indicator of therapy response. The TP53 gene is a tumour suppressor gene that is mutated in more than 90% of HGSOC patients; however, somatic variants are distributed across all exonic regions of the gene, necessitating mutational analysis using NGS technologies. Droplet digital PCR was used to confirm the identified mutations in Oncomine's somatic variants. Overall, the Oncomine panel with unique molecular identifiers (UMI) appears to be more useful for HGSOC ctDNA analysis and genomic profiling in precision oncology for advancing cancer research and improving cancer patient outcomes (75). In order to identify somatic anomalies in colorectal cancer, the Cancer Genome Atlas Network conducted a study and looked at the exome sequencing, DNA copy number, promoter methylation, messenger RNA, and microRNA expression in 276 samples. On 97 of these samples, low level whole-genome sequencing was carried out. In total, 16% of colorectal carcinomas had hypermutation (76).

About 400,000 individuals per year die from squamous cell carcinoma (SCC), or simply the common kind of lung cancer. The genomes of squamous cell lung tumours have not been completely defined, and no molecularly targeted medications have been developed specifically to treat this cancer (77). The sixth most prevalent type of cancer worldwide is head and neck squamous cell carcinoma (HNSCC). The scientist examined 32 primary tumours using whole-exome sequencing and gene copy number analysis to investigate the genetic causes of this malignancy and made a targeted medicine to treat the patients and care management (78, 79). Hepatocellular carcinoma, one of the most frequently occurring virus-associated tumours, is a further cause of cancer-related death on a global scale. Through massively parallel sequencing of a primary hepatitis C virus-positive hepatocellular carcinoma and matched lymphocytes from the same patient, the researchers found more than 11,000 somatic alterations in the tumour genome. The tumour genome changes revealed a preference for transition and substitution on the transcribed strand, which suggests preferred DNA repair. Patients' deaths are caused by these mutations, and only sophisticated NGS methodology can disclose this (80). Berger et al. conducted a study in Melanoma genome research and discovered that DNA mutations occur frequently. They used NGS technology to sequence the genomes of 25 metastatic melanomas and matched germline DNA to gain a comprehensive genomic view of melanoma in humans. The study revealed numerous point mutations, which were lowest in non-UV-exposed extremities melanomas with hairless primary sites. (81). Patients with acute myeloid leukaemia (AML) typically die as their condition worsens. The team performed deep sequencing of NGS to validate hundreds of somatic mutations in the initial tumour and relapse genomes of eight AML patients, and they discovered that these numerous mutations cause ongoing sickness and mortality (82).

NGS has the potential to change medical research, diagnosis, and treatment when paired with potent bioinformatics tools (84). The use of NGS, primarily through WGS and WES, has resulted in an explosion in the context and complexity of genomic alterations, including point mutations, small insertions or deletions, copy number variations, and structural variations (98). The previous study greatly increases our understanding of the genetic drivers of the most prevalent diseases and their subgroups. It was based on molecular analysis such as Sanger sequencing. Despite the fact that NGS has already helped researchers unearth a plethora of knowledge in the field of medicine, difficulties still exist in converting vast amounts of data into knowledge that is understandable and available for medical care. Many technical and statistical issues remain unresolved in terms of computationRepeated DNA, for example, is a significant impediment to the accuracy of read alignment and assembly, as well as the detection of structure variation. (85). Furthermore, it can be challenging to differentiate between sequencing and alignment errors and uncommon

mutations that cause disease. Although there have been advances in cataloguing genomic variants, the ability to forecast their functional impact and locate disease-causing variants is still in its infancy (86).

4. NGS and its cost-effectiveness

The genomic age has been transformed by sequencing technology, which also aid in our understanding and characterization of the genomes of people, animals, and plants. Any genomics question or DNA-based clinical activity can now be investigated using next-generation sequencing techniques that have been developed and put forth (87). The sequencing volume for these approaches has increased to several billion nucleotides in a very short period of time and at a reasonable cost because to technical advancements. DNA sequencing techniques have only been around for around 60 years, but in that time they have advanced incredibly quickly, making them a prime example of progress that has led to significant advancements in capability, applications, cost reduction, and high throughput (88). The NGS equipment can produce several billion nucleotides quickly and cheaply (89). NGS offers a number of advantages and significant benefits, including rapid speed, cheap cost, parallel generation of many short DNA sequences known as reads, and tremendous throughput (90,91) (Figure 2).

