

REVIEW ARTICLE

Metabolomics: An Emerging Tool for Precision Medicine

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Abstract

BACKGROUND: Metabolomics is a developed technology that comprehensively analyzes the metabolites in biological specimens. It appears to be a prospective method in the practice of precision medicine.

CONTENT: Metabolomic technologies currently surpass beyond the traditional clinical chemistry techniques. Metabolomic is capable to perform a precise analysis for hundreds to thousands of metabolites, therefore provide a detailed characterization of metabolic phenotypes and

metabolic derangements that underlie disease, to represent an individual's overall health status, furthermore to discover new precise therapeutic targets, and discovery of biomarkers, either for diagnosis or therapy monitoring purpose.

SUMMARY: Adequate data processing and quantification methods are still needed to be developed to boost integrated -omics as a powerful clinical practice platform.

KEYWORDS: metabolomic, precision medicine, phenotyping, biomarker, nutritional pattern

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Introduction

Precision medicine aims to design disease prevention and clinical care strategies based on individual environments, lifestyles, genetics, and molecular phenotype variability. Since 1957, the Central Dogma of molecular biology set an impact in life sciences. However, at that moment, Central Dogma didn't catch the idea of small molecule composition in the cells, which play the actual role in controlling the function of genes, transcripts, and proteins, as well as the macromolecules activities regulation in a complex feedback circuit. The interaction between small molecules and the cells' macromolecular components is the main determinant of cell function or dysfunction.(1)

Metabolomics is a comprehensive measurement of all metabolites in biology specimens, including parent compounds and their low-molecular weight molecules <1000 Da, such as amino acids, monosaccharides, small lipids, cofactors, vitamins, energy cycle intermediates,

nucleotides, and exogenous xenobiotics. The numbers of metabolites profiled by metabolomics are much larger than standard clinical laboratory techniques, so we can describe more comprehensive coverage of biological processes and metabolic pathways to consider the strategies for precision medicine.(2)

Currently, clinicians only have a small piece of information about human metabolism based on the routine blood chemistry analytes measurement.(1) Updated information from complex interactions between genotype, lifestyle, diet, nutrition, drug therapy, environmental exposure, and gut microflora at the molecular level, provide new insight into disease pathophysiology and drug response mechanism in clinical practice to predict both risk of toxicity and beneficial responses to drug treatment.(3-15) However, even after 20 years, there are still so many barriers to face in translating -omics technologies in clinical practice.(16)

In this narrative review, we discuss the current knowledge about metabolomic as the latest tool in -omics family, together with its challenges and future, especially

in its application for biomarker discoveries, nutritional management, and precision medicine related to metabolic diseases.

Metabolomics is An Emerging Technology

“Omics” approaches, including genomics, transcriptomics, proteomics and metabolomics, recently have become important tools to integrate genetic, protein, metabolite, cellular and pathway events in flux and interdependent in one system to develop a better understanding of the complex biological processes.(17-22) The “omics” approaches measure multiple genes, transcripts, proteins, or metabolites changes simultaneously and provide an overview of various physiological or pathological conditions.(23) These advanced techniques could bring a big change in clinical setting for human diseases’ diagnosis and treatments with full consideration of the limitations.(24) Metabolomics and metabonomics are two terms that are often used interchangeably.(25-28) In brief definition, metabolomics measure all metabolites in the cell, while metabonomics assess the change due to metabolic responses, or in other word, metabolomic profiling.(1,17,29-32) Metabolome profiling is typically performed either by targeted or untargeted methods. Targeted and semi-targeted metabolomics focus on selected metabolites based on a hypothesis-driven approach, while untargeted metabolomics pursues an unbiased screening of all metabolites and hypothesis-free. Therefore, a combination of both approaches is preferred for a thorough metabolome capture.(16,33)

As the changes in the genome, transcriptome and proteome do not always result in altered biochemical phenotypes, metabolomics may in fact provide the most “functional” information of the omics technologies, with the facts that the metabolome represents the final “omic” level in a biological system, and metabolites represent functional entities which have a clear function in the life of the biological system and are also contextual, reflecting the surrounding environment.(28) Metabolomics has the capacity to increase the readouts by orders of magnitude compared with traditional chemical or even genetic screening.

As the emerging omics tools, metabolomics is less evolved compared to the other family, and in practice send quite a challenge. It targets a big range of divergent molecule physical properties, with different polarity from very water-soluble organic acids to very nonpolar lipids.

(34) A comprehensive metabolomic technology platforms prepare the sample pre-analytic and analytical procedures specifically and divide metabolomes into subgroups of metabolites, either by the polarity, common functional groups, or structural similarity as illustrated in Figure 1, and the methods used in metabolomics still continue to evolve and improve each year.(35) Current techniques used in metabolomics such as liquid chromatography-mass spectrometry (LC-MS) can measure tens to hundreds of metabolites with excellent precision, make it possible for rapid discovery and validation of early metabolic indicators of human disease, even years before the symptoms are clinically seen, for example, pancreatic cancer (36), type 2 diabetes (17-20,37), memory impairment (20). Metabolomics studies also reveal our understanding of diet and disease relationships, such as the link between elevated branched-chain amino acids (BCAA) and obesity to insulin resistance.(34) A previous study brought microbiome into the metabolite profiling and found that elevated plasma levels of trimethylamine-N-oxide, which is abundant in red meat, was associated with the composition of the gut microbiome and risk for cardiac events.(2,35)

While metabolomics technology is still developing, recent studies are also observing what actually constitutes the human metabolome. The complete set of small molecules in human body may exceed 19,000 (38), consist of metabolites derived from endogenous enzymatic activities encoded by the human genome, and also exogenous metabolites from food, medications, the microbiota that inhabit the body, and the environment. Nine of the 20 amino acids our body needs was depend on diet, because even we have the codons in our body but not the endogenous biosynthetic route, and this fact highlight how the exogenous metabolites are important to be counted.(2) Liquid chromatography and gas chromatography coupled to mass spectrometry (GC-MS and LC-MS) and nuclear magnetic resonance (NMR) spectroscopy improve the capabilities for holistic metabolic profiling for precision medicine. The platform should be stable and reproducible, and recent studies showed the capability now possible for robust and high-quality data generation.(33,36)

