REVIEW ARTICLE

# Applications of nuclear magnetic resonance spectroscopy in pediatric clinical metabolomics: From research to future perspectives

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**Abstract**

**Metabolomics studies small-molecule metabolites to provide insights into health and disease, supporting early diagnosis and personalized medicine. Advances in mass spectrometry and nuclear magnetic resonance (NMR) have expanded its use in metabolic, cancer, and cardiovascular diseases. In pediatrics, high-resolution NMR metabolomics has been instrumental in identifying age-related metabolic changes during early childhood and their associations with growth, nutrition, and disease risk. However, a comprehensive review of its clinical applications and future potential remains limited. This review highlights how utilizing specific NMR pulse sequences, such as CPMG and NOESY, allows for precise and non-destructive metabolic profiling of diverse biofluids, supported by minimal sample preparation and high-throughput automated analysis. Data processing tools like NMRProcFlow and MetaboAnalyst facilitate spectral preprocessing, statistical analysis, and biological interpretation, streamlining metabolomics workflows. Clinically, NMR-based metabolomics has elucidated metabolic alterations in pediatric growth, prematurity, nutrition-related sensitizations, allergic diseases, lipid metabolism, infectious conditions, and neurobehavioral disorders. In particular, metabolomics has been applied to identify specific metabolic signatures underlying the molecular mechanisms of childhood allergic asthma. Despite limitations in detecting low-abundance metabolites, NMR's ability to preserve sample integrity and integrate multi-omics data, especially gut microbiota-derived metabolites, shows great promise in advancing precision pediatric medicine, early disease screening, and personalized therapeutic strategies.**

*Keywords:* Allergic diseases, Clinical metabolomics, Early childhood, Growth, Nuclear magnetic resonance

## 1. Introduction

Metabolomics is the study of the composition and changes in metabolites within biological systems, focusing primarily on how these small molecules interact with health states or disease processes. Metabolites reflect the physiological state of cells and organisms, metabolomics therefore can provide insights into the early stages of diseases, assist clinicians in predicting disease risks, evaluate treatment responses, and offer valuable support for personalized medicine [1–3]. With the development of mass spectrometry (MS) and nuclear magnetic resonance (NMR) technologies, the application of metabolomics in fields such as metabolic diseases, cancer, and cardiovascular diseases has increased significantly. However, individual metabolomics data often fail to provide a complete picture of the disease. Consequently, multi-omics approaches have emerged.

Received 5 September 2025; accepted 5 November 2025.
Available online 15 June 2026

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https://doi.org/10.38212/2224-6614.3574

Multi-omics integrates data from various levels, such as genomics, proteomics, and metabolomics, to obtain a comprehensive understanding of biological functions and disease mechanisms [4]. This interdisciplinary data integration can reveal how genes and environmental factors jointly influence disease development, contributing to the development of new diagnostic methods and therapeutic strategies. Metabolism encompasses all biological processes and pathways in the body, with enzymes playing a crucial role. Metabolomics can provide detailed data on patients' metabolic characteristics, and when combined with genomic and proteomic information, it assists clinicians in making more accurate diagnoses, thus enabling tailored treatment plans for individual patients.

During childhood growth and development, various physiological and metabolic changes occur, leading to conditions related to nutrition, immunity, and development, such as prematurity, metabolic disorders, and allergies. In recent years, the emergence of metabolomics has provided new insights into pediatric medicine. Particularly in childhood allergies, recent advancements in allergy metabolomics highlight distinct asthma metabotypes characterized by specific lipid metabolite patterns such as altered ceramides, triglycerides, phosphatidylcholines, and sphingomyelins, which correlate with asthma control, severity, and systemic inflammation [5]. These metabolomic profiles enhance the understanding of asthma heterogeneity and support precision medicine approaches for more accurate diagnosis and tailored treatments in pediatric asthma.

Understanding the complexity of age-related metabolic changes during early life is vital for managing health and disease in childhood [6]. Metabolic profiling, particularly using high-resolution NMR spectroscopy, offers insights into global metabolic effects and helps identify metabolites specifically associated with the growth spurt in early childhood. This review aims to summarize recent advances in pediatric metabolomics, with a particular focus on NMR-based studies, to elucidate developmental metabolic patterns. Metabolomic profiling is highlighted as a non-invasive tool to monitor growth, identify early biomarkers of disease risk, and guide personalized healthcare strategies in pediatric populations.

