Komal Uppal et al. JBC Genetics. 2025;8(1):036-047
DOI: 10.24911/JBCGenetics.11-2221

REVIEW ARTICLE

Bridging metabolomics and genomics: genetic counselling for IEMs

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ABSTRACT

Inborn errors of metabolism (IEMs) caused by a deficit of some specific metabolic pathways are phenotypically heterogeneous complex disorders. Although in recent years metabolomics has helped in understanding the pathophysiology of IEMs, its challenges and limitations such as false positives and negatives result in delayed diagnosis and postponed treatment. This leaves the physician with a large list of differential diagnosis among IEMs. Early and accurate diagnosis in case of suspicious IEMs can be lifesaving, especially for conditions those are treatable. Recently, next-generation sequencing (NGS)-based whole-exome sequencing proved to be an efficient technology in enhancing the accuracy of diagnosis of metabolic disorders, especially for complex disorders. It offers a broader range of disorders diagnosed at an affordable cost compared to metabolomics. The aim of this article is to provide insights into bridging metabolomics and NGS-based genomics to improve diagnostic yield in complex IEMs, while also emphasizing the role of genetic counselling in empowering families and improving patient quality life.

Keywords: Inborn errors of metabolism, metabolomic, genomics, genetic counselling.

Introduction

Inborn errors of metabolism (IEMs) are rare genetic disorders. They are often inherited as autosomal recessive traits or arise from *de novo* mutations, environmental, epigenetic, and microbiome factors. They result from metabolic defects affecting small molecules (e.g., amino acids, fatty acids) or larger ones (e.g., glycogen, sphingolipids, etc.). This defect leads to the accumulation or deficiency of energy, causing heterogeneous clinical spectrum and multiple organ damage depending on the metabolic pathway involved (Figure 1a,b) (1,2). These disorders primarily manifest with neurological symptoms, such as developmental delays, poor muscle tone, and seizures, as well as gastrointestinal issues, including vomiting, diarrhea, dehydration, and hepatomegaly. Additional signs may include features resembling unconfirmed sepsis, hypoglycemia, failure to thrive, autonomic instability, and behavioral or learning difficulties. Despite individual rarity, IEMs collectively affect 1 in 2,500 births, highlighting their clinical significance.

The varied phenotypes and ages of onset in IEMs pose significant diagnostic challenges for clinicians, requiring them to navigate an extensive list of differential diagnoses. This complexity arises from cases where a single gene may affect multiple pathways or multiple genes may lead to similar phenotypes. This complexity challenges the traditional "one gene–one enzyme–one disease" model (3). Therefore, understanding the full spectrum of these disorders and their evaluation is crucial for early recognition, which can help prevent mortality and long-term morbidity (4). Diversity of IEMs needs a personalized approach for each patient. Basic tests such as glucose, ammonia, and ketones offer initial clues, but advanced tools such as metabolomics and depending on its results and suspected disorder, and further next-generation sequencing (NGS) are essential for precise, personalized diagnoses.

Metabolomics in IEMs

Metabolomics is typically performed as a first-tier testing on dried blood spots (DBSs). Various methods,

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Received: 17 May 2025 | **Revised:** 09 June 2025 | **Accepted:** 16 June 2025

such as time-resolved fluorimmunoassay, plasma amino acid assay by high-performance liquid chromatography, urine organic acid assay by gas chromatography-mass spectrometry (GC-MS), and acyl carnitine and amino acid profile by mass spectrometry (MS/MS), are used to identify diagnostic biomarkers for certain IEMs. This approach improves understanding of pathophysiology, diagnosis, and management of diseases with specific biomarkers (8–50 different diseases). However, diagnosing complex IEMs with huge clinical overlapping and variability, nonspecific or unknown biomarkers, and inconclusive biochemical investigations remains challenging. These cases often leave physicians with broad differential diagnoses. The limited tools for accurate identification explain that advanced metabolomics aids some diagnoses but cannot address the entire spectrum of IEMs (5).

Enough literature showed that MS/MS offers advantages such as speed, convenience, early diagnosis, and cost-effectiveness in expanded newborn screening to improve IEM detection significantly (6–8). However, Tarini et al. has reported its limitations, including false positives/negatives, low sensitivity, and poor predictive value (9). Early testing for newborns before sufficient metabolite accumulation or post-transfusion can yield false negatives. Schnabel et al. (10) showed that (MS/MS) is also limited as a single-tier analysis, sometimes failing to distinguish between similar diseases or subtypes. In addition, repeating tests for unclear diagnoses adds emotional stress and financial burden.

Genomics in IEMs

Targeted NGS panels or whole-exome sequencing (WES) are cost-effective options for diagnosing IEMs supported by previous literature (11–15). Tang et al. (15) study shows that integrating NGS with MS/MS improves screening accuracy, diagnostic yield, and raising sensitivity to 91.3%–100% while Shen et al. (16) 2024 showed reduction in false-positive rate of MS/MS (1.4%). NGS enhances the specificity of biochemical evaluations by linking genetic and biochemical networks to address unexplained genotype–phenotype relationships. Furthermore, in regions with limited literacy or cultural taboos, integrating genomic testing along with biochemical methods greatly impacts diagnostic accuracy in case of false positives/negatives (17). In silico homology modeling tools, such as SWISS-MODEL, are instrumental in elucidating how disease-causing mutations - particularly missense and truncating variants - affect protein folding, stability, and function, thereby contributing to disease onset and progression. These tools simulate the structural ramifications of genetic alterations by aligning mutant sequences with wild-type templates to predict conformational changes that may compromise protein activity or stability. This computational approach significantly enhances our understanding of genotype-phenotype correlations and improves the clinical interpretation of mutations identified through WES. When integrated with experimental and biochemical data, such analyses aid in refining variant pathogenicity and informing clinical decision-making.

The SWISS-MODEL is a widely used automated homology modeling server that generates reliable 3D protein structures from amino acid sequences based on template alignments. It supports visualization of both tertiary and quaternary structures, making it valuable for exploring the structural impact of mutations (18).

