Emerging applications of metabolomics in drug discovery and precision medicine

David S. Wishart[1–3]

Abstract | Metabolomics is an emerging 'omics' science involving the comprehensive characterization of metabolites and metabolism in biological systems. Recent advances in metabolomics technologies are leading to a growing number of mainstream biomedical applications. In particular, metabolomics is increasingly being used to diagnose disease, understand disease mechanisms, identify novel drug targets, customize drug treatments and monitor therapeutic outcomes. This Review discusses some of the latest technological advances in metabolomics, focusing on the application of metabolomics towards uncovering the underlying causes of complex diseases (such as atherosclerosis, cancer and diabetes), the growing role of metabolomics in drug discovery and its potential effect on precision medicine.

Metabolic phenotyping
The characterization of a cell, organism or biological system using metabolomics or metabolic profiling. Metabolic phenotyping is a method of describing the phenotype using chemical or metabolite readouts as a proxy for an organism's observable biochemical traits.

[1]*Department of Biological Sciences, CW 405, Biological Sciences Building, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.*
[2]*Department of Computing Science, 2–21 Athabasca Hall University of Alberta, Edmonton, Alberta, Canada T6G 2E8.*
[3]*National Institute of Nanotechnology, National Research Council, Edmonton, Alberta, Canada T6G 2M9.*
dwishart@ualberta.ca

doi:10.1038/nrd.2016.32
Published online 11 Mar 2016

Metabolites represent both the downstream output of the genome and the upstream input from the environment. Therefore, the study of metabolites and metabolism — metabolomics — allows scientists to explore the nexus of gene–environment interactions. In contrast to genes and genetic risk scores that can be used to indicate what might happen, metabolic profiling and metabolic phenotyping indicate what is currently taking place. In this regard, metabolomics not only enables the identification of disease biomarkers in the form of endogenous metabolites (gene-derived metabolites) and exogenous metabolites (environmentally derived metabolites), it also provides unique insights into the fundamental causes of disease[1,2]. These new metabolic insights, along with the increasing ease with which metabolomic assays can now be performed, are leading to a paradigm shift in how drugs are being discovered, developed, delivered and dosed[3,4]. The growing accessibility of metabolomics is also leading to a phenomenon known as personalized metabolic phenotyping, which, in combination with personalized genomics, is widely expected to help advance the field of precision medicine[5]. The goal of precision medicine is to use advanced diagnostic testing to customize an individual's medical treatment according to their specific omic profiles. Given that metabolomics can be used in patient diagnosis, patient monitoring and patient omic profiling[2–5], there can be little doubt that metabolomics will play a key part in future precision medicine initiatives.

In this Review, I explore how recent developments in metabolomics are being used to identify more-informative disease biomarkers, to aid the design or development of improved treatments and to better assess health outcomes. On a population level, these developments are helping to advance drug discovery and development, and on an individual level these same developments are advancing the field of precision medicine. The key take-home message is that metabolomics, whether it is being used in the pharmaceutical industry or in the clinic, is becoming a vital tool for drug discovery and therapeutics.

Metabolomics techniques and technologies

Metabolomics is a field of omics science that uses cutting-edge analytical chemistry techniques and advanced computational methods to characterize complex biochemical mixtures. Metabolomics can trace its origins to clinical chemistry, nutritional chemistry and general metabolic biochemistry — all of which began in the early twentieth century[6,7]. Over the past 15 years, metabolomics researchers have also coined or co-opted many other names to describe their field, including metabonomics[8], targeted metabolomics[9], untargeted metabolomics[10], metabolic footprinting[11], metabolic fingerprinting[12], fluxomics[13], lipidomics[14], metallomics[15] and exposomics[16]. Regardless of its name, metabolomics is finding applications in many diverse areas, including human and animal health, biomarker discovery, drug discovery and development, plant biology, microbiology, food chemistry and environmental monitoring[2–16]. The diversity of applications of metabolomics arises from the fact that it can be used to analyse a wide range of substrates, including solids (such as tissues, soil and biological waste), liquids (such as biofluids, effluent and water)

and gases (such as breath, fumes or scents). Furthermore, metabolomics can be performed *in vivo* (using imaging or live cells) or *in vitro* (using extracts or biofluids).

