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Cover Story **PREVENTIVE WELLNESS** BY THE POWER OF GENOMICS

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Featured article CHALLENGES AND OPPORTUNITIES IN PRECISION MEDICINE USING BIG DATA- PART 2

WORDS FROM THE FRONTLINE



Greetings to all readers,

I am pleased to be able to share my experience and viewpoint on MedGenome's journey from a humble beginning a decade ago to a market leader today. This extraordinary journey would not have been achieved without the collaborative efforts of numerous teams, especially the often-unsung heroes of the software and IT infrastructure departments.

The Backbone of Modern Business Operations

In the fast-paced world of Genetic Testing services, Genomics Research, where accuracy, efficiency, and reliability are of paramount importance, the contributions of software and IT infrastructure teams cannot be overstated. From laboratory processes to operations, finance, and sales, these teams have played a pivotal role in driving the growth and success of MedGenome.

Laboratory Excellence with Software Solutions

Behind every accurate and timely genetic test result lies a sophisticated web of software applications and IT infrastructure. The software and IT teams have tirelessly worked to develop and maintain LIMS (Laboratory information management system) that ensure seamless sample tracking, data management, and result reporting. Their dedication has not only increased the efficiency of laboratory operations but has also minimized errors, leading to improved patient outcomes.



Sales, Finance, and Operations: Powered by IT

One of the key factors behind our success has been the extension of our software modules to support essential processes across various departments, including but not limited to Laboratory, Sales, Finance, and Operations. By integrating these modules seamlessly, we have been able to streamline our operations, enhance efficiency, and deliver an exceptional experience to our Customers and Clinicians.

Simultaneously, our IT infrastructure team has also played a crucial role in facilitating our growth. From managing a handful of servers during our early days, they now oversee a vast network of hundreds of servers and handle massive storage capacities across different laboratory locations in the country. Their expertise and meticulous planning have ensured that our systems are robust, reliable, and capable of handling the enormous amount of data generated by our facility.

MANGO: Bridging Gaps and Accelerating Solutions

In our quest for continuous improvement, we have developed a foundational framework called MAnGo (MedGenome Analytics for Genomics) that serves as the backbone of our IT infrastructure. MAnGo has proven to be an invaluable tool in bridging gaps across departments and promoting cross-functional collaboration.

This framework provides quick solutions by integrating different software applications, allowing data to flow seamlessly from one application to another. One distinctive use for MAnGo has been the automated upload of raw-data files and the subsequent provision for customers to download them from the Customer Portal. This has greatly enhanced efficiency and freed up a substantial amount of time for the clinical personnel.

Looking Ahead

As we reflect on our achievements, we remain focused on the future. We are committed to further strengthening our software and IT infrastructure teams, empowering them to drive innovation, efficiency, and excellence across our organization. We envision taking our important software applications, such as our MG LIMS, NxGen LIMS (Lab module) and interpretation applications such as Varminer and Oncominer, to the cloud. This will not only enhance accessibility and scalability but also enable us to offer them as Software-as-a-Service (SaaS) packages to other companies worldwide. By doing so, we aim to empower organizations globally with the tools they need to unlock the potential of genetic testing and personalized medicine, and thereby helping achieve MedGenome's objective of democratizing the access of quality and affordable genetic solutions to millions around the world.

Furthermore, our bioinformatics software teams are leveraging the power of Artificial Intelligence (AI) and Machine Learning (ML) to make the best use of the vast amount of genomic data at our disposal. These technologies enable us to derive meaningful insights, identify patterns, and optimize our testing processes, ultimately leading to better patient outcomes.

I extend my heartfelt gratitude to every member of the IT and Software teams, whose tireless work behind the scenes has propelled us to new heights. Their domain expertise, passion, and ability to adapt to evolving technologies have been the driving force behind our market leadership.



Contents



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As MedGenome continues to work towards its goal to leverage omics and develop an ecosystem that is more reflective of the healthcare consumers, we continue to drive our key messages and share our perspectives on the genomics industry with the media community. We are also proud to be recognised as a leader in the industry, which is a reflection of our good work and the reputation that we have built. Sharing some of the latest highlights in this PR Newsletter.



Tuberculosis: Is whole genome sequencing the gold standard for TB testing?

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Role of BRCA1 and BRCA2 Testing in **Understanding Cancer.**

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Most Talked About

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MedGenome Connect



The marketing collateral designed to create awareness about different offerings under Claria division.

Flyers – PGT-M Flyer, Claria Offerings





We launched FSHD1 test, which is India's first optical genome mapping based test for facioscapulohumeral muscular dystrophy (FSHD) Type 1. The test was launched during a conference at NIMHANS which was attended by leading clinicians of India and in Kolkata as well. We also had insightful recommendations for our services from Dr Nalini and Dr Senna, NIMHANS. They spoke about how MedGenome has been helping them with precise and cost-effective genomic diagnostic services.

FSHD1 is a common form of muscular dystrophy with an extremely complex genotype. It is a type of progressive myopathy which needs precise testing for diagnosis. MedGenome is the first and only lab in India & South Asia to perform this test based on optical-genome mapping. Empowering clinicians with precise diagnosis and accurate results and supporting patients with affordable tests has been the vision that has always inspired MedGenome.

View videos posted on linkedin:

#FSHD1 #MedGenome #PowerOfGenetics #PreciseTesting #Experts #Leaders #Pioneers #Accuracy #MedGenomeTestimonials

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MEDGENOME 61,707 followers 4mo

MedGenome is thrilled to have Prof. A. Nalini along with Dr **seena vengalil** and Dr. **Saraswati Nashi**, Department of Neurology, NIMHANS launch our latest offering, Facioscapulohumeral Muscular Dystrophy Type 1 (FSHD1), at the NIMHANS International Symposium on Neuromuscular Disorders - NISNMD 2023.