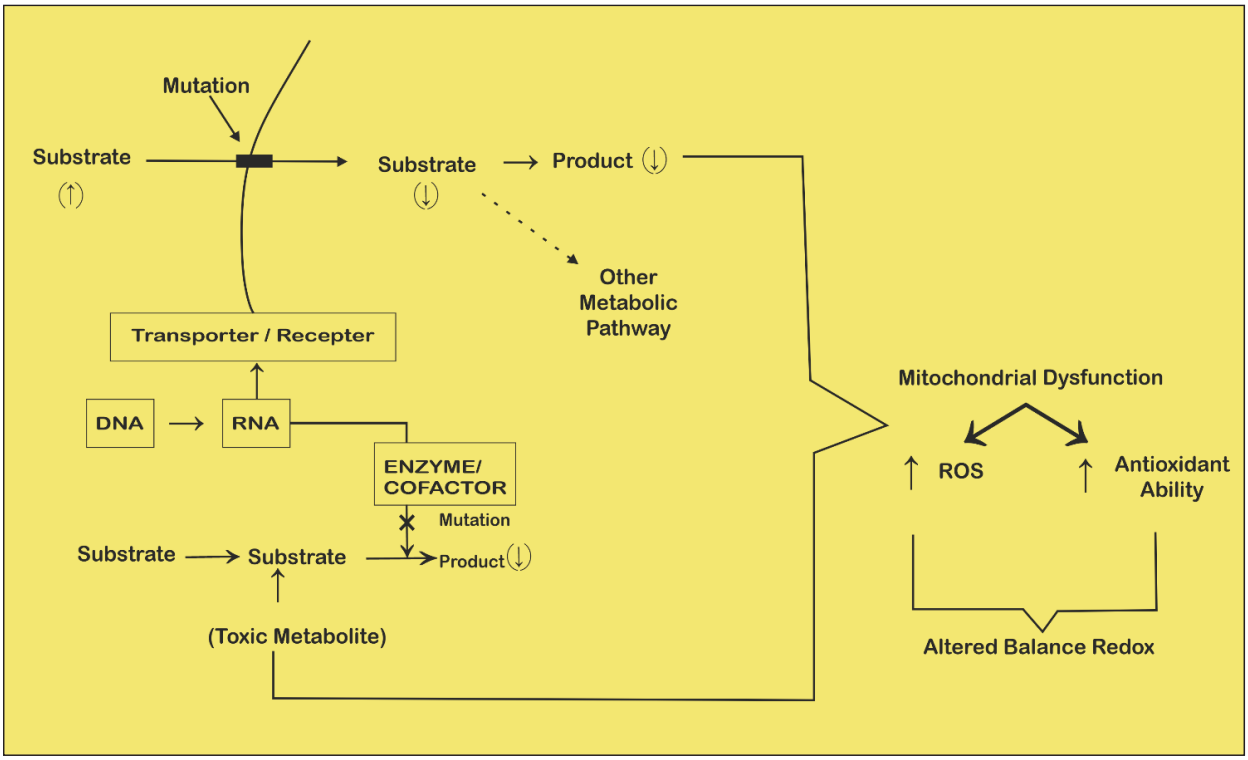
To further aid interpretation, molecular visualization software such as PyMOL plays a critical role. PyMOL allows detailed 3D visualization of modeled proteins, enabling researchers to compare wild-type and mutant structures. It analyzes changes in hydrogen bonding, surface accessibility, and binding interfaces. These visual insights can validate whether a variant disrupts the protein's active site, folding core, or interaction regions - essential for understanding disease mechanisms and therapeutic targeting (19). In our recent study involving a rare pathogenic variant in the STXBP1 gene (20), we used SWISS-MODEL and PyMOL to model and visualize the structural consequences of the variant. The observed conformational alterations supported its pathogenic classification and highlighted the utility of combining homology modeling with visual analysis in clinical genomics.

Beyond variant detection, structural modeling provides crucial functional insights, especially for variants of uncertain significance. For instance, in the current study, we identified a frameshift mutation (c.558_559delAT) in the PAH gene and assessed its structural implications via homology modeling. Sequence alignment revealed a significant divergence from the wild-type (WTPAH), resulting in a truncated mutant (MutPAH) protein. Homology models generated using SWISSMODEL, based on PDB template 6hyc.1.B, demonstrated that the mutation induced substantial alterations in the C-terminal domain. WTPAH showed a 92.57% identity with the template and a QMEANDisCo global score of 0.65 ± 0.06, indicating moderate model reliability. In contrast, the MutPAH model, truncated and structurally distorted, retained a QMEANDisCo score of 0.70 ± 0.06, yet exhibited local conformational instability. Structural visualization in PyMOL revealed disruption of helices and loops, suggesting potential loss of enzymatic function (Figure 2Ba–Bf). Such integrative modeling strengthens variant interpretation by linking molecular disruptions to biochemical phenotypes and provides a rational basis for reclassifying pathogenicity.

Ultimately, accurate interpretation of NGS data hinges on robust quality control at every step from deoxyribonucleic acid (DNA) extraction to variant annotation. Advances in exome capture technology, sequencing platforms, and bioinformatics pipelines have increased diagnostic precision but also complexity. Thus, the integration of standardized protocols, clinical phenotyping, and biochemical data remains essential to maximize the diagnostic utility of WES in IEMs.

NGS, including targeted panels, WES, and whole-genome sequencing (WGS), has significantly improved IEM diagnosis. It enables high-throughput massively parallel sequencing of numerous genomic sites simultaneously. WES, which focuses on coding regions where 80% of pathogenic variants occur, offers a

(Figure 1a)



(Figure 1b)

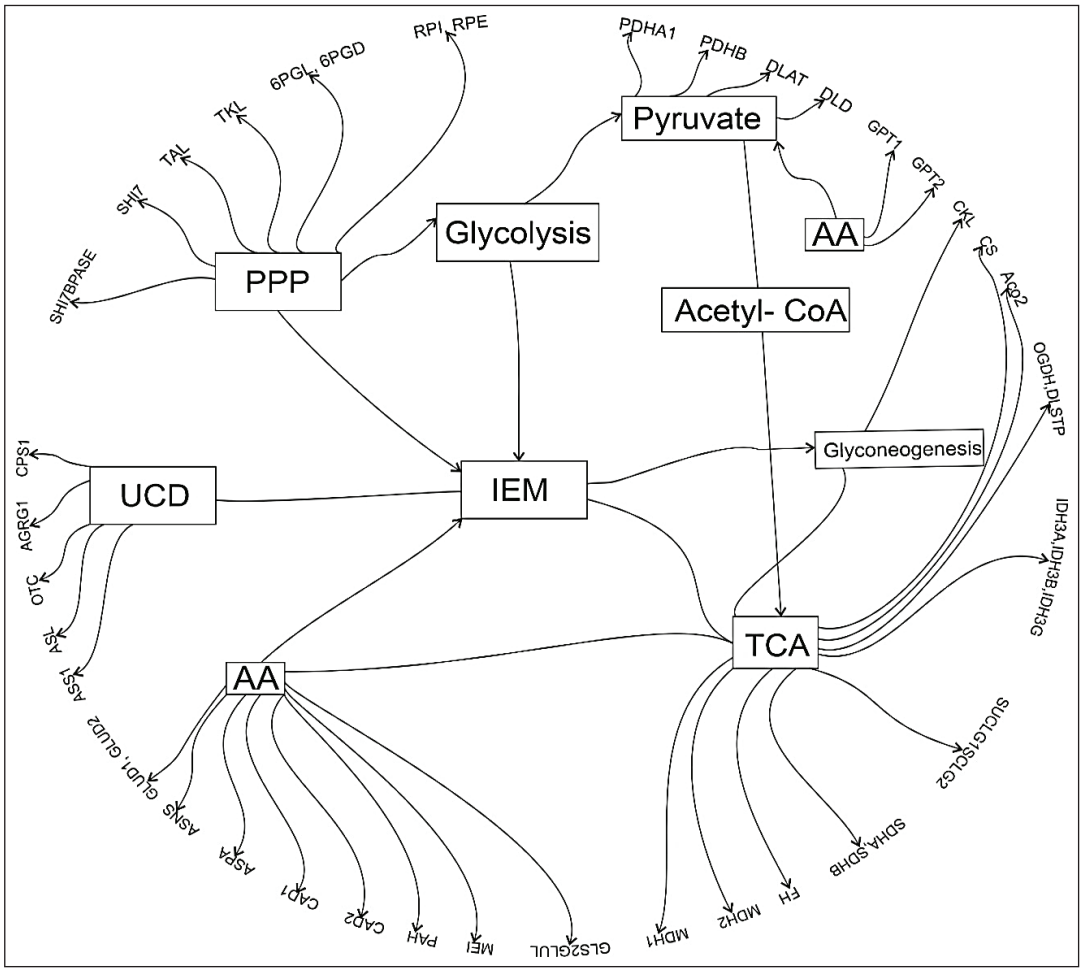
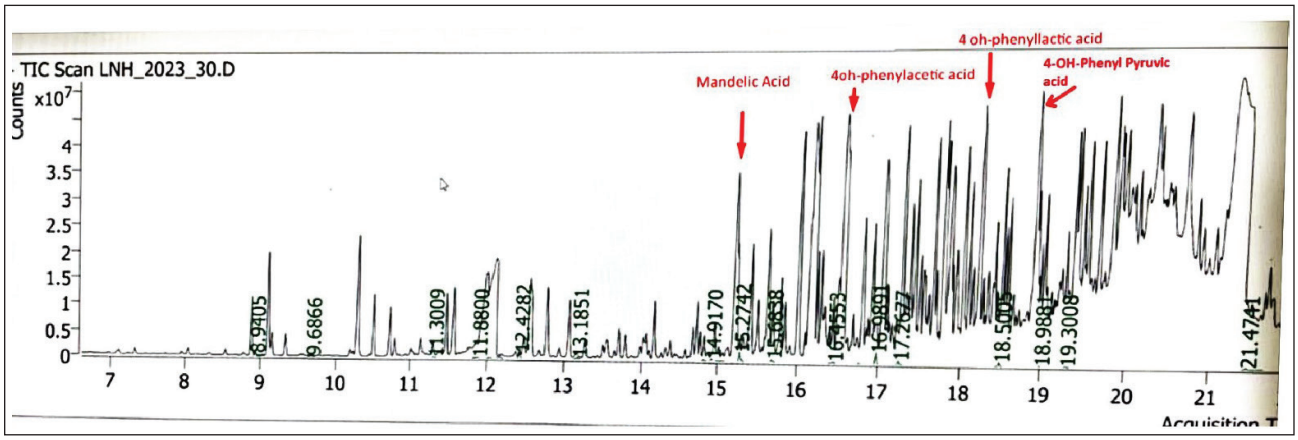


