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RESEARCH ARTICLE

REVOLUTIONIZING NEWBORN SCREENING: THE ROLE OF OMICS TECHNOLOGIES IN IDENTIFYING INBORN ERROR OF METABOLISM

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ABSTRACT

Over the last 20 years, improvement in development and application of omics technology has significantly improved newborn screening for inherited metabolic abnormalities. Early intervention was possible with traditional screening methods that are still in practice today and could largely rely on biochemical testing. This greatly decreased the risk of diseases and mortality from inborn errors of metabolism. These conventional approaches frequently lack the depth and precision needed to detect, at an early stage, a larger class of hereditary metabolic abnormalities. As a result, omics technologies will broaden and improve neonatal screening through analyses of transcriptomics, proteomics, metabolomics, and genomes. In genomics, next-generation sequencing makes it possible to identify genetic variants in precise order that are linked to hundreds of metabolic illnesses, even before symptoms appear. Proteomics and metabolomics provide the methods for quantifying proteins and metabolites respectively, thereby granting some very precious insights into the functional state of metabolism. This is also supported by transcriptomics i.e., study of RNA expression patterns as it aids in understanding changes that occur in gene expression due to IEMs. If these omics technologies are introduced into newborn screening programs, both better detection sensitivity and broader coverage offer the potential to identify more complex and rare metabolic diseases that also became leading causes for false negative results in past incarnations. Such an approach improves the accuracy of diagnosis and allows personalised therapy depending on which intervention works better as indicated by the metabolic profile. In addition, the exploitation of omics approaches might result in identification of entirely new markers further extending the list of diseases that can be screened using a non-invasive test In essence, this will signify a tectonic shift towards the adoption of omics technology in neonatal screening for uncovering inheritable metabolic disorders in novel ways that are extensive, precise and individualized. These technologies will lead to much better outcomes for the infants in this population given increased accuracy of diagnosis and earlier treatment, tailored to the specific cause of each patient.

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INTRODUCTION

Definition and prevalence of IEM - Garrod first described an inborn error of metabolism (IEM) in 1902 (1). IEMs refers to a class of inherited conditions known to be caused by abnormalities or deficiencies due to an insufficiency of an amount of either enzyme, cofactor or transporter in a product or acculates an abnormal quantity of a substrate. These deficiencies will lead to toxic substances building up in the blood and brain as a result of less than normal or absent enzyme activity; however other parts that are vital for good health will be depleted and have negative effects on health. (2),(3) Due to the advancement in medical treatment and testing facilities in laboratories, there has been an increased awareness of metabolic diseases in the country. The variations of such a wide range of IEM prevalence in different ethnic groups and geographical locations have been fuelled by the diversified tribal groups and the distinct religious communities of India as well as castes, subcastes, and a high level of endogamy (4,5).

Importance of early detection and treatment of IEM

Evolution of Newborn Screening (NBS): From PKU to WGS, the past sixty years have brought both possibilities and difficulties. "All screening programs do harm, some do good as well, and, of these, some do more good than harm at reasonable

cost" is a widely accepted truth. (6). More than 50 years ago, a newborn screening test using the bacterial-inhibition assay (BIA) on blood spots was initially made available. Since then, the test has developed into intricate public service scientific initiatives. Over the past few decades, early intervention has shown benefits for many people worldwide who suffer from hemoglobinopathy illnesses, congenital hypothyroidism (CD), cystic fibrosis (CF), and phenylketonuria (PKU). Thanks to significant advancements in laboratory methods and high throughput data processing over the past 20 years, a growing population can now be studied for a wide range of illnesses. This, along with the fact that there are more and more treatments available for uncommon illnesses, suggests that in the future, complex and costly demands may overwhelm health facilities (6,7).

Historical overview of NBS: Analytical techniques were created and utilised to begin for PKU, CH, and CAH screening in 1963. Guthrie created a quick turnaround, low- cost technique with high throughput wherein for newborn screening, the amount of phenylalanine in dried blood spots (DBS) with inherited errors of metabolism was evaluated(8). A little drop of blood is extracted from a baby's heel using the dried-up clots of blood (DBS),also known as the Guthrie card, and then easily transported to a testing laboratory using a filter paper card, may have been made popular by him (9). Furthermore, he utilised the globally acknowledged β-2-Thienylalanine test to inhibit bacterial growth (8).

Conventional techniques applied in NBS: When newborn screening was first introduced twenty years ago, it was also known as the baby's first test. According to reports, the following conventional techniques are used for NBS: tandem mass spectrometry (MS/MS), fluoro enzymatic tests, immunoassays, and so on. (10, 11) Tandem mass spectrophotometry (MS/MS) was the most widely used conventional technology in extended NBS programs worldwide because of its exceptional sensitivity and specificity.

On the other hand, the most popular method for genomic NBS used by research institutions and commercial businesses was gene-panel based sequencing.(12) For the first time, NBS used tandem mass spectrometry (MS/MS) technology in 1990. Due to its ability to quantify many metabolites simultaneously, it is essential to the detection of IEM. With a 2–3-minute turnaround time, a single MS/MS run has a lot of potential for high-throughput screening applications. (13)

OMICS TECHNOLOGIES IN NBS

OVERVIEW OF OMICS TECHNOLOGY - 2.1OVERVIEW OF OMICS TECHNOLOGY: The most distinctive feature of "OMICS" technology is the capability to analyse the proteome, metabolome, transcriptome, genome, and epigenome.(14) "OMICS,", which is the process of scanning through and evaluating enormous amounts of data encompassing the anatomy and function of a biological system at a specific level has drastically altered the methods employed in the study of biological systems. In other words, "top down" methods which are mainly associated with the development of "omics," in conjunction with "bottom up" techniques provide holistic means to effective research for systems of biology (15).

Definition and scope of genomics, proteomics, metabolomics

GENOMICS: This rapidly emerging science is concerned with the identification and annotation of all sequences found in a complete organism's genome.The word "genome" refers to the set of genes in a cell. As such, studying the genetic makeup of an organism is called genomics (16,17). Genomics provides a jumping off point to study the other "omics" fields. The genotype that is contained in an organism's genes determines the final physical makeup of the organism, more commonly referred to as its "phenotype."

PROTEOMICS: There are countless functions that proteins carry out for the cell. A proteome is termed as a cell's entire collection of proteins , and the investigation of protein structure, function, and the various roles that individual proteins perform within cells is known as proteomics(16).

METABOLOMICS: Metabolomics is the newest of the "omics" sciences. The whole collection of low molecular weight substances present in a sample is referred to as the "metabolome." The materials are byproducts and substrates of enzymatic processes that modify the phenotype of the cell directly. The only goal of metabolomics is to identify the composition of this environment (16).