Like any other its predecessors, there are still some bottlenecks in metabolomics to be translated clinically, especially in precision medicine, such as the general quality assurance and quality control standards, systematic errors assessment, universal workflow, big data integration and collection platform, reproducible data analysis and interpretation, and potential bioethical issues. Despite the current challenges, quantified metabolomics data

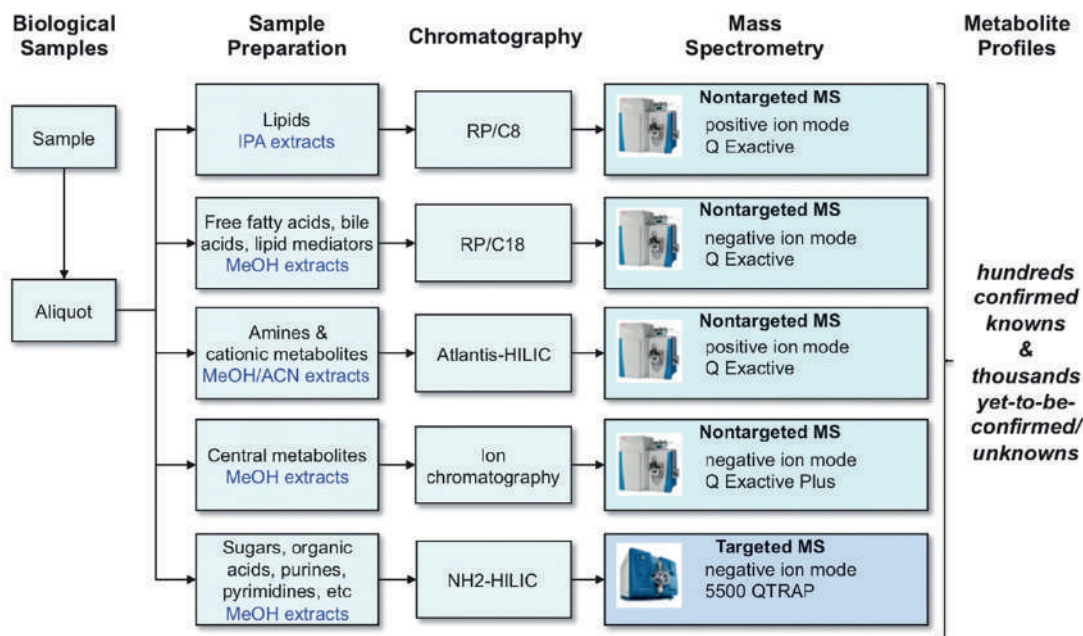


Figure 1. Illustration of the liquid chromatography-mass spectrometry (LC-MS)-based metabolomics platform.(2) (Adapted with permission from Cold Spring Harbor Laboratory Press).

have significant translational opportunities in biomarker discovery, precision nutrition and precision medicine, due to its advantages to detect responses even when growth phenotypes are lacking.

Metabolomics Analysis for Biomarker Discovery

Metabolome was first coined in 1998 as the set of metabolites (low molecular weight compounds) synthesized by cells, tissues, organs or organisms, dependent and varying according to the physiology.(29) Metabolome can be divided into several fractions: the endogenous metabolome, which is naturally produced by an organism, and the exogenous metabolome, which are chemicals that are not naturally produced by an organism, includes all foreign metabolites derived from drugs, pollutants and dietary compounds. (1,39,40) The endogenous metabolome is subdivided as primary metabolome includes all metabolites characteristic of each cell type, tissue, organism, or biological fluid, reflecting particular environmental conditions and evolving according to physiological demands, and secondary metabolome which is the microbial metabolome produced by the microflora.

Thus, the metabolic phenotype consists of the integration of individual's genetic, nutritional, pharmacological, and environmental status.(1,41,42)

Indeed, the biochemical pathways of metabolites, as well as knowledge of their intracellular fluxes ought to be understood because the production and degradation of metabolites are regulated in interconnected distinct metabolic pathways.(41-43)

The fact that metabolites is closely related to phenotype, and that metabolome is sensitive to many factors, make it feasible for a large range of applications including diagnosis and identification of certain metabolites which characterize distinct pathological and physiological states (11,32,44-46), added with the assumption that metabolites are important players in biological systems and the disruption of biochemical pathways induce diseases. Metabolomics results in multiple metabolites assessment, thus metabolomics offers potential advantages in sensitivity and specificity in the clinical area compared to classical diagnostic approaches and conventional clinical biomarkers.(47)

An ideal biomarker is expected to fulfill some criteria included: present in minimally invasive and readily available sources such as blood or urine; highly sensitive and specific, means allow early diagnosis and unaffected by external or comorbid conditions; precisely change in response to treatment and disease progression; it provide a better understanding about the disease mechanism; and helpful in disease risk stratification and prognosis.(27) The central dogma of molecular biology explains that DNA (genes) are transcribed to mRNA (transcripts) and translated into

proteins, then the protein activity results in small molecules (metabolites) formation.(29) Therefore, any changes in genes or proteins will affect a change in the metabolism and these were the “omics” complementary sciences all about (48), and metabolomics is applied for the nutritional and physiological status of the patient by measuring the variety of small molecules. The current metabolomics analysis tools allow a large number of samples measurement in a high-throughput manner, so it provides us the understanding of current molecular response of a biological system to any perturbation in its microenvironment (49,50), explain the basic mechanisms of diseases at once able to identify the molecular markers for the diseases (1). Metabolite biomarkers can be classified into three different classes: predictive biomarkers to determine who in the population might respond to specific treatment regimes, prognostic biomarkers to observe the prospect of the disease in a patient, and pharmacodynamics biomarkers to evaluate the outcome of the treatment.(16)

Metabolomics as A Tool in Nutritional Research

The comprehensive approach of metabolomics made possible of exploring the complex interaction between human and diet, complete with the implications and subtle changes in metabolism activated by foods, nutrients and disease. In general, metabolomics application in nutrition application is divided into three categories: studies for dietary biomarker discovery, studies of diet-related diseases, and studies for dietary intervention.(51)

Overall, most metabolomics studies recently observe the disturbances in metabolic pathways (52), to find out how nutrition influences metabolism and homeostatic control and to find the early phase disturbance on this regulation, related to early detection of diseases (53). Commonly the studies performed by three approaches: acute intervention, cohort, and dietary patterns and metabolic profiles association