Figure 1. *(a) Metabolic defects leading to the accumulation of toxic metabolites or deficiency of energy. (b) Key metabolic pathways involved in IEMs, including glycolysis, pentose phosphate pathway (PPP), pyruvate metabolism,* **tricarboxylic acid (TCA)** *cycle, amino acid (AA) metabolism, and urea cycle disorders (UCD). It highlights how disruptions in specific enzymes and genes across these pathways contribute to the complexity of IEMs.*

(Figure 2a)



(Figure-2b)

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PAHWT           MSTAVLENPGLGRKLSDFGQETSYIEDNCNQNGAISLIFSLKEEVGALAKVLRLFEENDV
MutPAH          MSTAVLENPGLGRKLSDFGQETSYIEDNCNQNGAISLIFSLKEEVGALAKVLRLFEENDV
                ************************************************************

PAHWT           NLTHIESRPSRLKKDEYEFFTHLDKRSLPALTNIIKILRHDIGATVHELSRDKKKDTVPW
MutPAH          NLTHIESRPSRLKKDEYEFFTHLDKRSLPALTNIIKILRHDIGATVHELSRDKKKDTVPW
                ************************************************************

PAHWT           FPRTIQELDRFANQILSYGAELDADHPGFKDPVYRARRKQFADIAYNYRHGQPIPRVEYM
MutPAH          FPRTIQELDRFANQILSYGAELDADHPGFKDPVYRARRKQFADIAYNYRHGQPIPRVEYM
                ************************************************************

PAHWT           EEEKKTWGTVFKTLKSLYKTHACYEYNHIFPLLEKYCGFHEDNIPQLEDVSQFLQIPAVL
MutPAH          EEEKKTGHSVQDSEVLV-NPCLL-VQSHFSTS-KVLWLP-R-HSPAGRRFSIPADSCCVM
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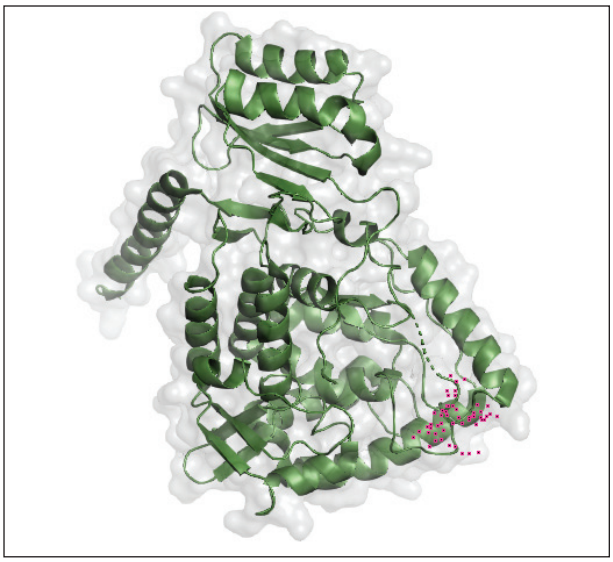
(Figure-2c)



(Figure-2d)