Advantages over traditional NBS methods (18)

GENOMICS IN NEWBORN SCREENING

Genomic Sequencing Techniques

Whole Exome Sequencing - In the human genome,each exon is denoted by the relatively new term 'exome'. The roughly 180,000 genetic sequences that both transcribe and persist in the mature RNA are referred to as 'exons'.

The exome, which makes up only 3% of the human genome, is made up of the approximately 22,000 protein-coding genes, mutations in which cause around 85% of our major clinical disorders.(19), (20), (21) Consequently, it is believed that exome sequencing is a useful technique for examining a patient's DNA to identify the underlying genetic etiology of their illness. In

consanguineous families, WES is very effective at finding uncommon mutations causing autosomal recessive disorders. The recently developed WES technology provides locus specific and gene panel testing as an additional analytical for individuals with wide spectrum symptoms (22).

CRITERIA	OMICS Tech	Traditional NBS
Comprehensive analysis vs targetedscreening	Omics (e. g., genomics, metabolomics) enable the quantification of a large number of biomolecules simultaneously and therefore offer an integrated view on genetic predispositions as well as metabolic settings and diseasemarkers in single individuals.	In the most conservative approach, NBS may screen for a relatively small number of pre-specified biomarkers or conditions (typically based on biochemical assays).
Early detection and predictive capability	omics can detect genetic variations and metabolic imbalances before onset of certain symptoms allowing for theaccurate prediction andprevention through early intervention as well personalized therapy.	Such standard methods of diagnosis may fail to catch a disorder until symptoms are present, which could slow down the time that it takes for one's condition to be diagnosed and treated
Precision	These latter and other omics investigations bring to light individual person differences in disease susceptibility, drug/diet response, or spectra of metabolites that together condition implementation of personalized (systems) medicine.	In contrast, conventionaltechniques give public- basedscreening diagnostics which in turn neglect the patients' geneticpeculiarities.
Data integration and interpretation	The global nature of omics enables integration and analysis by sophisticated bioinformatics tools, which help us to better understand complex disease mechanisms and biologicalpathways.	In traditional NBS, the interpretation of data is frequently restricted to predefined cutoff values for single biomarkers and much less focus on holistic pathway analysis.
Non-invasive andMinimal SampleRequirement	Non-invasive or minimally invasive sampling (e.g., blood, urine) is possible for many omic techniques, therefore making it less burdensome on animals and investigators; whole genome sequencing as readily obtained a few drops of newborn heel prick beyond neonatal screening.	The high-touch requirementof sampling on some traditional NBS methods is invasive and stressful,especially to the easy-to- stress newborn.
Cost-effectiveness and Long-termBenefits	Through earlier detection,elimination of complications and providing personalized treatment approaches that could potentially lower future healthcare costs - even if omics platforms requireincreased upfront cost.	The upfront cost of initial diagnosis might be lower with traditional methods, but the cumulative harm from both delayed and missed diagnosis could build up over time if omics technologies are no longer used for preventativehealthcare.

Detection of IEM applications

The genetic diagnostic confirmation of IEM categories made possible the use of a particular medical and/or dietary management: amino acid and organic acid issues, mucopolysaccharidosis, glycogen storage disorders, biotinidase deficiency, Wilson disease, and galactosemia (23).

Challenges and Limitations (21), (24), (25), (26), (27),

Only exons and the areas around them are covered by WES.

Limitations in identifying specific variants due to the characteristics of the genetic variations. As a result, it is unable to identify causal variants outside of the covered areas, such as regulatory regions that are non-coding and intronic. Certain variants including substantial insertion/deletion/duplication, copy number variation, somatic mosaic variants, uniparental disomy, structural genomic variants, and variants on the mitochondrial genome, cannot be discovered because of the methodology used. When analysing large amounts of data, if the wrong filters are used or not used at all, the right diagnosis will be missed.

Proteomics and Metabolomics in Newborn Screening: In order to comprehend human disorders, proteomics and metabolomics have evolved as complimentary approaches to genomics. These methods, which have advanced quickly in recent years, present the chance to identify putative biomarkers for patient categorization, prognosis, therapy, and diagnosis (9) Proteomics is the large-scale investigation of all the proteins found in an organism, tissue, or cell under predetermined conditions. This investigation covers both the physiological functions and structural makeup of the proteins (9) The study of metabolomics entails identifying and measuring the metabolites that are present in biological systems. Sample preparation, which is crucial for metabolomic analysis, depends on the platform to be utilised and the kind of metabolites to be investigated (21). Metabolites are any organic small molecules having a molecular weight of less than 1,800–1,500 Dalton. These may function in biochemical metabolic pathways as products or substrates (28). As byproducts of cellular metabolism, metabolites including lipids, carbohydrates, and amino acids regulate how a cell communicates or transfers energy to other cells. Indeed, in a biological system, metabolites exhibit sudden changes in quantity and composition in response to physio pathological disturbances or therapeutic interventions (29).

Proteomic and Metabolic Techniques Used

Mass Spectrometry Based Proteomics - Biomolecules known as proteins can more effectively connect genomic data with biological activities and disease symptoms. Protein interactions control important biological processes, which in turn regulate cellular functions, organismal systems, metabolic and signalling pathways, and disorganised networks and systems connected to both disease and health (30). Proteins are not isolated entities. The large-scale identification and quantification of proteins in biological material is the foundation of the integrated field of proteomics. Due to its high sensitivity and specificity, MS is a better

tool than immunoassays for the study of various types of drugs. (31), (32). The integrated field of proteomics relies on the exhaustive identification and measurement of proteins in biological specimens.(30), (33).Mass spectrometry (MS) has very high sensitivity and specificity, making it better than immunoassays for studying different types of drugs. (34) (35) Clinical proteomics based on mass spectrometry (MS) enhances medical practice by identifying novel targets for prognosis, therapeutic intervention, drug development, and potential biomarker identification. (36), (37).