Unlike genomics, transcriptomics or proteomics, in which a single instrument is often sufficient to perform the necessary measurements, metabolomics requires a broad array of instrumentation. This can include compound-specific instruments such as coulometric array detectors (for detecting redox active compounds), ultraviolet or fluorescence spectrometers (for detecting aromatic compounds), inductively coupled mass spectrometers (for detecting metals) and evaporative light-scattering detectors (for detecting lipids). However, over the past 15 years, three general purpose technologies have emerged as the primary workhorses in metabolomics: nuclear magnetic resonance (NMR) spectroscopy; gas chromatography mass spectrometry (GC-MS); and liquid chromatography MS (LC-MS). Each technique provides broad coverage of many classes of organic compounds, including lipids, amino acids, sugars, biogenic amines and organic acids. Many comprehensive reviews have been written regarding how each of these three technologies works and how each can be used in metabolomics[17–20]. Although these technologies each have their own advantages and disadvantages (TABLE 1), numerous studies have shown how they may be used to complement each other[17–22]. Indeed, the use of multiple technologies greatly broadens the level of metabolite coverage that can be achieved and the types of samples that can be studied[21–22].

Emerging metabolomics methods in biomedicine

Until recently, most metabolomic activities were limited to academic institutions where the primary focus is on research, discovery and prototype development. Under this type of environment, sample sizes are relatively small, protocols are diverse, student labour is cheap and, consequently, there is no compelling need for automation. Likewise, given the diversity of approaches and the relative lack of standards, metabolomics researchers, just as with proteomics and transcriptomics researchers, largely eschewed the idea of absolute quantification[23–25]. However, given the need for rapid and accurate absolute quantification in clinical settings, a recent emerging trend in metabolomics is an increased emphasis on quantification and automation. Automation and quantification not only increase throughput, but they also increase reliability and reproducibility across laboratories and among countries[26]. Additionally, they are essential for eventual clinical test adoption and regulatory approval. Indeed, this move towards automation and clinical adoption is now well underway with LC-MS-based metabolomics[26–28].

Several exciting developments in automated, quantitative, NMR-based metabolomics have also recently been described. These include both commercial products, such as Bruker's food screeners for wine and fruit juice, as well as notable efforts by several academic labs[29,30]. These new metabolomic techniques make use of advances in NMR automation along with novel machine learning techniques to perform automated compound identification and quantification. As a result, comprehensive NMR analyses can now be completed in less than a few minutes per sample[30]. These developments could lead to a substantial rejuvenation in the use of NMR in metabolomics studies. Similar advancements have occurred for GC-MS-based metabolomics using comparable techniques and concepts[31,32]. LC-MS is also becoming increasingly automated and far more quantitative through the introduction of commercial kits (produced by Biocrates Life Sciences) and 'black-box' systems (such as AB Sciex's Lipidyzer). Both the LC-MS and GC-MS systems typically require isotopically labelled or derivatized reference standards, automated liquid-handling systems, strict standard operating protocols and sophisticated software.

This shift towards kits, black-box systems, automation and greater standardization, especially in MS-based metabolomics, will certainly reduce costs, increase throughput, ensure greater reproducibility and substantially cut down on sample-handling errors, and it is likely to encourage a greater focus on absolute quantification. However, the associated simplification of metabolomics may discourage methodological innovation, lead to excessive industry and/or commercial reliance, reduce experimental flexibility, instil a limited view of the metabolome and encourage inexperienced researchers to enter the field. Nevertheless, given the inexorable trends towards automation, miniaturization and kit development seen in so many other scientific fields, it is likely that a similar trend will be introduced in metabolomics in the coming years.

Another area in which exciting technological advances in metabolomics are occurring is the field of metabolite imaging. Metabolite imaging involves the *in vivo* or *in vitro* detection and visualization of metabolites in tissues using NMR, magnetic resonance spectroscopy (MRS), positron emission tomography (PET), matrix-assisted laser desorption/ionization (MALDI)-MS, secondary ion MS (SIMS) or desorption electrospray ionization MS (DESI-MS) techniques[33,34]. MRS and PET are particularly appealing because they permit non-invasive metabolic imaging for medical diagnosis and metabolic phenotyping. Improvements in NMR pulse sequences, magnetic field strength, chemical labelling protocols and computer processing speed are now permitting a wider number of compounds at lower concentrations to be detected through MRS. In some cases, up to 20 different metabolites can be identified and partially quantified in certain tissues[35]. These improvements are leading to far more precise diagnoses and customized therapies. Recent advances in isotope preparation are opening the door to PET, not only of ^{18}F glucose but also ^{11}C acetate, methionine, choline and glutamine metabolism[36,37]. These advances are resulting in more-accurate tumour phenotyping and enabling greater customization of cancer therapies. Not to be outdone, the field of MS imaging is now entering into a real renaissance. The use of DESI-MS, in conjunction with electro-incision techniques (that is, the iKnife) is now permitting real-time tissue-typing during surgery[38]. In particular, the gases generated from vaporized or ionized tissue during electro-incision are vacuumed up