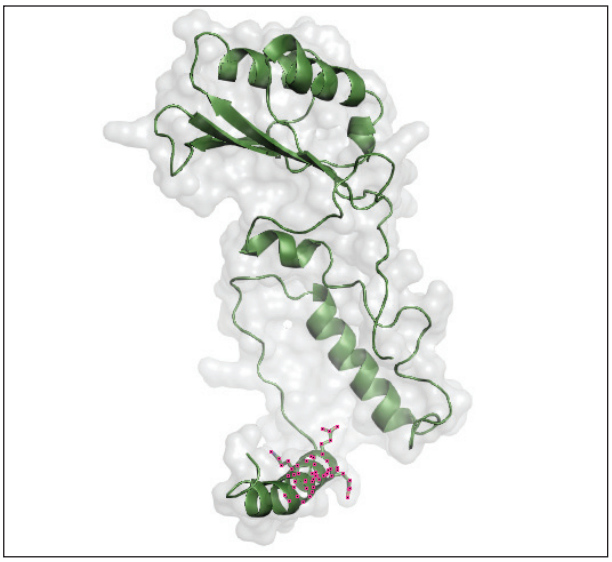
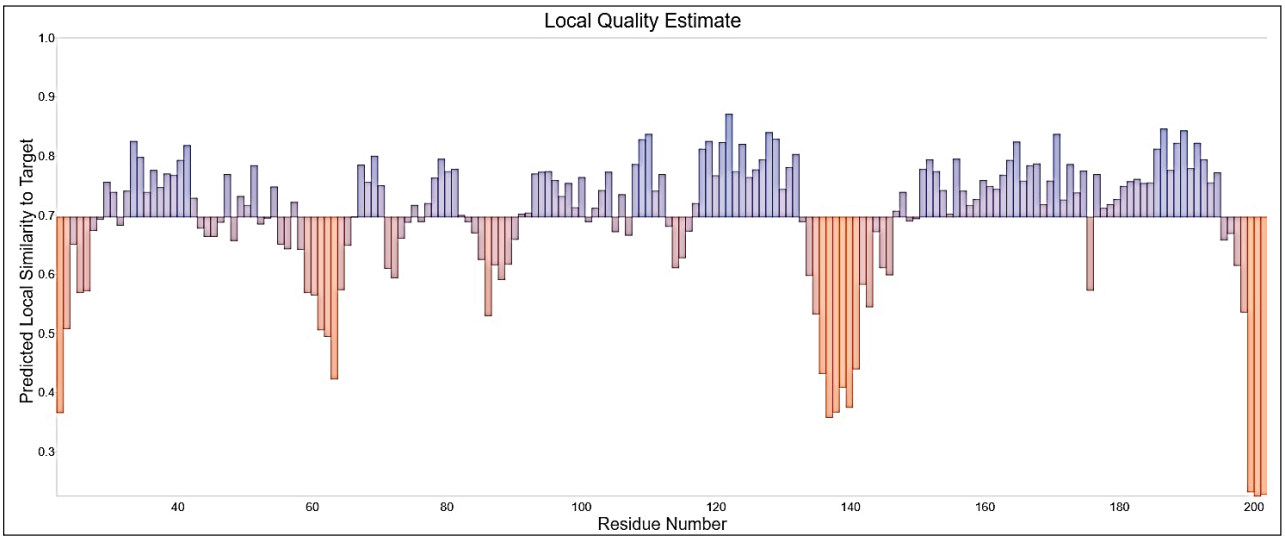
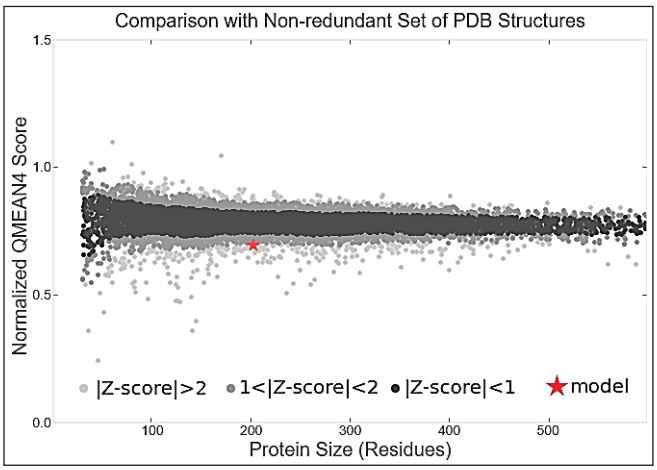


Figure 2. *Continued*

(Figure-2e)



(Figure-2f)



(Figure-2g)

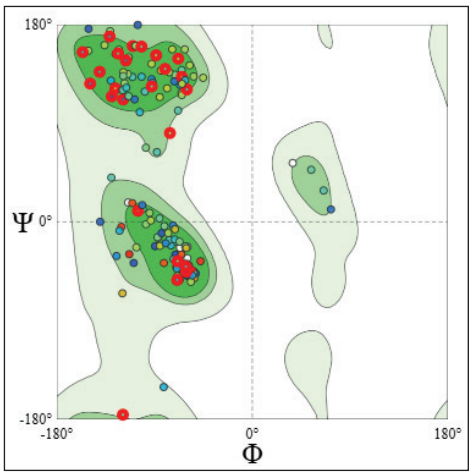


Figure 2. *(a) GC-MS chromatogram of urine from Case no.5 with phenylketonuria (PKU). The chromatogram displays the separation of urinary metabolites. Peaks corresponding to phenylalanine and its metabolites are highlighted, showing elevated levels of phenylalanine. The presence of abnormal metabolites such as phenylpyruvic acid and phenylacetic acid supports the diagnosis of PKU. Peak identification was confirmed by retention time and mass spectral analysis. The chromatogram illustrates the characteristic metabolic disruption observed in PKU. (b) Sequence alignment and structural modeling of PAHWT and MutPAH (c.558_559delAT) revealed significant differences in the C-terminal region, with altered residues indicated by colons (:) and periods (.), and conserved regions marked by asterisks (**). The mutant protein showed a frameshift-induced truncation. (c, d) Homology models generated using SWISS-MODEL (PDB ID: 6hyc.1.B) showed the wild-type PAH spanned residues 22–202, with 92.57% identity and moderate structural reliability (GMQE: 0.60; QMEANDisCo: 0.65 ± 0.06). (e-g) The mutant model, though retaining a similar QMEAN score (0.70 ± 0.06), displayed local structural instability, suggesting loss of function. Validation metrics (Z-scores: global –1.91, Cβ –2.23, all-atom –0.58; MolProbity score: 1.18; 95.53% favored Ramachandran residues) supported moderate model confidence, with minor deviations indicating potential functional impairment in the mutant PAH.*

practical diagnostic advantage over Sanger sequencing (which has the limitation of analyzing only a small genomic region). It is less labor-intensive and more cost-effective. While WGS can identify novel genes, its high cost and complexity limit clinical use. WES remains a preferred option, facilitating early, accurate diagnosis and treatment of complex metabolic disorders, even from DBSs (21). Scherer et al. (22) reported that integration of genomics and metabolomics can identify new variants in incompletely recognized human metabolic pathways and reveal the underlying metabolic causes of human traits and diseases. Heinken et al. (23) proposed a method to create personalized genome-scale models for IEM patients for the prediction of biomarkers and tailored therapeutic or dietary interventions. Another study used a multi-omics integrating approach to discover the presence of anaplerosis in patients with methylmalonic acidemia (MMA) (24). A metabolite-genome-wide association study for two large cohorts identified a graph-based network of causal metabolite-gene variant associations linked to IEMs (25).

WES has shown a diagnostic yield of 16%–68% for neurometabolic disorders, influenced by factors like population diversity, phenotype variation, technical

differences, and advances in analysis (26–28). Cautious data analysis and genotype–phenotype relationship not only aid in identifying causative genes but also uncover novel variants. It also identifies the deleterious effects of these novel variants on gene function, novel diseases, and phenotypes, improving future diagnoses for similar phenotypes (29). Its integration with copy number variations and mitochondrial sequencing has positioned WES as a first-tier tool for many genetic disorders, particularly for complex or blended phenotypes for precise diagnosis and treatment planning (30–32).

The diagnostic accuracy of WES relies on robust quality control, from DNA extraction to variant interpretation. Advances in exome-capturing systems, sequencing platforms, and bioinformatics pipelines add complexity to data analysis. Hence, proper standards, protocols, and clinical and biochemical information are required to get accurate results. Collaboration among bioinformaticians, clinicians, geneticists, and families ensures reliable results. A clear understanding of WES's details and efficacy helps manage expectations for definitive diagnosis, specific treatment, and family planning. A definitive diagnosis supports understanding natural history of disease, precision management, guiding management decisions, reducing psychosocial stress, and optimizing healthcare resources between curative and palliative and rehabilitative care (33).

