MOLECULAR MEDICINE
ASSIGNMENT

Mutation detection and DNA sequencing for disease association

INTRODUCTION

DNA sequencing and mutation detection are fundamental techniques in genetics and molecular biology. They played a pivotal role in understanding disease mechanisms and genetic predisposition.
These technologies work together to enable the identification of genetic variations, such as mutations and polymorphisms, that contribute to the onset, advancement, and severity of various diseases.

DNA sequencing is the laboratory technique that aims at determining the exact sequence of nucleotides or bases, in a DNA molecule. In other words DNA sequencing means determining the order of the bases that make up the DNA molecule. The sequence of the bases tells scientists the kind of genetic information is found or carried in a particular segment of the DNA.
This sequence encodes the biological information essential for cells to develop and operate. Determining the DNA sequence is essential to understand the roles played by genes and other genomic components.
Since DNA sequencing provides an elaborate and comprehensive view of one's genetic makeup, it permits researchers to pinpoint specific change in the DNA sequence that may be linked to hereditary conditions.
There are various techniques or methods for carrying out DNA sequencing. Ranging from polymerase based DNA sequencing such as Sanger sequencing and Massive parallel sequencing to Single molecule DNA sequencing such as Nanopore DNA sequencing techniques.

In contrast, Mutation detection focuses on identifying alterations in the DNA sequence that may occur due to environmental factors or errors during processes such as DNA replication.

These techniques, together, play a key role in elucidating the genetic foundations of diseases, guiding personalised medicine approaches and for carrying out targeted therapy.

Following the growth of our knowledge in terms of the human genome, the integration of mutation detection and DNA sequencing in research helps enhance our ability to monitor, diagnose and treat a wide array of genetic disorders and complex diseases.

HUMAN GENOME PROJECT

The HUman Genome Project is a groundbreaking initiative that focused on decoding the entire human genome, which includes understanding the sequence and composition of all human genetic material. This massive initiative relies on the idea that isolating and analysing the genetic material found in DNA will give researchers significant fresh insights into how diseases originate and drive novel techniques to both prevent and treat them.

One of its main goals is to develop research tools that help scientists identify genes associated with various diseases, in both rare and common diseases. These tools are known as positional cloning.[1]

This enables researchers to identify the disease linked genes without having to identify the gene's protein product.

Diseases such as Cystic Fibrosis, Wilm's Tumour, Fragile X Syndrome, Wilson's Disease, Bloom Syndrome, Early Onset Breast Cancer and so on were identified using Positional cloning.

A significant aspect of this project includes addressing the ethical, legal and social implications of genetic technologies and raising public awareness regarding the issue.

With respect to mutation detection, the human genome project has significantly advanced these fields by providing genetic maps and sequences. This progress has hence enabled researchers to detect mutations more accurately and to efficiently sequence DNA, thus aiding in identification of genetic variants linked to diseases.

The Human Genome Project also mapped the genomes of model organisms like mice and rats, which play a crucial role in studying and understanding complex genetic disorders such as diabetes, hypertension and alcoholism.

These advancements are improving our understanding of disease mechanisms and contributing to the development of targeted therapies and personalised medicine.

TYPES OF MUTATIONS AND THEIR IMPLICATIONS

Single-gene, chromosomal, and multifactorial illnesses are the three primary categories into which genetic disorders are generally divided.

Mendelian disorders, often known as single genes, are caused by mistakes in a gene's DNA sequence. These disorders include X-linked recessive (XR), X-linked dominant, Y-linked, and autosomal dominant (AD) and autosomal recessive (AR).

Chromosome aberrations, such as structural and numerical damage, are the cause of chromosomal diseases.

Genetic mutations causing diseases have been found using molecular and cytogenetic methods.

Precise illness identification is necessary for effective patient care, genetic counselling, and preventative measures.