Endogenous metabolites
Metabolites that are biosynthesized or potentially biosynthesized by the host organism and/or its endogenous microflora. Endogenous metabolites also include xenobiotics that have been metabolically transformed by the host.

Exogenous metabolites
Xenobiotic metabolites or chemicals that the host (and/or its endogenous microflora) is not capable of biosynthesizing or that have not yet been metabolically transformed.

Exposomics
A branch of omics science that involves the study of the complete collection of environmental exposures (chemicals, foods, pollutants and pathogens) that a human is exposed to from conception onwards, which is referred to as the exposome.

Coulometric array detectors
Multi-array electrochemical detection systems for detecting redox-active compounds as they elute from a high performance liquid chromatography (HPLC) column. Chemicals or metabolites react with specific electrodes in the detector depending on their redox potential.

Inductively coupled plasma mass spectrometers
Mass spectrometers that are specifically designed to detect and quantify metals at very low concentrations. Metal ions are ionized by inductive heating to create an electrically conductive plasma that is then sent to a conventional mass spectrometer for detection.

Evaporative light-scattering detectors
(ELSDs). Instruments that detect compounds eluting from a high-performance liquid chromatography (HPLC) system on the basis of light scattering rather than ultraviolet absorption or fluorescence. ELSDs permit the detection of far more compounds than other optical techniques.

Table 1 | **A comparison of different metabolomics technologies**

Technology	Advantages	Disadvantages	Refs
NMR spectroscopy	• Quantitative • Non-destructive • Fast (2–3 min per sample) • Requires no derivatization • Requires no separation • Detects most organic classes • Allows identification of novel chemicals • Most spectral features are identifiable • Robust, mature technology • Can be used for metabolite imaging (fMRI or MRS) • Can be fully automated • Compatible with liquids and solids • Long instrument lifetime (over 20 years)	• Not sensitive (LOD = 5 μM) • High start-up cost (>US$1 million) • Large instrument footprint • Cannot detect or identify salts and inorganic ions • Cannot detect non-protonated compounds • Requires larger sample volumes (0.1–0.5 mL)	17,18,35
GC-MS	• Robust, mature technology • Modest start-up cost (~$150,000) • Quantitative (with calibration) • Modest sample volume (0.1–0.2 mL) • Good sensitivity (LOD = 0.5 μM) • Large body of software and databases for metabolite identification • Detects most organic and some inorganic molecules • Excellent separation reproducibility • Many spectral features are identifiable • Can be mostly automated • Compatible with gases and liquids	• Destructive (sample not recoverable) • Requires sample derivatization • Requires separation • Slow (20–40 min per sample) • Cannot be used in imaging • Not compatible with solids • Novel compound identification is difficult	18–20
LC-MS	• Superb sensitivity (LOD = 0.5 nM) • Very flexible technology • Detects most organic and some inorganic molecules • Small sample volumes (10–100 μL) • Can be used in metabolite imaging (MALDI or DESI) • Can be done without separation (direct injection) • Has the potential to detect the largest portion of metabolome • Can be mostly automated • Compatible with solids and liquids	• Destructive (sample not recoverable) • Not very quantitative • Higher start-up cost (>$300,000) • Slow (15–40 min per sample) • Usually requires separation • Poor separation resolution and lower reproducibility versus GC-MS • Less-robust instrumentation than NMR or GC-MS • Not compatible with gases • Most spectral features are not yet identifiable • Novel compound identification is difficult • Short instrument lifetime (<9 years)	19,20,33,38

DESI, desorption electrospray ionization; fMRI, functional MRI; GC-MS, gas chromatography mass spectrometry; LOD, limit of detection; LC-MS, liquid chromatography mass spectrometry; MRS, magnetic resonance spectroscopy; MALDI, matrix-assisted laser desorption/ionization; NMR, nuclear magnetic resonance.