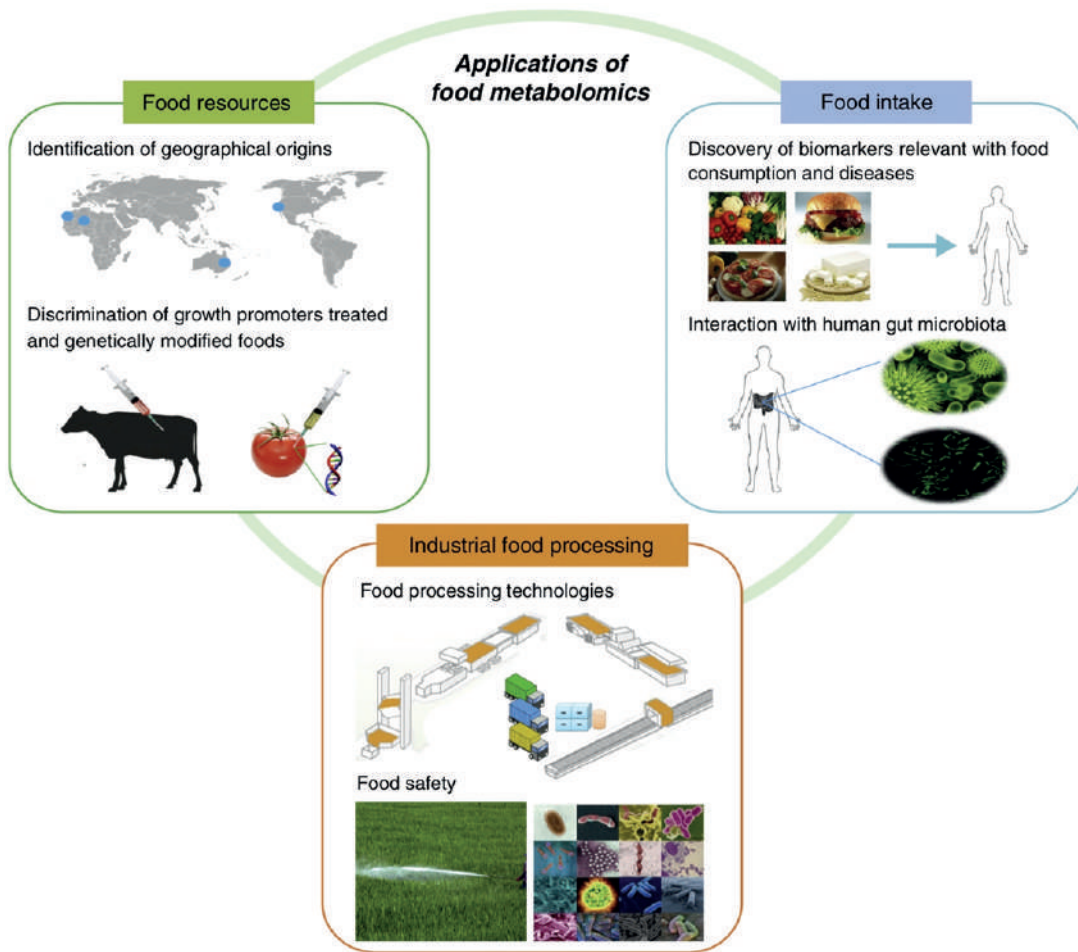


Figure 2. The three main applications of food metabolomics, from farm to human: food resource production, industrial food processing and food intake by humans.(60) (Adapted with permission from Wolter Kluwer Health).

analysis. Attempts on self-reporting dietary assessment currently such as food-frequency questionnaires (FFQs), 24-h recalls, weighed food records cannot be fully reliable due to energy under-reporting, recall errors and difficulty in assessment of portion sizes.(54,55) Thus, biomarkers for dietary intake are needed to improve the dietary assessment. Currently, we have biomarkers for salt, protein, sucrose and fructose intake (sodium/nitrogen/sucrose and fructose measured in 24 h urine samples), and for energy expenditure (the doubly labeled water technique) (56,57), which were extremely useful to validate the previous self-reports (57). Food metabolomics is the application of metabolomics application in human food systems, including food resources, food processing and diet. Food systems are evidently related to nutrition and human health directly. Hence, metabolomic not only useful for biomarker discoveries but also to elucidate the underlying mechanisms of disease development.(58) For instance, a number of possible risk factors of type 2 diabetes mellitus have been identified by metabolomics involving lipid molecules such as free fatty acids, bile acids and amino acids, and notably BCAA, which is correlated with insulin resistance in human and rodents. (34,59) A study found an elevated levels of BCAA in obese subjects, suggest due to increased catabolism of BCAAs or

'BCAA overload'.(34) The three main applications of food metabolomics, from farm to human include food resource production, industrial food processing and food intake by humans (Figure 2).(60)

Food processing in food system used in food industries to increase food safety; to change or modify food textures, tastes, flavors, colors, etc.; and to provide nutritional or balanced diets for customers by using all techniques and methods to convert food resources such as crops, vegetables, fruits, meat and milk into valuable food products.(61) Figure 3 shows one example of the workflow of food metabolomics.

Many studies on new metabolite biomarkers correlated with dietary intake patterns such as juice (62), fruits, vegetables (63), grain, fish (64), wine (65) coffee (66) and the complex ones (67,68) revealed that either specific food intake or the complex dietary pattern significantly affect human metabolism like amino acid and lipid metabolism, related to human health. Therefore, metabolomics is useful for nutritional epidemiological studies to effectively analyze the effect of specific food consumption and dietary patterns on metabolic changes. The application of metabolomics will be wider in food sciences and technology, delivering food from the farm to human, including food resource