Facioscapulohumeral Muscular Dystrophy Type 1 (FSHD1) is India's First Optical Genome Mapping based test. We are thrilled to share that we are the 1st & only genetic lab in India & South Asia to perform FSHD1 test.

FSHD1 accounts for 95% of the FSHD cases, which is caused by a contraction of the polymorphic macrosatellite repeat D4Z4 on chromosome 4q35.





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MedGenome is proud to receive this insightful recommendation from Prof. Dr. A. Nalini, Department of Neurology, NIMHANS, on Facioscapulohumeral Muscular Dystrophy Type 1 (FSHD1), India's First Optical Genome Mapping based test.

Dr. A. Nalini, spoke about how this test is going to benefit many patients with Facioscapulohumeral Muscular Dystrophy Type 1. She also appreciated our contribution to genomics research and introducing many innovative and much needed genetic tests. We thank Dr. Nalini for sharing her valuable thoughts regarding the **#FSHD1** test.



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MedGenome was thrilled to have Dr Arijit Chattopadhyay, Senior Consultant Paediatric Neurologist, Apollo Hospitals, Dr.Kalpana Datta -Professor and HOD of Department of Paediatrics-Calcutta Medical College, Dr.Mandira Roy-Assistant Professor, Department of Paediatrics, Santiniketan Medical College & Hospital along with Dr Parag Tamhankar, Consultant Geneticist, MedGenome Labs, launch our latest offering, Facioscapulohumeral Muscular Dystrophy Type 1 (FSHD1), at the 3rd National CME on Paediatric Neurology at the Park Hotel, Kolkata.

Facioscapulohumeral Muscular Dystrophy Type 1 (FSHD1) is India's First Optical Genome Mapping based test. We are thrilled to share that we are the 1st & only genetic lab in India & South Asia to perform FSHD1 test.

FSHD1 accounts for 95% of the FSHD cases, which is caused by a contraction of the polymorphic macrosatellite repeat D4Z4 on chromosome 4q35.



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We also ran online campaign on Neurogenetics, Epilepsy, newborn screening and a research paper on Neuro Muscular disorder. #MedGenome #Epilepsy #NeuroGenetics #PrecisionMedicine #GeneticTesting #Leader #Pioneer



MEDGENOME 61,707 followers 2mo

Epilepsy is a neurological disorder characterized by recurrent seizures. In significant cases, it has an underlying genetic factor. Genetic testing provides useful information of the genetic causes, contributors, prognosis of the disease, and precision medicine strategies to manage epilepsy.

To know more about Genetic Tests for Epilepsy and NeuroGenetics please visit: https://lnkd.in/e5stKvmc or Call 1800 103 3691 or write to us at techsupport@medgenome.com.

Executed several activities in the last quarter. We had a photobooth engagement at AOCN Pune conference and successfully executed multi-dimensional **Rare Disease Day** campaign. This campaign was spread across all zones and external & internal KOLs were engaged for these activities. Webinars were conducted both for the customers and the doctors. We also launched FSHD1 test, which is India's first



Past guarter digital campaigns were focused on Liguid biopsy, ThyroTrack, Blood cancer & Endometrial cancer prognostication panel. We had an insightful real life case study on LungTrack Advance which was promoted across all platforms. On offline engagement perspective we had more than 25 CMEs and RTM spread across all location.



Liquid Biopsy test helps in screening and early detection, treatment selection, treatment monitoring, determining recurrence in cancer patients, and detection of Minimal Residual Disease (MRD). It can be used as an alternative, #Empowering clinicians with precise diagnosis and accurate results has been the vision that has always inspired

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#ProstateCancer #BreastCancer #OvarianCancer #PancreaticCancer1m

serial, reflex, and complementary test to Tissue Biopsy test.

MEDGENOME 61,709 followers 3mo

MedGenome. We are dedicated to delivering the power of genetics to help you lead healthy and happy lives.

#MedGenomePrima #GeneticTesting #Genomics #LiquidBiopsy #GeneExperts #PrecisionTesting #PowerOfGenetics #CancerDiagnosis #GenomicProfiling #SolidTumors #LungCancer #HRRGene

> ThyroTrack is India's first Next Generation Sequencing (NGS) based test that screens genetic alterations in thyroid cancer-related genes curated according to ATA (2015) & NCCN (2022) guidelines, covering SNVs, InDels & Fusions. It helps to understand thyroid tumor genetics with accurate diagnostics, allowing precise & personalized treatment delivery, and detection of therapeutic targets.

> > ...

#ThyroTrack #ThyroidCancer #AccurateDiagnosis #Precision #GeneticTesting **#Experts #Pioneers**



MEDGENOME 61,705 followers 4mo · Edited

This is the story of Sujata who was battling with Lung Adenocarcinoma. Sujata had an episode of cancer and was responding well to the treatment but faced a relapse in 2023.

Re-biopsy was not feasible. Hence, as an alternative the clinician recommended to go for #MedGenome #LungTrackAdvance, an NGS-based Liquid Biopsy test. Watch the video to know how the clinician was empowered with precise information, and helped to put Sujata on an improved treatment plan.

#Genomics #GeneticTestingIndia #PrecisionTesting #Experts #Pioneers #LiquidBiopsy #LungCancer #LungAdenocarcinoma #PrecisionMedicine





The past quarter was very encouraging and has been a very active for Micra with a focus to engage more clinicians with CME, conference participation, and test specific mailers and social media posts. We have also posted Video on Gastrointestinal Pathogen Panel and Pan Viral Neuro Panel. The team also completed a comprehensive Micra Brochure which contains in depth. information about all the tests along new tests launched in Micra.