Secondary ion MS
(SIMS). A mass spectrometry (MS) technique that can be used to analyse and image the composition of thin films. Ions (that is, secondary ions) are generated by sputtering the surface of the sample with an intense ion beam.

Desorption electrospray ionization MS
(DESI-MS). A mass spectrometry technique (MS) that uses atmospheric pressure ion sources to ionize samples in open air under ambient conditions. It is a combination of both electrospray and desorption ionization techniques wherein ionization occurs by spraying an electrically charged mist onto the sample surface.

by the iKnife, and the mass spectrum of the ionized gas is acquired. This DESI-MS spectrum is compared in real time, using sophisticated pattern recognition software, against a database of similarly acquired DESI-MS spectra of diseased and healthy tissues. Using a colour-coding scheme (with red for diseased tissue and green for healthy tissue) automatically generated by the iKnife software and a video monitor, surgeons can precisely characterize the tissues they have just cut as well as determine where and what else needs to be cut. In many surgeries, the visual distinction of healthy and diseased tissue is often difficult and requires time-consuming biopsies and immunostaining procedures to be performed by histologists during surgery. By eliminating the need for external tissue typing, the iKnife could open the door to true real-time 'precision surgery'.

Continuing developments in MALDI-based imaging, such as matrix-free approaches, improved software, faster scan rates and smaller spot sizes (<1 μm), are leading to more-detailed images with much greater metabolite coverage[39,40]. It seems likely that MS imaging could revolutionize the field of histology the same way that immuno-fluorescence staining fundamentally changed the field in the 1990s. These technological developments in metabolite imaging and metabolomic automation, along with other emerging techniques in metabolomics, are having a marked effect on several areas of biomedicine. Not only are these advances making metabolomics more accessible and more powerful, they are also changing our understanding of disease, our approach to how drugs are discovered, and our view on how healthcare should be delivered.

New insights into disease processes

For most of the past 40 years the working assumption has been that many chronic or serious illnesses are genetic in origin. Very large, population-wide efforts aimed at whole genome sequencing, transcript profiling and single nucleotide polymorphism (SNP) characterization have been conducted to search for disease genes or disease SNPs[41,42]. Although tremendous and fundamentally important insights into biology have been gained, the expected genetic 'gold mine' has yielded far fewer disease genes, mutations or SNPs than originally expected[43,44].

Indeed, this dearth of targetable disease genes is one of the reasons why many drug-discovery pipelines have been drying up over the past decade[45,46]. More-recent epidemiological surveys into the environmental causes of death and disease[47,48], along with revelations about the importance of the microbiome[49] and the epigenome[50], are revealing the effect of the environment on disease development. Metabolomics is helping to lead this paradigm shift[1,2]. Not only has it helped us to understand cellular metabolism (and its major external influences) to a degree that was not previously obtainable[47,51], metabolomics has also helped to reveal the importance of the microbiome (and its major internal influences) on human health and disease[52,53]. Indeed, with its unique ability to probe complex biochemistry at both a cellular and an organismal level, combined with its environmental or 'small-molecule centric' view (that is, its ability to measure small molecules from both the body and the environment), metabolomics has already helped to identify a number of unexpected chemical causes for several important chronic and complex diseases, including atherosclerosis, cancer and diabetes[1,47]. Such studies are revealing that metabolites (both exogenous and endogenous) have a far more central role in disease development, cellular signalling and physiological control than previously thought.