## 2. The principles and fundamentals of NMR

NMR, since its inception in the 1940s, has primarily been used for chemical structure identification. Beginning in the 1990s, with the development of high-field magnets and advances in automated analytical techniques, NMR began to be widely applied in the field of metabolomics, becoming one of the key tools for analyzing changes in metabolites within biological systems [7].

The application of NMR in metabolomics is not based on a single approach but requires the selection of an appropriate pulse sequence depending on the physicochemical properties of the sample. For biological samples with high protein content, such as plasma and serum, when general proton spectra are directly collected, the metabolite signals can easily be overshadowed by broad peak signals generated by proteins and lipoproteins. In this case, a one-dimensional pulse using the Carr-Purcell-Meiboom-Gill (CPMG) sequence is selected, which effectively filters out signals from macromolecules with short $T_2$ relaxation times through a series of echo pulses. This allows the resonance peaks of small molecules such as lactate, pyruvate, choline, and short-chain fatty acids to be clearly displayed [8].

For samples such as urine or tissue extracts, which have very low protein content, no macromolecule signal suppression is necessary. In this case, NOESY is the primary sequence used, as it effectively suppresses water signals while preserving small molecule metabolite information across the entire spectrum. This is suitable for high-throughput metabolic profiling and provides stable, high-quality data for subsequent statistical and pattern recognition analysis [9,10].

Based on these two primary pulse sequences, one-dimensional proton (1D 1H) spectra have become the most commonly used spectra in metabolomics due to their speed, ease of operation, and ease of data processing. These spectra enable researchers to rapidly identify 50–100 metabolites. Additionally, modern NMR equipment, assisted by automated devices, allows for the detection of large sample volumes using automatic sample exchangers.

In terms of sample preparation for NMR metabolomics, minimal treatment is required. For instance, when analyzing urine, it is simply mixed with a buffer containing deuterium oxide (D2O) and centrifuged to remove precipitates. Moreover, NMR detects molecular resonance in the sample through pulse sequences, making it non-destructive. Researchers can reuse the samples for other experiments after analysis. Furthermore, the metabolite signal intensity detected by this method shows a linear correlation with the concentration, ensuring high reproducibility [11].

## 3. Data processing methods

NMR data analysis, particularly for metabolite identification, often utilizes specialized analytical software, such as the Chenomx NMR Suite. However, raw data is first preprocessed before being used for data comparison. In the laboratory, the initial step usually involves using software like NMRProcFlow for spectral preprocessing, which includes parts per million (ppm) calibration, baseline correction, and bucketing, followed by subsequent data analysis. During the analysis, processes such as metabolite clustering comparisons, metabolic pathway exploration, and statistical analysis are conducted, all of which are crucial steps in metabolomics research. The MetaboAnalyst analysis platform is commonly used for these purposes. The following sections will introduce the relevant software and analysis methods, mainly NMRProcFlow and MetaboAnalyst.

### *3.1. NMRProcFlow*

NMRProcFlow is a visual analysis software specifically designed for one-dimensional NMR spectra, supporting common formats such as Bruker and Varian [12]. Its key feature is the provision of a graphical interface that requires no programming, allowing users to intuitively perform spectral preprocessing. The main functions include: 1. ppm calibration: Using internal standards like trimethylsilyl propionic acid (TSP) for ppm calibration, ensuring that the spectra of each sample align accurately for subsequent analysis. 2. Baseline correction: The baseline stability has a significant impact on integration area. The software provides both global and local correction methods, allowing adjustments to the entire spectrum or specific regions. 3. Bucketing: Offering both equal-width and manually customizable bucketing modes, the software integrates the spectrum into metabolomics data (bucket table) and supports the exclusion of interference signals, such as water peaks and urea. The modular design and real-time graphical feedback of NMRProcFlow make it especially suitable for researchers in fields unrelated to coding.

