Chapter

Pharmacometabolomics: A New Horizon in Personalized Medicine

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Abstract

Pharmacology is the predominant first-line treatment for most pathologies. However, various factors, such as genetics, gender, diet, and health status, significantly influence the efficacy of drugs in different patients, sometimes with fatal consequences. Personalized diagnosis substantially improves treatment efficacy but requires a more comprehensive process for health assessment. Pharmacometabolomics combines metabolomic, genomic, transcriptomic and proteomic approaches and therefore offers data that other analytical methods cannot provide. In this way, pharmacometabolomics more accurately guides medical professionals in predicting an individual's response to selected drugs. In this chapter, we discuss the potentials and the advantages of metabolomics approaches for designing innovative and personalized drug treatments.

Keywords: Personalized Medicine, Pharmacometabolomics, Metabolomics, NMR, metabolites

1. Introduction

Conventional drugs are developed as standard treatments for all patients diagnosed with particular diseases regardless of any differences between those patients. Consequently, this universal approach comes with a high degree of uncertainty regarding the treatment outcome. It is well-established that individuals can be differentially affected by the same disease due to factors such as general health status, genetics, gender, diet habits, smoking, alcoholic intake, *etc.* [1, 2]. The global COVID-19 pandemic has demonstrated clearly that a single disease can have different outcomes in different people, and the choice of therapeutic strategies needs to be calibrated to an individual rather than using a standard protocol for heterogenous populations. Indeed, the increasing incidence of treatment failure, especially with life threatening diseases such as cancer relapse, evidences a need for personalized drug regimens.

Each pathological state in humans affects multiple organs/systems and leads to the perturbation of metabolites and protein concentration levels. Thus, analysis of biomarkers (such as unique metabolites or proteins) is an effective way to monitor human health [3, 4]. Biomarkers can be used for disease prediction, diagnosis, and to screen the efficacy of treatment intervention. For example, the glucose level in blood is a biomarker of diabetes and can be used to monitor

drug efficacy [5–7]. **Table 1** summarizes the most prominent examples of protein biomarkers discovered recently.

Among all '-omics' approaches, metabolomics is the most effective of qualifying and quantifying the perturbation of metabolite concentrations under external and internal factors. Thus, joining metabolomics with other '-omic' sciences (e.g. genomics) is essential for a comprehensive understanding of disease onset and pathogenesis, and provides a better diagnosis and treatment.

The total number of endogenous metabolites (although it is not completely determined yet) in human bio-fluid and tissues are lower than the total number of expressed proteins, giving metabolomics an extra advantage in monitoring disease pathology. Moreover, the perturbation of metabolite levels in human bio-fluids is usually greater than that of protein concentrations, providing an easy and clearer bio-marker role [18–20]. For instance, cancer leads to changes in affected cells, which cause an up-regulation in metabolite concentration levels during carcinogenesis [21]. For example, increased lactate levels have long been associated with different types of cancer [22]. Recently, the development of computational methods, such as bioinformatics and human metabolome databases establishing large scale bio-banks and computer programs, have facilitated the employment of metabolomics in stratified medicine. Pharmacometabolomics is a new subset of the metabolomics field aiming to predict the response of an individual to a drug or to develop optimized treatment strategies based on previous knowledge of subject

Protein biomarkers	**Useful for:**	**Ref**
Apolipoprotein H, ApoCI, Complement C3a, Transthyretin, ApoAI	Prediction of recurrence-free survival in women with estrogen receptor-negative tumors	[8]
S100 calcium-binding protein B, Neuron-specific enolase, Glial fibrillary acidic protein, Ubiquitin Carboxy-terminal hydrolase-L1, Tau, Neurofilament-light	Prediction of outcome and severity in traumatic brain injury patients	[9]
S100A9, ThioredoxiN, Cadherin-related family member 2	Diagnosis (presence) of cholangiocarcinoma	[10]
TFF1, ADAM (male only), BARD (female only)	Early diagnosis of gastric cancer	[11]
Acidic nuclear phosphoprotein 32 family member B, Thrombospondin-4, Cardiac muscle troponin T, Glucocorticoid-induced TNFR-related protein, NAD-dependent deacetylase sirtuin-2	Creating new utrophin modulation strategies that could help patients with Duchenne muscular dystrophy	[12]
C-reactive protein, S100A8, S100A9, S100A12	Prognosis of the severity of rheumatoid arthritis.	[13]
S100A4, S100A8, S100A9, Carbonic anhydrase I, Annexin V	Diagnosis of urinary bladder cancer and prognosis of patient outcome.	[14]
Gelsolin, Fibronectin, Angiotensinogen, Haptoglobin	Detection of lymph node metastasis of oral squamous cell carcinoma.	[15]
Neurotrophic factor, Angiotensinogen, Insulin-like growth factor binding protein 2, Osteopontin, Cathepsin D, Serum amyloid P component, Complement C4, Prealbumin (transthyretin)	Diagnosis of Alzheimer's disease in Han Chinese.	[16]
Alpha-2-macroglobulin, Chromogranin-A, Glutathione pertidase 3	Obtaining qualitative and quantitative assessments of radiation exposure.	[17]

Table 1.
Examples of biomarkers and their use in medicine.

Year	Number of approved novel drug by FDA
1993	25
1994	21
1995	29
1996	53
1997	39
1998	30
1999	35
2000	27
2001	24
2002	17
2003	21
2004	36
2005	20
2006	22
2007	18
2008	24
2009	26
2010	21
2011	30
2012	39
2013	27
2014	41
2015	45
2016	22
2017	46
2018	59
2019	48
2020	53