Although NGS provides a confirmatory diagnosis for IEMs, several challenges persist. These include lack of trained clinicians, ethical concerns (reporting uncertain or late-onset findings, maintaining confidentiality and autonomy, sharing of reports with relatives at risk), legal risks (errors in testing), and psychological, societal impacts, and insurance debates. Technical issues include data storage, analysis costs, and limitations like missing certain gene regions, large structural variations, repeat expansions, epigenetics, deep intronic variants, multifactorial disorders, mitochondrial DNA variants, and identification of pseudogenes, uncertain variants for WGS. In addition, the lack of extensive functional studies and requirement of high through output sequencers for WGS are also big challenge. Recognizing these challenges is crucial for evaluating NGS's effectiveness and addressing gaps in clinical practice (34–36).

The advancements in genomic and metabolomic integration methodologies can significantly impact the field of genetic counseling for IEMs

The integration of multi-omic approaches beyond genomics and metabolomics has enabled researchers to uncover complex biological pathways and interactions, advancing our understanding of metabolic disorders and paving the way for innovative therapeutic strategies.

One compelling avenue to explore is the incorporation of transcriptomics, proteomics, and epigenomics, alongside metabolomics, to create a comprehensive multi-omic profile of individuals affected by IEMs. This combined approach allows for a more nuanced understanding of how genetic variations translate into metabolic phenotypes, ultimately influencing patient outcomes (37,38). For instance, as discussed by Kuile and Westerhoff (37), considering the interactions between the transcriptome and metabolome provides deeper insights into metabolic regulation, which is critical for identifying personalized treatment options. The holistic view offered by these multi-omic strategies not only enhances diagnostic accuracy but also lays the groundwork for targeted and novel therapies, especially as we begin to generate and analyze high-dimensional data produced by these methods (38,39).

Emerging technologies in metabolomics, such as single-cell metabolomics and advanced mass spectrometry techniques, provide new opportunities for understanding metabolic dynamics at unprecedented resolution. These techniques facilitate the detection of metabolic changes at the cellular level, revealing heterogeneity that traditional methods may overlook (40,41). For instance, single-cell approaches could illuminate specific metabolic alterations occurring within distinct cell populations in the context of IEMs, which is essential for accurate phenotyping and subsequent intervention strategies (42,43).

Furthermore, recent developments in bioinformatics tools and artificial intelligence (AI) applications for data integration are reshaping how we approach metabolomic datasets in relation to genomic data. Machine learning algorithms can sift through massive datasets to identify patterns, correlations, and potential biomarkers that can inform clinical practice (39,44). An exemplary study illustrates the power of AI in analyzing complex datasets derived from multi-omics platforms, highlighting the potential for identifying novel therapeutic targets and methodologies that could be adapted for metabolic disorders (45).

As our work progresses, it becomes paramount to articulate future directions that focus on translating these emerging technologies into clinical applications. The integration of metabolomics with advancements in CRISPR technology and gene-editing may enable not only the exploration of gene function but also the capability to manipulate metabolic pathways for therapeutic gain (39,43). Coupled with advancements in personalized medicine, there is great potential for metabolomics to guide clinicians in tailoring treatments based on individual metabolic profiles and responses to therapeutics (40,44).

Role of genetic counselling in IEMs

Due to the complexity, criticality and lack of expertise and awareness in IEMs and about the diagnostic technology, general physicians hesitate to treat and counsel the parents of the patients. Hence, it is making the genetic counselling a valuable resource to educate the families of patients with IEMs at the time of the initial diagnosis and later on.

Genetic counselling for IEMs by a person who is an expert in both clinical and counselling aspects is important not only to reduce the burden of these disorders but also to improve the patient's quality of life.

The objective of the genetic counselling is to provide information about its fundamental elements of counselling, to make aware the family about the natural

course of IEMs and management of the disease. It also provides the opportunity for clinicians to understand the family's knowledge, acceptance, and attitude toward the disorder, which helps them to accept and adapt to the situation. To achieve this, genetic counselling in a step-wise manner, which first includes pretest counselling (i.e., before making the definitive diagnosis) regarding the importance of getting a definitive diagnosis, all the details of genetic and miscellaneous tests, is done. In the second phase, post-test counselling (at the time of disclosure of definitive diagnosis) includes: what does the genetic testing report and disease diagnosed means, counselling regarding co-morbidities and prognosis, need of special, précised and holistic disease management by a multidisciplinary team, importance of early therapeutic intervention to prevent significant neurological damage, long-term longitudinal management, recurrence risk (25%), preconception and prenatal management counselling, including preimplantation genetic testing and ***in-vitro*** fertilization options. Psychosocial counselling and counselling during follow-up visits after establishing the diagnosis is very influential in bringing the difference in the attitude of family toward acceptance of such patients. Counselling of affected teenage children supports a smooth shift of the teenage patient to an adult health care unit, helping them in understanding their condition and in taking charge of their care independently, which is also a crucial component of the genetic counselling. Additionally, making the family aware about various metabolic organizations in India, support networks, advocacy groups, the government initiative like the National Policy for Rare Diseases, 2021 can help them in facing the social, mental, emotional, and financial challenges (46,49). Furthermore, cultural sensitivity in genetic counselling should be considered to respect different beliefs and values. Ethical considerations, such as informed consent and handling uncertain findings, must be addressed. Legal aspects, including patient privacy and protection from genetic discrimination, should also be highlighted.

Lastly, encouraging participation in research or clinical trials provides access to emerging therapies and strengthens the understanding of IEMs, contributing to better care and outcomes. With these added components, genetic counselling becomes a comprehensive process that supports the family through every step of diagnosis, treatment, and beyond.

List of a few cases to show the role of integration of genomics and genetic counselling in IEMs

Case scenario 1: global development delay and seizure disorder

A 9-month-old male born full-term to non-consanguineous parents with normal initial growth and development presented with global developmental delay, irritability, hyperactivity, and seizures. Examination and blood investigations showed exaggerated deep tendon reflexes, mild hypoglycemia, elevated ammonia, and metabolic acidosis. TMS showed low glutamic acid levels (89 nmol/l normal: 149–515), high glycine (855 nmol/l normal: 2.0–745), and high levels of propionyl carnitine (C3): 19.9 nmol/l (0.08–4.8), gas chromatography/mass spectrometry (GCMS) urine showed high peak of 3 OH-propionic acid, methyl citric acid, proponyl glycine, triglycine, and 3-OH Isovaleric suggestive of propionic aciduria. MRI brain revealed abnormal signal in periventricular bilateral parietal white matter and subcortical bilateral parieto-occipital white matter consistent with hypoxic-ischemic encephalopathy sequelae. WES confirmed compound heterozygous mutation in exon 14 c.1396T>A and intron c. 101060-3C>A in the PCCB gene, making the diagnosis of propionic acidemia (Figure 3). Management, such as dietary protein restriction, carnitine, and metronidazole, leads to clinical improvement in the patient. Genetic counseling was provided for the natural history of disease, 25% of recurrence risk, and family screening.