### *3.2. MetaboAnalyst*

MetaboAnalyst is a comprehensive online metabolomics data analysis platform developed by McGill University in Canada, designed to assist researchers in performing statistical analysis and biological interpretation of metabolomics data in a simple and visual manner [13,14]. It covers a wide range of functions, including data preprocessing, multivariate and univariate statistical analysis, feature selection, and pathway analysis, addressing various research design and analysis needs. During the preprocessing stage, the platform offers options such as missing value imputation, data filtering, normalization, and transformation, helping users improve data quality and reduce batch effects or non-biological variations.

For subsequent analysis, MetaboAnalyst provides statistical tools such as principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), clustering analysis, *t*-tests, ANOVA, and random forests, which help in exploring sample grouping, identifying differential metabolites, and building classification models [15]. In terms of biological interpretation, the platform integrates databases such as Kyoto Encyclopedia of Genes and Genomes (KEGG) and Human Metabolome Database (HMDB), enabling metabolic pathway analysis for differential metabolites and assisting users in understanding the biological significance of the statistical results. Furthermore, all analysis results can be output as high-quality charts and tables, which can be exported and directly applied to academic reports or manuscript writing. The greatest feature of MetaboAnalyst is its user-friendly interface, which continues to expand its functionality in response to advances in metabolomics research. It gradually incorporates time-series analysis, multi-group data integration, and machine learning methods, making it one of the most complete and flexible analysis tools in modern metabolomics research.

## 4. Clinical applications of using NMR spectroscopy

Metabolomics findings have broad clinical applications, including identifying disease-specific metabolic signatures and biomarkers that enable early diagnosis, prognosis, and monitoring of therapeutic responses. Additionally, metabolomics facilitates personalized medicine by characterizing individual metabolic profiles, guiding targeted treatments and improving patient stratification in diverse pediatric diseases. Clinical applications of NMR spectroscopy in pediatrics, including growth and development, prematurity, nutrition, allergy, and common pediatric disorders, will be demonstrated accordingly.

### *4.1. Growth and development: A birth cohort study*

A birth cohort study refers to a longitudinal research design that tracks the health, behavior,

living environment, and other data of the same group of individuals from birth at different time points. This type of study is characterized by its temporal and continuous nature [16–18]. When applied to metabolomics, the primary benefit of this approach is its ability to clarify the temporal sequence of metabolic changes and disease occurrence, which aids in establishing causal relationships.

Initially, this birth cohort has investigated age-related metabolic changes in healthy children during early childhood [19]. Urinary metabolites were analyzed using NMR spectroscopy in this cohort from 6 months to 4 years of age. The results revealed significant variations in metabolite levels, such as higher trimethylamine N-oxide (TMAO) and betaine in children aged 6 months, and changes in amino acid and carbohydrate metabolism pathways at different ages. These findings suggest that urinary metabolomics can provide valuable insights into dynamic metabolic changes during early life, offering potential insights into infant nutrition and growth.

Recently, 144 children from this birth cohort were tracked from birth to 5 years of age to analyze the changes in urinary metabolites. The results revealed that longitudinal metabolomics analysis showed metabolic patterns changing with age, with an increase in amino acid metabolism and a decrease in carbohydrate metabolism. Additionally, metabolites related to gut microbiota were closely associated with feeding patterns, with formula feeding significantly linked to overweight/obesity during infancy. However, overweight/obese children were more likely to develop pediatric allergic diseases [20].

### 4.2. Prematurity

Preterm birth, affecting 10.6% globally, is associated with complications such as bronchopulmonary dysplasia (BPD), especially in very and extremely preterm infants, due to inflammation and lung immaturity [21]. Despite symptom improvement over time, understanding the molecular mechanisms behind BPD remains incomplete. A preterm cohort designed by our group enrolled 89 infants born before 32 weeks of gestation and 50 term-born controls to investigate the molecular mechanisms of BPD using $^{1}$H-NMR-based urinary metabolomics. According to the 2019 NICHD criteria, preterm infants were categorized into no/mild and moderate/severe BPD groups, identifying 21 metabolites linked to gestational age and 24 associated with BPD severity. The results indicate that disruptions in gut microbiota and impaired antioxidative defenses play key roles in the development of BPD, particularly in extremely preterm infants with more severe forms [22].