Powered by 🖧 MEDGENOME

We continued to evolve the various tests being offered under the Genessense portfolio in this quarter. We focussed on forging partnerships with a few aggregators & conversations were initiated with some of the major players in this space. To ensure awareness about this brand, we posted on social media. The version 1 of the Comprehensive Genessense, Kardiogen, Curegen, Diabetogen, Hypersense, Neurosense, Visigen, Carriergen and Oncogen brochure and report were created, and the test was made available for internal testing with a prospective partner organisation. We are also revamping our Genessense Website with packages details, Scientific Insights on staging. We have collaborated with Tata 1mg for marketing Genessense Individuals tests.



Marketing of Genessense Tests on TATA 1mg Website



What's new

Research Publications

- South Asian medical cohorts reveal strong founder effects and high rates of homozygosity <u>Read more</u> Journal : Nature Communication PMID: 37291107
- The genetic drivers of juvenile, young, and early-onset Parkinson's Disease in India (Preprint version) <u>Read more</u> Journal : Movement Disorders

Tests launched

Karyoseq

2

- Respiratory panel + Pneumocystis jirovecii combo Qualitative PCR
- **3** Post Transplant viral Panel

Imp. Announcement MedGenome acquired Prognosis labs

MedGenome has acquired Prognosis labs, a diagnostics lab/company based in NewDelhi. https://www.prlworld.com/. This acquisition will help us to expand our cover age and offerings in the Northern region of the country and shot in the arm for our business teams to achieve their goals and also support in logistics. Established in 2013, Prognosis Laboratories is NABL accredited and provides diagnostics, microbiology and radiology services. The company has a test catalogue of over 700 tests spread across pathology, radiology with a specialization in molecular diagnostics, histopathology, immunology, biochemistry, microbiology and serology.



From Our US Office

We successfully completed the past quarter by acquiring some new clients. With new plans in place, we are looking forward for a fruitful year that focuses on our novel solutions in the areas of Single Cell, Immune Profiling and Bioinformatics. Our team is geared up in promoting and exploring new arenas such as spatial transcriptomics.

Towards this end, we will be attending many events and planning to forge new partnerships.

We have recently published new articles on https://research.medgenome.com/blog/

- 1. Introduction to Single Cell Sequencing Cite-Seq Series 3
- 2. Single Cell Sequencing New Insights

We encourage you to share your viewpoints and articles of interest at mgus-blog@medgenome.com

dGenome

Sneak Peek into the World of Science

Preventive wellness by the **POWE** of genomics.

"Now, predict your genetic risk for a health condition".



Venu Seenappa Ph.D Senior Scientist

An Overview

Preventive wellness is the proactive process of being aware and making decisions and choices to attain a healthy wellbeing. It can be achieved by predictive testing that alerts an individual for any future risk associated with the various health conditions. Existing risk predictions are based on factors such as weight, lifestyle and blood measurements, which are variable through a person's lifetime and cannot be used to predict risk accurately. On the other hand, genetic screening tests are an independent risk predictor, free from variability and are based on a person's genetic makeup (DNA) Figure 1.



Figure 1: Disease risk prediction by genetic screening test.

Genetic research over the past decade has realized that our risk for many common conditions such as heart disease and diabetes are not influenced by just one gene, instead, multiple genes work in tandem to influence our risk for common diseases. Growing healthcare awareness, advantages of predictive genetic testing and the emergence of whole genome genotyping arrays and next-generation sequencing (NGS) technology is accelerating the demand for disease risk prediction testing. Knowing a person's risk for certain common diseases can help in timely lifestyle changes, health monitoring and disease management.

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The high disease prevalence urges for genetic risk screening tests

The rising prevalence of non-communicable diseases (NCDs) such as lifestyle associated disease and cancers remains a serious and growing challenge to public health. These chronic disorders put a huge burden on individuals affected and their families. Further, it also has an economic and societal impact. People living with these chronic ailments are at risk of developing several life-threatening complications, leading to an increased need for medical care, reduced quality of life and premature death.

Genetic variations in your DNA predispose to common diseases like coronary artery disease (CAD), type 2 diabetes (T2DM), hypertension, obesity, neurological disease, hereditary cancer and few more. These



diseases are at high prevalence in the population across different ethnicities and geographical regions. The recent stats are indeed alarming, to look few i) as per 2021 data from International diabetes federation (IDF), 537 million adults have diabetes globally, India accounting 74.2 million people living with diabetes and crossing 100 million as per latest ICMR data ^{1,2} ii) an estimated 17.9 million people died from Cardiovascular diseases (CVDs) in 2019, and the prevalence of total CVD reached 523 million in 2019 ^{3,4} iii) as per latest WHO data, 1.28 billion adults have hypertension globally ⁵.

The science behind predicting the genetic risk for a health condition



Though multiple genetic tests are in practice for screening a health condition, the prediction of genetic risk for a common disease is majorly driven by polygenic risk score (PRS) based genetic tests. Post human genome sequencing project, the genome wide association studies (GWAS) gained researcher attention and started publishing data since 2005 onwards to identify genomic variants that are statistically associated with a risk for a health condition/disease or a particular trait. In this approach, researchers started looking for genomic variants that occur more frequently in those with a specific disease or trait compared to those without the disease or trait. Although GWAS studies

generated huge genetic data, clinical translation was not delivered as estimated. In parallel, the concept of a genetic/polygenic risk score (PRS) was circulating among researchers for several years for its potential clinical use. However, in 2018, a group of researchers from Broad Institute led by Amit V. Khera used the data generated from the GWAS and utilized imputation methods to assess millions of common genetic variations associated with 5 common diseases (e.g. CAD, T2DM). Further, the research team applied a computational algorithm that combines information from all the variants into a number, or PRS, that reflects a person's inherited susceptibility to the specific disease ⁶. The PRS score identified people at normal and high risk based on their genome. Additionally, in 2020, PRS for CAD in South Asians was published by collaborative work between MedGenome labs and Broad Institute ⁷.