Case scenario 2: global development delay and seizure disorder

A 4-month-old male born full-term to non-consanguineous parents presented with severe anemia with history of two blood transfusions, global developmental delay, microcephaly, pneumonia, and failure to thrive at the age of 3.5 months. Examination showed anthropometric measurements < 3rd SD. Investigations revealed microcytic hypochromic anemia with Hb of 3.8 gm/dl, normal B12 (878.00 pg/ml) and normal folic acid levels (17.00 ng/ml), and MRI findings of cortical atrophy. Ophthalmology assessment showed an atrophic patch in the right iris, not following/ fixating focal light. TMS, urine GC-MS showed elevated C3 propionylcarnitine, methylmalonic acid, and methylcitric acid. WES identified a homozygous pathogenic mutation in exon 3 c.394C>T in MMACHC gene, confirming methylmalonic aciduria and homocystinuria (cblC type) (Figure 3). Treatment with hydroxocobalamin injection, betaine, folinic acid, carnitine, and dietary protein restriction of isoleucine, valine, threonine, and methionine improved outcomes. Genetic counseling addressed natural history of the disease, recurrence risks, and family screening.

Case scenario 3: seizure disorder with developmental delay leading to infant mortality

A non-consanguineous couple sought genetic counselling after two babies were born during infancy. Both children were full-term delivered, had developmental delays (lack of social smile), generalized tonic-clonic seizures, and microcytic hypochromic anemia. MRI brain was normal. TMS and urine GCMS did not show any metabolic abnormality. Biotinidase enzyme assay showed low levels 1.3 nmol/min/ml of biotinidase and WES revealed a homozygous mutation in exon 4 c. and 104_110delinsTCC of BTD gene in the second child (Figure 3). Despite treatment with biotin, the child later passed away. Genetic counseling regarding natural history of biotinidase deficiency, about the risk of recurrence, prenatal diagnosis and need for genetic testing for parents and other family members at risk was done. Prenatal diagnosis in a subsequent pregnancy confirmed the mutation, but the parents opted to continue the pregnancy

Figure 3. *Sequence alignment of wild-type and mutated gene sequences.*

with early biotin treatment for the newborn. The child is now healthy, with only sparse hair, following timely intervention. Genetic counseling and prenatal planning helped manage the condition effectively.

Case scenario 4: global development delay and dystonia

An 8-month-old female born full-term to non-consanguineous presented with a significant global developmental delay, excessive crying, irritability, and generalized dystonia with normal hearing and ophthalmological examination. Examination revealed dystonia, hypertonia and exaggerated deep tendon reflexes. Blood and urine tests were normal. TMS showed high levels of C5-DC (glutarylcarnitine) which can be increased in glutaric acidemia and MADD. GCMS urine was positive for glutaric acid, 3-OH glutaric acid, and glutaconic acid. MRI brain was normal. Based on history, symptoms, and metabolic evaluation, a high suspicion of organic academia especially GA1 was kept. WES showed homozygous mutation in exon 8 c.881G>C of GCDH gene confirming Glutaric acidemia 1 (Figure 3). Treatment with a diet restricted in lysine and tryptophan with carnitine supplements improved outcome. Genetic counseling regarding the natural history of Glutaric acidemia, the risk for future pregnancies, and the need for genetic testing for the parents and other family members at risk was done.

Case scenario 5: global development delay (GDD) with microcephaly and encephalopathy, hypopigmented hair, and atopic dermatitis

A 6-month-old female born full-term to non-consanguineous parents presented with a significant global developmental delay and encephalopathy with

a past history of pneumonia and seizures. The family gave the history of two sibling deaths who also had a history of GDD. Anthropometric measurements were less than 3rd centile suggesting severe acute malnutrition and microcephaly. Examination showed sparse hypopigmented hair, perioral rash, discharging ear, atopic dermatitis, generalized hypotonia and hyporeflexia. Clinical suspicion of biotinidase deficiency and infantile holocarboxylase deficiency was kept. Complete hemogram showed Pancytopenia. Thyroid function tests were normal. Fundus examination showed macular atrophy. MRI Brain showed diffuse b/l symmetrical T2/FLAIR hypertrophy, showing diffusion restriction involving subcortical and deep periventricular white matter, centrum semiovale as well as corona radiata along with involvement of b/l globus pallidus with mild cerebral atrophy, suggestive of hypoxic ischemic injury, metabolic disorder, and bilirubin encephalopathy. The TMS report showed high phenylalanine (1034.1 nmol/l) and low Gln/Lys ratio (217.97). GCMS urine showed phenyl acetic acid, phenyl pyruvic acid, and phenyl lactic acid (Figure 2A). Provisional diagnosis of phenylketonuria (PKU) and biopterin cofactor defects was kept and WES showed pathogenic homozygous mutation in exon 6 c.558_559delAT of PAH gene and confirming phenylketonuria (Figure 3). Homology model of wild-type and mutant human PAH protein model resulting in truncation and structural disruption of protein showed deviation in local quality scores in the mutant model suggesting altered protein folding and possible functional loss of protein (Figure 2Ba–2Bf). Treatment with low protein diet and Phe free formula diet improved clinical outcome. Genetic counseling regarding natural history of PKU, about recurrence risk for and the need for genetic testing for the parents and other family members at risk was given.

The figure illustrates nucleotide and corresponding amino acid sequence alignments, comparing wild-type (WTPCCB, WTMMACHC, WTGCDH, WTBTD, WTPAH) and mutant variants (c.1396T>A, c.394C>T, c.881G>C, c.104_110delinsTCC, c.558_559delAT). Conserved regions are marked by asterisks (*), while mutations are highlighted in yellow. Notably, the BTD c.104_110delinsTCC mutation involves deletion of ACGT and insertion of TCC, highlighted in red. The alignment emphasizes sequence divergence and conservation for variant interpretation.

WES in confirming diagnosis and treatment initiation

The WES test was useful to confirm the definitive diagnosis and initiate the treatment which was crucial to prevent long-term complications in the abovementioned cases. The WES testing report was important for providing recurrence risk of 25% in the next pregnancy and a prenatal diagnosis option during next pregnancy. The abovementioned cases show the importance of WES in getting a confirmed diagnosis and the role of genetic counselling in taking the right decision by the family. In case, if we do not have the affected child's sample for WES, parental WES can be done to know the carrier status of the parents for IEMs or many other genetic disorders that mimic IEMs, which can help in changing the approach and outcome of these complex disorders.

Conclusion

In conclusion, this article highlights a pivotal shift in IEM diagnostics from a metabolomics-centered approach to one grounded in genomics, particularly through NGS and WES. While metabolomics offers valuable biochemical clues, genomic tools provide the depth needed to uncover root causes and enable mechanism-based therapies. Moving beyond NGS, we emphasize the promise of multi-omic integration, AI-driven analytics, and CRISPR-based functional studies to address unresolved cases and personalize interventions. By positioning genomics as the foundation of a future-ready diagnostic framework supported by clinical infrastructure, policy reform, and robust genetic counseling, this work advocates for a predictive, proactive, and personalized model of IEM care. Our manuscript's aim is to bridge current diagnostic gaps and catalyze next-generation innovations that will transform outcomes and redefine standards of care for patients with metabolic disorders.

Diagnostic approach and genetic testing methodology

WES for genetic diagnosis was performed using IDT's xGen™ DNA Library Prep EZ technology on NovaSeq/ NextSeq platforms, with parental consent. Raw reads were aligned to hg38, and variants were filtered, ranked, annotated, and prioritized using the SMART-One™ algorithm (Sequence and Meta-analysis Research Toolkit/ GeneUIS®) Compute Genomics Pvt. Ltd.