Sl.No.	AminoAcid Panel	Increase	Decrease
1.	Alanine (Ala)	Hyperammonemia, Pyruvate/Lactate defects, mitochondrial disorders, Glucagon receptor defect, MSUD	Ketosis, Prolonged fasting, Fasting, ,
2.	Arginine (Arg)	Hyperargininemia Disorder, Arginase deficiency, Glucagon receptor defect.	Lysinuric Protein Intolerance (LPI), Urea Cycle defects, HHH syndrome, Hemolytic plasma
3.	ASA total (Argininosuccinic Academia)	ASA-Lyase deficiency	
4.	Citrulline (Cit)	Citrullinemia 1 and 2, ASA lyase deficiency, Pyruvate carboxylase deficiency Type –B.	NAGS, CPS, OCT deficiency, Pyrroline-5-carboxylate (P5CS), synthase deficiency, Mitochondrial disorders, LPI.
5.	Gln/Lys (Glutamine / lysine)	Hyperammonemia, UCD, Glutaminase deficiency/Hyperlysinemia, Sacchropinuria, UCD, Pyruvate carboxylase Type-B.	Glutamine synthase deficiency, PPA, MMA, MSUD, Pyruvate carboxylase deficiency / LPI, HHH syndrome, Ornithine aminotransferase deficiency.
6.	Glutamic Acid(Glu)	Hemolytic plasma Stored Plasma Ca-levlunate treatment, Glutamic academia, glutamine synthase deficiency	
7.	Glycine (Gly)	NKH, Ketotic Hyperglycinemia Valproate treatment, Glycine transporter deficiency, PPA, MMA, D-Glyceric aciduria.	Hypoglycinemia (?), Serine deficiency disorders.
8.	Leu and Ile/Pro-oH	MSUD, Lipoamide Dehydrogenase deficiency, E3 deficiency / Prolinemia type 2,	Severe protein malnutrition
9.	Methionine(Met)	Methionine adenosyltransferase, Glycine N-methyltransferase SAH hydrolase deficiency CBS deficiency Tyrosinemia type I Liver disease	Cobalamin defects MTHFR deficiency Severe protein malnutrition
10.	Ornithine(Orn)	Hypernonithnemia, Ornithine amino trasnferase deficiency, HHH syndrome Hemolytic plasma	Urea cycle defects, P5C synthase deficiency.
11.	Phenylalanine (Phe)	PKU and BH_4 defects, Hyper Phenylalaninemia, Sever liver disease (Tyrosinemia type 1)	NTBC treatment
12.	Proline (Pro)	Prolinemia type 1 and 2, Lactic acidemias, Multiple acyl – CoA, Dehydrogenase deficiency.	P-5-C synthase deficiency
13.	Tyrosine (Tyr)	Tyrosinemia type 1,2,3, Transsient tyrosinemia of the newborn, Liver disease, Prematurity.	PKU
14.	Valine (Val)	MSUD Iipoamide dehydrogenase deficiency, Prolonged fasting, ketosis Leucine, Isoleucine, E3 deficiency, branched chain amino transferase 2 deficiency.	Severe protein malnutrition

Implementation of proteomics based on mass spectrometry

- Comparative proteomics in health-related studies
- Clinical pathology based on mass spectrometry
- Imaging using mass spectrometry (MSI)
- New developments in vivo methods

List of Analytes for analysis of blood spots collected in filter paper (38, 39)

Sl.No.	Acyl carnitine Panel	Increase	Decrease
1.	Free carnitines (C0)	CPIT, Carnitine supplementation	CTD, Carnitine uptake defect, Secondary carnitine deficiencies
2.	Acetyl carnitine (C2)	Carnitine supplementation, Ketosis	Carnitine Transporter Defect or Insufficiency
3.	Propionyl carnitine (C3)	MMA, PA, MCD, SUCLA2, treatment with hepatonic acid.	
4.	Malonyl carnitine (C3DC/C4-OH)	Malonic aciduria, Malonyl-CoA carboxylase deficiency /	
5.	Butyryl carnitine (C4)/ Isobutyryl	SCAD deficiency, variant SCAD deficiency, Isobutryl-CoA dehydrogenase deficiency, Formimnoglutamic aciduria (with more prominent peak at m/z 287), Ethylmalonic encephalopathy, Isobutyryl-CoA dehydrogenase (IBD) deficiency, MADD, Antibiotics derived artifact and treatment with hepatonic acid.	

Continue ….

6.	Methylmalonyl (C4-DC)	MMA, Succinyl-CoA synthetase (SUCLA2) deficiency.	
7.	3-Hydroxybutyryl (C4-OH)	SCHAD deficiency, ketosis	
8.	Isovaleryl ½	IVA, 2-methylbutryl-CoA dehydrogenase deficiency, MAD	
9.	C5:1 TigIyI	3MCC, 2-methyl-3-OH butryl CoA dehydrogenase. SKAT, 3-Oxothiolase deficiency.	
10.	C5DC	GA-1, MAD	
11.	Hydroxyisovaleryl C5-OH / 3-Hydroxy 2-methylbutyryl	3MCC, 3-methylglutaconyl CoA hydratase deficiency, HMG-CoA lyse, MCD, 2-methyl-3OH butryl CoA dehydrogenase, Biotinidase deficiency, 3-<ethygultaconyl-CoA hydratase deficiency, 3-Oxothiolase deficiency, MAT, MHBD.	
12.	Hexanoyl carnitine (C6)	MCAD, MADD, MKAT	
13.	Adipyl carnitine (C6DC)		
14.	Octanoyl carnitine (C8)	MCAD, M/SCHAD, MKAT, MADD	
15.	Decanoyl carnitine (C10)	MCAD, MADD	
16.	3-Hydroxy decanoyl (C10-OH)	M/SCHAD deficiency, MCKAT Deficiency	
17.	C10:1	MCAD	
18.	C10:2	DCR	
19.	Dodecanoyl carnitine (C12)	VLCAD, MADD	
20.	C12:1		
21.	Tetradecanoyl carnitine (C14)	VLCAD, CACT, CPT-II, MADD,LCHAD/TFP; CPT-I	
22.	C14:1	VLCAD, MADD, LCHAD/TFP	
23.	Tetradodecenoyl (C14:2)	VLCAD	
24.	3-OH-tetradecanoyl carnitine (C14-OH)	LCHAD/TFP	
25.	Hexanoyl carnitin e (C16)	VLCAD, LCHAD, CPTII, CAT, MADD, Carnitine /acylcarnitine translocase (CACT) deficiency, CPT-1.	CPTI
26.	C16:1-OH	Antibiotics derived artifact	
27.	C16OH	LCHAD, TFP deficiency	
28.	C16:10H/C17		
29.	Stearoyl carnitine (C18)	VLCAD, CACT, CPT-II, MADD, VLCAD, LCHAD/TFP	
30.	Oleyl carnitine (C18:1)	VLCAD, CPTII, CAT, LCHAD, MADD, TFP, CPT-1.	CPTI
31.	C18:2	VLCAD, CPTII, CAT, LCHAD, MADD, TFP.	
Ketones			
32.	Succinyl Acetone (SA)	Tyrosinemia type 1	
Nucleosides			
33.	Adenosine (ADO)	Severe combined immunodeficiency	
34.	2'-Deoxyadenosine(D-ADO)	Severe combined immunodeficiency	