Prediction by PRS based genetic tests

There are millions of genetic variations, with independent minor effects that have a combined influence on your chance of developing disease (Figure 2). PRS prediction quantifies the contributing effects into a score and estimates whether the tested individual is at a high, moderate or average risk of a specific disease. The PRS based tests must follow ethnic specific validation to generate the background distribution plot to ensure the PRS score accuracy to the specific genetic markers for the generation of PRS provides a highly precise score. To provide an example, the MedGenome CAD study comprised about 3000 samples with clinically well characterized cases and controls ⁷. The genetic risk for CAD is calculated from 6.6 million genetic markers which are implicated in the CAD and is given as a validated PRS. The positive predictive value (PPV) of this validation study showed 71% of the individuals who came in the high-risk zone turned up to be diagnosed with CAD condition. While the remaining 29% individuals in the high-risk zone who did not have CAD condition or showed symptoms but may be at a higher chance of developing CAD later on. Similarly, MedGenome labs have recently developed and launched multiple PRS based tests upon validation studies for common disease under the portfolio of Genessense (Table 1).



Single gene disorders, where changes in a single gene causes disease e.g Cystic Fibrosis, Beta Thalassemia



Complex disorders where many small changes to multiple genes can cause disease e.g. CAD, diabetes, hypertension

Figure 2: Schematic representation of Monogenic disease identification and Polygenic Risk Score (PRS) based disease risk prediction.

Table 1: MedGenome scientific validation of PRS based tests at a glimpse

Disease	Validation study details	No. of genetic marker in the model 6.6 million	
Coronary Artery Disease (CAD)	Validated in the South Asian cohort of about 3000 case-control samples. Study is published.		
Type 2 Diabetes (T2DM)	Validated in the South Asian cohort of about 11,000 case-control samples from 3 independent cohorts. The study manuscript is in the process of getting published.	6.9 million	
Obesity/BMI	Validated in the South Asian cohort of 14,000 individuals from 3 independent cohorts. The study manuscript is in the process of getting published.	2 million	
B Hypertension	Validated in the South Asian cohort of about 2700 case-control samples. The study manuscript is in the process of getting published.	10,000	
Parkinson's Disease (PD)	Validated in the South Asian cohort about 2000 case-control samples. The pilot study is published.	1805	
Age-related Macular Degeneration	Validated in the South Asian cohort about 600 case-control samples. The study manuscript is in the process of getting published.	47	

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AHA and ACMG statement on clinical applications of PRS based tests



Recently American heart association (AHA) has released a statement on the addition of PRS to the AHA/ACC ASCVD risk calculator significantly improves prediction for CVD events ⁸. Similarly, American college of medical genetics and genomics (ACMG) has released a statement on the clinical application of PRS ⁹. ACMG statement outlines the general considerations for the clinical application of PRS, to summarize few important points:



A Scientific Statement from American Heart Association (AHA)



The inclusion of PRS in the AHA/ACC ASCVD risk calculator significantly improves prediction.....

Above excerpt is from the latest Scientific statement published by American Heart Association (AHA)

01

PRS tests are not a diagnostic test, instead the PRS test provides a statistical prediction of increased genetic risk for a health condition. These tests are genetic risk screening tests.

02

PRS is one part of the disease story, A low PRS does not completely rule out significant risk for the developing disease or condition in question, which may depend on other factors like lifestyle, environmental and monogenic variants.

03

PRS models must be optimized and validated for testing population, because if the risk prediction of a PRS is derived from a population that is different from the individual being tested, then the PRS results may have a poor predictive value for the tested individual.

04

Before testing, genetic counselling must be offered by a test provider to explain the utility of the test and implications of the results.

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Various clinical studies showed proven benefits of PRS

In a study conducted by Merino and team on combined association of genetic risk and diet quality with the risk of type 2 diabetes showed risk gradient for diabetes with increasing genetic risk and decreasing diet quality ¹⁰. The same study also suggested that a healthy diet is associated with lower diabetes risk across all levels of genetic risk. Similarly, Vaura and team studied association of polygenic risk scores for blood pressure with incident hypertension in about 2,00,000 individuals and observed a high PRS conferred 2.3-fold risk of hypertension and 10.6 years earlier hypertension onset ¹¹. An interesting study on coronary artery disease involving 55,685 participants, indicated adherence to a favorable/healthy lifestyle was associated with a 46% lower relative risk of coronary events than an unfavorable lifestyle in high genetic risk participants ¹². This research observation also showed reduction in the standardized 10-year incidence of coronary events from 10.7% for an unfavorable lifestyle to 5.1% for a favorable lifestyle in one of the cohorts. Furthermore, a study on statin therapy for coronary heart disease was associated with reduced risk for a first CHD event by 46% in high-risk group vs 26% among all others ¹³. These research findings also demonstrated that individuals at high genetic risk have a greater risk of CHD and can derive greater benefit from statin therapy to prevent a first CHD event.