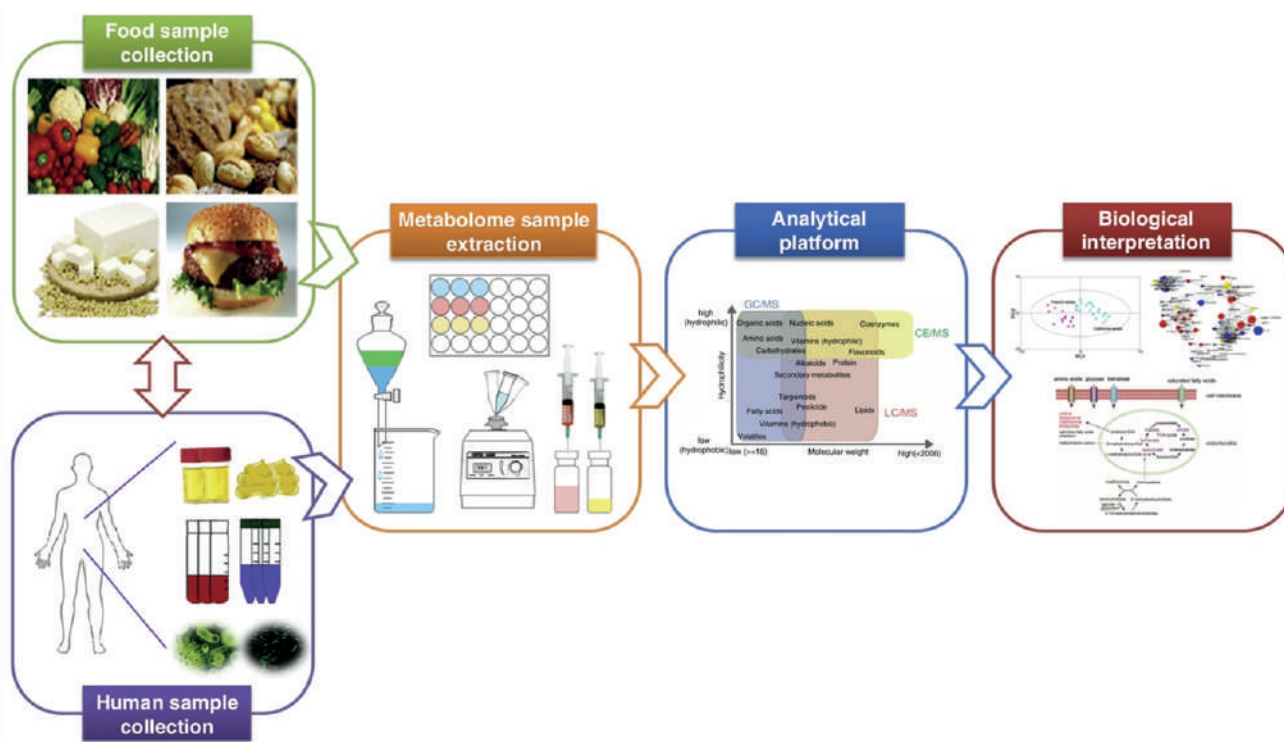


Figure 3. The workflow of food metabolomics consisting of food and human sample collection, metabolome sample extraction, instrumental analysis using analytical tools and statistical analysis of metabolic data and their biological interpretation.(60) (Adapted with permission from Wolter Kluwer Health).

production, industrial food processing and food intake. Comprehensively, metabolomics will result in personalized food practice from design up to final products.

Metabolomics Profiles and Nutritional Patterns

Nutrition is essential in human metabolism and health, thus considered as one major factor contributes in metabolic diseases. Metabolomics is used for nutritional studies to profile all low-molecular-weight metabolites in biological samples to better understand how a stimulus could affect metabolic pathways.(69,70) Many studies observed the impact of single nutrients or single foods, up to global diets or dietary patterns such as the Mediterranean diet on chronic diseases to find the link between diet and the diseases.(67)

Principal component analysis (PCA) is utilized to depict a portrait of a population's dietary patterns (71) such as the Prudent (or Healthy pattern), the Western dietary pattern, and so on, which are positively or inversely correlated with cardiovascular disease risk factors and mortality (72-74). A dietary pattern such as foods that high in sugar-sweetened soft drinks, or refined grain, processed meats and low vegetable intake was associated with the risk of type 2 diabetes mellitus.(75) Though we have not had a full understanding of the pathways underlying metabolic risk factors in humans, yet we know those are likely related to derangements in primary metabolism. More recent technologies apply LC-MS to acquire high-throughput metabolic status profiles of organisms and a comprehensive assessment of molecules of substrates or products of metabolic pathways (*i.e.*, metabolomics).(76-80) By having a complete profile of individual metabolites, we can elucidate specific metabolites' role in metabolic disorders development and its sequelae.(81-84)

There are many dietary guidelines to promote well-being and prevent chronic diseases. Most researches focus only on individual food or nutrient, while in fact quality of a diet is influenced by the combination of foods, its quantity, and also interrelations.(68,85,86) Diets in their complexities can be summarized in patterns.(87-91) Therefore, studies should focus more on these patterns in relation to the health benefits.(92) Due to its potential to measure a range of small molecules present in a biological system, nutritional metabolomics combined with multivariate statistical analysis, can characterize and define the normal physiologic variation profiles and the changes in different biofluids as a result of specific dietary interventions.(93-95) A recent

application on nutritional metabolomics has shown its potential in the identification of dietary biomarkers (96), as long as it's qualified to be replicated in future studies and comply to a classical measurement model. Moreover, metabolomics has the advantages of highlighting which metabolites and pathways are influenced by diet, thus dietary pattern-disease relations could be further explored.(97,98) Biocrates Life Science AG (Innsbruck, Austria) developed AbsoluteIDZ kit, which allows more than 160 targeted metabolites in over 4 compound classes quantification in an easy, reliable and robust way. The 4 months reproducibility is good, and data showed a single measurement is sufficient for risk assessment in epidemiologic studies using healthy subjects.(99) Hence, we could collect data on metabolite properties to create a dietary biomarker classification (*i.e.*, recovery, concentration, replacement, or predictive biomarkers).(59,93,100)

Metabolomics Profiles and Obesity

Obesity is a worldwide problem. The technology development spoiled most people's lifestyle and contributed to obesity increasing number, in line with its metabolic comorbidities, especially the risk of insulin resistance, hypertension and dyslipidemia, and mortality rate.(100) However, 10-30% of obese population have a relatively healthy metabolic status, with normal insulin sensitivity, blood pressure and lipid profiles.(101,102) These metabolically healthy obesity (MHO) group compared to the metabolically abnormal obesity (MAO), also shown a lower mortality rate and lower risk of developing metabolic disease.(103-105) Many studies aim to determine the underlying mechanisms that differentiate normal subjects, MHO and MAO subjects. Metabolomics could be used to quantitatively analyze the substantial differences in circulating markers of amino acid (106-109) and lipid (107,109) metabolism, to understand how metabolites reflect physiological states. Perturbation in these metabolic pathways also reflects not only established cardiometabolic risk biomarkers, independently of weight status (110,111), but were also predictive of future disease (112-114).