In comparison to the conventional single-gene testing (SGT) approach, the cost-effectiveness and budget impact of NGS have come under scrutiny. Sanger and next-generation sequencing (NGS) technologies are conceptually comparable. DNA polymerase sequentially adds fluorescent nucleotides to a developing DNA template strand in both NGS and Sanger sequencing. The fluorescent tag on each inserted nucleotide serves as an indicator. Sanger sequencing and NGS differ significantly in terms of sequencing volume. In contrast to the Sanger method, which can only sequence one DNA fragment at a time, next-generation sequencing (NGS) can sequence millions of DNA fragments at once. This procedure results in the simultaneous sequencing of hundreds to thousands of genes (92). When combined with deep sequencing, NGS also gives better discovery capability to find new or uncommon variants. NGS is a useful tool for finding mutations in people with cancer and other genetic disorders. To ascertain whether NGS can cost-effectively improve patient outcomes, more thorough cost-effectiveness studies of NGS applied to patient care management are required (93).

A review publication by Tan et al. with the title "A review of the clinical effectiveness and cost-effectiveness" made it evident that in-house NGS sequencing is less expensive than outsourcing. Thus, it is evident that NGS will soon become a standard protocol for clinical studies (94). Cancer treatment may be made more affordable by NGS testing, but the benefit of NGS-directed therapy depends on the decision-willingness makers to pay. When compared to previous approaches, NGS increased the discovery of actionable biomarkers by 74.4% from 40.5%. It may become more expensive in the future due to its accuracy in identifying brief alterations in cells and its integration into therapeutic practices (95). Comparing NGS testing tactics to PCR testing strategies for newly diagnosed cancer patients, NGS was related with the