### 4.3. Nutrients

#### 4.3.1. Infant formula feeding

Early exposure to formula milk enhances the probability of cow's milk sensitization and food allergies in later childhood. However, the underlying mechanisms are multifactorial and not well understood. In our previous follow-up birth cohort study, children were divided into exclusive breastfeeding (EBF) and formula feeding (EFF) groups. Longitudinal analysis of urinary metabolites using NMR spectroscopy revealed significant differences between the two groups at different ages [23]. Metabolic profiling identified metabolites associated with formula feeding and milk sensitization, including gut microbial-derived metabolites and immunoglobulin E (IgE)-associated metabolites, such as 3-indoxysulfate, N-phenylacetylglycine, and N,N-dimethylglycine. The findings suggest that formula feeding impacts gut microbial metabolism and contributes to milk sensitization, with specific metabolites playing a role in the development of sensitization and allergic symptoms.

#### 4.3.2. Vitamin D

The relationship between serum vitamin D levels, allergic IgE responses, and atopic diseases has been explored in various studies. However, the specific effects of vitamin D on allergic reactions using a metabolomics-based approach are still not well understood. A total of 111 children in our cohort were followed for three years and classified according to their longitudinal vitamin D levels. A significant correlation was observed between vitamin D-related hippuric acid and asthma-related, gut microbiota-derived formic acid, while vitamin D was negatively correlated with AEC. This study suggests that vitamin D-associated metabolites are linked to the gut microbiome and immune responses underlying childhood allergic airway diseases [24].

#### 4.3.3. Hypercholesterolemia

Dyslipidemia, characterized by abnormal blood lipid levels, is a major risk factor for cardiovascular disease that often begins in childhood [25]. Early detection and management through lifestyle changes and monitoring of lipid markers like total cholesterol, LDL, triglycerides, HDL, and Apo-B are crucial [26]. However, despite growing awareness,

challenges remain in consistently identifying and treating dyslipidemia in children to effectively prevent future cardiovascular complications. One hundred twenty-five children were grouped by cholesterol levels and analyzed using $^{1}$H-NMR metabolomics to identify metabolites linked to cholesterol status. Eight metabolites, especially glutamic acid and tyrosine, were significantly associated with cholesterol levels, highlighting the role of amino acid and carbohydrate metabolism in hypercholesterolemia. Further metabolic pathway analysis identified glutamic acid-related metabolism as playing a key role in regulating lipid levels [27].

### 4.4. Allergy

#### 4.4.1. Gut microbiota health and childhood allergic asthma

The association of asthma with specific metabolites and altered metabolic pathways has been investigated, but a comprehensive understanding of the longitudinal dynamics of these metabolites in relation to asthma development is still lacking. A longitudinal urinary metabolomic profiling was performed to investigate the metabolic mechanisms underlying childhood asthma development [28]. Four metabolites, including dimethylamine, 1-methylnicotinamide, and allantoin, were identified as being significantly associated with asthma development, suggesting a link between microbe-environment interactions, allergic reactions, and asthma.

In healthy children, the gut microbiota efficiently breaks down dietary fibers, producing short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, which account for approximately 60% of the total gut metabolites. Most importantly, our further metabolomic study has observed that butyrate levels are significantly reduced in the feces of children with asthma, which is associated with gut dysbiosis, particularly a reduction in carbohydrate-active enzyme (CAZyme) genes [29]. This reduction impairs the breakdown of dietary fibers, leading to decreased butyrate production. Evidence has demonstrated that butyrate is essential for regulating gut immune responses, particularly in maintaining gut barrier function and reducing allergic reactions [30]. Reduced butyrate levels can compromise the intestinal barrier, which facilitates allergen penetration and promotes allergic responses and asthma symptoms.