Application of genetic risk score in central eye disorder also known as Age-related macular degeneration (AMD) showed significant benefits in early predictions. A research study showed addition of genetic risk score (GRS) to the demographic/environmental risk factors considerably improved the prediction performance for AMD ¹⁴. A similar research investigation in a Japanese cohort showed that high genetic risk score patients displayed a 51% risk for second eye involvement as compared to 2.3% risk in low-risk counterparts within 10 years from their first visit, indicating the genetic risk score has high predictive ability for second eye involvement of AMD ¹⁵.



Although PRS based genetic risk score tests are emerging, the sequencing of full-length targeted genes, whole exome, and whole genome are beneficial for risk assessment of various health conditions. Sequencing an individual DNA will provide information on presence or absence of pathogenic/likely pathogenic variants in the coding or non-coding region of the gene which are associated with the disease susceptibility, carrier status or predisposition for common disease. To get more insights on such tests, few examples are listed below:

Hereditary cancer: About 5-10% of all cancers can be caused by inherited genetic changes ¹⁶. Individuals who have inherited a variant/mutation in cancer-susceptibility gene are at a significantly higher chance of developing certain types of hereditary cancer compared to those without variant. It's usually recommended when certain types of cancer run in a family and a gene mutation is suspected.

Carrier testing: In India alone over a million babies are born with genetic diseases each year and globally the incidence of genetic disorder is estimated as 5.32% in newborns when followed up to 25 years ^{17, 18}. In the NGS technology era, high coverage DNA sequencing helped individuals and families to uncover their previously unknown genetic risk for their carrier status of rare genetic disorders (only recessive). The carrier screening test helps individuals to find out whether they or/and their partner carry any genetic defect in their genes that could cause an inherited genetic disorder in their child.



Pharmacogenetics (PGx) and Personalized medicine: The science of how genes affect a person's response to drugs is called Pharmacogenomics. Research studies showed that people respond differently to the same drug based on an individual's genetic profile indicating the necessity of personalized medicine. With the help of advanced technology, using an individual's genetic profile the PGx tests can now tell a) Is a medication effective, b) whether any need of different doses than average and c) are individuals at risk for serious side effects due to altered metabolism of the medication. As an example, Asians are poor and intermediate metabolizers of CYP2C19 gene compared with African Americans and Caucasians¹⁹. CYP2C19 is a prevalent hepatic enzyme that metabolizes at least 10% of all commonly prescribed drugs. This results in reduced activity of drugs like clopidogrel, with Asians being at higher risk of adverse effects from this drug such as heart attack and stroke. Clinical Pharmacogenetics Implementation Consortium (CPIC) and PharmaGKB provide evidence-based guidelines and recommendations for many drugs.

Conclusion

Preventive wellness by utilizing the genetic information of an individual, to assess the genetic risks for a disease with cutting-edge technologies and well-validated scientific approaches will empower an individual for a true well-being. Predicting the genetic risk of an individual before presenting the symptoms will forewarn, help an individual to prevent and mitigate the disease related complications. The PRS based risk scoring is gaining momentum along with the existing sequencing-based disease risk prediction. With great innovation in the field of genomic medicine, we at MedGenome, providing scientifically validated direct to consumer (DTC) genomic wellness preventive genetic tests under Genessense portfolio with the broad coverage of above discussed health conditions.



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Sneak Peek into the World of Science

Introduction to Single Cell Sequencing – Cite-Seq – Series 3

By MedGenome Inc., Scientific Affairs

Cite-Seq, short for Cellular Indexing of Transcriptomes and Epitopes by sequencing, is a powerful technology that has revolutionized single-cell sequencing. With its ability to analyze transcriptomes and protein expression at a single-cell level, Cite-Seq has the potential to greatly advance our understanding of cellular heterogeneity and function in biological systems. In this article, we will discuss the workings of Cite-Seq, its current and potential applications in various fields of research, and its limitations.

How Cite-Seq Works?

Cite-Seq is a technique that combines single-cell RNA sequencing (scRNA-seq) with antibody-based surface protein detection. The goal is to analyze the transcriptome of each individual cell, along with the surface proteins that are expressed on the cell membrane. By doing so, researchers can get a better understanding of the diversity and functionality of individual cells in a population. The Cite-Seq workflow involves several key steps:

Surface Protein Staining with Antibody-oligonucleotide Conjugates

The first step is to dissociate the tissue into a single cell suspension. Next, the cells are stained with a panel of antibodies targeting specific surface proteins of interest. Each antibody is conjugated to a unique oligonucleotide barcode, which allows for the identification of the protein that is bound to each cell. The cells are then sorted based on the presence or absence of each surface protein, and the RNA and protein are isolated from each individual cell.

Single Cell RNA Sequencing & Cell surface protein detection

The next step is to generate gel bead in emulsion (GEM) with antibody labeled cells. Because the antibodies attached to the individual cell, they end up together in one GEM. This can be done using a droplet-based method developed by 10X Genomics. The cells are encapsulated in tiny droplets, along with a bead that contains a unique barcode. The reverse transcriptase then adds the barcode into the mRNA transcripts of the cell, allowing for its identification during downstream analysis. The cell barcodes are also added to the antibody-oligonucleotide conjugate.

Sequencing and Data Analysis

The RNA and protein from each individual cell are then sequenced using standard techniques. The sequencing data is then analyzed using bioinformatic tools that allow for the identification of individual cells based on their gene expression and protein markers. By analyzing the transcriptomes and protein expression of individual cells, researchers can identify new cell types, characterize the heterogeneity of cell populations, and study the relationships between different cell types.



Link a cell's RNA profile with its surface proteins. Profiles multiple surface proteins simultaneously. Combines long standing knowledge of surface protein analysis with ever more complete RNA -Seq data.