Acknowledgments
Gratitude to the Division of Genetics, Lok Nayak Hospital, New Delhi & patient's family.

List of Abbreviations

AA	amino acid
AI	artificial intelligence
DBS	dried blood spots
DNA	deoxyribonucleic acid
GCMS	gas chromatography/mass spectrometry
GC-MS	gas chromatography-mass spectrometry
GDD	global developmental delay
IEMs	inborn errors of metabolism
LD	linear dichroism
MMA	methylmalonic acidemia
MRI	magnetic resonance imaging
MS/MS	mass spectrometry
MutPAH	mutant
NGS	next-generation sequencing
NPRD	National Policy for Rare Diseases
PKU	provisional diagnosis of phenylketonuria
PPP	pentose phosphate pathway
TCA	tricarboxylic acid
TLA	three-letter acronym
UCD	urea cycle disorder
WES	whole-exome sequencing
WGS	whole-genome sequencing
WTPAH	wild-type

Declaration of conflicting interests
The authors of this article have no affiliations with or involvement in any organization or entity with any financial

interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Funding
Not applicable.

Consent for publication
The authors certify that they have obtained all appropriate patient consent forms. In the form, the parents have given their consent for the patient's clinical information to be reported in the journal. The parents understand that the patient's name and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Ethical approval
Given the observational design of the study, formal ethics approval was not sought.

Institutional Review Board Statement
Not applicable.

Informed Consent
Not applicable.

Author contributions
Conceptualization—K.U and S.K; original manuscript draft preparation—K.U, H.K, N.B, S.S; review and editing—S.K and H.K; visualization—K.S and S.K.P; supervision—K.S and S.K.P; project administration—S.K. All authors have read and agreed to the published version of the manuscript. All authors reviewed and approved the final draft of the manuscript, along with final approval of this version to be published.

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References

1. Jeanmonod R, Asuka E, Jeanmonod D. Inborn errors of metabolism. Treasure Island, FL: StatPearls Publishing; 2022 [cited 2025 Jul 14]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459455/
2. Ismail IT, Showalter MR, Fiehn O. Inborn errors of metabolism in the era of untargeted metabolomics and lipidomics. Metabolites. 2019;9(11):242. https://doi.org/10.3390/metabo9110242
3. Mussap M, Zaffanello M, Fanos V. Metabolomics: a challenge for detecting and monitoring inborn errors of metabolism. Ann Transl Med. 2018;6(17):338. https://doi.org/10.21037/atm.2018.08.09
4. Stenton SL, Kremer LS, Kopajtich R, Ludwig C, Prokisch H. The diagnosis of inborn errors of metabolism by an integrative "multi-omics" approach: a perspective encompassing genomics, transcriptomics, and proteomics. J Inherit Metab Dis. 2020;43(1):25–35. https://doi.org/10.1002/jimd.12042
5. Balakrishnan U. Inborn errors of metabolism—approach to diagnosis and management in neonates. Indian J Pediatr. 2021;88(7):679–89. https://doi.org/10.1007/s12098-021-03640-0
6. Messina M, Meli C, Raudino F, Pittalà A, Arena A, Barone R, et al. Expanded newborn screening using tandem mass spectrometry: seven years of experience in Eastern Sicily. Int J Neonatal Screen. 2018;4(2):12. https://doi.org/10.3390/ijns4020012
7. Tang C, Liu S, Wu M, Lin S, Lin Y, Su L, et al. Clinical and molecular characteristics of carnitine-acylcarnitine translocase deficiency: experience with six patients in Guangdong, China. Clin Chim Acta. 2019;495:476–80. https://doi.org/10.1016/j.cca.2019.04.042
8. Zhao Z, Chen C, Sun X, Zhou D, Huang X, Dong H. Newborn screening for inherited metabolic diseases using tandem mass spectrometry in China: outcome and cost-utility analysis. J Med Screen. 2022;29(1):12–20. https://doi.org/10.1177/09691413221078113
9. Tarini BA, Christakis DA, Welch HG. State newborn screening in the tandem mass spectrometry era: more tests, more false-positive results. Pediatrics. 2006;118(2):448–56. https://doi.org/10.1542/peds.2005-2632
10. Schnabel E, Kölker S, Gleich F, Feyh P, Hörster F, Haas D, et al. Combined newborn screening allows comprehensive identification also of attenuated phenotypes for methylmalonic acidurias and homocystinuria. Nutrients. 2023;15(15):3355. https://doi.org/10.3390/nu15153355
11. Adhikari AN, Gallagher RC, Wang Y, Currier RJ, Amatuni G, Bassaganyas L, et al. The role of exome sequencing in newborn screening for inborn errors of metabolism. Nat Med. 2020;26(9):1392–7. https://doi.org/10.1038/s41591-020-0966-5
12. Tong F, Wang J, Xiao R, Wu BB, Zou CC, Wu DW, et al. Application of next generation sequencing in the screening of monogenic diseases in China, 2021: a consensus among Chinese newborn screening experts. World J Pediatr. 2022;18(3):235–42. https://doi.org/10.1007/s12519-022-00549-0
13. Huang X, Wu D, Zhu L, Wang W, Yang R, Yang J, et al. Application of a next-generation sequencing (NGS) panel in newborn screening efficiently identifies inborn disorders of neonates. Orphanet J Rare Dis. 2022;17:66. https://doi.org/10.1186/s13023-022-02248-9
14. Chen T, Fan C, Huang Y, Feng J, Zhang Y, Miao J, et al. Genomic sequencing as a first-tier screening test and outcomes of newborn screening. JAMA Netw Open. 2023;6(11):e2331162. https://doi.org/10.1001/jamanetworkopen.2023.31162
15. Tang C, Li L, Chen T, Li Y, Zhu B, Zhang Y, et al. Newborn screening for inborn errors of metabolism by next-generation sequencing combined with tandem mass spectrometry. Int J Neonatal Screen. 2024;10(1):28. https://doi.org/10.3390/ijns10010028
16. Shen G, Li W, Zhang Y, Chen L. Next-generation sequencing based newborn screening and comparative analysis with MS/MS. BMC Pediatr. 2024;24:230. https://doi.org/10.1186/s12887-024-04608-8
17. Christy AL, Das E, Surana J, Padhye P, Shirodkar K, Dixit RB, et al. Expanding the screening of newborns for detecting inborn errors in metabolism using next generation sequencing following mass spectrometry/immunoassay. Int J Clin Biochem Res. 2023;10(3):332–8. https://doi.org/10.18231/j.ijcbr.2023.057
18. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res.