Case study 1 — trimethylamine* N*-oxide and atherosclerosis. Atherosclerosis is normally attributed to 'bad genes' and high levels of cholesterol[54]. However, in a series of articles beginning in 2011, Stanley Hazen and colleagues used metabolomics to identify an unexpected but compelling connection between diet, the microbiome and a plaque-inducing atherotoxin called trimethylamine *N*-oxide (TMAO)[55–58] (FIG. 1a). Using an untargeted metabolomics approach, Hazen *et al.* identified elevated plasma levels of TMAO in rats that had developed atherosclerotic plaques. By performing targeted metabolomic studies, they determined that TMAO is a liver by-product of trimethylamine (TMA). TMA, in turn, is a microbial breakdown product of carnitine, betaine and choline, which are largely derived from meat and phospholipids in the diet[55–57]. Rats injected with TMAO showed a rapid build-up of arterial plaques, which clarified the role of TMAO as an atherotoxin. Subsequent studies in humans showed a strong correlation between high plasma TMAO levels and subsequent adverse myocardial events[57]. Further studies in mice also identified certain strains of gut microorganisms that produce high levels of TMA and other strains that produce low levels of TMA[58]. More-recent studies have implicated TMAO as a molecule that disrupts cholesterol balance through modifying the activity of flavin monooxygenase 3 (also known as dimethylaniline monooxygenase [*N*-oxide-forming] 3)[59]. Although the TMAO story is still evolving, it seems that endogenously produced TMAO, in sufficiently high levels, is probably a disease-causing metabolite.

Case study 2 — cancer and the discovery of oncometabolites. Cancer is widely known as a genetic disease arising from mutations in key oncogenes or tumour suppressors. However, over the past 5 years a substantial paradigm shift has occurred in our understanding of this disease. Indeed, cancer is now increasingly viewed as a metabolic disorder[60]. This has arisen, in part, through the 'rediscovery' of two metabolic processes, aerobic glycolysis and glutaminolysis, that are found in essentially all tumours and are also closely linked to many known oncogenes and tumour suppressors[61]. The discovery, through metabolomics, of 'oncometabolites' has also had a substantial effect on our understanding of cancer. Oncometabolites are endogenous metabolites and their accumulation initiates or sustains tumour growth and metastasis. The first oncometabolite to be discovered was 2-hydroxyglutarate, a natural metabolite that is found in high concentrations in gliomas[62]. This compound seems to indirectly alter histone methylation patterns, which ultimately leads to tumorigenesis. Since the discovery of 2-hydroxyglutarate, many other oncometabolites have been identified or subsequently 'reclassified' (REFS 63,64) (TABLE 2). As the causal links are being further explored, many of these oncometabolites seem to act as signalling molecules or allosteric regulators that control important cell division processes[65,66].

Case study 3 — amino acids and diabetes. Type 2 diabetes is a well-known metabolic disorder, but its underlying molecular causes remain poorly understood. A sugar-rich diet and physical inactivity can contribute to disease development, but physically fit individuals may also develop diabetes[67] suggesting that genetics may play a part. However, over the past 4 years, several groups using both targeted and untargeted metabolomic techniques have identified an unexpected causal agent — amino acids. In particular, high serum levels of branched-chain amino acids (Ile, Leu and Val), aromatic amino acids (Phe and Tyr) and a little-known amino acid (aminoadipic acid) can be used to identify individuals at risk of developing type 2 diabetes[68–72]. Levels of these markers are relevant up to 15 years before disease onset and have been found to be substantially more predictive than genome-wide association studies (GWAS) or other genetic data[68]. High levels of these amino acids can come from the diet, but the gut microbiome also seems to affect the abundance of amino acids, especially essential amino acids such as Ile, Leu, Val and Phe[72]. It is not widely appreciated that branched-chain amino acids are insulin analogues. These amino acids specifically act on the mammalian target of rapamycin (mTOR) receptor and upregulate the same pathways and physiological processes as insulin[68,73] (FIG. 1b). If chronically high levels of an insulin analogue are present this could eventually lead to insulin resistance and diabetes.

Although only three examples have been highlighted here, the list of diseases for which their aetiology is being 'rewritten' through metabolomics is growing rapidly. Many of these diseases are now catalogued in the Human Metabolome Database[74]. Among the most interesting examples are those diseases with previously unknown or unexplained causes, including: autism and schizophrenia[75], mild cognitive impairment or pre-Alzheimer disease[76], as well as asthma and inflammatory bowel disease[77]. Some of these metabolic links are still at

Microbiome
The collection of microorganisms that reside in or on a larger organism, a larger organ or within a specific environmental niche.

Epigenome
The collection of chemical compounds that act on DNA as well as the collection of chemical modifications to DNA (and histones) that direct and/or alter the original instructions in the genome.

Atherotoxin
An agent (specifically, a chemical, protein or pathogen) that damages arteries leading to atherosclerosis or cardiovascular disease.