Abbreviations

- 3MCC-3-Methyl crotonyl CoA carboxylase
- 3MCC-3-Methyl crotonyl CoA carboxylase
- ADO-Adenosine
- ASA-Argininosuccinic Aciduria
- BH4-Tetrahydrobiopterin
- CACT-Carnitine acylcarnitine translocase deficiency
- CAT-carnitine acylcarnitine translocase deficiency
- CBS-cystathionine β-synthase
- CPIT-Carnitine palmitoyltransferase
- CPS-carbamoyl phosphate synthetase
- CPTII-Carnitine palmitoyl transferase II
- CTD-Creatine transporter deficiency
- D-ADO-2'-Deoxyadenosine
- IVA-Isovaleric academia
- LCHAD-Long-chain L3-hydroxyacyl-CoA dehydrogenase
- M/SCHAD-Medium/short-chain acyl-CoA dehydrogenase deficiency
- MADD-Multiple Acyl CoA Dehydrogenase Deficiency
- MAT-Methionine Adenosyl Transferase
- MCD -Multiple carboxylase (holocarboxylase synthetase and biotinidase) deficiency.
- MCKAT-Medium-chain ketoacyl-CoA thiolase deficiency
- MHBD-2-Methyl-3-Hydroxybutyryl-CoA Dehydrogenase (MHBD) Deficiency
- MKAT-Medium-chain ketoacyl-CoA thiolase deficiency.
- MMA – Methylmalonic acidemia
- MSUD-Maple syrup urine disease
- MTHFR-Methylenetetrahydrofolate reductase

- NAGS-N-acetyl glutamate synthetase
- NKH-Nonketotic hyperglycinemia
- NTBC-2-(2-Nitro-4-Trifluoromethylbenzoyl)-1,3cyclohexanedione
- OCT-Ornithine transcarbamylase
- P5C-1-pyrroline-5-carboxylic acid dehydrogenase
- PA- Propionic acidemia
- PKU-Phenylketonuria
- SAH-S-adenosylhomocysteine hydrolase
- SCAD-Short-chain acyl-CoA dehydrogenase deficiency
- SKAT-Short -chain ketoacyl-CoA thiolase deficiency.
- SUCLA2-Succinyl CoA synthetase deficiency
- TFP-Trifunctional protein deficiency
- UCD-Urea Cycle defect
- VLCAD-Very long-chain acyl-CoA dehydrogenase

METABOLOMIC TECHNIQUES APPLIED - An intricate, dynamic, and incredibly sensitive system is the human metabolome. As such, it presents distinct analytical hurdles in contrast to other omics analysis methodologies that rely on the profiling of big molecules constructed from a small and basic set of subunits, including nucleotides for genomes and transcriptomics and amino acids for proteomics. As a result, the order combination of the subunits determines the identity and functional analysis of proteins, RNAs, and DNA (40).

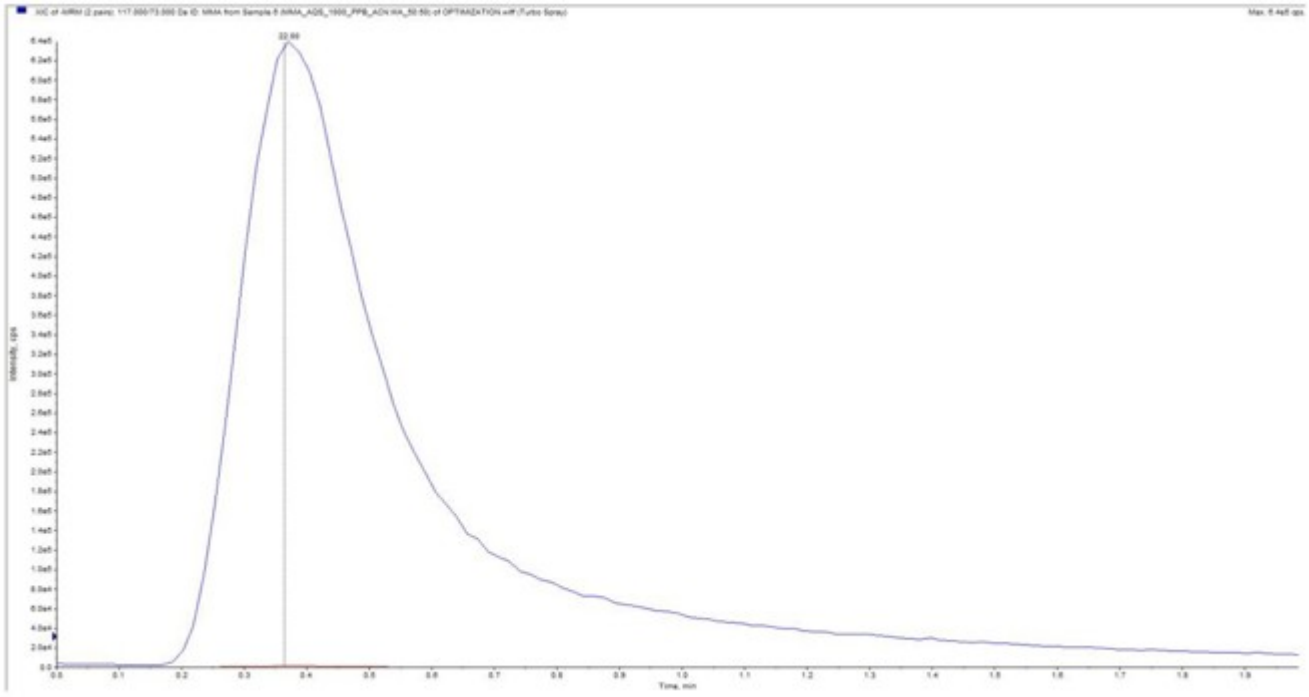

Figure 1. Chromatogram of Methylmalonic Acidemia (Mma) And D3-Mma -

The metabolic disorder methylmalonic acidemia (MMA) is characterised by multiorgan dysfunction and pleiotropic metabolic abnormalities. The inborn metabolic mistake known as methylmalonic aciduria (MMA) lacks effective treatments due to its poorly understood etiology and various monogenic origins. Liquid chromatography coupled with mass spectrometry (LC-MS) The most popular technology for accurately identifying and quantifying proteins is liquid chromatography coupled to tandem mass spectrometry, or LC- MS/MS. This method separates proteins from biological materials, breaks them down into peptides using an enzyme called trypsin, and then separates the peptides using LC and electrospray ionisation so they can be injected into a mass spectrometer. In order to identify peptides, precursor ions must be chosen and fragmented in a collision cell. The mass to charge ratio (m/z) of the precursor ions is then measured in the MS1 spectra, and the m/z of the collision-produced product ions is measured in the MS2 spectra.(41). Clinical applications of MS-based proteomics include understanding the pathophysiology of a wide range of diseases, including phylogenetic classification, metabolic screening in neonates, urine toxicology screening, clinical metabolic profiling, and non-communicable pathological conditions like cancer, metabolic disorders, amyloidosis, immune system disorders, and characterization of renal diseases, reproductive diseases, blood disorders, and ocular diseases are just a few examples of the pathophysiology of a wide range of diseases.(42), (43) (44).

Gas chromatography-mass spectrometry (GC-MS) - Since 2000, there has been gas chromatography-mass spectrometry (GC-MS). China, including Beijing, Shanghai, Wuhan, and Guangzhou, has access to the GC-MS urine organic acid analysis and the tandem mass spectrometry blood acryl carnitine assay techniques(45).Analysts employ a method known as gas chromatography-

mass spectrometry (GC-MS), which combines the benefits of mass spectrometry and gas chromatography, to detect different compounds within a test sample. (46).