2018;46(W1):W296–303. https://doi.org/10.1093/nar/gky427

19. DeLano WL. The PyMOL molecular graphics system. San Carlos, CA: DeLano Scientific; 2002.
20. Uppal K, Rana L, Kaushik H, Polipalli S, Kumar S, Kapoor S. Clinical phenotype of a rare pathogenic variant in STXBP1 gene. 2024.
21. Need AC, Shashi V, Hitomi Y, Schoch K, Shianna KV, McDonald MT, et al. Clinical application of exome sequencing in undiagnosed genetic conditions. J Med Genet. 2012;49(6):353–61. https://doi.org/10.1136/jmedgenet-2012-100819
22. Scherer N, Fässler D, Borisov O, Cheng Y, Schlosser P, Wuttke M, et al. Coupling metabolomics and exome sequencing reveals graded effects of rare damaging heterozygous variants on gene function and human traits. Nat Genet. 2025;57(2):193–205. https://doi.org/10.1038/s41588-024-01714-y
23. Heinken A, El Kouche S, Guéant-Rodriguez RM, Guéant JL. Towards personalized genome-scale modeling of inborn errors of metabolism for systems medicine applications. Metabolism. 2024;150:155738. https://doi.org/10.1016/j.metabol.2024.155738
24. Forny P, Bonilla X, Lamparter D, Shao W, Plessl T, Frei C, et al. Integrated multi-omics reveals anaplerotic rewiring in methylmalonyl-CoA mutase deficiency. Nat Metab. 2023;5(1):80–95. https://doi.org/10.1038/s42255-022-00683-5
25. Surendran P, Stewart ID, Au Yeung VPW, Pietzner M, Raffler J, Wörheide MA, et al. Rare and common genetic determinants of metabolic individuality and their effects on human health. Nat Med. 2022;28(11):2321–37. https://doi.org/10.1038/s41591-022-02019-w
26. Shakiba M, Keramatipour M. Effect of whole exome sequencing in diagnosis of inborn errors of metabolism and neurogenetic disorders. Iran J Child Neurol. 2018;12(4):7–15. https://doi.org/10.22037/ijcn.v12i4.23240
27. Yubero D, Brandi N, Ormazabal A, Garcia-Cazorla À, Pérez-Dueñas B, Campistol J, et al. Targeted next generation sequencing in patients with inborn errors of metabolism. PLoS One. 2016;11(5):e0156359. https://doi.org/10.1371/journal.pone.0156359
28. Salman DO, Mahfouz R, Bitar ER, Samaha J, Karam PE. Challenges of genetic diagnosis of inborn errors of metabolism in a major tertiary care center in Lebanon. Front Genet. 2022;13:1029947. https://doi.org/10.3389/fgene.2022.1029947
29. Chand RP, Wankhede V, Vaidya V, Iyer AS, Shelke M, Aggarwal S, et al. Proband only exome sequencing in 403 Indian children with neurodevelopmental disorders: diagnostic yield, utility and challenges in a resource-limited setting. Eur J Med Genet. 2023;66:104730. https://doi.org/10.1016/j.ejmg.2023.104730
30. Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, et al. Molecular findings among patients referred for clinical whole-exome sequencing. JAMA. 2014;312(18):1870–9. https://doi.org/10.1001/jama.2014.14601
31. Marchuk DS, Crooks K, Strande N, Kaiser-Rogers K, Milko LV, Brandt A, et al. Increasing the diagnostic yield of exome sequencing by copy number variant analysis. PLoS One. 2018;13(12):e0209185. https://doi.org/10.1371/journal.pone.0209185
32. Srivastava S, Love-Nichols JA, Dies KA, Ledbetter DH, Martin CL, Chung WK, et al. Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders. Genet Med. 2019;21(11):2413–21. https://doi.org/10.1038/s41436-019-0554-6
33. Tarailo-Graovac M, Shyr C, Ross CJ, Horvath GA, Salvarinova R, Ye XC, et al. Exome sequencing and the management of neurometabolic disorders. N Engl J Med. 2016;374(23):2246–55. https://doi.org/10.1056/NEJMoa1515792
34. Trier C, Fournous G, Strand JM, Stray-Pedersen A, Pettersen RD, Rowe AD. Next-generation sequencing of newborn screening genes: the accuracy of short-read mapping. NPJ Genom Med. 2020;5:36. https://doi.org/10.1038/s41525-020-0124-1
35. Tada H, Kawashiri MA, Nomura A, Teramoto R, Hosomichi K, Nohara A, et al. Oligogenic familial hypercholesterolemia, LDL cholesterol, and coronary artery disease. J Clin Lipidol. 2018;12(6):1436–44. https://doi.org/10.1016/j.jacl.2018.07.014
36. Remec ŽI, Trebušak Podkrajšek K, Lampret BR, Kovač J, Grošelj U, Tesovnik T, et al. Next-generation sequencing in newborn screening: a review of current state. Front Genet. 2021;12:662254. https://doi.org/10.3389/fgene.2021.662254
37. Kuile B, Westerhoff H. Transcriptome meets metabolome: hierarchical and metabolic regulation of the glycolytic pathway. FEBS Lett. 2001;500(3):169–71. https://doi.org/10.1016/s0014-5793(01)02613-8
38. Ferrara M. Future perspectives for metabolomics in nutrition research. In: Capozzi F, Bordoni A, editors. Metabolomics as a tool in nutrition research. Cambridge, MA; 2015. pp 231–6. https://doi.org/10.1016/b978-1-78242-084-2.00012-5
39. Shaath R, Al-Maraghi A, Ali H, AlRayahi J, Kennedy A, DeBalsi K, et al. Integrating genome sequencing and untargeted metabolomics in monozygotic twins with a rare complex neurological disorder. Metabolites. 2024;14(3):152. https://doi.org/10.3390/metabo14030152
40. Beger R. A review of applications of metabolomics in cancer. Metabolites. 2013;3(3):552–74. https://doi.org/10.3390/metabo3030552
41. Hu C, Xu G. Mass-spectrometry-based metabolomics analysis for foodomics. TrAC Trends Anal Chem. 2013;52:36–46. https://doi.org/10.1016/j.trac.2013.09.005
42. Fang C, Luo J, Wang S. The diversity of nutritional metabolites: origin, dissection, and application in crop breeding. Front Plant Sci. 2019;10:1028. https://doi.org/10.3389/fpls.2019.01028
43. Sirangelo T, Rogers H, Spadafora N. Multi-omic approaches to investigate molecular mechanisms in peach post-harvest ripening. Agriculture. 2022;12(4):553. https://doi.org/10.3390/agriculture12040553
44. Lu M, Zhan X. The crucial role of multiomic approach in cancer research and clinically relevant outcomes. EPMA J. 2018;9(1):77–102. https://doi.org/10.1007/s13167-018-0128-8
45. Ghazal R, Wang M, Liu D, Tschumperlin D, Pereira N. Cardiac fibrosis in the multi-omics era: implications for

heart failure. Circ Res. 2025;136(7):773–802. https://doi.org/10.1161/circresaha.124.325402

46. Veach PM, Leroy BS, Callanan N. Facilitating the genetic counseling process. Abingdon UK: ResearchGate; 2018.

47. Stein QP, Vockley CW, Edick MJ, Zhai S, Hiner SJ, Loman RS, et al. An exploration of genetic test utilization, genetic counseling, and consanguinity within the Inborn Errors of Metabolism Collaborative (IBEMC). J Genet Couns. 2017;26:1238–43. https://doi.org/10.1007/s10897-017-0094-9

48. Hartley JN, Greenberg CR, Mhanni AA. Genetic counseling in a busy pediatric metabolic practice. J Genet Couns. 2011;20:20–2. https://doi.org/10.1007/s10897-010-9330-6

49. Beck N, Applegate C. Elements of genetic counseling for inborn errors of metabolism. Transl Sci Rare Dis. 2019;4:197–208. https://doi.org/10.3233/TRD-190043