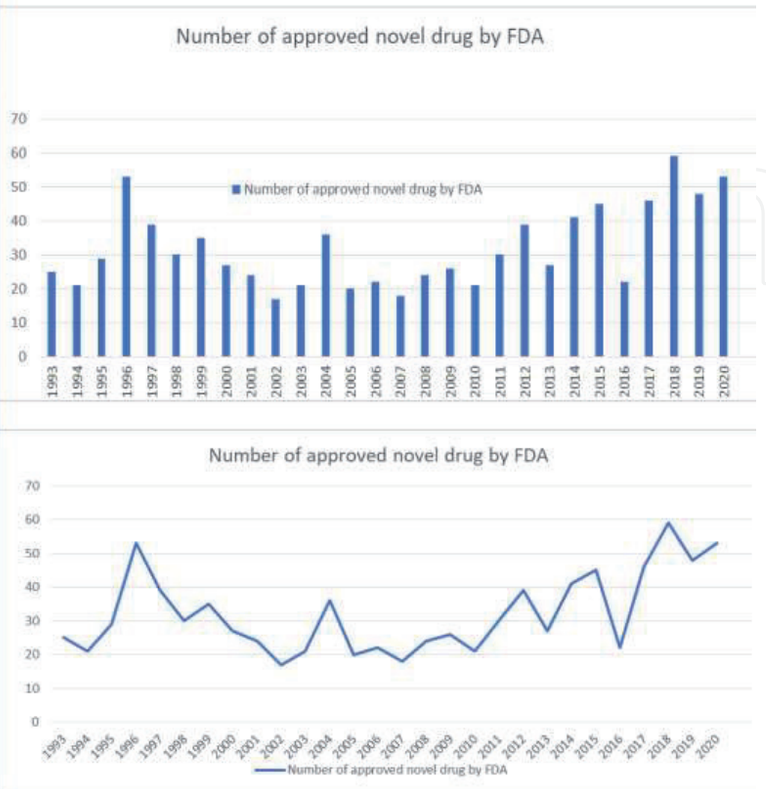

Figure 1.
Number of novel drugs approved annually by the FDA between 1993 and 2020 with graphical representation.

metabolomics information (individual's metabolic profile). One should keep in mind that aerosolized treatment would never lead to the discovery of a novel drug for each individual subject. Indeed, the number of new drugs is almost constant in the last decades (**Figure 1**).

In this chapter, we briefly introduce metabolomics along with common metabolomics analytical platforms regarding the development of a personalized medicine approach and factors that will empower advances in personalized medicine.

2. FDA approved drugs since 1975

Over the past few decades, pharmaceutical product intervention has improved significantly resulting in more saved lives and enhanced public health. The annual number of newly approved drugs applicable for human use has varied greatly over the years (**Figure 1**). The Food and Drug Administration (FDA) is an agency within

Years	Average FDA novel drug approval per year
1970s	10
1980s	10
1990s	20-25
2000-2010	25.6
2010-2020	39.2

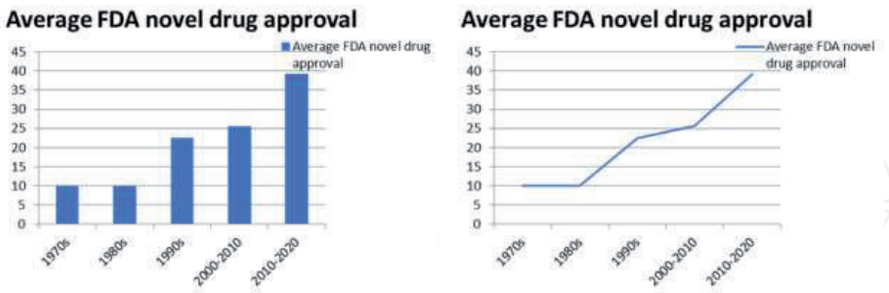

Figure 2.
Average numbers of novel drug approvals by the FDA over the last five decades with graphical representation.

the United States. One of its primary responsibilities is the approval of human pharmaceutical products based on safety and efficacy. Regulating and managing the human pharmaceutical industry and the approval of new drugs is the responsibility of the Center for Drug Evaluation and Research (CDER) [23].

The FDA catalog contains most of the approved drug products since 1939. However, since 1998 a complete human drug database is available, known as the Orange Book, which includes patient information, drug labels, and drug reviews. The Orange Book is considered a comprehensive detailed list of all pharmaceutical products approved in the U.S. by the FDA. However, studying the number of pharmaceutical products approved annually is not straightforward. First, the number of approved human drugs was not accurate before 1981, as the Orange Book did not report pharmaceutical drug approval data until after 1981, including new molecular entities (NMWE), the pharmaceutically active ingredient, drug dosage form, combination, formulation, and indication [24]. In addition, the Orange Book excludes any withdrawn drug or 'no-longer marketed' pharmaceutical products due to either drug efficacy concerns or safety concerns. Below, the reader can find **Figure 1** summarizing the number of FDA-approved novel human drugs per year from 1993 to 2020 [24, 25].

As is apparent from **Figure 1**, the year 2020 represents the second-highest number of FDA-approved novel human drugs over the past twenty years (53 drugs), while 2018 was the year when the highest number of drugs were approved by the FDA (57 drugs). In 2017, only 46 drugs were approved [26].

The average rate of new drug approvals by the FDA has increased over the years (**Figure 2**). Before 1950, the average annual drug approval was less than four, while the average annual drug approval in the 1960s and 1970s was 10. However, in the 1980s the average approval rate increased to more than twenty per year. It has continued to increase to reach more than twenty-five approvals per year from 2000 to 2010. Over the last several years there have been further increases, reaching an average of more than 39 approved compounds per year from 2010 till 2020 [26, 27]. The average novel drug approval by the FDA over the decades is listed in **Figure 2**.

3. Metabolomics

Metabolomics is defined as "the measurement of metabolite concentrations and fluxes and secretion in cells and tissues in which there is a direct connection between the genetic activity, protein activity, and the metabolic activity itself" [28]. It is a

relatively new field and is employed in a wide range of applications that monitor biological systems [3, 29, 30]. Integrating metabolomics with other '-omics', including proteomics, transcriptomics, and genomics, provides an exhaustive description of the biological system under study. Metabolomics provides a snapshot of the metabolite dynamics, and is a powerful tool when investigating numerous perturbations in biological systems, including pathophysiological events, environmental stimuli, and genetic modifications [31–34]. Moreover, metabolomics investigates every perturbation in metabolite compositions and/or concentrations, and it has already been applied in different fields such as biomedicine, environmental science, nutrition and diet studies, microbiology, and drug toxicology, as well as marine and plant sciences [35–39].

Metabolomics is usually classified into two main categories: (1) untargeted, and (2) targeted. Untargeted metabolomics is focused on the entire pool of "detectable" metabolites in a biological sample and makes no assumptions about metabolite(s) or class of metabolites, nor their concentrations. Untargeted metabolomics relies on fingerprinting approaches, where a group or different classes of samples (e.g., healthy control vs. pathological samples) are compared, and where absolute metabolite quantifications are not necessary. In contrast, targeted metabolomics focuses both on the identification and quantification of a specific number of metabolites. Targeted metabolomics approaches are relevant for drug development, where the drug mechanism (including drug absorption and drug distribution) needs to be precisely monitored.

The choice of proper analytical technique(s) in metabolomics is the crucial step, and particularly targeted metabolomics requires accurate metabolite quantification. Metabolomics applies different analytical techniques, including mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, Fourier transformed infrared (FT-IR) spectroscopy, and high-performance liquid chromatography (HPLC). Among them, NMR spectroscopy and MS spectrometry are the most common and powerful analytical tools [40, 41].

