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EDITORIAL

Next Generation Sequencing and it's Clinical Applications

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Next-Generation Sequencing (NGS) is a powerful, fast growing technology in genetic research, and plays a key role in clinical practice. NGS entails rapid sequencing of millions of DNA and RNA fragments with comprehensive insight and understanding of genomic structure, genetic variations and gene expression. It has brought a paradigm shift in molecular techniques, facilitating studies on constitutional disorders, cancers and infectious diseases. The use of NGS has opened up new avenues in research, diagnostics, and personalized medicine and has not only improved the diagnostic methods, but plays a key role in development of targeted therapy and precision medicine. One of the most significant developments in NGS is the notable reduction in sequencing costs, high throughput, better resolution and improved data analysis. These advancements have been instrumental in our understanding of complex genomic regions with greater precision and better understanding of diseases.

Various approaches can be used to analyze DNA using NGS techniques, such as whole-genome sequencing, whole-exome sequencing, and Targeted (panel) sequencing. **Whole-genome sequencing (WGS)** is a comprehensive genetic technique that involves assessment of whole DNA sequence of an individual's genome. It provides a comprehensive blueprint of an individual's genetic makeup, inclusive of all the genes, their coding and non-coding regions and regulatory regions. WGS helps in the identification of all genetic variations in a genome, from single-nucleotide polymorphisms (SNPs) to larger structural changes. However, due to high cost, it's use is less frequent as compared to whole exome sequencing and panel sequencing. **Whole-exome sequencing (WES)** is an approach that mainly targets protein-coding regions of the genome, known as the exome. The exome constitutes approximately 1–2% of the entire genome and carries the information of majority of disease-causing variants. WES is a cost-effective alternative to WGS for detection of genetic variants in constitutional diseases, cancers and for identification of specific variant in population genetics. **Targeted sequencing (Panel sequencing)** is most frequently used technique in diagnostics as it targets specific regions of the gene, but has limited role in identification of unknown variants. It is however cost effective and provides detailed disease-related information with much deeper coverage, thus helping in making precise diagnosis and robust clinical decisions. Many targeted panels are available commercially, that are used for specific diseases, in particular for various hematological malignancies, cancers and infectious diseases.

Sequencing Technologies: The technology for DNA sequencing has progressed rapidly leading to the development of three generations of sequencing technologies. **The first-generation platform**, **Sanger sequencing**, developed by a scientist Fred Sanger in 1977, revolutionized the field of genetics, by enabling rapid sequencing of DNA and RNA. In 1987, the first commercial automated sequencing

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machine, the Applied Biosystems ABI 370, was launched in the United States. **Second-generation sequencing** methods further revolutionized this field of molecular biology by enabling real-time sequencing of millions of DNA or RNA fragments. The advantages of NGS compared to traditional sequencing methods include higher throughput, rapid turnaround time, multiplexing and higher sensitivity in detection of less frequent variants in relatively less cost. The second-generation sequencing technologies offer short read sequencing and have a widespread application in diagnostics and clinical research.

Third-generation sequencing technologies represent the latest developments in sequencing techniques, with an advantage to overcome the limitations of techniques used in former generations. The third-generation technologies, such as PacBio and Nanopore, enable sequencing of much larger DNA fragments (long-read analysis) as compared to earlier methods (employing short read analysis). Among third generation sequencing techniques, Oxford Nanopore is the most sensitive and latest technology. It is based on nanopore technology, where a single-stranded DNA molecule passes through a nanopore, and changes in electrical current are measured to determine the DNA sequence. The benefits of Oxford Nanopore sequencing include analysis of long-read in real-time and easy portability.

Clinical applications of Next Generation Sequencing: NGS has a wide spectrum of applications in laboratory medicine and has become an integral part of precision medicine. The clinical usefulness of NGS is expanding rapidly. The technique is being widely used for diagnosis of various genetic disorders, cancers, infectious diseases, and is extremely useful for selection of targeted therapy. The technique is highly efficient, providing thorough genetic information in short time.

Inherited Genetic Diseases: Several studies have compared the diagnostic yield and cost of NGS with other types of genetic testing techniques, and it has been reported that the diagnostic yield of NGS for Mendelian and other constitutional disorders is much higher than G banding and high-resolution chromosomal microarray analysis (CMA). Besides, NGS is also used for prenatal screening, evaluation of non-invasive prenatal testing, preimplantation genetic disease testing, and other genetic disorders related to developmental delay, metabolic syndromes etc. Moreover, NGS-based HLA typing provides a complete information on all the HLA loci with better resolution and high-throughput, as compared to conventional HLA typing

Cancers: NGS can be used to identify different genetic variations, present in various malignancies. Identification of characteristic genetic abnormalities in cancers has become an integral part of precision medicine, helping in planning of personalized treatment strategies. Although the availability and use of whole genome sequencing and whole exome sequencing is increasing, targeted gene sequencing is the method of choice for diagnosis of cancers, due to its high read depth, cost-effectiveness, and rapid turnaround time. Various NGS panels are available and are used, not only for diagnosis of cancers, but are extremely useful for selection of targeted therapy and assessment of disease prognosis. NGS is also being used for MRD (Minimal residual disease) with much higher sensitivity and yield than other technologies used for MRD. Another application of NGS in oncology is identifying and enrolling patients into the appropriate clinical trials.

Microbiological Research: NGS plays a significant role in microbiological research. NGS panels have been developed and these panels can not only pick the causative organism, but can also be used to identify drug-resistant mutations including antimicrobial drug-resistant mutations and antiviral drug-resistant mutations. The data produced through NGS on microbial identification and drug resistance has been vital for disease surveillance, disease control, early therapeutic interventions and policy making (as was evident during the COVID-19

outbreak). During the epidemics of COVID-19, NGS technology was used to identify mutant stains of virus which played a vital role in limiting the spread of disease and monitoring of vaccine response. However, due to financial constraints and need for rapid detection of pathogen, NGS has not replaced existing standard point of care testing by PCR in diagnostic microbiology.