Applications of Proteomics in Identifying IEM

Biomarker discovery and validation: Typically, a biomarker is a protein associated with a disease or a biochemical signal that can be applied in a medical environment to guide treatment for a molecular target or assess the therapeutic response, as well as to diagnose or track the course, prognosis, and progression of an illness.(47), (48). The field of biomarker research is structured along a continuum, starting with the potential discovery of a biomarker and continuing through candidate prioritization, validation, and verification before reaching clinical application and post- implementation monitoring. In the discovery phase, biomarker candidates that provide information about proteins that exhibit statistically significant changes in response to a particular environmental change or medication treatment must be simultaneously identified with high confidence and quantified (41).

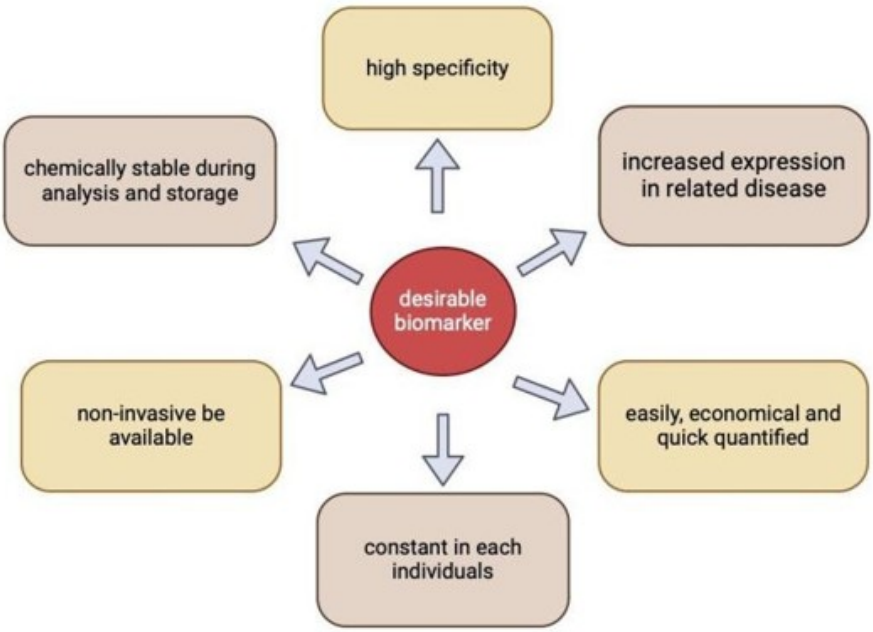

Figure 2. Appropriate Biomarker Attributes - Proteomics technology is an effective method for identifying biomarkers by characterising and assessing the global protein profiling of a particular condition. For a given illness condition, a high specificity biomarker is optimal [49]

Reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6269565/figure/F2/

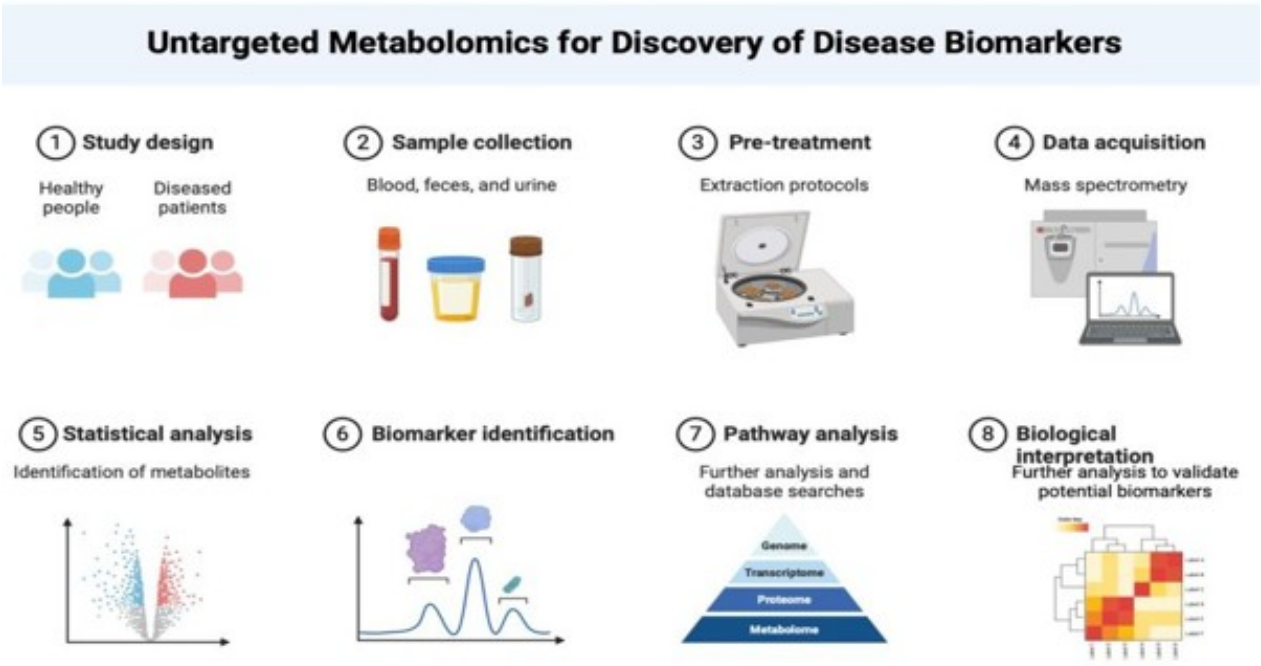

Figure 3. Metabolomics Untargeted for the Detection of Disease

Biomarkers - Untargeted metabolomics-based illness biomarker discovery is useful for pathogenic mechanism investigations and promotes early disease detection. It has been established that metabolites are critical for both the diagnosis and prevention of disease, and that they have a major function in the onset of disease. More and more research is being done on metabolomics in a variety of fields, including pathological mechanism investigations, pathway analysis, and the search for new illness biomarkers. Reference :https://images.app.goo.gl/EkSDmkV7ecbf4Jki5

Applications of Metabolomics in Identifying IEM

Metabolic profiling and early detection- Since urine is sterile, readily obtained in large quantities, and minimally intrusive, it is the perfect biological fluid for metabolomics in neonatology. More importantly, the urine metabolome characterization plays a major role in identifying the molecular fingerprints metabolic metabolism within cells that are implicated in numerous IEMs. (50). Urine is also the substrate that should be watched for the diagnosis, development, or commencement of many IEMs. Nevertheless, proteomic approaches are used to more thoroughly examine the molecular underpinnings and disrupted pathways of metabolic diseases.(51) The metabolomics analysis of urine employing the combined procedures of urease pretreatment, stable-isotope dilution, and capillary gas chromatography/mass spectrometry delivers accurate and measurable data for the simultaneous screening or molecular diagnosis of over 130 IEMs including enzyme failure caused by aberrant alteration that occurs after transcription or translation, aberrant subcellular localization, or aberrant regulatory gene (52).