Mutations of DNA, which can occur in coding regions that alter amino acid sequences of proteins or in noncoding regions that affect gene expression, such as by altering promoter strength. Mutations are broadly classified into chromosomal and DNA-based mutations and can also be categorised by their functional impact:

- Loss-of-function mutations result in reduced or absent gene product activity.
- Gain-of-function mutations increase gene product activity or confer a new, potentially harmful property.
- Haploinsufficiency occurs when one allele's product is insufficient for normal function, as seen in LDLR mutations causing familial hypercholesterolemia.
- Dominant negative mutations involve a mutated allele interfering with the normal allele's function, such as in collagen-related disorders.
- Gain-of-function mutations can lead to new or excessive activity, as observed in FGFR3 mutations in achondroplasia.

Mutation detection plays a crucial role in genetic diagnosis, utilising advanced technologies for applications like genetic diagnosis, prenatal diagnosis, and forensic testing.

These mutations can be detected by techniques such as direct sequencing, genetic linkage analysis using DNA markers, restriction enzyme digestion sand so on.

There are many approaches utilised for the identification of known mutations. Generally starting with Polymerase Chain Reaction, various techniques can be performed such:-

- DNA microarray:- These chips or microarrays are significant for testing multiple mutations. In this technology, single DNA strands containing sequences of various target genes are attached to a solid surface in an array

format. The sample DNA or complementary DNA (cDNA), labelled with fluorescent dyes, is then hybridised to the chip. A laser system is used to detect fluorescence, which reveals the presence of specific sequences and their quantities in the sample.

- Multiplex ligation-dependent probe amplification (MLPA) - It is a technique used to detect deletions and duplications of up to 50 different DNA or RNA sequences by hybridising a set of probes to the genomic DNA, where each probe consists of two halves. After ligation of adjacent probes, the target sequence is amplified using PCR. The presence and quantity of deletions or duplications are determined by analysing the relative peak heights.
- DNA sequencing:- It is a powerful technique in molecular genetics, where sequencing is carried out at the nucleotide or base level. In this method, the sequence of a region of interest is obtained by using PCR product as a template.

DNA SEQUENCING TECHNOLOGIES

The knowledge of DNA sequences allows us to understand a wide range of biological processes and provides critical information regarding the molecular basis of life. The sequence or order of nucleotides in DNA, specifies the order of nucleotides in RNA which in turn encodes the informational content of proteins. The base order governs DNA shape and thus its function as well. I t provides a molecular program that can determine normal development, expression of hereditary illness, or cancer. Understanding DNA and the ability to control them has sped up the development of biotechnological processes and led to the production of molecular techniques that gives scientists the tools to ask and answer crucial questions.

There are two main techniques that are typically used to determine the DNA sequence, the first uses chemicals to specifically degrade the DNA strand, and is referred to as Maxam–Gilbert DNA sequencing, while the second method makes use of specific inhibition of enzymatic DNA synthesis and is referred to as Sanger Sequencing.

Maxam-Gilbert sequencing was an early approach that was important in the development of DNA sequencing since it gave a foundational way for sequencing small DNA segments through selective chemical cleavage. Unfortunately, its application is now limited due to its technical complexity, labour-intensive nature, and use of toxic chemicals. Sanger sequencing was created subsequently and was the industry standard for many years due to its excellent accuracy and dependability. Because of its accuracy, it is still useful for clinical applications and small-scale

sequencing initiatives. Sanger sequencing is less appropriate for large-scale genomic research due to its relative slowness and high cost, especially when contrasted with contemporary high-throughput technologies, notwithstanding these benefits.

DNA sequencing technologies have now evolved to more accurate high throughput techniques, such as Next-Generation SEquencing(NGS).

NGS was developed in the early 2000s, by several companies including Illumina, Roche and so on. It makes use of large scale parallel sequencing, allowing millions of DNA fragments to be sequenced simultaneously. Methods differ between platforms, but , in general sense, it involves fragmenting the DNA, attaching adapters, amplifying the fragments, and sequencing them in parallel using techniques such as sequencing-by-synthesis or sequencing-by-ligation.
NGS enables the rapid and cost-effective sequencing of entire genomes. This technology has revolutionised genomics by facilitating comprehensive studies in personalised medicine, cancer research, and evolutionary biology. However, NGS platforms can exhibit slightly lower per-read accuracy compared to Sanger sequencing, though this is often offset by deep sequencing coverage.