Glutaminolysis
A metabolic process involving the catabolism of glutamine to generate energy as well as nitrogen and carbon byproducts. It is an important energy pathway for tumour cells.

Mammalian target of rapamycin
(mTOR). A serine/threonine kinase that acts as a master controller of cell metabolism, cell growth, cell proliferation, cell survival and protein synthesis.

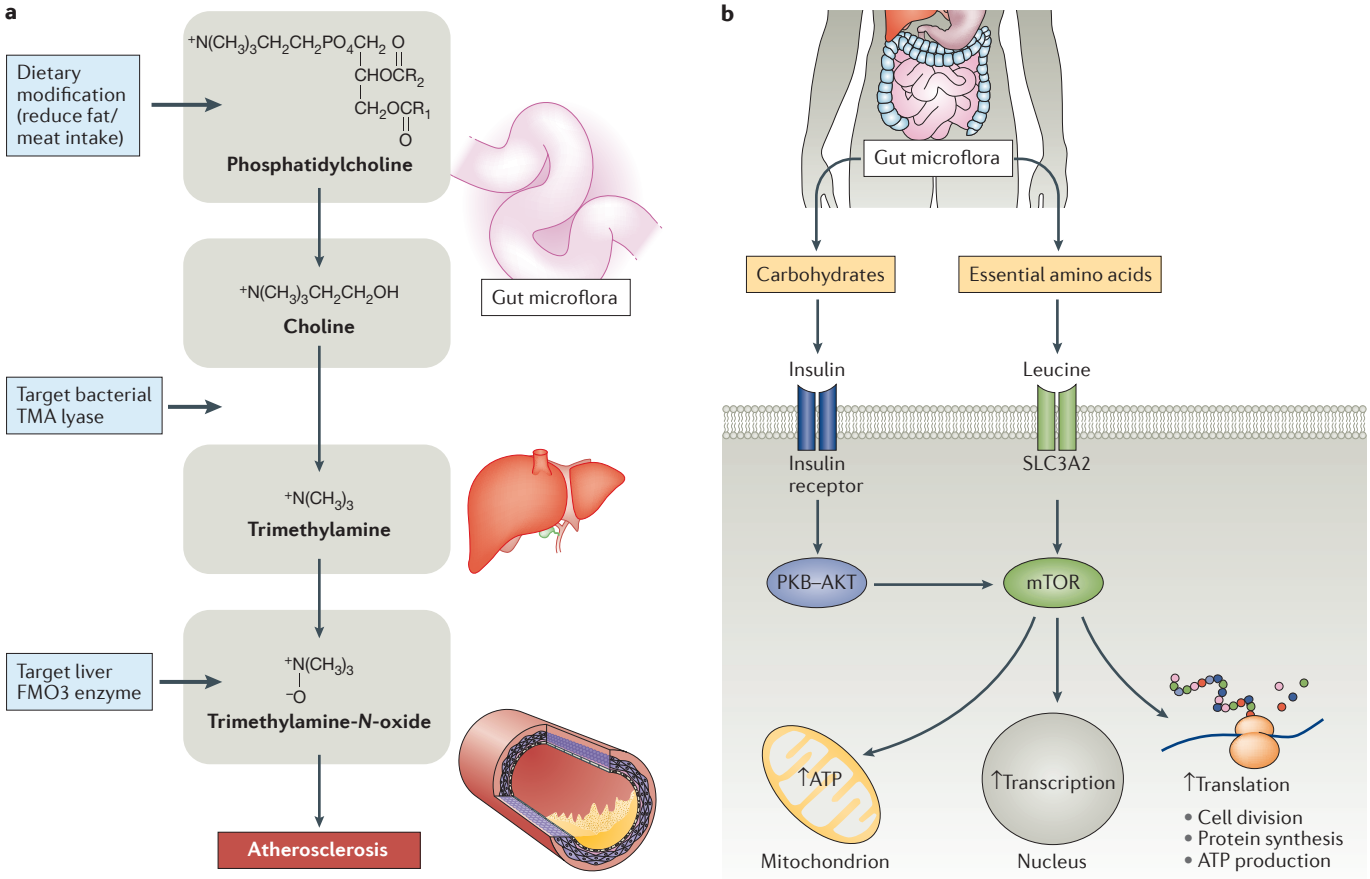

Figure 1 | **Metabolites play a central part in disease development. a** | An illustration of how the consumption of choline (through fatty foods) can lead to the production of trimethylamine *N*-oxide (TMAO). Also indicated are the routes for potential therapeutic development (blue boxes), including diet modification, targeting specific microbial enzymes and targeting specific liver enzymes. **b** | An illustration of how carbohydrate signalling (from the diet) and amino acid signalling (from the diet and gut microbial metabolism) can lead to similar cellular consequences. Both signalling pathways lead to activation of mammalian target of rapamycin (mTOR), which is a central regulator of cellular metabolism. In particular, mTOR activates protein synthesis through phosphorylation of the eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1; not shown), enhances ATP synthesis in the mitochondria and promotes cell growth and proliferation. Chronic exposure to high levels of carbohydrates and/or high levels of essential amino acids eventually overwhelm the insulin-signalling process, leading to insulin resistance, which is a hallmark of type 2 diabetes. AMPK, 5′-AMP-activated protein kinase; FMO3, flavin monooxygenase 3; PKB, protein kinase B; SLC3A2, solute carrier family 3 member 2.

the associative stage, whereas others are demonstrating clear cause and effect. In almost all cases, endogenously produced metabolites seem to function either as direct toxins or as signalling molecules that cause a cascade of adverse consequences.

Metabolomics in drug research and development

For the past 40 years, the standard paradigm for drug discovery and development has been comprised of the identification of disease-causing genes through pedigree analysis, GWAS or whole-genome sequencing, cloning of identified genes, purification of target proteins and high-throughput screening to identify potential drug leads, which are then optimized and tested in animal models and eventually human trials. Unfortunately, this paradigm is not working very well. Current drug-development programmes are lengthy and costly[78]. Only a small percentage of the drugs that enter development programmes are making it past Phase I trials[79], with some of these eventually failing in Phase III trials[80]. Issues associated with the existing model are that relatively few diseases have a strong genetic basis (perhaps less than 10%), many diseases are a result of exposures (that is, the exposome) and not all disease-causing genes are amenable to high-throughput screens or targetable with drugs[43,44,46,48,78].