Given that IEM and metabolism are closely related, the underlying pathophysiological alterations are primarily responsible for the metabolome and functional comprehension of the illness. Therefore, in the era of precision and post-genomic medicine, metabolomics analysis is a viable approach to gain enhanced knowledge and better diagnosis of IEM because it is inherently multidisciplinary, combining advanced statistics, bioinformatics, analytical chemistry, and biochemistry (40).

Challenges and Limitations

Metabolite Identification: The primary obstacle preventing metabolomics from being widely used in translational and clinical contexts is metabolite identification. Metabolite identification is a difficult task even with the availability of spectrum information in spectral databases or the literature (53).

Standardisation - The broad acceptance of every new technology depends on standardisation and harmonisation. Thus, using proven and standardised methodologies to standardise sample preparation, handling, reporting, and analysis can lead to accurate results (54), (55).

Clinical Actionability, Automation, and Data Visualization- Instrument, pre- and post- analytical automation is an essential component of any diagnostic innovation that is widely adopted in the clinical setting. Automation of the metabolic omics workflow is essential for achieving high throughput, repeatability, and reliability—three qualities that are fundamental to the practice of modern laboratory medicine (56).

Obstacles in Clinical and Translational Metabolomics: Small molecules (metabolites) found in a cell, a tissue, or an organism are included in metabolic analysis. One of the main causes of these restrictions is the metabolome's complexity, which is made up of thousands of chemical structures with concentrations and routes connected to many biological processes. To properly utilise metabolomics in clinical and translational research, we must address these issues by coordinating efforts in standardisation, technological innovation, interdisciplinary collaboration, and ethical considerations (figure 4).

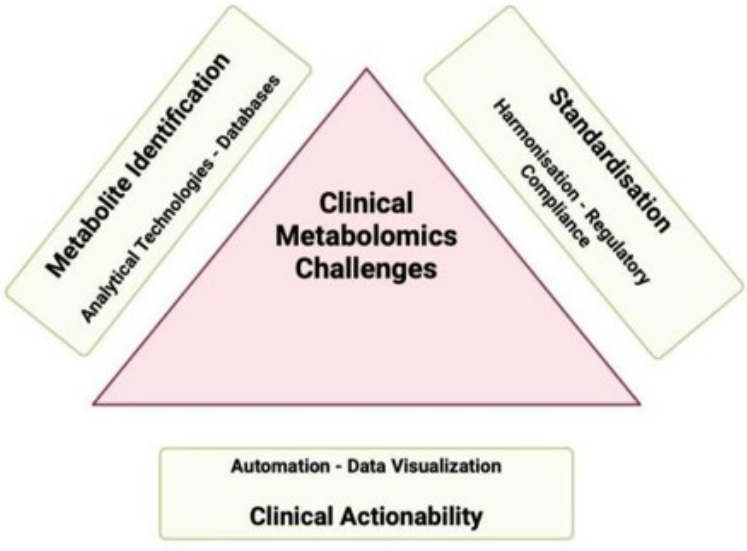

Figure 4. Obstacles in Clinical and Translational Metabolomics

Reference: https://www.mdpi.com/1422-0067/17/7/1167

Integrated Omics Approach

Multimodal Omics: The field of personalised medicine is entering a new phase, offering a model of personalised healthcare that includes medical target treatment and management that is specific to each patient. (57). Recent years have seen a considerable advancement in high-throughput omics technologies, which are based on mass spectrometry and next-generation sequencing.Very detailed molecular physiology investigations of organismal homeostasis of whole-tissue are made possible by these technologies. Among the technical developments are enhanced methods for computational analysis (58), enhanced instrument sensitivity (29), and simplified workflows for sample preparation (59). The growth of screening programs to include more conditions has been fueled by new technologies. In the present day, including omics technologies like untargeted metabolomics and genomes could increase the scope of examined circumstances even further (60). Potential disease-related concerns that multi-omics research may address come in many forms. A phenotype-first strategy might start with a proteomic, metabolomic, or even phenomics approach, whereas a genome-first approach first begins at the genomic level and later includes other types of omics. Many omics technologies in concert might more fully illuminate the elements associated with disease pathways (61) (63).

Integrated Omics Approach: There are various approaches one may use toward multi-omic investigation of disease-associated problem-solving. The first one is phenotype based approach including proteomic, metabolomic, or phonemic analysis and the second one is a genome based approach which starts at the genomic level and add other omic types sequentially. Integrative multiple omic technologies may lead to better understanding of the variable effecting disease processes (Figure 5).

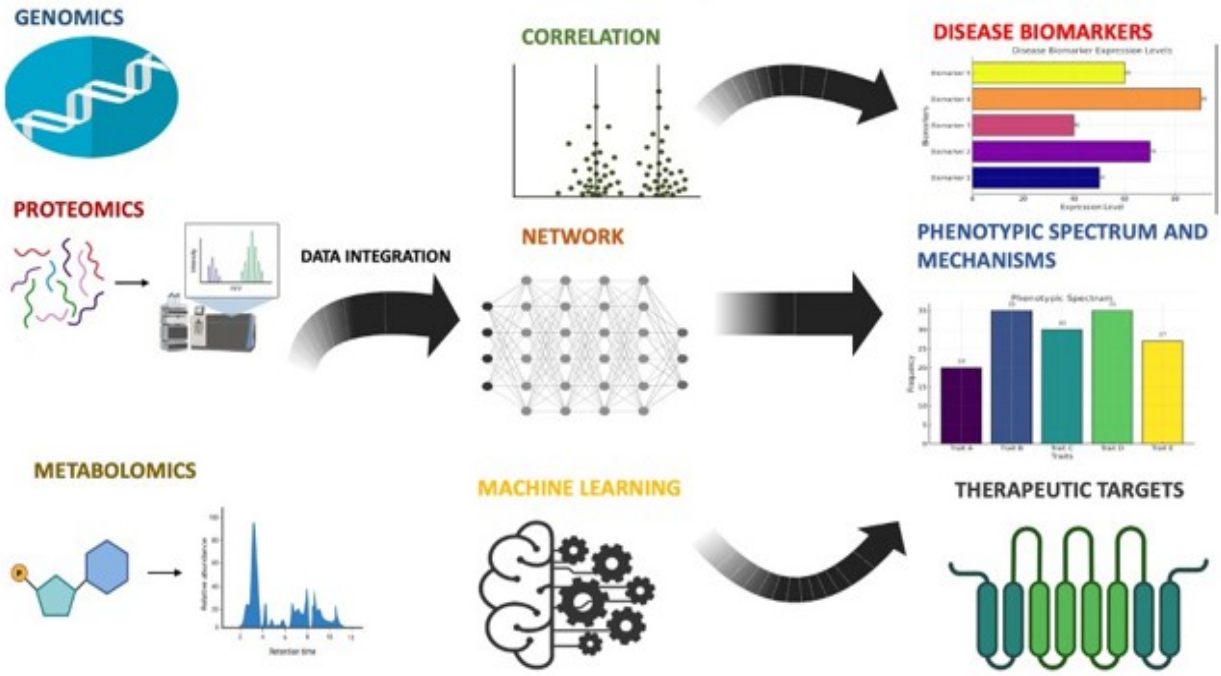

Figure 5. Integrated Omics Approach

Reference-https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10390758/figure/mco2315-fig- 0003/

Implications of integrative Approach

Applications of Integrated Technologies: The most benefit from these technologies may come from a multi-pronged approach that uses information from multiple omics data to more accurately estimate illness risk and lowers the likelihood of over interpreting omics screening results (62).