Applications of Cite-Seq in Biological Research

Cite-Seq has a wide range of applications in biological research. One of the most significant applications is in the identification of new cell types and the characterization of cellular heterogeneity. By analyzing the transcriptomes and protein expression of individual cells, researchers can identify rare or previously unknown cell types and explore the differences between cell populations. This has important implications for understanding disease states and developing new treatments.

For example, Cite-Seq has been used to identify new immune cell subsets and characterize their roles in the immune response. Researchers used Cite-Seq to investigate the heterogeneity of T cells in the lung tissue of mice infected with influenza virus. They identified a new subset of T cells that expressed a specific set of surface proteins and had a unique gene expression profile. This subset was found to be important for controlling viral replication and preventing lung tissue damage.

Cite-Seq has also been used to investigate the differentiation of stem cells into specific cell types. By analyzing the transcriptomes and protein expression of individual cells during the differentiation process, researchers can identify the genes and proteins that are important for cell fate determination. This has important implications for regenerative medicine and the development of cell-based therapies.

MedGenome offers end-to-end project support for Cite-Seq (TotalSeq A, B & C) experiments. Our high-throughput lab can take fresh tissue samples, dissociate them into single-cell suspensions, then stain with oligonucleotide-conjugated antibodies (we recommend Biolegend's universal cocktail for maximum coverage). We generate scRNA-Seq libraries using the 10X Genomics platform.

Our bioinformatics team is specialized in providing cutting-edge analysis of single-cell data, using the latest technology and techniques to help you gain deep insights into your biological samples. Whether you are working in genomics, transcriptomics, or other fields, our team of expert analysts can help you interpret your data with precision and efficiency. We use advanced algorithms and machine learning techniques to analyze your data and provide customized reports that meet your unique needs. With our comprehensive approach to single-cell data analysis, you can be sure that you are getting the most accurate and reliable results possible.

Sneak Peek into the World of Science

Challenges and Opportunities in Precision Medicine using Big data- Part 2



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Part 1 summary

In the part 1 of this article (April'23 edition), we went through various challenges involving managing and processing Big data in healthcare organization especially a NGS based diagnostic lab. Now, we want to dive into the opportunities as well as BIG data solutions that can alleviate some of the plaguing problems. There are many solutions developed in the past decade to efficiently manage big data in NGS labs but these solutions are like precision medicine and should be targeted at a specific problem as opposed to a generalized solution to every big data issue. The part 2 also touches upon sensitive issues of data ownership and protection but this topic needs an elaborate discussion separately.

Big data solutions play a crucial role in handling and analyzing the massive amount of data generated by next-generation sequencing (NGS) technologies. NGS produces vast volumes of genomic data, including DNA sequences, RNA sequences, and other associated information. Here, in **Part 2**, sharing some key big data solutions used in NGS:

Data Storage and Management

Efficient storage and management of NGS data are critical. Traditional file systems may not be capable of handling the scale and complexity of NGS data. Distributed file systems like Hadoop Distributed File System (HDFS) or object storage systems like Amazon S3 are often used to store and manage large-scale NGS datasets. These systems provide fault tolerance, scalability, and parallel processing capabilities.

Data Analysis using various frameworks

Big data analytics techniques enable the pre-processing and secondary analysis of NGS data to derive meaningful biological insights. This involves various computational methods, such as quality control, adapter trimming, read alignment, variant calling, gene expression quantification, differential expression analysis, functional annotation, and pathway analysis. Tools and frameworks like Hadoop MapReduce, Apache Spark, and specialized bioinformatics software packages are commonly used for scalable data analysis.

- **Parallelization:** allows for simultaneous processing of multiple samples or genomic regions, reducing analysis time and increasing throughput.
- Cloud Computing: Utilizing cloud computing platforms like Amazon Web Services (AWS), Microsoft Azure, or Google Cloud Platform can offer cost-effective and scalable resources for NGS data analysis. Cloud providers offer pay-asyou-go models, allowing users to scale up or down based on their computational needs. Additionally, cloud-based platforms often have pre-configured NGS analysis pipelines and tools, simplifying the setup and reducing analysis time.

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• **GPU Acceleration**: Graphics Processing Units (GPUs) are highly efficient for certain types of NGS data analysis tasks, such as read alignment or variant calling. Utilizing GPU-accelerated tools, such as GATK-GPU or BWA-MEM-GPU, can significanly speed up these computationally intensive tasks, thereby reducing analysis time.

Automated Data Pipelines

Developing streamlined and automated data analysis pipelines can save time and improve efficiency. Workflow management systems like Snakemake or Nextflow can be used to define and execute complex NGS analysis workflows. These tools handle dependencies between analysis steps, enable parallelization, and allow for easy reproducibility and compliance, accelerating the analysis process.

Data Integration into warehouse or data lake



NGS data for e.g variant annotation is typically generated from multiple sources and may need to be integrated with other relevant biological or clinical data. Big data integration techniques, such as data warehousing and data integration frameworks, can be utilized to consolidate and harmonize diverse datasets for comprehensive analysis.

Big data solutions facilitate the integration of genomic data with electronic health records (EHRs), patient-reported outcomes, medical imaging, and other clinical data. Integrating and analyzing diverse data sources can uncover correlations between genomic variations, disease progression, treatment response, and patient outcomes. This integrated approach enables the identification of genetic markers, biomarkers, and therapeutic targets specific to individual patients.

Machine Learning and Artificial Intelligence (AI)

Machine learning and AI techniques are increasingly employed in NGS data analysis. These techniques can assist in tasks such as variant classification, variant prioritization, disease prediction, and biomarker discovery. Deep learning algorithms, such as convolutional neural networks and recurrent neural networks, are utilized for tasks like variant calling, treatment response prediction, patient stratification, and transcriptome analysis.