3.1 Analytical techniques in metabolomics

Similar to other '-omics' disciplines, metabolomics uses different analytical platforms, separately or in combination (two or more techniques) [32, 42]. Although several analytical platforms are employed in metabolomics studies, including FT-IR spectroscopy [43–45], HPLC [46, 47], NMR spectroscopy [48–53], and MS [54–57] combined with gas or liquid chromatography [58–62], MS and NMR are the most common approaches [3, 50, 63–65]. There is no single optimum analytical technique that can elucidate all metabolites equally. Each analytical method has its advantages and limitations. For example, NMR is a non-destructive and highly reproducible technique where metabolic pathways or metabolic flux can be studied by using isotopic nuclei (such as ^{13}C and ^{15}N NMR), thus monitoring the flow of compounds through metabolic pathways [66–69].

Nevertheless, it has two main drawbacks that must be kept in mind: inherently low sensitivity and potential signal overlap. Different technical approaches have been developed to overcome these two drawbacks, contributing to the development of new and more efficient NMR techniques. For example, dynamic nuclear polarization (DNP) can be used to increase the NMR signal enhancement [70, 71], and the use of cryoprobes and the introduction of ultra-high magnetic fields (e.g., 1GHz) helps to overcome the low sensitivity limitation [72, 73]. The peak overlap problem can be minimized by the use of the highest magnetic fields and multi-dimensional NMR methods such as HSQC, TOCSY, COSY, and HMBC techniques [66, 74–78].

As stated, no singular analytical technique can perform a complete quantification and identification of all the metabolites in one analysis. Therefore, in addition to one and two-dimensional NMR experiments, different complementary techniques are required, such as liquid chromatography-mass spectrometry (LC–MS) and gas chromatography–mass spectrometry (GC–MS), which help to maximize the number of identified and quantified metabolites [32, 65, 79, 80].

For instance, the human urine metabolome was analyzed by Wishart *et al.* with several different analytical tools (ICP-MS, NMR, GC–MS, DFI/LC–MS/MS, HPLC) to facilitate the detection of the highest possible number of human urine metabolites. Among all metabolites, 209 were identified by NMR, 179 by GC–MS, 127 by DFI/LC–MS/MS, 40 by ICP-MS, and 10 by HPLC [81].

Based on the ability to separate and detect a wide range of metabolites, LC–MS is one of the most widely used tools for carrying out metabolite profiling studies [82–86]. LC–MS combines HPLC and mass spectrometry, and provides a powerful analytical tool for the separation, identification, and quantification of metabolites in a studied sample [65, 87–90]. HPLC separates molecules based on different physical and chemical properties such as charge, polarity, molecular size, and affinity towards column matrices [91–94]. Thus, different successful chromatography methods have been developed, such as reversed-phase (RP) gradient chromatography [85, 86, 95, 96]. To obtain the best separation, and presumably the highest number of detected metabolites, each sample can be analyzed twice using RP and normal phase chromatography. Moreover, the column switching approach of 2-dimensional analysis in an "orthogonal" combination of hydrophilic interaction liquid chromatography (HILIC) and RP-L, in conjunction with utilizing different electro spray ionization (ESI) modes can also be used [85, 86, 97–99]. In addition to using different separation methods and/or ionization methods, LC–MS is inherently far more sensitive than NMR and enables researchers to detect secondary metabolites at lower concentrations [100, 101]. The drawback occurs with the consistency of the separation performance. For example, columns can degrade non-linearly over time, requiring constant monitoring, determination of effect(s), and compensation in the final analysis. Solvent purity, pump performance, and injector consistency can all come into play. The inclusion of quality control samples at the beginning, end, and randomly inserted into the experimental samples should allow the compensation and quality control of any introduced confounder(s), but adds material costs, extends batch run times, and introduces complexity to the analyses.

3.2 Development of '-omics' in personalized medicine approach

Over the last decades, various fields of bioresearch (genetics, genomics, proteomics, and metabolomics) have quickly evolved and revealed mechanisms of diseases, and most importantly delivered new therapeutic outcomes. Although the current tenet regarding the uniformity of the drug response seems to be widely accepted, it does not take into account the individual differences. Individuals may not respond in the same way to the pharmacological treatments or present minor and serious side effects. For example, antidepressants [102], statins [103, 104], or antipsychotic drugs [105] have been shown to have reduced effects on some individuals, even to the extent that only a quarter of patients can achieve a functional remission of the disease [105]. Pharmaceutical treatments are ineffective for 30 to 60% of patients [106]. Moreover, a significant number of patients may develop adverse drug reactions (ADR) related to their treatment, with the incidence of fatal ADR being 0.32% [107]. In order to minimize the negative effects of pharmaceutical treatments, and at the same time optimize the drug therapy in terms of its

efficiency, a more personalized approach has been proposed, which assesses various factors prior to the treatment through the application of the different '-omics' [108].

This approach is not entirely new, as some characteristics (age, weight, co-morbidity, family history, and biochemical parameters) are already commonly considered. However, technological progress allows us to analyze individuals in more detail – from different genes, and single-nucleotide polymorphism (SNPs) genomics, to small, biologically active molecules (proteomics, metabolomics) and even the metabolic pathways of individuals (metabolomics, fluxomics) [109, 110]. In addition, personalized medicine not only takes into account the physiological status of a person's body - it also considers the unique, psychosocial situation of the individual, which may have a direct effect when a given health condition manifests in that individual and how he/she will respond to treatment [111]. Although these aspects are taken into consideration for a more complete picture of a person's medical status, separate approaches could also be used to focus on precise problems. For example, a fairly new field called pharmacogenomics tries to assess and validate the impact of human genetic variation on drug responses [112, 113]. Currently, we know that inherited variations in approximately 20 genes can affect around 80 medications and the way the body responds to them [114]. Another young field that has become a prominent branch of metabolomics is pharmacometabolomics, which is the subject of this review.

Personalized medicine has already shown its value in therapies to combat diabetes and cancer [115–119]. For example, the management of blood glucose in diabetes requires proper medication, for which the dosage and efficiency is suited to the individual patient. The efficacy of one of the drugs used in type 2 diabetes, metformin, has been associated with polymorphisms in several genes, specifically solute carrier family (SLC) 22 (an organic cation transporter) member 1 (*SLC22A1*), *SLC22A2*, *SLC47A1*, organic cation transporter 1 and 2 (*OCT1* and *OCT2*), and the gene encoding for multidrug and toxin extrusion 1 protein [MATE1] [115, 120]. Sulfonylureas which are another class of drugs used to treat type 2 diabetes, have been shown to have a variable response effect depending on the genomic profile of the patient, *e.g.*, the variant 'TT' at rs12255372 in the *TCF7L2* gene results in a weaker response compared to the 'GG' version [116, 121]. Those genetic factors are usually not considered when therapy is administered, despite the fact that the information they provide can have direct and substantial effects on therapy optimization and the success of treatment.