#### 4.4.2. Atopic dermatitis

Atopic dermatitis (AD) is a chronic inflammatory skin disorder influenced by genetic factors like filaggrin (*FLG*) gene mutations and immunoglobulin E (IgE)-mediated allergic reactions, which contribute to skin barrier dysfunction and disease severity [31]. AD commonly begins in infancy and can persist into adolescence, with genetic predisposition and aeroallergen sensitization predicting its course. Although metabolomics offers insights into gene-environment interactions in complex diseases, studies on metabolic pathways related to *FLG* mutations and allergen exposure in adolescent AD are still limited. Eighty-one children with adolescent AD and healthy controls were studied using whole-exome sequencing and $^{1}$H-NMR plasma metabolomics to explore associations with *FLG* mutations and IgE levels. Metabolic pathways involving nitrogen, amino acids, methane, and propanoate were significantly associated with AD, while microbial-derived metabolites dimethylamine and isopropanol showed strong links to *FLG* mutations and IgE levels. These results suggest that host genetics impact the microbiome, which subsequently influences susceptibility to allergic responses in the development of AD [32].

### 4.5. Pediatric disorders: Metabolomics approaches

#### 4.5.1. Parapneumonic effusions caused by pneumonia

Pneumococcal pneumonia, a leading cause of community-acquired pneumonia, often leads to parapneumonic effusions, which can progress to complicated effusions requiring aggressive treatment if inflammation is not controlled [33]. While traditional biochemical markers in pleural fluid guide management, identifying specific protein biomarkers remains challenging. Metabolomics using NMR spectroscopy offers a promising approach to detect metabolic changes in pleural fluids, but metabolic alterations related to host-microbe interactions in pneumococcal parapneumonic effusions have not yet been studied. Forty children with pneumococcal pneumonia had their pleural fluid metabolites analyzed by $^{1}$H-NMR spectroscopy to compare complicated (CPE) and non-complicated effusions. Ten metabolites differed significantly, with lower glucose and higher bacterial biosynthesis and fermentation metabolites observed in CPE; glucose and 3-hydroxybutyric acid effectively predicted the need for intervention. These findings reveal bacterial metabolic activity in pleural fluid and suggest increased butyric acid fermentation may indicate a requirement for aggressive pleural drainage [34].

Furthermore, pneumonia in children can lead to infectious parapneumonic effusion (IPE), where ongoing pleural inflammation causes fibrin

deposition and fluid accumulation, often requiring aggressive surgical intervention [35]. Tissue-type plasminogen activators and inflammatory cytokines affect fibrinolytic activity and fibrin formation, yet the metabolic processes involved in fibrinous infectious parapneumonic effusion are still not well understood [36,37]. Pleural fluid metabolites analyzed by $^{1}$H-NMR spectroscopy differentiated fibrinous from nonfibrinous infectious parapneumonic effusions (IPE), showing significant correlations between inflammatory cytokine IL-1β, glucose, and several metabolites. Four metabolites including glucose, lactic acid, 3-hydroxybutyric acid, and hypoxanthine were associated with fibrinolytic enzyme activity and fibrin formation. These findings indicate that increased anaerobic bacterial fermentation and hypoxanthine buildup under low oxygen conditions contribute to advanced pleural inflammation and fibrin deposition in children with pneumonia-related IPE [38].

*4.5.2. Nocturnal enuresis*

Nocturnal enuresis (NE), in children over 5 years old, affects about 10% of school-aged children globally and can be categorized into monosymptomatic and non-monosymptomatic types [39,40]. While standard treatments like desmopressin and bedwetting alarms help many, around 40% of children, especially with non-monosymptomatic NE, show poor response or relapse, indicating additional factors beyond the classic pathophysiology [41]. Urine samples from 41 NE children and 27 healthy controls were analyzed using $^{1}$H-NMR spectroscopy, identifying eight metabolites that differed significantly. Increased levels of betaine, creatine, and guanidinoacetate, linked to amino acid metabolism, were associated with neurobehavioral disorders like attention deficient hyperactivity disorder (ADHD) and anxiety in children with refractory nocturnal enuresis. Betaine, due to its role as a renal osmolyte and methyl donor, may influence renal or central circadian clocks, suggesting its potential as a urinary marker for diagnosing treatment-resistant NE in affected children [42]. This review summarizes the current applications of NMR experimental methods in children's growth and development, prematurity, nutrition, allergy, and common diseases, as shown in Table 1.