Data Visualization

NGS data analysis results can be complex and challenging to interpret. Data visualization techniques, including interactive visualizations, heatmaps, and network representations, are employed to present the results in a comprehensive and interpretable manner. Visualization tools like R, Python libraries (e.g., Matplotlib, Plotly), and dedicated genomics visualization platforms are commonly used. The performance of these tools can be further scaled up using cloud technologies or parallel computing.



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Data ownership, Security and Privacy

Given the sensitive nature of clinical and genomic data, ensuring data security and privacy is crucial. Encryption, access controls, and secure data transfer protocols are implemented to protect the data throughout the processing and analysis pipeline. Globally accepted policies such as HIPAA, 21CFRPart11, GDPR, ISO/IEC 27017, data encryption methods etc.

Data Security and privacy tools and policies are relatively easier to implement in cloud-based solutions as most cloud service provides offer compliance services, tools, operational controls and auto-audits. For example, AWS provides HIPAA business associate agreement (BAA) under which patient data protection policies and privacy can be implemented and audited easily. The program further helps implement organization wide HIPAA guidelines. Globally Data ownership, processing and storage is currently guided by GDPR policies and these need to be implemented top down in a healthcare organization to ensure the data is protected and shared in a secure manner either at rest or in transit.

Overall, big data solutions provide the infrastructure, tools, and techniques necessary to handle the large-scale, high-dimensional data generated by next-generation sequencing technologies, enabling researchers to extract valuable insights from genomic data. Big data solutions along with high performance computing or cloud computing allows for Cost-effective and fast analysis of next-generation sequencing (NGS) data which is quintessential to efficient clinical diagnostics applications. Big data solutions can deliver the ultimate promise of precision medicine by tailoring interventions to individual patients based on their unique genetic profiles, clinical characteristics, and environmental factors.



Book Review



Book

Journey of a civilization Indus to Vaigai- Part 2

Author - R. Balakrishnan



Book review by

Soumittra N, Ph.D. Disease Head - Ophthalmology

In the first part of the review, I presented chapters 1-6, in the second and final part of the review, I present chapters 7-17. Toponyms, archaeological materials with reference to Sangam literature has been the main methodology used by the author to substantiate his hypothesis of Dravidian authorship of IVC.

The chapter seven is very long and elaborate. Here the IVC place names, similar names in Southern India (SI) and certain descriptions in Sangam texts are presented as proof of Dravidian authorship of IVC. The Korakai-Vanji-Tondi (KVT) complex of place names detailed in Sangam texts are to be seen in IVC and is not known is Vedic texts as described by the author. A systemic search of place names in SI and Pakistan/Afghanistan (PK/AF) has yielded 899 unique names that are common in these regions. Further, the suffix names, "Ur", "Patti", "Palli", "Kay/Kai/Gai", "Cheri" as seen in current SI, Pakistan and Afghanistan and in Indian states of Punjab, Himachal Pradesh, Uttar Pradesh, Rajasthan, Andhra Pradesh, and Maharashtra which were either parts of IVC or possible routes of migration of the IVC people. The concept of five physical cultural zones of Kurinci (mountain), Mullai (forests), Marutam (agricultural wet land), Neytal (coastal are), Palai (dessert) is considered unique to Sangam literature. The names of these regions like "malai" or "malay" for hills, "kad" for forest and similarly for other zones are observed as suffixes in toponyms in IVC and several places of PK/AF. The Sangam text describes the river Pahruli and mount Kumari as "lost Tamil homeland". The Pahruli as river and kavatapuram are not seen in current Tamil Nadu but plenty in IVC toponym. The Pohru river is in PK which is a tributary of Jhelum. The place name Khumari are aplenty in PK/AF and in places which are home to Dravidian speaking Gond tribes in India. The names of mythic lost land of Tamils and place names in PK are similar indicating these lost lands are retained in the collective consciousness and recorded in Sangam texts. The Tamil grammar text Tolkapiyam describes 12 dialects, and these dialect names are traceable to places in IVC and continuity of these names are seen in Indian geographies like Madhya Pradesh, Gujarat, Orissa, Rajasthan, Bihar, Himachal Pradesh, Maharashtra and the Southern Indian states indicating migration and settlement routes. Similarly, the names of the Chieftains and the names of the kings of the three major Tamil dynasties, the Chola, the Chera, the Pandiya as described in Sangam text are observed as place names in IVC either exactly or derivative. The place names details seem very convincing for the author's hypothesis of Dravidian authorship of IVC.

The high west and low east dichotomy were observed in all excavated sites in Harappa and Mohenjo-daro sites indicating community identity, social order and status recognised and maintained. The author mentions that this high west and low east urban organization would have probably arisen from the Kilhar mountain range located in Bolochistan and Sindh and the lower Indus plain in the east.

These high west and low east names as "mel", and "kil" are observed in all Dravidian languages to denote directions and well as social and economic stratification as described in Sangam texts. These are well presented in the chapter 8.

The ninth chapter is titled the Dravidian Red, which seems to be the colour of IVC. The brunt red bricks, copper, pottery, all in red colour, the paintings in pottery with predominant red colour. The IVC had the finest brunt brick of the proportion 1:2:4, with excellent quality, practically indestructible as identified in the excavations. They were used to build artificial platforms for the high west settings, to avoid floodings, used in the excellent underground drainage system with smooth bricks to make it watertight. Extensive reference to burnt bricks, brick walls and forts are described in Sangam texts and not in the Rig Veda as described by the author and was used only later in Yajur Veda tradition for the Agnicayana ritual. The excavations at Keeladi near Madurai in TN revealed pots, terracotta drains, toys, dice and remind the IVC. The black and red ware pottery unearthed in the Harappa/Mohenjadaro sites are observed in Gujarat, Maharashtra as well as in Keeladi and Adichannallur in Tamil Nadu probably indicating the migration routes of the IVC people. The Sangam texts describes pottery and the socioeconomic status of potters which the author explains as indicative of that the IVC was Dravidian. Similarly, the copper and copper alloy used in IVC as identified from the excavations and descriptions in the Sangam literature reiterate that IVC was populated by Dravidian people.