Many factors can modulate metabolites in its pathways, either genetic factors, environmental factors or gene-environment interactions.(115) Mass spectrometry-based metabolite profiling was applied to investigation of serum metabolite concentrations between normal (Nw), overweight (Ov), and obese (Ob) group with metabolic disturbance (MetS) and not, and showed that there were three

principal factors explaining a maximum of variance between groups. First, the score of long-chain glycerophospholipids metabolite is higher in Ov/Ob with MetS compare to Ov/Ob and Nw subjects without MetS. This factor also positively correlated with plasma total cholesterol (total-C) and triglyceride levels in the three groups, and high-density lipoprotein-cholesterol (HDL-C) only in subjects without MetS. The second factor is amino acids and short to long-chain acylcarnitine which was positively correlated with HDL-C and negatively correlated with insulin levels in Nw participants. The third factor is medium-chain acylcarnitines profile scores which was higher in Nw subjects than other groups independent to MetS. It was negatively associated with glucose levels among the Ov/Ob with MetS. It seems like factor 1 have a relationship effect to deteriorated metabolic profile in obesity, while factor 2 and 3 related to the healthy metabolic profile.(116)

Other interesting metabolites associated with obesity are choline and betaine. They are quaternary ammonium compounds obtained from food or synthesized *de novo* in tissues. Choline roles as the major source of methyl groups in the diet and can be found in eggs, beef, pork, liver, soybean, and wheat germ.(117,118) Phosphatidylcholine (lecithin) is the most abundant choline species, and an important source of choline relative to dietary intake, moreover in premenopausal women.(119) Lecithin is formed endogenously from phosphatidylethanolamine by a S-adenosylmethionine-dependent methylation reaction catalyzed by phosphatidylethanolamine N-methyltransferase. The biological function of choline, including gene expression epigenetic regulator (120), as a lipoprotein precursor, membrane phospholipids, and the

neurotransmitter acetylcholine; thus, it was important for lipid metabolism, the integrity of cell membranes, and nerve function (117,121).

Betaine is a modified amino acid found in the highest content in whole grains such as wheat bran, wheat germ, and spinach. It is also formed in the kidney and liver by choline oxidation catalyzed by the mitochondrial enzyme, choline dehydrogenase.(117,122,123) Betaine is a key organic osmolyte due to its dipolar zwitterion structure. It accumulates in a variety of cells, including renal medullary cells, under the condition of hypertonicity.(124) Betaine metabolism is dominant in the liver in mammals, where it functions as a methyl donor in the betaine-homocysteine methyltransferase (BHMT) reaction (125), for homocysteine betaine-dependent remethylation yielding dimethylglycine and methionine (126). That's why betaine supplementation can reduce total homocysteine (tHcy) level and they're inversely correlated; therefore betaine used as a treatment for homocysteinemia.(127-130)

Central obesity and excessive flux of fatty acids in the visceral tissue are regarded as the main factors of metabolic syndrome, which leads to insulin resistance and atherogenic dyslipidemia.(131) Betaine intake and plasma levels were inversely correlated with several metabolic syndrome markers.(121,132) Insulin decrease both BHMT and choline dehydrogenase in rat liver, while diabetes increased them (133), human with insulin resistance also found to have lower N,N,N-trimethylglycine, or glycine betaine level. Inversely, betaine decreases hepatic lipid content and improves glucose tolerance in rodents.(134-137) Mitochondrial dysfunction found in metabolic syndrome (138-140), suggests the involvement of choline oxidation to betaine that takes place

Table 1. Compilation of metabolomic profiling studies on obesity patients conducted in various countries.

Metabolite	Sample	Analytical Tools	Country	References
Fatty acids pathway				
Carnitine (+)	Plasma	LC-MS	USA	(108)
Carnitine (-)	Plasma	LC-MS	USA	(142)
Acylcarnitine (+)	Plasma and Urine	Flow injection-MS	UK	(34)
Acylcarnitine (+)	Plasma	LC-MS	USA	(108)
Amino acids pathway				
Leucine (+)	Plasma	LC-MS	UK	(143)
Valine (+)	Blood and Urine	GC-MS	UK	(34)
Tyrosine (+)	Blood	UPLC-MS	South Korea	(144)
Glutamine (-)	Plasma	LC-MS	Italy	(145)
Kynurenic acid (-)	Urine	LC-MS	UK	(143)
Xanthurenic acid (-)	Urine	LC-MS	UK	(143)
Uric Acid (-)	Plasma	LC-MS	UK	(143)

in the inner mitochondrial membrane (139). Thus, choline and betaine dehydrogenase pathways might be associated in insulin resistance, and could be used to predict the response of prevention strategies. Another study showed that dietary betaine increase Fgf21 and improve metabolic health in mice, suggest that betaine supplementation merits further investigation for type 2 diabetes mellitus prevention in humans.(141) Table 1 shows some examples of metabolites profiling on human subjects that have been identified in obesity cases utilizing the metabolomics approaches.