APPLICATIONS
The recent advent of next-generation sequencing technologies has dramatically changed the nature of biomedical research. Human genetics is no exception-it has never been easier to interrogate human patient genomes at the nucleotide level to identify disease-associated variants. To further facilitate the efficiency of this approach, whole exome sequencing (WES) was first developed in 2009. Over the past three years, multiple groups have demonstrated the power of WES through robust disease-associated variant discoveries across a diverse spectrum of human diseases.[2]

Whole exome sequencing (WES) is a technique to selectively capture and sequence the cistros of all annotated protein-coding genes. Together with next-generation sequencing (NGS) platforms, it facilitates the analysis of functional regions of the human genome. WES has emerged as a powerful and popular tool for researchers elucidating genetic variants underlying human diseases overcoming certain limitations. This technique has been used in identifying genetic variants associated with various diseases.

DNA sequencing techniques such as WES, has significantly advanced the detection of mutations in diseases inherited in a recessive pattern. Traditionally, recessive diseases were analysed through linkage analysis using large family pedigrees to identify genomic intervals containing disease-causing variants.

With NGS, it is now possible to directly sequence entire genomes or exomes to detect homozygous variants, especially in individuals from consanguineous marriages. This approach helps filter out common variants, focusing on rare ones more likely to cause disease.
For instance, WES was used to identify recessive mutations in the WDR62 gene in Turkish patients with developmental brain defects, demonstrating its effectiveness in discovering novel disease-associated genes and enhancing our understanding of genetic disorders.[3]

Another application is in Mendelian diseases with dominant inheritance patterns, which are more challenging to analyse genetically because heterozygous variants are numerous and harder to detect than homozygous ones, and they are also more prone to errors. However, Whole Exome Sequencing (WES) can still be a powerful tool for investigating these diseases, particularly in familial cohorts. When large families with the disease are available, sequencing at least two affected members can help identify shared variants that may be disease-causing.
This approach was effectively demonstrated in a study on familial amyotrophic lateral sclerosis (FALS), where WES was performed on two distantly related affected members from each of two large families.
By focusing on rare functional variants that segregated with the disease, researchers were able to pinpoint a common gene, PFN1, associated with FALS, highlighting WES's utility in uncovering genetic causes of dominant diseases.[4]

CONCLUSION

In conclusion, mutation detection and DNA sequencing are integral to understanding the genetic basis of diseases. These technologies have evolved from basic sequencing techniques to advanced high-throughput methods, significantly enhancing our ability to identify genetic mutations associated with a wide range of diseases. The Human Genome Project has laid the foundation for these advancements, enabling detailed exploration of genetic variations. Techniques such as Whole Exome Sequencing (WES) and Next-Generation Sequencing (NGS) have revolutionized genetic research, facilitating the discovery of disease-associated variants, even in complex genetic disorders. As these technologies continue to advance, they hold immense potential for personalized medicine, targeted therapies, and improving our overall understanding of human genetics.

REFERENCES

1. Collins, Francis S, and Leslie Fink. “The Human Genome Project.” Alcohol health and research world vol. 19,3 (1995): 190-195.
2. Goh, Gerald, and Murim Choi. “Application of whole exome sequencing to identify disease-causing variants in inherited human diseases.” Genomics & informatics vol. 10,4 (2012): 214-9. doi:10.5808/GI.2012.10.4.214
3. Dixon-Salazar TJ, Silhavy JL, Udpa N, Schroth J, Bielas S, Schaffer AE, et al. Exome sequencing can improve diagnosis and alter patient management. Sci Transl Med. 2012;4:138ra178.
4. Wu CH, Fallini C, Ticozzi N, Keagle PJ, Sapp PC, Piotrowska K, et al. Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature. 2012;488:499–503.