Similar benefits from personalized medicine have been observed in the treatment of various types of cancer. One of the best examples that highlights recent progress is breast cancer. Based on the biomarkers present in tumors, such as the estrogen receptor, progesterone receptor, antigen Ki-67, human epidermal receptor 2 [122], and mutations in genes such as Breast cancer gene 1 and 2 (*BRCA1*, *BRCA2*), which are related to carcinogenesis [123], breast cancer can be divided into different subgroups [122]. Each of the cancer types has its own characteristics and requires a specific, more personalized approach to maximize treatment efficacy while minimizing the adverse effects [122, 124]. The decision regarding which therapy to choose becomes even more complicated when we also consider the genetic profile of an individual (the susceptibility to the treatment) [118, 122, 125]. For example, different variants of CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6), which interacts with tamoxifen (a standard drug used in steroid receptor positive breast cancer) have been shown to have direct impacts on the treatment. The impaired version of the protein could also be associated with the recurrence of breast cancer [118, 122]. On the other hand, a personalized approach could also be used in a preventive way. As an example, genetic testing with a focus on the identification of potential, carcinogenic mutations in the *BRCA1* and *BRCA2*

genes could be used to create a proactive strategy (MRI, chemoprevention, bilateral mastectomy), thus significantly decreasing the chances of developing a more severe disease [126].

3.3 Metabolomics databases

The demand for functional and inclusive metabolomics databases is driven by the need for fast data analysis including metabolite identification, quantification, and subsequent interpretation of complex metabolite data, and possibly from multiple instrument sources. As a result of collective efforts in this area, several different databases have been established, including the Human Metabolome Database (HMDB) (https://hmdb.ca/) [127, 128], Platform for RIKEN Metabolomics (PRIMe) [129], Biological Magnetic Resonance Data Bank (BMRB) [130], and the Madison Metabolomics Consortium Database (MMCD) [131]. The existing information on the human urine metabolome was published recently with detailed information on each reported metabolite, including concentration perturbation at normal and disease-related levels (http://www.urinemetabolome.ca). The human urine metabolome along with the human serum metabolome represent a significant development and resource for researchers, which may be critical when employing metabolomics approaches in clinical applications including stratified medicine. Furthermore, the human metabolome database serves as a cross-referencing and benchmarking tool for general metabolomics studies, including metabolite identification, quantification, and newly discovered disease biomarkers. The Madison-Qingdao Metabolomics Consortium Database (http://mmcd.nmrfam.wisc.edu/) contains information on more than 20,000 compounds, including NMR and MS data that are valuable in the identification and quantification of metabolites present in biological samples [131]. Among different freely available metabolomic databases, the HMDB (www.hmdb.ca) [128, 132] (University of Alberta, Canada, David Wishart group) is becoming the de-facto standard reference for the metabolomics community. The HMDB contains information on 74,462 metabolite entries gathered and summarized from literature-derived data and also contains an extensive collection of experimental metabolite concentration information compiled from hundreds of MS and NMR metabolomics analyses performed on urine, blood, and cerebrospinal fluid samples. The data entries encompass a wide range of information, including structural, chemical, clinical, and biological information for many of the reported metabolites.

In 2012, the MetaboLights database (http://www.ebi.ac.uk/metabolights) [133] was established for the online storage of metabolomic experiments, associated raw data, and metadata, to interrogate databases of collected experimental information in publications. This database was first developed and maintained by the European Bioinformatics Institute [134], and later it has been endorsed and developed by the COSMOS consortium [135]. The continuous development of metabolomics databases alongside the uninterrupted advancements in software and supercomputer capabilities may lead to better clinical practices, including diagnosis, disease prognosis, and, ultimately, effective personalized treatments.

3.4 Biobanks and their impact on personalized medicine studies

Over the past decade, several high-capacity biobanks have been established to serve as baseline research and clinical studies tools in use by scientific institutions, clinics, private companies, and regulators at both national and international levels, encompassing a high number (*i.e.*, millions) of samples necessary for medical research. Furthermore, the standardization of sample collection and storage

conditions will help reduce sample collection bias and overcome the limitations afforded by variations between different studies, protocols, and practices. Biobanks usually also collect relevant data, such as whole-genome, genotype, geographic location, dietary preference(s), proteomic, and medical image information [136–138]. In addition to national registries, the incorporation of existing electronic health records (EHRs) is becoming more common, making large biobank datasets more applicable for a greater number of users [139, 140]. The availability of this additional information, combined with the collection of multiple samples over longer periods from the same individual, facilitates improved interpretation of experimental data and provides controls for possible confounders. Establishing large scale national and international biobanks therefore is an essential step and a valuable resource for clinical practitioners and in the development of public health policies, in addition to being crucial for the development of personalized treatments. These megabanks have the capacity to store samples from the same person over the course of many years, which in the future may be collected from childhood and followed up with the periodic collection of new samples throughout life [141].

As biobanks represent a major resource in large-scale global studies, we believe that the impact of metabolomics approaches will become ever more important in future medical research and public health efforts, including personalized health care and stratified medicine.

3.5 Pharmacometabolomics

As mentioned, pharmacometabolomics is a fairly new addition to the '-omics" family of studies. One of the pioneering works that helped create this novel field of science was carried out in 2006 by Clayton *et al.* on paracetamol [142]. Their main goal was to check if the metabolite profile of an animal, prior to the administration of a drug, would allow for the prediction of the metabolism of a drug as well as its toxic effects on an animal. For this purpose, the team collected urine samples from 65 rats, both before and after the administration of paracetamol. Later, samples were analyzed by ^{1}H 1D NOESY NMR spectroscopy. After analyzing the spectra, researchers identified four paracetamol-related metabolites, specifically paracetamol sulphate, paracetamol glucuronide, mercapturic acid derived from paracetamol, and paracetamol. Compared to the histopathological results obtained from the same rats, a substantial model for predicting post-dose histology of the liver could not be established, but they discovered a relationship between the pre-dose metabolic profile of urine and the histological outcome. The main factors predicting that relationship were identified as taurine, trimethylamine-N-oxide (TMAO), and betaine, where higher pre-dose levels of taurine indicated less damage to the liver while higher levels of TMAO and betaine were associated with greater damage [142]. This pioneering work paved the way for the establishment of organizations focused on pharmacometabolomics, such as the Pharmacometabolomics Research Network (PMRN), where the main aim is to "integrate the rapidly evolving science of metabolomics with molecular pharmacology and pharmacogenomics" [143]. So far, PMRN has produced numerous publications, many of them pertaining to lifestyle disorders and diseases. One example concerns the lipidomic response to treatment with simvastatin [144]. The authors of this paper identified metabolites that could predict the outcome of treatment with simvastatin – phosphatidylcholine, including 18 carbon fatty acids with two double bonds at the n6 position, cholesterol esters with 18 carbon fatty acids with one double bond at the n7 position, and 18 carbon-free fatty acids with three double bonds at the n3 position [144]. Additionally, the authors discovered a group of metabolites that may help to predict the changes of C-reactive protein (CRP)