Metabolite Profiles and Risk of Diabetes

Early identification of functional β -cell mass will help to prevent diabetes, unfortunately early asymptomatic β -cell defect identification cannot yet be successfully performed. Metabolomics could be proposed to readout the early disease states before the clinical manifestation by identifying novel plasma biomarkers representing functional pancreatic β -cell insufficiency before it reaches the clinical symptomatic stage.(146) Recent clinical and laboratory markers can be helpful to indicate the diabetes and its risk progression but provide only a few insight regarding pathophysiologic mechanisms.(147) Nonetheless, identification of individual risk could provide effective interventions for delaying or preventing the onset of T2D and lessen the burden.(148-151)

BCAAs, aromatic amino acids, fructose, mannose, α -hydroxybutyrate, and phospholipids are the main metabolites that are consistently appearing in human T2D studies.(106,112,152-158) Most of them are also associated with insulin resistance and obesity (159,160) but not directly to β -cells alteration. Thus, they still cannot answer the need for biomarkers reflecting the loss of functional β -cell mass.(161,162)

A study documenting the differences in blood metabolite profiles before and after glucose loading in obese vs. lean individuals noted differences in amount of C3 and C5 acylcarnitines, glutamine and glutamate, and other small molecules.(81,83) This provides a complementary information to identify at-risk individuals over standard clinical markers.(106) Identification of 1,5-anhydroglucitol showed association with the loss of functional β -cell mass, raise a possibility of this marker for early detection on β -cells decline.(163) Another study utilizing LC-MS analyzing amino acids, amines and some polar metabolites, BMI and fasting glucose, found five branched-chain and aromatic amino acids that might significantly predict future diabetes

i.e.: isoleucine, leucine, valine, tyrosine and phenylalanine. A combination of three showed more than fivefold higher diabetes risk for individuals in the top quartile, and replicated result has been performed in an independent, prospective cohort. Thus, we can suggest that amino acid profiles could aid in diabetes risk assessment.(112) Additional study in this observed, and a lower ratio of circulating glutamine-to-glutamate concentrations (glutamine/glutamate) in Europe population (84,164) proposed for up to 12 years prediction of diabetes in the future.

PGC-1 α is an important protein in human known to regulate energy metabolism. It induces broad genetic programs in skeletal muscles, including mitochondrial biogenesis and fatty acid β -oxidation. PGC-1 α induces the paracrine activation for angiogenesis, by coordinating a link for fatty acids transport across plasma and mitochondrial membranes, deliver fatty acids to muscle, and also increase trans-endothelial fatty acid transport from the blood vessel lumen to the extraluminal myofibers, which mechanism is not fully understood.

BCAA comprising up to 30% of muscle protein. Valine is one of BCAAs that is essential in dietary components. In skeletal muscle as well as many other organs, BCAA catabolic flux is tightly regulated. All catabolic products are trapped inside the cell by covalent linkage to coenzyme A, except for 3-hydroxyisobutyrate (3-HIB). Therefore, 3-HIB can be utilized as a secreted reporter of BCAA catabolic flux in muscle.(106,112,165) High 3-HIB secretion from muscle represents the increased catabolic flux of BCAAs, leads to excess trans-endothelial fatty acid import into muscle, accumulate the lipotoxic and incompletely esterified intermediates, such as DAG, finally blunted insulin signaling. Excess BCAA then implicates the progression to diabetes. The levels of 3-HIB in skeletal muscle from db/db mice, as well as the muscle biopsies from diabetic patients, were significantly increased. Some studies also reported the increased serum level of 3-HIB in diabetic patients. (166,167) Taken together, paracrine secretion of 3-HIB in the cross-regulatory link between the catabolism of BCAAs and fatty acids, causes excess accumulation of incompletely esterified lipids in skeletal muscle, which finally blunted AKT signaling and glucose intolerance.(168)

Table 2 shows some examples of metabolites profiling on human subjects that have been identified in diabetes cases utilizing the metabolomics approaches. In the past few years, many metabolomic studies were conducted in various countries. Unfortunately, we cannot find a metabolite profiling study from Indonesia performed on human subjects.

Table 2. Compilation of metabolomic profiling studies on diabetes patients conducted in various countries.

Metabolite	Sample	Analytical Tools	Country	References
Fatty acids pathway				
Fatty Acids (+)	Serum	GC-MS	China	(169)
Fatty Acids (+)	Plasma	LC-MS, GC-MS	Singapore	(170)
Carnitine (+)	Plasma	LC-MS	USA	(108)
Carnitine (-)	Plasma	LC-MS	USA	(142)
Acylcarnitine (+/-)	Plasma	LC-MS	USA	(171)
Acylcarnitine (+)	Plasma	LC-MS	USA	(108)
α -hydroxybutyrate (+)	Plasma	LC-MS, GC-MS	Singapore	(170)
3-hydroxybutyrate (+)	Serum	GC-MS	China	(169)
Acetate (+)	Plasma	LC-MS	Germany	(172)
Amino acids pathway				
Leucine (+)	Plasma	UPLC-MS	Germany	(172)
Leucine (+)	Serum	NMR, LC-MS, GC-MS	Germany	(173)
Isoleucine (+)	Serum	NMR, LC-MS, GC-MS	Germany	(173)
Valine (+)	Serum	NMR, LC-MS, GC-MS	Germany	(173)
Methionine (+)	Serum	GC-MS	China	(169)
Glycine (-)	Serum	LC-MS	Germany	(174)
Serine (+)	Plasma	UPLC-MS	Germany	(172)
Phenylalanine (+)	Serum	LC-MS	Germany	(174)
Phenylalanine (+)	Plasma	LC-MS, GC-MS	Singapore	(170)
Glutamate (-)	Serum	GC-MS	China	(169)

Pharmacometabolomics and Precision Medicine

Metabolic disorders occur as a result of dysregulation in multiple biochemical pathways at the molecular level. Disease heterogeneity contributes to drug response variability, and getting more complicated with genetic variability, environment and gut microbiome activity. New approaches were developed to understand drug effects. Pharmacometabolomics and its union with pharmacogenomics provide new insight to discover new biomarkers in clinical pharmacology.(8,175)

Recent medical treatment refers to “average patients” based on one-size-fits-all approached therapy, which could be successful for some patients and not for others. On January 2015 “President Obama’s Precision Medicine Initiative” released by the White House defines precision medicine as, “a new model of patient-powered research that promises to accelerate biomedical discoveries and provide clinicians with new tools, knowledge, and therapies to select which treatments will work best for which patients.” In a simple statement, precision medicine is an individually-tailored treatment based on the patient’s illness, given at the right time (Figure 4).(176,177)

