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Invited critical review

The role of metabolomics in neonatal and pediatric laboratory medicine

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ABSTRACT

Metabolomics consists of the quantitative analysis of a large number of low molecular mass metabolites involving substrates or products in metabolic pathways existing in all living systems. The analysis of the metabolic profile detectable in a human biological fluid allows to instantly identify changes in the composition of endogenous and exogenous metabolites caused by the interaction between specific physiopathological states, gene expression, and environment. In pediatrics and neonatology, metabolomics offers new encouraging perspectives for the improvement of critically ill patient outcome, for the early recognition of metabolic profiles associated with the development of diseases in the adult life, and for delivery of individualized medicine. In this view, nutrimetabolomics, based on the recognition of specific cluster of metabolites associated with nutrition and pharmacometabolomics, based on the capacity to personalize drug therapy by analyzing metabolic modifications due to therapeutic treatment may open new frontiers in the prevention and in the treatment of pediatric and neonatal diseases. This review summarizes the most relevant results published in the literature on the application of metabolomics in pediatric and neonatal clinical settings. However, there is the urgent need to standardize physiological and preanalytical variables, analytical methods, data processing, and result presentation, before establishing the definitive clinical value of results.

Contents

Abbreviations: ^{1}H NMR, proton nuclear magnetic resonance; ADMA, asymmetric dimethylarginine; ADP, adenosine diphosphate; AGA, appropriate for gestational age; AKD, acute kidney disease; AKI, acute kidney injury; AMP, adenosine monophosphate; BALF, bronchoalveolar lavage fluid; BMI, body mass index; CE, capillary electrophoresis; CKD, chronic kidney disease; CLD, chronic lung disease; CSF, cerebrospinal fluid; DIMS, direct injection mass spectrometry; ELBW, extreme low birth weight; ESRD, end stage renal disease; FTIR, Fourier transform infrared spectroscopy; GC–MS, gas chromatography–mass spectrometry; HIE, hypoxic ischemic encephalopathy; HMDB, human metabolome data base; HVA-SO_4, homovanillic acid sulfate; ICU, intensive care unit; IQ, intelligence quotient; IUGR, intrauterine growth restricted; LBW, low birth weight; LC–MS, liquid chromatography–mass spectrometry; MDA, malondialdehyde; MMA, methylmalonic acidemia; MS, mass spectrometry; OPLS-DA, orthogonal projections to latent structures for discriminant analysis; PA, propionic acidemia; PCA, principal component analysis; PDA, patent ductus arteriosus (Botallo's duct); PLS-DA, projection to latent structures discriminant analysis; RCDs, respiratory chain deficiencies; RDS, Respiratory Distress Syndrome (hyaline membrane disease); SGA, small for gestational age; TMAO, trimethylamine-N-oxide; VLBW, very low birth weight; VOCs, volatile organic compounds.

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1. Introduction

Over recent decades, the contributions of laboratory medicine in improving patient care have increasingly become essential, because of the advent of new generation of laboratory diagnostics consisting of sophisticated tests with potentially profound implications for the delivery of personalized health care. The “genomic revolution” started with the mapping of the entire sequence of human genoma [1,2] and accelerated the development of system biology studies based on various disciplines like genomics, transcriptomics and proteomics. These “omics” may be considered the most relevant driver in changing the face of laboratory medicine, opening new challenges for patients, clinicians, and clinical pathologists. These perspectives seem to be of extreme importance in neonatal care to improve diagnosis, prognosis, and clinical outcome in critically ill newborns by the routine application of emerging molecular technologies. In addition, such disciplines seem to open new hopes for the identification of molecular patterns (proteins, metabolites); each of them is specifically associated with a severe pathological condition (sepsis, acute kidney injury, etc.). The identification of these “clusters” of substances could lead to the development of very low-cost devices like a dipstick, easily usable in low-income countries, representing an excellent example of how to convert translational research results in low-cost medical device [3].

2. Metabolomics: historical background

The presence of a metabolic pattern extremely variable between individuals, but relatively constant for a given individual, was firstly reported by Roger Wlilliams in 1951 [4]. By examining data from over 200,000 paper chromatograms belonging to a variety of subjects, including alcoholics, schizophrenics, and residents of mental hospitals, Williams found characteristic metabolic patterns associated with each of these groups. Despite these results giving rise to the idea that the metabolic pattern analysis might have clinical utility, no further investigation was performed until the '70s, when a boost came by the rapid increase of technology progresses in gas chromatography (GC), liquid chromatography (LC), and mass spectrometry (MS) methods. In 1971, the term “metabolic profile” was introduced, with the intent to describe chromatographic patterns observed in biological fluids characterizing both normal and pathologic states and potentially useful for studies of drug metabolism and for human developmental studies [5]. Simultaneously, Linus Pauling realized that information-rich data reflecting the functional status of a complex biological system resides in the quantitative and qualitative pattern of metabolites in body fluids [6]. This emerging approach to the quantitative metabolic profiling of large numbers of small molecules in biofluids was experimented by several research groups [7] and ultimately was termed metabonomics by Nicholson et al. in 1999 [8] and metabolomics by Fiehn in 2002 [9]. Metabolic profiles include endogenous and exogenous chemical entities such as peptides, amino acids, nucleic acids, carbohydrates, fatty acids, organic acids, vitamins, hormones, drugs, food additives, phytochemicals, toxins, and other chemicals ingested or synthesized by a cell or organism. This heterogeneous multitude of molecules was called metabolome by Oliver et al. [10]: the metabolome is the holistic quantitative set of low molecular-weight compounds (<1000 Da). The first draft of the human metabolome was completed on January 23, 2007 and consisted of a database of about 2500 metabolites [11]. Despite the very high overall number of endogenous metabolites (~100,000), the number of major metabolites relevant for clinical diagnostics and drug development has been estimated at 1400–3000 molecules [12], which means less data to manipulate and interpret, being genes (~25,000), transcripts (~85,000), and proteins (>10,000,000) greatly outnumbered. Most endogenous metabolites are tied to specific biochemical pathways such as glycolysis, Krebs' cycle, lipid or amino acid metabolism, signaling pathways such as transmitters and hormones and specific pathobiochemical processes like oxidative stress. Thus, changes in specific metabolite patterns reflect changes in pathways and processes; it is reasonable to argue that metabolome is typically more closely associated with a disease process or drug effect than proteins, mRNA or genes [13].

3. Metabolomics workflow

3.1. Targeted and non-targeted approach

The metabolomics workflow includes sample preparation, analysis using various instruments, data processing and data analysis, as condensed in Fig. 1. The power of metabolomics lies on the acquisition of analytical data in which metabolites in a cellular system are quantified, and the extraction of the most meaningful elements of the data by using various data analysis tool. Two strategies configure metabolomics studies: the targeted and the non-targeted approach [14]. The latter may be defined as a “nonspecific approach”, investigating all the metabolites (both endogenous and exogenous) detectable in a fluid or tissue; this analysis is focused on capture as much information as possible, providing a functional fingerprint of the physiological and pathological state of the body. The former is focused on the investigation of several well defined compounds (for example those discovered in a new metabolic pathway); it is only used when the target of a drug or disease process is at least partially understood. Metabolic fingerprinting describes the unbiased analysis of the metabolome by examination of metabolite patterns in different experimental groups with the subsequent classification of these patterns into a fingerprint. Samples can be classified if the metabolite fingerprints differ between groups allowing for sample clustering. Metabolite identification relies on public databases [15]: the human metabolome Data Base (HMDB) is the metabolomic equivalent