after the treatment - five of them were plasmalogens (a specific group of glycerophospholipids containing a vinyl ether moiety at the sn-1-position of the glycerol backbone) [145], and the others were phosphatidylcholines and cholesterol esters [144]. Another interesting study worth mentioning is related to changes in lipids levels in schizophrenia and upon treatment with antipsychotics [146]. The authors measured the changes in the lipid profiles of patients before and after treatment with olanzapine, risperidone, and aripiprazole. They discovered that treatment

Type of Biomarker	Definition	Example
Diagnostic Biomarker	Biomarker used to detect or confirm the presence of disease or to identify individuals with a subtype of the disease.	Sweat chloride can be used to confirm cystic fibrosis [148].
Monitoring Biomarker	Biomarker measured constantly to assess the status of the disease or for evidence of exposure to (or effect of) a medical product or an environmental agent.	HIV-RNA can be used as a monitoring biomarker to measure and guide treatment with antiretroviral therapy (ART) [149].
Pharmacodynamic/Response Biomarker	Biomarker used to show a biological response of an individual exposed to a medical product or an environmental agent.	Serum LDL cholesterol can be used for evaluating response to a lipid-lowering agent in patients with hypercholesterolemia [150].
Predictive Biomarker	Biomarker used to identify individuals who will experience positive or negative outcome from exposure to a medical product or an environmental agent.	Mutations in BRCA 1/2 genes can be used to identify women with platinum-sensitive ovarian cancer that will most likely respond to PARP inhibitors [151].
Prognostic Biomarker	Biomarker used to identify the likelihood of a clinical event such as disease recurrence or progression.	Mutations in BRCA 1/2 genes can be used to evaluate the likelihood of a future second breast cancer in patients currently diagnosed with one [152].
Safety Biomarker	Biomarker used for indicating the likelihood or presence of a toxic effect, measured before or after the exposure to a medical product or an environmental agent.	Hepatic aminotransferases and bilirubin can be used to evaluate potential hepatotoxicity [153]
Susceptibility/Risk Biomarker	Biomarker used for the estimation of a chance of disease or other medical condition in an individual who currently does not have clinically apparent disease or condition.	Mutation in BRCA 1/2 genes can be used to identify individuals with a predisposition to develop breast cancer [154].

Table 2.
Types of biomarkers with examples of practical applications. Based on the BEST (Biomarkers, EndpointS, and other Tools) resource by the FDA-NIH Biomarker Working Group [147].

with olanzapine and risperidone increased the levels of 50 lipids, raised the concentration of triacylglycerols and generally decreased free fatty acids. Moreover, the concentration of phosphatidylethanolamine that is suppressed in patients with schizophrenia was raised by all three drugs [146].

Presently, most of the pharmacometabolomics studies focus on identifying specific biomarkers related to administered medications. Those biomarkers can provide information ranging from predicting patient treatment response, to monitoring the changes during the treatment, or evaluating the end effects of treatment (i.e., if the patient responded positively or negatively to the therapy) (see **Table 2** and **Figure 3**). Examples of pharmacometabolomic studies are shown in **Table 3**.

The successful isolation of a metabolite that may become a biomarker depends on the type of sample and the approach. In addition to easily and commonly accessed samples like urine and blood serum, pharmacometabolomics studies can also utilize feces, saliva, human breast milk, and even breath [175–177]. Samples are usually collected before, during, and after the treatment, and can be further divided by type of response from an individual (*e.g.*, mostly positive, mostly negative, or intermediate) [175, 178]. After obtaining data from a set of samples using various techniques adapted to the particular type [36, 175, 178], a database for each individual is created, with metabolites detected and identified before and after the treatment [178]. Lastly, a statistical analysis is applied to obtain information ranging from differences that can distinguish good and poor responders prior to the treatment, to changes in metabolites due to drug application that can be correlated with response phenotypes and assumptions of pathways connected to variants of response [178].

For example, Wikoff and colleagues [179] investigated atenolol-induced changes in Caucasians and African Americans. Atenolol is a beta-adrenergic receptor blocker used in a first line antihypertensive treatment. However, various patients responded quite differently. The main objective of this study was to obtain metabolic signatures of atenolol treatment that provided insight into racial differences in response to beta blockers. They found that atenolol has a strong impact on fatty acids in blood serum, but the results were different for different groups (*e.g.*, effects of treatment were highly significant in Caucasians but minimal in African Americans). Furthermore, the authors examined associations between oleic acid and SNPs on the 16 genes encoding lipases. They discovered that a SNP in the *LIPC* (rs9652472) and *PLA2G4C* (rs7250148) genes were associated with the change in oleic acid concentration in Caucasians and African Americans, respectively [179]. Another example of utilizing a combined approach

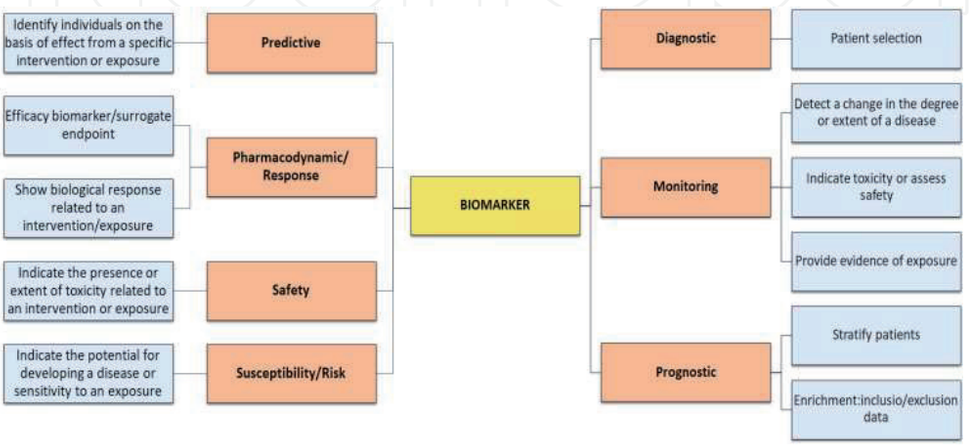

Figure 3.
A brief description of biomarkers of specific use in the drug development process. Based on "Context of use (COU) for a biomarker" by U.S. Food and Drug Administration [155].