The Human Genome Project published a reference for human genome sequence (178) contribute big milestone in biology research, particular in cancer. Metabolomics in the last decade added the understanding of cancer biology. Small molecules studied in metabolomics can be formed by numerous biosynthetic and catabolic pathways within a biological system which present in a cell, tissue, or biofluids such as urine, blood, or saliva, originating from host’s microbiota, nutrient, and pharmaceuticals intake to describe a physiological or pathological condition.(179-182) The Human Metabolome Project (HMP) in 2004 equivalent the Human Genome Project, archived 2500 small molecules produced by metabolic reactions in the body’s tissues and biofluids (183) include lipids, sugars, ions, metabolic intermediates, and products of biochemical reactions, and also proteins, nucleic acids, and cell membranes as the building blocks for all other biochemical species. Integrating multivariate omics technology including genomics, epigenetics, transcriptomics, proteomics and metabolomics with advanced computation, statistics and bioinformatics will develop mechanistic understanding within molecular biology at the genome, gene transcription, protein and metabolite levels, respectively.(184)

Personalized medicine or precision medicine predict the safest drug treatments for each person, involving

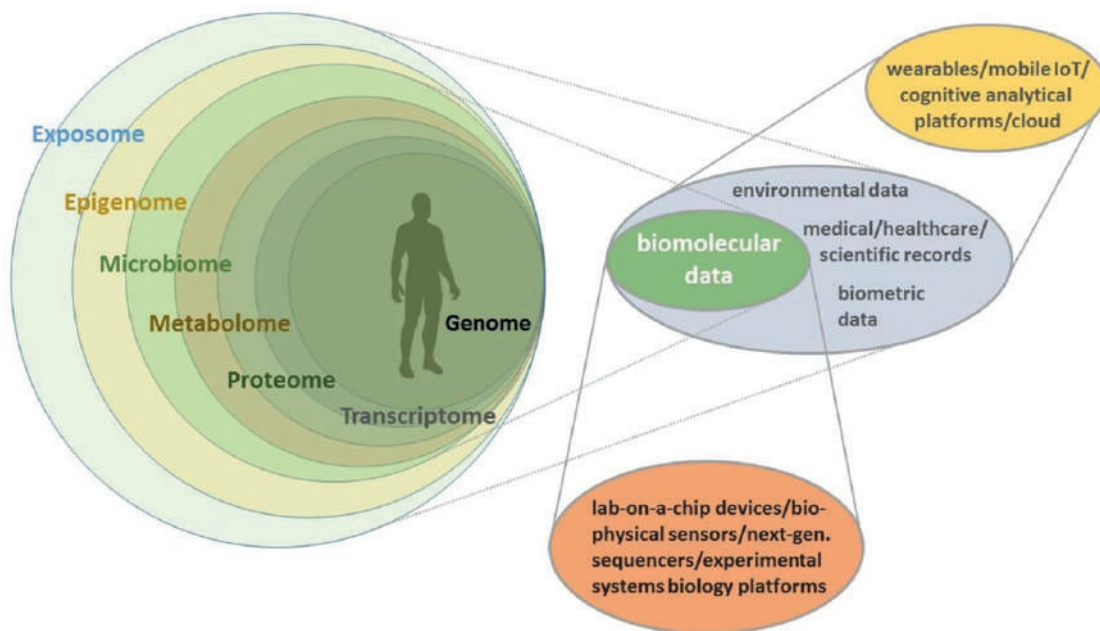


Figure 4. Precision medicine contextualizes data from genome to exposome. Thereby genomic, transcriptomic, proteomic, metabolomic and microbiomic data is biomolecular by nature, while epigenomic and exposomic data also takes environmental, biometric and medical metadata sources into account.(177) (Adapted with permission from SPIE).

individual’s genetic, or known as pharmacogenetics and/or pharmacogenomics.(185-189) In fact, many factors more than genetics, such as environmental or some personal factors (*i.e.*, the “exposome”), alcohol consumption, the gut microbiome, nutrients intake, another medications and supplements, age, and other comorbidities (such as obesity, cancer, pregnancy) make the clinical implementation of pharmacogenomic face lots of challenges.(190-192) The determination of individual metabolic state refer as “metabotype” (7,193-196) could be employed to define someone’s metabolic signatures and provide a better prediction of the potential for drug safety issues (197-199), together this fields called as pharmacometabolomics (6) and pharmacometabonomics (193).

Pharmacogenomics classify patients as poor or rapid drug metabolizers, or responder and non-responder. (186,200,201) However, patient’s genotype does not always have a clear definition of the phenotype or the current (patho) physiological state of the individual (190), because the genotype cannot describe the environmental factors and/or disease-related factors influence. Phenotypic status of an individual results from complex factors such as demographic, environmental interaction, gut microbiota, and any comorbidities, then the endogenous metabolite profile describes a snapshot of these all. Metabolomics application provides a direct readout of individual current state, so it can better explain the inter-individual

variability of drug pharmacokinetic and pharmacodynamic. Pharmacometabolomics evaluate the metabolism of pharmaceutical compounds of the patients, and better understand the pharmacokinetic profile of a drug (Figure 5). Study of pharmacometabolomics involving the application of pharmacology, includes pharmacokinetics to define metabolic influences on the drug concentration reaching its target, pharmacodynamics to define metabolic influences on target signaling downstream, clinical pharmacology, drug discovery and development, clinical trials and precision medicine. The application of “metabotypes” sub-classified patients based on their metabolic profiles and useful for grouping the subjects in trial inclusion (8,202,203). When we interplay this baseline to pharmacometabolomic, we

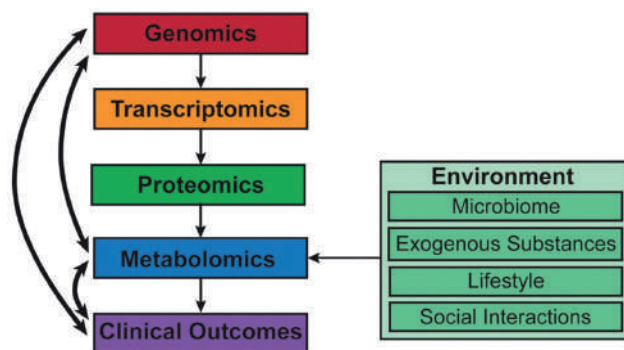


Figure 5. Pharmacometabolomics-informed pharmacogenomics.(202) (Adapted from permission from PMC).