Though much of the focus of NGS has been on human genomics, NGS technology has made significant contributions in other areas as well, including agriculture, environmental monitoring, and microbial genomics. These applications point towards the multifaceted potential of NGS. NGS has also been increasingly integrated with other technologies, such as proteomics, metabolomics, and epigenomics. By retrieving the genomic data from these technologies, researchers can get deeper understandings of various molecular mechanisms and functional genomics. The integration of artificial intelligence (AI) and machine learning with NGS data analysis also holds a great potential. AI can predict disease risks, and enhance the interpretation of complex genetic data. This interaction between AI and NGS has a great potential to accelerate discoveries and improve diagnostic accuracy. However, as NGS becomes more widespread, ethical and privacy concerns become more and more important with regards to data ownership, and confidentiality. Responsible use of genomic information and maintenance of data confidentiality are critical for ethical use of NGS.

Challenges for Low- & Middle-income countries: Establishing NGS technology in low- and middle-income countries poses several significant challenges. The high initial investment for sequencing equipment, reagents, and consumables may be unaffordable. Even running and maintenance of NGS facilities require significant financial resources. NGS requires highly trained personnel including molecular biologists, and technicians, and many underdeveloped countries lack the necessary laboratory infrastructure to support this advanced technology. Moreover, a huge amount of raw data is generated by NGS and a highly competent and skilled bioinformatician is required for its analysis and interpretation. There is often a shortage of such professionals or lack of their collaboration with diagnosticians and clinicians. Thus, despite the vital role of NGS in personalized medicine, its Integration into existing healthcare systems and practices may be difficult, especially in regions where basic healthcare infrastructure is lacking. Besides this, awareness of the benefits of NGS among clinicians hampers its acceptance and use.

Sustainability of NGS programs for research and diagnostic purpose requires continuous funding, which can be difficult to secure in resource-limited settings. It is recommended that such settings, following the example of developing countries, may develop dedicated referral centers for NGS and all molecular tests requiring use of NGS technique may be sent to these referral centers. Keeping in view all these challenges, a multifaceted approach is required to overcome financial and infrastructural barriers. This includes training programs and workshops for capacity building, strengthening of referral centers and international collaborations. By implementing a combination of these strategies, we can secure the necessary resources to establish and sustain NGS capabilities.

To summarize, Next-Generation Sequencing has made a profound impact on diagnostics, research and precision medicine, and its potential use continues to expand. With its application in point-of-care testing in routine clinical practice, and cost reduction, it will become more accessible. This will open new avenues of knowledge and advancement and will have a profound impact on human health.

References

1. Hua T, Chitnis N, Monos D, Dinh A. Next-generation sequencing technologies: An overview. Human Immunology 82 (2021) 801–811. https://doi.org/10.1016/j.humimm.2021.02.012.

2. Kishore R. Kumar, Mark J. Cowley, Ryan L. Davis. Next-Generation Sequencing and Emerging Technologies. Semin Thromb Hemost 2019;45: 661–673. https://doi.org/10.1055/s-0039 1688446.
3. Dumschott K, Maximilian H.-W. Schmidt, Chawla H, Snowdon R and Usadel B. Oxford Nanopore sequencing: new opportunities for plant genomics? Journal of Experimental Botany, Vol. 71, No. 18 pp. 5313–5322, 2020 https://doi.org/10.1093/jxb/eraa263
4. Satam H, Joshi K, Mangrolia U, Waghoo S, Zaidi G and Rawoo S et al. Next-Generation Sequencing Technology: Current Trends and Advancements Biology 2023;12(997):2-25. https://doi.org/10.3390/biology12070997
5. Zhong Y, Xu F, Wu J, Schubert J and Marilyn M. Li. Application of Next Generation Sequencing in Laboratory Medicine. Ann Lab Med 2021;41: 25-43. https://doi.org/10.3343/alm.2021.41.1.25.
6. Naito Y, Aburatani H, Amano T, Baba E, Furukawa T and Hayashida T et al. Clinical practice guidance for next-generation sequencing in cancer diagnosis and treatment. International Journal of Clinical Oncology (2021) 26:233–283. https://doi.org/10.1007/s10147-020-01831-6.
7. SalkJ. and Kennedy S. Next-Generation Genotoxicology:Using Modern Sequencing Technologies to Assess Somatic Mutagenesis and Cancer Risk. Environmental and Molecular Mutagenesis 2020; 61:135-151. https://doi.org/10.1002/em.22342
8. John G, Sahajpal N, Mondal A, Ananth S, Williams C and Chaubey A et al. Next-Generation Sequencing (NGS) in COVID-19: A Tool for SARS-CoV-2 Diagnosis, Monitoring New Strains and Phylodynamic Modeling in Molecular Epidemiology. Curr. Issues Mol. Biol. 2021, 43, 845–867. https://doi.org/10.3390/ cimb43020061.
9. Gill S, Xu M, Ottaviani C, Patros P, Bahsoon R and Shaghaghi A. AI for next generation computing: Emerging trends and future Directions. Internet of Things 19 (2022) 100514. https://doi.org/10.1016/j.iot.2022.100514.
10. Rute Pereira 1,2, *, Jorge Oliveira 2,3, y and Mário Sousa. Bioinformatics and Computational Tools for Next-Generation Sequencing Analysis in Clinical Genetics. J. Clin. Med. 2020, 9, 132; https://doi.org/10.3390/jcm9010132