Chemical Compound	Goal of the study	Main changes in metabolites post exposure	Conclusions	Ref
Aspirin	To investigate the mechanism of aspirin resistance.	↑ Inosine, adenosine, guanosine ↓ Hypoxanthine, xanthine	• Higher levels of adenosine and inosine were observed in the group categorized as "poor responders". • A pharmacogenomics approach pinpointed an SNP in the adenosine kinase (ADK) intronic variant - rs16931294, where the G allele of this variant was associated with poor response to the treatment.	[156]
	To define pathways implicated in variation of response to treatment with a focus on metabolites containing an amine functional group.	↑ O-Phosphoethanolamine, serotonin ↓ Glycylglycine, L-aspartic acid, L-glutamic acid, L-leucine, L-phenylalanine, L-serine, ethanolamine, glycine, ornithine, taurine, L-asparagine, L-valine, beta-alanine, L-lysine, L-histidine, L-tyrosine, L-glutamine	• The changes in metabolite profiles of healthy individuals treated with low dosage of aspirin cannot be directly attributed to COX-1 inhibition. • Increased levels of serotonin correlated with higher post-aspirin platelet reactivity.	[157]
	To investigate: • The effects of low-dose aspirin therapy on the oxylipid metabolic pathways, • the sex differences in aspirin-induced oxylipid changes, and • potential association of oxylipid on aspirin-induced inhibition of platelet aggregation.	↑ 13,14-dihydroPGF2 ↓ TXB2, 12-HHTrE, 11-HETE, 5-HETE, 12-HETE, 8-HETE, 15-HETE, 9-HODE, 13-HODE, 5-HETrE, 5-HEPE, 12-HEPE, 15-HEPE, 9-HOTrE, EpOMEs, DiHOMEs, DiHETrEs, 20-HETE.	• Aspirin does not show any sex-specific effects on oxylipid levels. • Aspirin decreases almost all of the oxylipids measured in the samples. • Several LA-derived oxylipid (3-HODE, 9-HODE, 12,13-DiHOME, and 12,13-EpOME) metabolites might contribute to the variability of non-COX1-mediated response to aspirin.	[158]
	To assess the metabolic pathways affected by aspirin administration that are potentially involved in cardiovascular and antitumoral protection.	↑ 3-methylglutarylcarnitine ↓ L-histidine, hydantoin-5-propionate, 4-imidazolone-5-propanoate, N-formimino-L-glutamate, xanthosine, L-glutamine, 5-aminoimidazole-4-carboxamide-1-β-D ribofuranoside, butyryl-L-carnitine, tiglylcarnitine, isovalerylcarnitine, heptanoylcarnitine,	• Aspirin decreases the levels of glutamine and metabolites involved in histidine and purine metabolism. • The ability of aspirin to increase the β-oxidation of fatty acids and decrease glutamine levels implicates reduced synthesis of acetyl-Co-A that could help explain aspirins potential anticancer effects.	[159]

Chemical Compound	Goal of the study	Main changes in metabolites post exposure	Conclusions	Ref
Aspirin eugenol ester (AEE)	To evaluate the protective effect of AEE on paraquat-induced acute liver injury (ALI) in rats.	↑ L-histidine, D-asparagine, L-phenylalanine, pipecolic acid, acetylglycine, N-(2-methylpropyl)acetamide, inosine, xanthosine, melatonin radical, ophthalmic acid, glutamylarginine, S-(PGJ2)-glutathione, L-octanoylcarnitine, lysoPC(P^{-16}:0), argininic acid, N-undecanoylglycine, chenodeoxyglycocholic acid, ↓ Glycerophosphocholine, hypoxanthine, nonyl isovalerate, glutamylleucine, pipecolic acid, deoxycholic acid glycine conjugate, dephospho-CoA, taurochenodesoxycholic acid, lysoPC(14:1), PA(22:2), cholic acid, 5,9,11-trihydroxyprosta-6E,14Z-dien-1-oate, lysoPE(18:2), lysoPE(20:4), lysoPE(16:0)	• AEE shows protective effects against PQ-induced ALI. • The mechanisms in which aspirin eugenol ester protects against the effects on PQ-induced ALI are correlated with antioxidants that regulate amino acid, phospholipid, and energy metabolism metabolic pathway disorders and attenuate liver mitochondria apoptosis.	[160]
	To identify the different proteins and small molecules in plasma to explore the mechanism of action of AEE against thrombosis.	↑ Oleamide, palmitic amide, linoleic acid, L-acetylcarnitine, creatine, proline betaine, arachidonic acid ↓ L-carnitine, L-methionine, L-proline, L-pipecolic acid, allantoin, palmitic acid, citric acid, L-tryptophan*	• Metabolomics results suggested that the therapeutic mechanism of action of AEE (as well as for aspirin and eugenol) could be involved with energy metabolism, amino acid metabolism, and fatty acid metabolism. • A total number of 38 (AEE), 41 (aspirin) and 54 (eugenol) proteins were differentially regulated in rats treated with those compounds.	[161]
Busulfan	To investigate biomarkers for predicting busulfan optimal dosage.	↑ Deferoxamine-derived metabolites ↓ Carnitine C9:1, carnitine C12:1-OH, phenylacetylglutamine**	• Busulfan metabolism is decreased in patients with high ferritin levels and reduced liver function.	[162]
Gemcitabine	To investigate potential predictive biomarkers for the efficacy of gemcitabine-based chemotherapy while obtaining the most optimal therapeutic results in patients with pancreatic cancer.	A total number of 38 and 26 different metabolites were identified between the gemcitabine resistant and gemcitabine sensitive pancreatic carcinomas from whom four of them: 3-hydroxyadipic acid, D-galactose, lysophosphatidylcholine (LysoPC) (P-16:0) and tetradecenoyl-L-carnitine, were significantly different between the carcinoma types.	• 3-hydroxyadipic acid, D-galactose, lysophosphatidylcholine (LysoPC) (P-16:0) and tetradecenoyl-L-carnitine could be used as biomarkers for evaluating the efficacy of chemotherapy in pancreatic carcinoma.	[163]