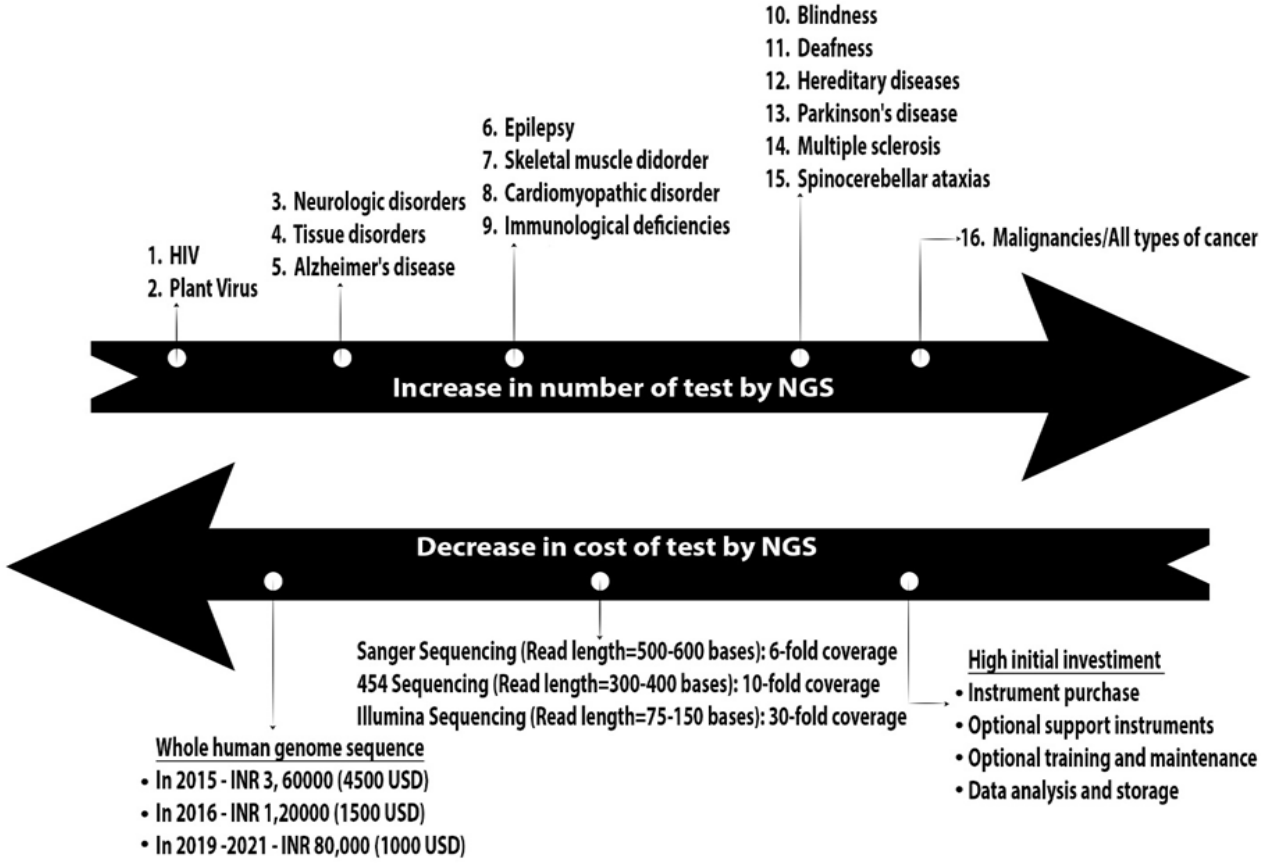

Figure 2. Increase in test and decrease in cost of NGS

quickest time to suitable targeted therapy initiation and the lowest overall cost of testing (96). An NGS-based strategy can be less expensive than an SGT-based strategy; additionally, created savings rise with the quantity of patients and various molecular variations examined. Although the initial investment for NGS implementation may be slightly greater than for SGT due to the cost of the NGS instrument and consumables, overall the NGS-based strategy is less expensive than the SGT-based one due to the lower minimum patient requirement (97).

Precision oncology has the potential to enhance patient wellbeing and lower medical expenses. On the other hand, the upfront cost of genetic testing using next-generation sequencing (NGS) technologies can be exorbitant. From single-gene tests to more comprehensive methods utilising next-generation sequencing (NGS) technologies, precision oncology diagnostics are available. Although the speed and throughput of NGS technologies have increased as a result of recent scientific developments,5 the state of the economic data now available to support resource allocation choices in the fight against cancer is unknown (98). The speed and throughput of NGS technologies have improved as a result of recent scientific developments, but it is unknown what the state of the economic data is currently supporting decisions regarding resource allocation in the fight against cancer. Research to date has mostly concentrated on calculating the cost effectiveness of multiplex panels for predicting illness prognosis, despite the fact that there is an increasing amount of economic evidence examining NGS in precision oncology available. To inform health policy, economic evidence for more extensive NGS is required, as is proof that precision oncology can find patients' most effective treatment options (99,100).

Conclusion

NGS genome sequencing, which would have a significant impact on public health, is still far from being accomplished, according to this review, but it will soon be a part of routine patient care management. In ways that were previously impossible, NGS has allowed us to uncover and study genomes. Bottlenecks in the management, analysis, and storage of the datasets have been revealed by the complexity of the sample processing for NGS. The processing power necessary for the assembly, annotation, and analysis of sequencing data is one of the major obstacles. Another significant difficulty is the large amount of data that NGS analysis produces. Since the early 2000s, NGS has developed into an invaluable tool in both research and clinical/diagnostic settings for contemporary medicine and drug discovery, with the use of techniques like WGS, WES, targeted sequencing, transcriptome, epigenome, and metagenome sequencing seeing a dramatic increase. NGS technology has transformed every area of the life sciences and medical science, providing a wealth of advantages in terms of huge parallel sequencing. The high cost of sequencing used to be a barrier, but it has significantly decreased in price recently, drawing academics in and making it possible for them to schedule their research around sequencing. Further study is therefore required to support these expenses in light of the added benefit for the patients.

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Author's contributions

PMS conducted the literature survey and initially drafted the manuscript. SK and RKV contributed to the literature survey, particularly focusing on clinical efficacy. ABV assisted in reviewing literature regarding cost-effectiveness and regulatory measures. PNN played a role in designing the paper, creating diagrammatic representations, and finalizing the review article. All authors have reviewed and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflicts of interest

Ethical issues: None.

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