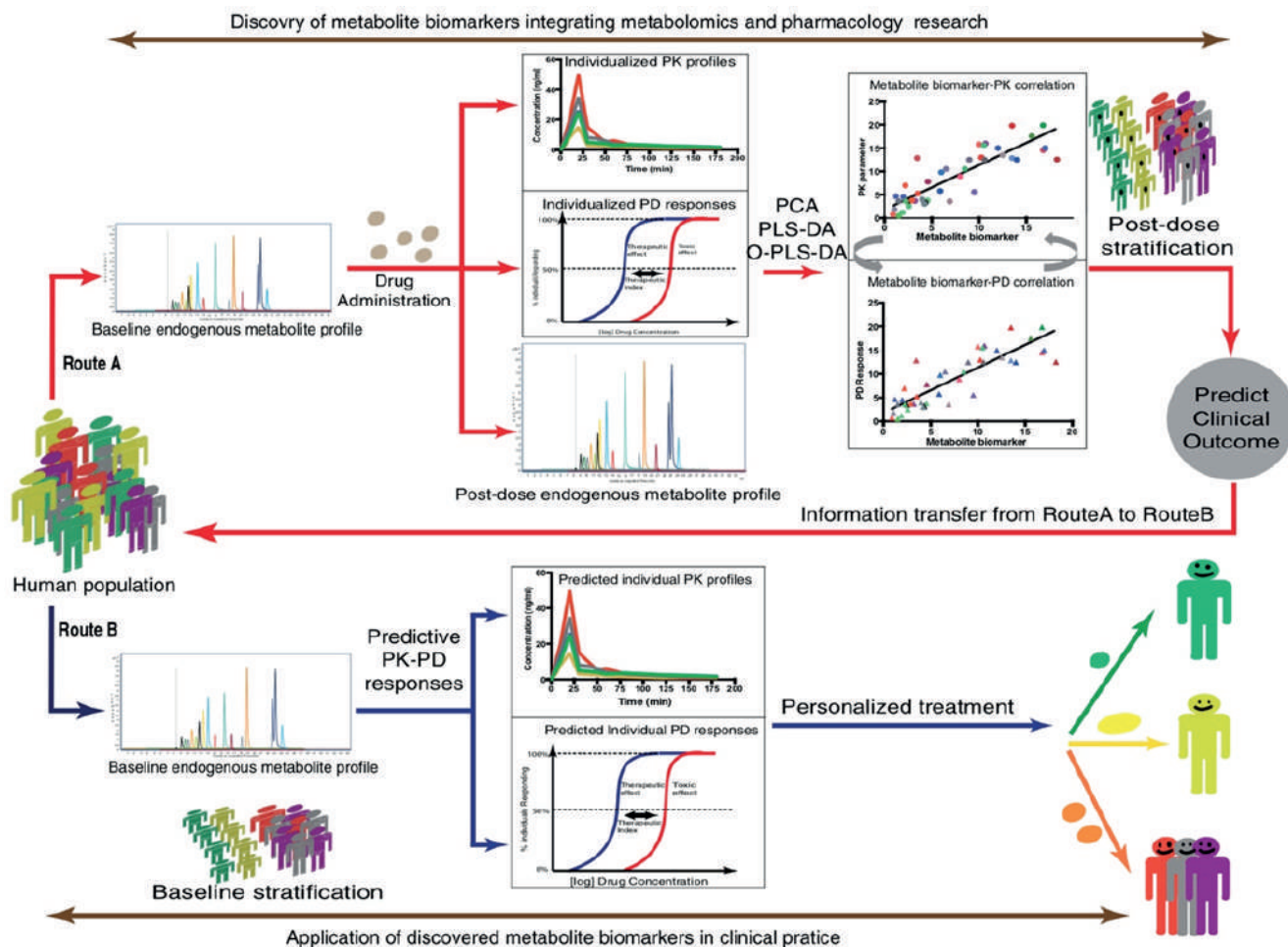


Figure 6. Pharmacometabolomics in research (route A) and clinical practice (route B).(206) (Adapted with permission from Springer).

can better define mechanisms of pharmacodynamic and pharmacokinetic variation between individual in response to drug therapy by measuring endogenous metabolites. (204–209) Figure 6 shows how pharmacometabolomics in research and in clinical practice works.

In humans and most complex systems, interdependent changes in the gene, protein and metabolite levels can occur on very different time scales. These become more complicated because an intestinal system has an internal ecosystem where the host interacting with many species of gut microbial organisms (210-212), and also the impact of environmental factors making the individual “metabotype” individual is highly responsive to time and the environment. (213) Metabolomic studies can be utilized to examine and understand this changes (214-218) and improve the therapies selection for particular patient classes, at once assess the drugs’ efficacy and toxic side effects.(184,218) it can be proposed from recent studies to create a patient metabolic phenotyping, together with the prognosis and pre-

treatment metabolic information to predict post-treatment outcomes and refine the strategy for personalized medicine (4,213).

Metabolomics give a better understanding about the metabolic signatures of diseases and then will provide predictive, prognostic, diagnostic, and surrogate markers of diverse disease states; expand our understanding on underlying molecular mechanisms of diseases; allow for stratification of patients based on metabolic pathways impacted; reveal biomarkers for drug response phenotypes, so we can predict the variation in a subject’s response to treatment (pharmacometabolomics) effectively; define a metabotype for each specific genotype, offering a functional readout for genetic variants: allow us to monitor response and recurrence of diseases; and describe the molecular landscape in human performance applications and extreme environments. Overall, this will change the way we treat diseases and find out the optimal therapeutic regimen precisely for each individual.(1)

Conclusion

Metabolome give important opportunities at present to revolutionize the strategy of precision medicine. Metabolomics is potential to be a key profile and phenotyping platform to predict patients' responses to different treatments. Equipped with other omics methodologies, metabolomic develop a decision support tool for selecting or recommending optimal treatment regimens and lifestyle changes. The goal is by using the personalized profiles to enhance the therapy effectivity and contribute to better patient outcomes. An integrated omics platform in the future will take account to complement the next-generation healthcare system, and there is a need to develop the methods for absolute quantification of metabolites, miniaturized metabolomics instruments, enhancing the automated data processing, to boost the translation of metabolomics either in new biomarkers discovery or in pharmacometabolomics.

Authors Contribution

AM planned, drafted and wrote the manuscript, NMD edited the manuscript and tables, while AW provided the main idea of the manuscript and proof read the final manuscript.

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