The chapter 10 and 11 talks about Dravidian Gujarat and Dravidian Maharashtra. The bone eating camels, wild Asian ass all in context of coastal region, lion and elephants together as described in Tamil Sangam literature can only be mapped to the geography of Gujarat indicating Tamil prehistory that must have come by oral traditions. Likewise, the Hala's Gatha Sattasai attributed to Hala of Satavahana dynasty of Maharashtra has many similarities with the Sangam literature indicative of influence of the Harappan people.

The chapter 12 details two communities in the current Tamil Nadu, the Kongu region and the Nagarathar/ Nattukottai chettiars who are agropastoral people and traders, respectively. Their clan, territory and settlement names matching to IVC and Gujarat, Maharashtra and Kanchipuram plausibly demonstrating the migratory route. The Chettiras seems to have a long oral history of how long they have lived, where they lived. It is said in Kaliyugam year 204 they migrated to Kanchi where they lived for 2108 years and in the year 2312 they migrated to Chola country where they lived for 1463 years etc. This Kaliyugam calendar is based on Sanskrit text though as I understand.

One of the major works of literature in Tamil is Cilapathikaram, which narrates the story of Kannagi and the work actually connects all the three kingdoms of Chola, Pandiya and Chera is elaborately described in chapter 13. The names of all characters in this Tamil epic have toponomy to IVC.



The sacred "Vanni tree" and the variations of its name as place names in IVC and currently in PK/AF, Rajasthan, Punjab and the description and significance in Sangam text are detailed by the author (Chapter 14). Similarly, the bull fight seal excavated in IVC and other seals and evidence from IVC and description of these sports in Tamil literature is another evidence to the Dravidian authorship of IVC. The terracotta, ivory and bone dice obtained from Harappa, Mohanja-Daro and the Keeladi sites, description of the dice games in Sangam literature, the toy figurines in the Harappan sites and similar sports and activities described in Sangam text indicate the probable sports, plays, leisure activities of the IVC.

In the final chapter 17, the author connects the Adichanallur and Keeladi excavations as the umbilical cord connecting the IVC. The potteries, graffiti on them, beads, jewelleries, urban architecture seen in the excavations all match the IVC and dates to 3rd BCE and 1-2nd century BCE. Tamil Brahmi, prakrit and converted prakrit words are seen in the graffiti, while some of the scripts are same as the IVC script. The place names in the Keeladi/Vaigai/Madurai region of the TN are same or similar to the IVC toponyms. All these does put forth and supports the journey of civilization from IVC/Indus to Vaigai through Gujarat and Maharashtra.

The author has presented the evidence for the Dravidian authorship of IVC with exhaustive toponyms, excavations from the IVC, Gujarat, Rajasthan, Maharashtra and Tamil Nadu sites and abundantly referred to the Tamil Sangam literature to substantiate his hypothesis. The book is a very interesting read, a proud moment if one's native language is Tamil like mine. Though the author says Dravidian authorship of IVC, the literature or language of other Dravidian languages like Telugu, Kannada and Malayalam are not considered as old as Tamil, also the phonetics of other Dravidian languages have lot in common with Sanskrit. The author does at the end, in the epilogue questions "Is it Dravidian or Tamil"?, but I am not convinced with the decision of calling it Dravidian in terms of language, if all the evidence are from Tamil literature, then it is Tamil to begin with and probably other languages developed later. The author comments very critically about Sanskrit texts, that the early Sanskrit texts, Rig Veda, do not have descriptions as vivid as in Sangam texts. He also mentions Aryan invasion theory whereas the theory itself is questioned now. The genetic data reveal that the IVC people themselves were admixture of Ancestors of Ancestor South Indians (who were hunter gatherers) (AASI) and Iranian Agropastoral. These people had further admixed with Steppe pastoralists which form the ancestors of the present Indian population. If the Steppe pastoralists brought the Indo-European language, then how long were these two populations separate and when the admixture began. How long it must have taken to maintain the non-influence of language or culture or practices such as religion.

Further, the current archaeological evidence defines IVC to the regions of Afghanistan, Pakistan, Northwestern India, however, we do not know if the IVC was much beyond that including the Southern India as currently archaeological evidence are not there. These are some of the questions that arise when I read the book. Of course, further studies, archaeological, genetics and comparative literature would throw further light on these.

I would like to thank Dr. Sekar Seshagiri for suggesting this book. I thoroughly enjoyed reading it.

From our Colleagues

Art meets Science

The most beautiful thing we can experience is the mysterious. It is the source of all true art and science. — Albert Einstein



By: Dhriti C Nathan

Genetic Counsellor and Clinical Reporting Coordination





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From our Colleagues

Our employee's little Picasso :)



By: **Aaditri** 7 years DNA of Dr Sandhya Nair, Operations Dept.







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Murali



Kommanapalli Bala Tanushree Sharma



A Tirumalesh

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M Pooja Shri









Photo Feature

International Yoga day

Let the power of yoga bring the best in you!

MedGenome organised an rejuvenating Yoga session to relax our body and mind and to improve our poster. Yoga challenge was organised and some of the best poses were recognised.





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