Comprehensive disease understanding: This will enhance the prevention of complications, improvement of long-term health, and most importantly prevent progression of a disease by early diagnosis and intervention(62).

Improved diagnostic accuracy: Therefore, using the genetic, transcriptomic, proteomic, and metabolomic markers of the disease, it becomes possible to diagnose the disorder long before the symptoms appear (63).

Challenges And Opportunities For Multi Omic Approach: Due to notable advancements in this area, algorithmic meta-analysis frameworks and techniques are now primary instruments for doing an extensive analysis of multi omics data. However, there are also significant potential and challenges for multi-omics integration analysis. A few of these include handling missing information, omics heterogeniety , interpretability limitations in multi omics models, and issues with data annotation, storage, and processing capacity Figure 6.

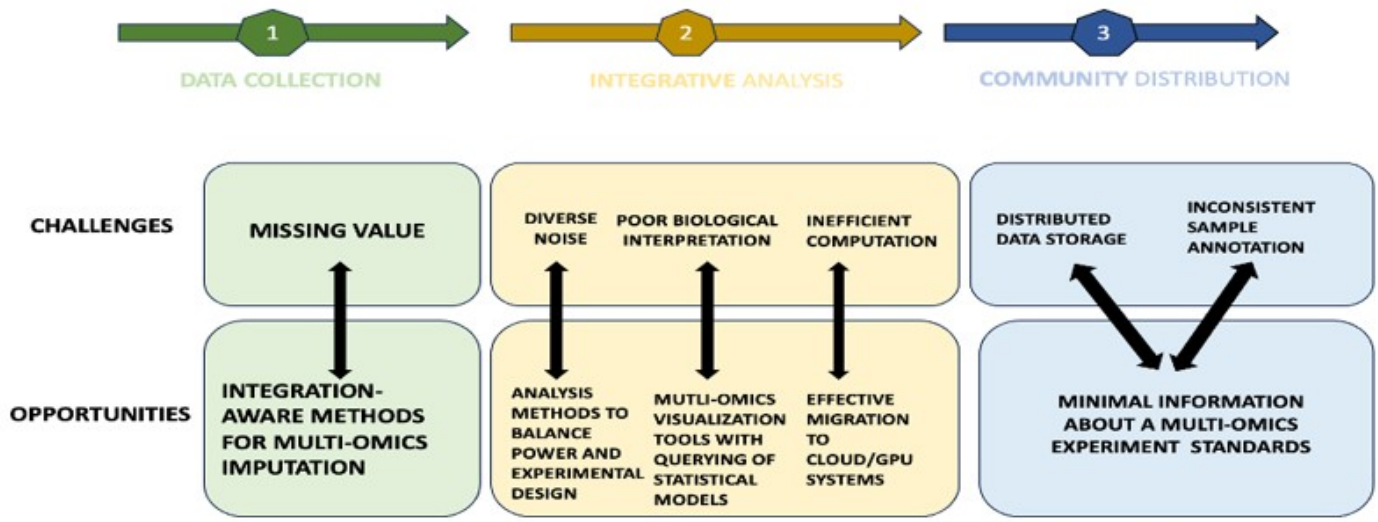

Figure 6. Challenges And Opportunities For Multi Omic Approach

Reference:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10390758/figure/mco2315-fig- 0007/

Implications For Ethics, Law and Society - In accordance with NABL & NABH guidelines, services related to newborn screening are upheld in terms of quality and checks.

Ethical Considerations

Informed consent and parental rights - Guaranteeing that parents have sufficient information about newborn screening's goals, advantages, and possible consequences. This entails the need to get prior permission from the subjects before any tests are conducted on them.

Confidentiality and Privacy - Ensuring the confidentiality of the newborn and the family by safeguarding all the records of the baby's medical history and results of tests conducted.

Implication to genetic information - It should be made clear to parents that they will be given all the details about the screening including how the results will be used and how the information will be kept.

Legal and Regulatory Frameworks

Regulation Compliance: Compliance with national and international standards and guidelines for newborn screening. It is mandatory to follow the National Accreditation Board for Testing and Calibration Laboratories (NABL) and National Accreditation Board for Hospitals & Healthcare Providers (NABH) standards in India.

Data Protection Laws: Using legal requirements for the protection of personal health information.

Mandatory vs. Voluntary Screening: Determining the exact legal requirements for newborn screening as well as establishing whether it is a mandated or optional procedure, and if the parents are informed of this status.

Social Impact

Public perception and acceptance - Educating the society and healthcare providers on the importance and drawbacks of newborn screening.

Education and awareness - Concerns with possible stigma or discrimination that may be associated with identification of some conditions in newborns.

Future Perspective and Conclusions

Technological Advancements

Emerging technologies and trends – It could have been difficult to imagine the condition of inborn errors of metabolism diagnosis and treatment a century ago. But these changes also bring with them issues for society and medicine. In the twenty-first century, we anticipate the day when morbidity and death are reduced by improved detection and treatment from inherited metabolic abnormalities a thing of the past. The nature of inborn errors of metabolism will likely continue to evolve as the

importance of epigenetics is explored and more complex lipid and other cellular metabolic pathways are identified. More advancements in the identification and treatment of secondary metabolic disorders resulting from immune system dysfunction, aging, cancer, and other illnesses are expected. To assist us be ready for an exciting future, the Online Metabolic and Molecular basis of Inherited Disease now includes chapters on each of these subjects in anticipation of this time (64).

Potential for artificial intelligence and machine learning: Since screened diseases are rare and screening technologies must be highly sensitive, developing and maintaining newborn screening (NBS) programs is still a significant and difficult task. Various machine learning (ML) techniques have been used recently to help NBS. ML techniques may generally lower the false positive rate and find previously undiscovered metabolic trends in NBS data (65).