Apart from NMR spectroscopy, MS (mass spectrometry) is another key platform in metabolomics. MS-based metabolomics offers higher sensitivity and broader metabolite coverage, which are essential for detecting subtle metabolic changes in diseases; however, it requires more complex sample preparation and stringent control of variability [43]. Conversely, although NMR has lower sensitivity and limited ability to detect low-abundance metabolites, it provides high reproducibility, simple and minimal sample preparation, non-destructive analysis, and quantitative results, making it ideal for pediatric clinical studies with limited sample volumes while preserving biological integrity through consistent outcomes.

## 5. Conclusions and future prospects

The use of NMR spectroscopy, combined with clinical metabolomics statistical analysis methods, enables the accurate detection and quantification of metabolic changes in various body fluids. This approach further reveals disease-related metabolic pathways and biomarkers, which aids in early disease diagnosis, risk assessment, and the development of personalized treatment strategies. As the application of NMR technology in clinical practice increases, particularly in pediatrics, it provides new opportunities for the development of precision medicine. However, despite its many advantages, NMR spectroscopy still has its limitations. It may not be able to detect metabolites present at low concentrations or in small quantities, which presents a challenge compared to other platforms. Nevertheless, the significant advantage of NMR is that its sample processing does not disrupt the sample, helping to preserve the integrity of biological data and providing a more representative insight into the overall status of various tissues.

In pediatric research, NMR metabolomics can be applied in multiple fields, including growth and development, preterm infants, common pediatric diseases, allergies, and the impact of nutrients on metabolism. This method offers a non-invasive approach to understanding how these factors influence metabolic pathways in children and helps to better understand their physiological state. Furthermore, the integration of multi-omics approaches with NMR technology shows great potential. In particular, combining gut microbiota-derived metabolites with NMR analysis provides deeper insights into how gut microbiota influences disease development and immune responses, especially in pediatric allergies. Research in this area not only identifies new biomarkers but also offers important clues for early diagnosis and personalized treatment of diseases. Apart from pediatrics, NMR-based metabolomics and integrative multi-omics approaches could further expand into medical, surgical medicine, and oncology for early disease screening, and precision medicine. As

*Table 1. Summary of NMR-based metabolomics studies associated on pediatric diseases and related metabolic pathways.*

| Clinical applications | Year | Author | Sample | Diseases | Target Metabolites | Metabolic Pathways | Reference |
|---|---|---|---|---|---|---|---|
| **Growth and development** | 2016 | Chiu et al. | Urine | Age-related metabolic changes (healthy early childhood growth) | Trimethylamine N-oxide (TMAO), betaine, glycine, glutamine, creatine, creatinine, galactose, lysine, lactose, formic acid, citric acid, succinic acid, N-phenylacetylglycine | Nitrogen metabolism, aminoacyl-tRNA biosynthesis, glycine/serine/threonine metabolism, galactose metabolism | [19] |
| | 2025 | Chiu et al. | Urine | Overweight/obesity, atopic diseases (eczema, allergic rhinitis, asthma) | Glycine, 2-hydroxyisobutyric acid, galactose, alanine, glutamine, 3-methyl-2-oxovaleric acid, tyrosine, trigonelline | Amino acid metabolism (phenylalanine, arginine, proline), carbohydrate metabolism (galactose, glyoxylate, dicarboxylate metabolism) | [20] |
| **Prematurity** | 2024 | Chiu et al. | Urine | Severe bronchopulmonary dysplasia (BPD) | N-Phenylacetylglycine, hippurate, acetylsalicylate, gluconate, indoxyl sulfate, betaine, N,N-dimethylglycine, ribose | Gut microbiota metabolism, oxidative stress response, pentose phosphate pathway, glycine/serine/threonine metabolism, amino acid metabolism | [22] |
| **Nutrients** | | | | | | | |
| Infant formula feeding | 2022 | Tang et al. | Urine | Milk sensitization (related to formula feeding) | 3-Indoxysulfate, N-phenylacetylglycine, N,N-dimethylglycine, 3-methyl-2-oxovaleric acid, glutarate, lysine, 2-oxoglutaric acid, pantothenate | Branched-chain amino acid metabolism, gut microbial metabolism, TCA cycle metabolism, Amino acid metabolism (Lysine degradation), cofactors and vitamin metabolism | [23] |
| Vitamin D | 2022 | Chang et al. | Urine | Allergic airway diseases (rhinitis, asthma) associated with vitamin D levels | 3-Hydroxyisobutyric acid, glutamine, hippuric acid, formic acid, succinic acid, alanine, glycine, 3-methyl-2-oxovaleric acid, acetylsalicylate, trans-aconitic acid | Amino acid metabolism (alanine/aspartate/glutamate, glycine/serine/threonine), glyoxylate and dicarboxylate metabolism, phenylalanine metabolism | [24] |
| Hypercholesterolemia | 2023 | Gu et al. | Plasma | Hypercholesterolemia | Tyrosine, glutamic acid, ornithine, lysine, alanine, creatinine, oxoglutaric acid, creatine | Amino acid metabolism (arginine/proline, alanine/aspartate/glutamate), arginine biosynthesis, glycolysis/gluconeogenesis, TCA cycle | [27] |