Metabolomics may offer a far more cost-effective and productive route to drug discovery, testing and development. First, many of today's most prominent diseases (such as heart disease, diabetes, obesity, hypertension, depression and inflammatory bowel disease) have a strong metabolic basis or a clear metabolic cause[47,48]. Second, as also highlighted above, many chronic diseases (including autism, schizophrenia, asthma, cancer and Alzheimer disease) are being found to have unexpected or unappreciated metabolic causes of associations[74–77,81–84]. For instance, the importance of dysregulated metabolism

Table 2 | **Oncometabolites and their roles in cancer**

Oncometabolite*	Mechanism or role	Refs
2-hydroxyglutarate	• Inhibits ATP synthase and mTOR signalling • Inhibits 2-oxoglutarate-dependent oxygenases, which activate oncogenic hypoxia-induced factor pathways and alter DNA methylation patterns • Produced by gain-of-function mutations in the gene encoding isocitrate dehydrogenase • Elevated in gliomas and acute myeloid leukemia	63,144
Fumarate	• Inhibits 2-oxoglutarate-dependent oxygenases, which activate oncogenic hypoxia induced factor pathways and alter DNA methylation patterns • Leads to protein succination and disrupted metabolism • Produced by loss-of-function mutations in the gene encoding fumarate hydratase • Elevated in renal carcinoma	144,145
Succinate	• Inhibits 2-oxoglutarate-dependent oxygenases which activate oncogenic hypoxia induced factor pathways and alter DNA methylation • Produced by loss-of-function mutations in the genes encoding succinate dehydrogenase • Elevated in paraganglioma and renal and thyroid tumours	144,146
Sarcosine	• Activates mTOR signalling pathway • Elevated by mutant glycine *N*-methyl transferase • Elevated in metastatic prostate cancer	147,148
Glucose	• Essential source of carbon to support cancer cell anabolism, TCA anaplerosis and aerobic glycolysis • Activates hexokinase II • Activates glucose-regulated proteins that alter signalling, proliferation, invasion and apoptosis • Elevated in most cancers	149,150
Glutamine	• Essential source of nitrogen to support cancer cell anabolism and aerobic glycolysis • Essential source of carbon for TCA anaplerosis • Elevated in MYC-dependent cancers	151,152
Asparagine	• Essential source of nitrogen to support cancer cell anabolism and aerobic glycolysis • Anti-apoptotic agent • Elevated in acute lymphoblastic leukemia	153
Choline	• Serves as a methyl donor for DNA methylation which disrupts DNA repair and gene expression • Modifies lipid signalling • Essential source of carbon and nitrogen to support phospholipid synthesis in rapidly dividing cells • Elevated in breast, brain and prostate cancer	154
Lactate	• Lowers extracellular pH and induces metastasis • Induces local immunosuppression • Elevated in most cancers	155,156