Line of Work for Machine Learning in Newborn Screening – A: promising development that has the potential to improve the precision, effectiveness, and scope of these screening programs is the incorporation of machine learning (ML) into newborn screening by enhancing the precision, effectiveness, and reach of these initiatives, machine learning holds the potential to completely transform newborn screening. As technology advances and more data becomes available, the application of machine learning in newborn screening is anticipated to improve the health and lives of countless babies by enabling early identification and improved outcomes for several congenital and genetic abnormalities (figure 7).

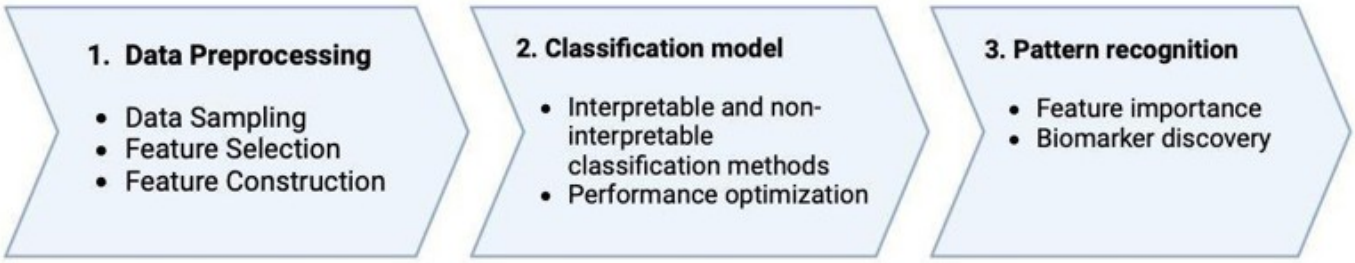

Figure 7. Line of Work for Machine Learning in Newborn Screening

Reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8995842/figure/jmd212285-fig- 0001/

7.2Implementation Challenges - NBS entails more than just testing. Six steps make up the NBS process, which is often coordinated and carried out by public health institutions with the funding and power to do universal screening.(68) It is a process involving many parties, such as the patients, families, POs, and healthcare professionals. All stakeholders must communicate and interact with each other before, during, and after the test. Resources, proper training, standardized and accredited methods, and quality controls that meet international standards are required for each step of the screening process. The phases of NBS can be summarized in the figure (8) (67). An illustration of the Newborn Screening stages : The goal of this significant public health strategy for newborn screening is to identify certain genetic, metabolic, hormonal, and functional problems early on and treat them. The complete workflow of the newborn screening program typically consists of a few crucial processes, ranging from sample collection to diagnosis and follow-up. A screening program aims to provide early detection and intervention for illnesses that are likely to be critically ill by means of a synchronized collection of prices in its workflow. Every step, from fast collection and sufficient laboratory testing of samples to proper follow-up and long-term care contributes to the health and well-being of infants. The successful implementation of these programs requires full cooperation between public health organizations, laboratories, and medical professionals. Modern technologies and extremely rigorous quality assurance procedures are also essential (FIGURE 8) (66) (67).

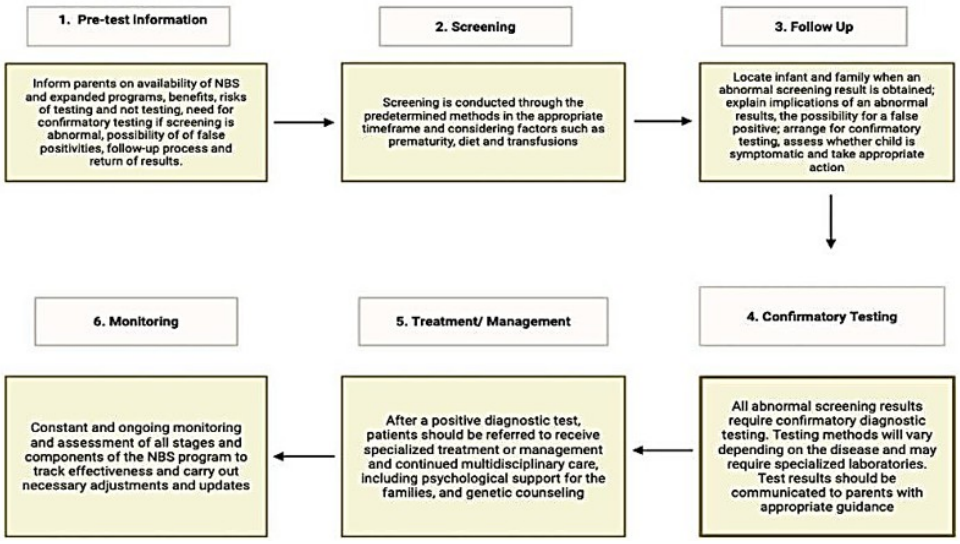

Figure 8. An illustration of the Newborn Screening stages

Reference: https://www.frontiersin.org/files/Articles/1053559/fgene-13-1053559- HTML/image_m/fgene-13-1053559-g002.jpg

CONCLUSION

Key points summarized: NBS enables the early detection of many congenital conditions and genetic illnesses in newborns. Screening can detect these issues in infants so that prompt treatment and interventions can be put in place. Using NBS, medical professionals may implement the proper care and preventive measures to identify diseases at an early stage. This can minimize the severity of the ailment, avoid problems, and enhance the affected infants' long-term health results. By allowing for early medical intervention, the start of specialised care, and therapeutic interventions, early discovery can significantly improve the quality of life for the affected newborn. NBS program setup may need an initial financial outlay, but it is typically not expensive over time. (6). Thus, NBS can help with early illness identification and treatment, which will lower the expense of long-term care, hospital stays, and problems from unknown ailments. In this sense, NBS program can improve public health generally by lowering the prevalence of genetic anomalies and congenital illnesses in a community. NBS program helps parents understand the need of early diagnosis and treatment. It empowers parents to take a more active role in their kid's care and empowers them to make informed decisions by giving them access to critical health information about their child. It means NBS of India has great potential to make the health of newborns much better and healthier. The process of early detection, treatment, and prevention of congenital diseases and genetic disorders is achieved. It enhances not only the health outcome but may also reduce the burden on healthcare systems of the nation.

Future directions - Newborn screening will continue to expand by the addition of disorders for which early intervention can significantly modify the outcome. This expansion is driven by the development of new therapies for larger numbers of inborn errors of metabolism and the advancement in testing methodologies. The integration of proteome, metabolomics, and genome technologies—either separately or in combination for a multi-omics approach— looks potential for future NBS endeavors. Any new testing methodologies presented must undergo sufficient feasibility and acceptability reviews in order for the NBS programs to remain a viable public health initiative. Applying a multi- pronged strategy that makes use of information from multiple-omics data to more precisely forecast illness risk and so reduce the risk of over interpreting omics screening results may yield the greatest value from these technologies. It needs to be seen how well a multi-omics NBS approach would work overall and how well it would fit in with the present NBS standard of care (60).

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

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