Chemical Compound	Goal of the study	Main changes in metabolites post exposure	Conclusions	Ref
Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZA), Ethambutol (EMB) - DOTS treatment program	To identify metabolites that describe the changes related to tuberculosis therapy	↑ Dodecyl acrylate, pyrazinamide, 1,6-hexylene glycol, ribitol, 1-decene, 2,4-dimethylbenzaldehyde, 2,6-dimethylnonane, 3,4-dihydroxybutyric acid, 5-hydroxyindoleacetic acid, alfa-isosaccharinic 1,4-lactone, beta-Isosaccharinic 1,4-lactone, decane, fumaric acid, hippuric acid, N-formylglycine, sebacic acid, threonic acid, undecane, urea, 3-ethyl-4-methyl-1Hpyrrole-2,5-dione, D-lyxose, phosphoric acid, ↓ Pyrazinoic acid, ethylene glycol, oleic acid, 5-oxoproline, citric acid, ethyl ester, cumene, hemimellitene, hexadecane, indane, isocumene, o-ethyltoluene, oxalic acid, p-ethyltoluene, sorbose, vannilic acid, cyclobutanamine***	• Metabolite markers that are associated with oxidative stress decline between weeks 2 and 4 of treatment – a sign of patient recovery. • During the tuberculosis therapy several enzymes (CYP2E1, CYP3A4, alcohol dehydrogenase, aminocarboxymuconate-semialdehyde decarboxylase) undergo inhibition in a time-dependent manner. • During treatment, the urea cycle is upregulated, and the production of insulin is altered.	[164]
Paclitaxel	To investigate the association between pretreatment metabolome, early treatment-induced metabolic changes, and the development of paclitaxel-induced peripheral neuropathy for breast cancer patients.	↑ Pyruvate, alanine, threonine, phenylalanine, tyrosine, asparagine, lysine, o-acetylcarnitine, proline, lactate, glutamine, leucine ↓ 3-hydroxy-butyrate, 2-hydroxybutyrate****	• Pre-treatment levels of histidine, phenyl-alanine, and threonine may predict severity of potential peripheral neuropathy.	[165]
	To investigate metabolite signatures prior to the treatment, in order to explain the variability of paclitaxel-induced pharmacokinetics.	↑ Creatinine, glucose, lysine, lactate ↓ Betaine	• Pre-treatment levels of creatinine, glucose, lysine, lactate and betaine could be associ-ated with variability of paclitaxel-induced pharmacokinetics	[166]

Chemical Compound	Goal of the study	Main changes in metabolites post exposure	Conclusions	Ref
Irinotecan	To identify metabolite changes that could have potential implications on the mechanism of action of irinote and could serve as biomarkers for efficiency of a treatment.	↑ N-α-acetyllysine, 2-aminoadipic acid, asymmetric dimethylarginine, cystathionine, propionylcarnitine,L-acetylcarnitine, malonylcarnitine, valerylcarnitine, thymine, uracil, xanthine	• The increased levels of purine and pyrimidine nucleobase metabolites could be the result of purine/pyrimidine nucleotide degradation (break of double stranded DNA in cancer cells) as a response to the treatment with irinotecan. • The increased levels of acylcarnitines and amino acid metabolites could reflect dysfunction of mitochondria and oxidative stress in the liver.	[167]
Docetaxel (DTX)	To evaluate the response of MCF7 tumor cells to high (5uM) and low (1 nM) doses of DTX.	For high dosage (5uM): ↑ Phosphoethanolamine, cytidinediphosphocholine, polyunsaturated fatty acid, ↓ Phosphatidylcholine, glycerophosphocholine, glycerophosphoethanolamine, total glutathione, glutamate, arginine, lysine, lactate, acetate, For low dosage (1 nM): ↑ Phosphoethanolamine, cytidinediphosphocholine, homocysteine, aspartate, ↓ Phosphatidyl-choline, glycerophosphocholine, hypotaurine, taurine, total glutathione, arginine, alanine, threonine, lysine, acetate,	• Both dosages result in inhibition of phosphatidylcholine biosynthesis and decreased levels of glutathione. • The mechanisms responsible for decreased glutathione levels are different. At high dosage, the extensive consumption and precursor starvation was the main reason, while for low dosage, it was the inhibition of trans-sulfuration that inhibited glutathione biosynthesis.	[168]
Metformin	To identify urinary markers of metformin responses in patients with type 2 diabetes mellitus.	↑ Myoinositol, hypoxanthine ↓ Citric acid, pseudouridine, p-hydroxyphenylacetic acid, hippuric acid*****	• Citric acid, myoinositol and hippuric acid have the potential to become biomarkers that could predict the response to metformin in patients with type 2 diabetes mellitus.	[169]

Chemical Compound	Goal of the study	Main changes in metabolites post exposure	Conclusions	Ref
Simvastatin	To investigate the metabolic changes connected with the increased risk of developing hyperglycemia as an adverse response to simvastatin.	↑ Glucose, glutamic acid, alanine, ↓ Lauric acid, myristic acid, linoleic acid, glycine¸ palmitoleic acid, 3-hydroxybutanoic acid¸ aminomalonate, oleic acid, N-methylalanine[******]	• Patients showing a mild resistance to insulin tend to develop full insulin resistance after simvastatin treatment. • Branched-chain amino acids, and other metabolites such as ketoleucine, hydroxylamine and ethanolamine could predict type 2 diabetes mellitus risk following simvastatin therapy.	[170]
Olanzapin	To reveal the pharmacodynamics and mechanism of action of olanzapine.	↑ Tyrosine, succinic acid semialdehyde, homovanillic acid, 3,4-dihydroxyphenylacetic acid, L-asparagine ↓ 5-hydroxytryptamine, −5- hydroxyindoleacetic acid, L-3,4-dihydroxyphenylalanine, γ-aminobutyric acid, kynurenine, kynurenine acid, tryptophan, glutamic acid, taurine, acetylcholine	• Olanzapin alters glycerophospholipid metabolism, sphingolipid metabolism and the citrate cycle.	[171]
Losartan	To predict inter-individual variations in the metabolism of losartan.	↑ Lipid CH3 (LDL/VLDL), lipid CH2 (LDL), lactate, citrate, creatine, α-glucose ↓ Lipid CH3 (HDL), creatinine, choline, glycine, phosphorylcholine	• Identification of 11 potential biomarkers from whom lactic acid, creatinine, glucose, and choline showed a good score for prediction of metabolic processes of losartan.	[172]
Midazolam, Ketoconazole, Rifampicin,	To predict biomarkers related to midazolam sum of the clearance related to the induction and inhibition of CYP3A.	↑ 6β-hydroxycortisol/cortisol, 6β-hydroxycortisone/cortisone, 16α-hydroxy-DHEA/DHEA, 16α-hydroxyandrostenedione/androstenedione, 4-hydroxyandrostenedione/androstenedione, 7β-hydroxy-DHEA/DHE,6β-hydroxyandrostenedione/androstenedione, 2-hydroxyestrone/estrone, 2-hydroxyestradiol/estradiol, 11β-hydroxyandrosterone/androsteron, 11β-hydroxyandrostenedione/androstenedione ↓16α-Hydroxytestosterone/testosterone, 11β-Hydroxytestosterone/testosterone[*******]	• Urinary DHEA levels, 7β-hydroxy-DHEA:DHEA ratios, 6β-hydroxycortisone: cortisone ratios could be used to predict sum of the clearance for midazolam	[173]