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|---|---|---|---|---|---|---|---|
| **Allergy** | | | | | | | |
| Asthma | 2018 | Chiu et al. | Urine | Asthma development in early childhood | Dimethylamine, 1-methylnicotinamide, allantoin, guanidoacetic acid | Microbial metabolism, choline metabolism, purine metabolism | [28] |
| | 2023 | Chiu et al. | Feces, Urine | Childhood airway allergies (asthma and rhinitis) | Butyrate, fumarate, β-alanine, 5-aminopentanoic acid | Short-chain fatty acid metabolism, carbohydrate metabolism, amino acid metabolism | [29] |
| Atopic dermatitis | 2021 | Chiu et al. | Plasma | Atopic dermatitis (*FLG* mutations related IgE response) | Dimethylamine, isopropanol, glutamic acid, succinic acid, lactic acid, formic acid, glucose, methanol, creatine, alanine, glycine, hypoxanthine, glutamine, betaine | Nitrogen metabolism, amino acid metabolism, methane metabolism, propanoate metabolism | [32] |
| **Common pediatric disorders** | | | | | | | |
| Pneumonia | 2016 | Chiu et al. | Pleural fluid | Infectious parapneumonic effusions (*Streptococcus pneumoniae* pneumonia) | Glucose, lactic acid, thymine, succinic acid, tryptophan, 3-hydroxybutyric acid, phenylalanine, threonine, leucine/isoleucine | Amino acid metabolism, glycolysis/gluconeogenesis, propanoate metabolism, butanoate metabolism | [34] |
| | 2019 | Chiu et al. | Pleural fluid | Fibrinous infectious parapneumonic effusions (fibrin formation) | Glucose, lactic acid, thymine, phenylalanine, leucine/isoleucine, tryptophan, glutamic acid, hypoxanthine, 3-hydroxybutyric acid | Glycolysis/gluconeogenesis, propanoate metabolism, butanoate metabolism, anaerobic bacterial fermentation, ATP hydrolysis | [38] |
| Nocturnal enuresis | 2021 | Yu et al. | Urine | Nocturnal enuresis (with ADHD or anxiety comorbidity) | Betaine, creatine, guanidinoacetate, 3-aminoisobutyrate, 3-hydroxyisobutyrate, formate, taurine, valine | Glycine, serine, threonine metabolism; arginine/proline metabolism; valine/leucine/isoleucine metabolism; taurine metabolism | [42] |

NMR, nuclear magnetic resonance; *FLG*, filaggrin; ADHD, attention-deficit/hyperactivity disorder; TCA, tricarboxylic acid; ATP, adenosine triphosphate.

advances in metabolomics research technologies continue, pediatric clinical metabolomics will play an increasingly important role in disease prevention, diagnosis, and treatment.

## Funding information

This study was supported by research grants of CMRPG3P0631-2 and CMRPG3N0701-2 from the Chang Gung Memorial Hospital, Taiwan.

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