Chemical Compound	Goal of the study	Main changes in metabolites post exposure	Conclusions	Ref
DA-9701 (extract from *Pharbitis nil* seed and *Corydalis yanhusuo* tube)	To monitor the changes of endogenous metabolites in order to understand better the mechanism of action.	For 0–4 h after exposure: ↑ Uric acid, L-acetylcarnitine ↓ Azelaic acid, ophthalmic acid, suberic acid, ε-(γ-glutamyl)-lysine, pimelic acid For 12–24 h after exposure********: ↑ Ophthalmic acid, pimelic acid, suberic acid, azelaic acid, ↓ Uric acid, ε-(γ-glutamyl)-lysine, L-acetylcarnitine	• Application of DA-9701 affects purine metabolic pathway, lipid, fatty acid metabolism and lipid peroxidation. DA-9701 improves gastrointestinal motility.	[174]

*Rats treated with AEE versus model.
**Patients from high busulfan concentration-time curve (high-AUC) compared with low-AUC group.
***The differences between 2 weeks and 4 weeks of treatment.
****Pre-treatment levels of metabolites compared to 24 hrs after the first infusion.
*****Differences between responders and non-responders.
******Type of association between post-treatment metabolites levels and post-treatment insulin measures.
*******Fold change of mean urinary metabolite ratios in the induction phase.
********When compared to mean fold-changes of 0–4 hours exposure.

Table 3.
Examples of pharmacometabolomic studies.

is the evaluation of aspirin response variability during antiplatelet therapy [180]. Lewis and colleagues identified that metabolites related to aspirin (salicylic acid and 2-hydroxyhippuric acid) were significantly increased, but exposure to aspirin also changed the levels of purines, fatty acids, glycerol metabolites, amino acids, and carbohydrate-related metabolites. Moreover, a substantial difference could be observed between good and poor responders in purine metabolites - higher levels of inosine and adenosine were observed in poor responders after aspirin intervention. Later, the authors identified 51 SNPs in the *ADK* gene region that had associations with platelet aggregation in response to aspirin exposure, the strongest of which was the rs16931294 variant. To confirm their findings, the authors compared their results to previously obtained metabolomic data and observed that rs16931294 was significantly associated with adenosine monophosphate, xanthine, and hypoxanthine levels before aspirin exposure. When compared with post-exposure results, this SNP was strongly associated with levels of inosine and guanosine [180]. The examples presented above [142, 144, 146, 179, 180] as well as other available literature [36, 175, 178] demonstrate the importance of pharmacometabolomics in drug design studies. Combined with other approaches, *e.g.*, pharmacogenomic, pharmacometabolomics can greatly contribute to our understanding of individual differences in responses to drug treatment and thus directly aid us in the development of new generations of drugs. There is also potential for significantly extending our understanding of health sustenance and disease development, and thus reduce drug-dependent therapies. Perhaps not the most profitable news for the pharma industry, but good news for health workers and the general population who will be able to identify at risk individuals and indeed tailor health management strategies to prevent and/or reduce the impact of disease.

4. Future perspectives

An intense research on '-omics' approaches, devoted to human health, led to the development of pharmacometabolomics, which is a new horizon in personalized medicine. Numerous research data on metabolomics, genomics, and transcriptomics can be combined and compared with health records around the world due to potent databases and biobanks collecting data and samples. Nowadays, software and informatics systems with sophisticated algorithms of artificial intelligence allow for deeper analyses of pharmacometabolomics data, and transform general medicine into a personalized approach.

The analytical techniques, databases, and biobanks presented here are the general trends, which need to be further developed. The sensitivity of the analytical platforms needs to be improved, and additional ameliorations related to time and overall costs must be done. Particular attention must be paid to the standardization of study protocols. The number of data and samples deposited in databases and biobank must be extended.

Up to now, major efforts in pharmacometabolomics have been concentrated on research aspects and method validation for medical applications. The results presented here show undoubtedly that pharmacometabolomics is key for personalized medicine and needs to be transferred 'from bench to bedside'. Nevertheless, medical personnel can source from pharmacometabolomics only if the data are presented in a simple and comprehensive way. In the future, more effort is needed to increase the broad awareness of pharmacometabolomics among patients and healthcare system staff, and to introduce the benefits of pharmacometabolomics into clinical practice.

5. Conclusion

Human genetics and lifestyle variation directly influence pharmacological treatments, whose effect can be enhanced positively or negatively in some individuals over the statistical population used in clinical trials.

This chapter has described pharmacometabolomics as an innovative tool capable of assisting researchers and frontline medical personnel in establishing personalized therapeutic strategies. Pharmacometabolomics can be used to personalize treatment type, dosage, duration, and to monitor metabolites' profiles during pharmacotherapy. The existing '-omics' and health records databases, and biobanks of human fluid samples and tissues are a precious resource for pharmacometabolomics, which identify biomarkers of therapeutic effects over a disease course. The metabolomics databases are increasing their data pool every day, and are priceless for researchers combining '-omics' knowledge for better and personalized pharmacotherapy.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

ADK	Adenosine kinase
ADR	Adverse drug reactions
AEE	Aspirin eugenol ester
ALI	Acute liver injury
ART	Antiretroviral therapy
BMRB	Biological Magnetic Resonance Data Bank
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CDER	Center for Drug Evaluation and Research
CYP2D6	Cytochrome P450 2D6
DTX	Docetaxel
EHRs	Existing electronic health records
EMB	Ethambutol
ESI	Electro spray ionization
FDA	Food and Drug Administration

FT-IR	Fourier transformed infrared spectroscopy
HILIC	Hydrophilic interaction liquid chromatography
HMDB	Human Metabolome Database
HPLC	High-performance liquid chromatography
INH	Isoniazid
MATE1	Multidrug and toxin extrusion 1
MMCD	Madison Metabolomics Consortium Database
MS	Mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
OCT1	Organic cation transporter 1
OCT2	Organic cation transporter 2
PMRN	Pharmacometabolomics Research Network
PRIMe	Platform for RIKEN Metabolomics
PZA	Pyrazinamide
RIF	Rifampicin
RP	Reversed-phase gradient chromatography
SLC22A1	Solute carrier family 22 member 1
SLC22A2	Solute carrier family 22 member 2
SLC47A1	Solute carrier family 47, member 1
SNP	Single-nucleotide polymorphism
TMAO	Trimethylamine-N-oxide

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