mTOR, mammalian target of rapamycin; TCA, tricarboxylic acid. *All of the listed metabolites are required for tumour survival or tumour propagation. All are locally elevated in tumours but not in surrounding tissues. Most alter important cell signalling and cell division pathways.

in cancer was never mentioned when Hanahan and Weinberg first published their landmark paper on the hallmarks of cancer in 2000 (REF. 81). However, the weight of metabolic evidence gathered through numerous studies[61–64], as well as growing appreciation of the Warburg effect in tumorigenesis[82], led them to include dysregulated metabolism as one of the hallmarks of cancer in their updated version of the paper[83]. More recently, the discovery of disturbed glucose and lipid metabolism in patients with Alzheimer disease[84] and pre-Alzheimer disease[76] has led to a number of new theories on the development and aetiology of this disease. Indeed, these findings, along with the striking similarity of experimental brain diabetes to Alzheimer disease, have led many to consider renaming Alzheimer disease 'type 3 diabetes' (REF. 85). The discovery of novel metabolic connections to diseases is also leading to the identification of novel drug targets. For example, the identification of TMA as a gut microbial by-product of choline and carnitine metabolism[55,56], combined with the knowledge that TMA is the precursor to TMAO, led to the identification of two novel protein targets — flavin monooxygenase 3 (in the liver) and bacterial choline TMA-lyase — that could be used to reduce TMAO levels and thereby potentially treat or prevent atherosclerosis[86].

Generally, if a metabolite or a set of metabolites is identified as being causal, it usually means that the drug target is an enzyme and its biosynthetic pathway is known. For instance, with regard to TMAO and atherosclerosis, both flavin monooxygenase 3 and choline TMA-lyase are enzymes. Likewise, the pathway for TMA generation and its conversion to TMAO by these two enzymes is now well understood[87,88]. This makes drug discovery, synthesis and testing both simple and cost effective[78]. Indeed, within a few months of the TMAO discovery, Hazen *et al.* were able to identify a potent inhibitor of choline TMA-lyase called 3,3-dimethylbutanol (a natural product found in olive oil)[55]. Therefore, atherosclerosis provides an example of how metabolomics-based drug discovery and development may be applied to the identification and testing of novel therapies (FIG. 2).

Many of the most-effective and best-selling drugs are enzyme inhibitors, and thousands of metabolite-inspired enzyme inhibitors or antimetabolites are already known[89]. Often, the discovery of a metabolic basis to a disease leads to a simple therapeutic solution, in that if the level of a metabolite is too high or too low its intake may be reduced or increased, respectively. Medical foods and dietary supplements have served as an effective solution to treat many inborn errors of metabolism, dietary deficiency diseases (such as rickets, scurvy and goitre) and other medical conditions such as coeliac disease (through gluten-free diets) and epilepsy (through ketogenic diets)[90–92].

Metabolomics can also play a part in reducing drug failures that are due to toxicity in early-phase trials or drug withdrawals in late-phase trials. Indeed, the earliest applications of metabolomics in the pharmaceutical industry were in animal toxicology screens and ADMET studies (absorption, distribution, metabolism, excretion and toxicity studies)[93,94]. Perhaps the best-known example of these early applications is COMET (the Consortium for Metabonomic Toxicity), which brought together five major pharmaceutical companies along with academics from Imperial College London to use metabolomics as a means of creating an expert system for predicting kidney and liver toxicity of drug-like compounds in rodents[95]. Over a period of 5 years and nearly 150 studies, COMET clearly demonstrated that it was possible to use metabolomics to provide fast, inexpensive, non-invasive approaches to assessing and even predicting liver

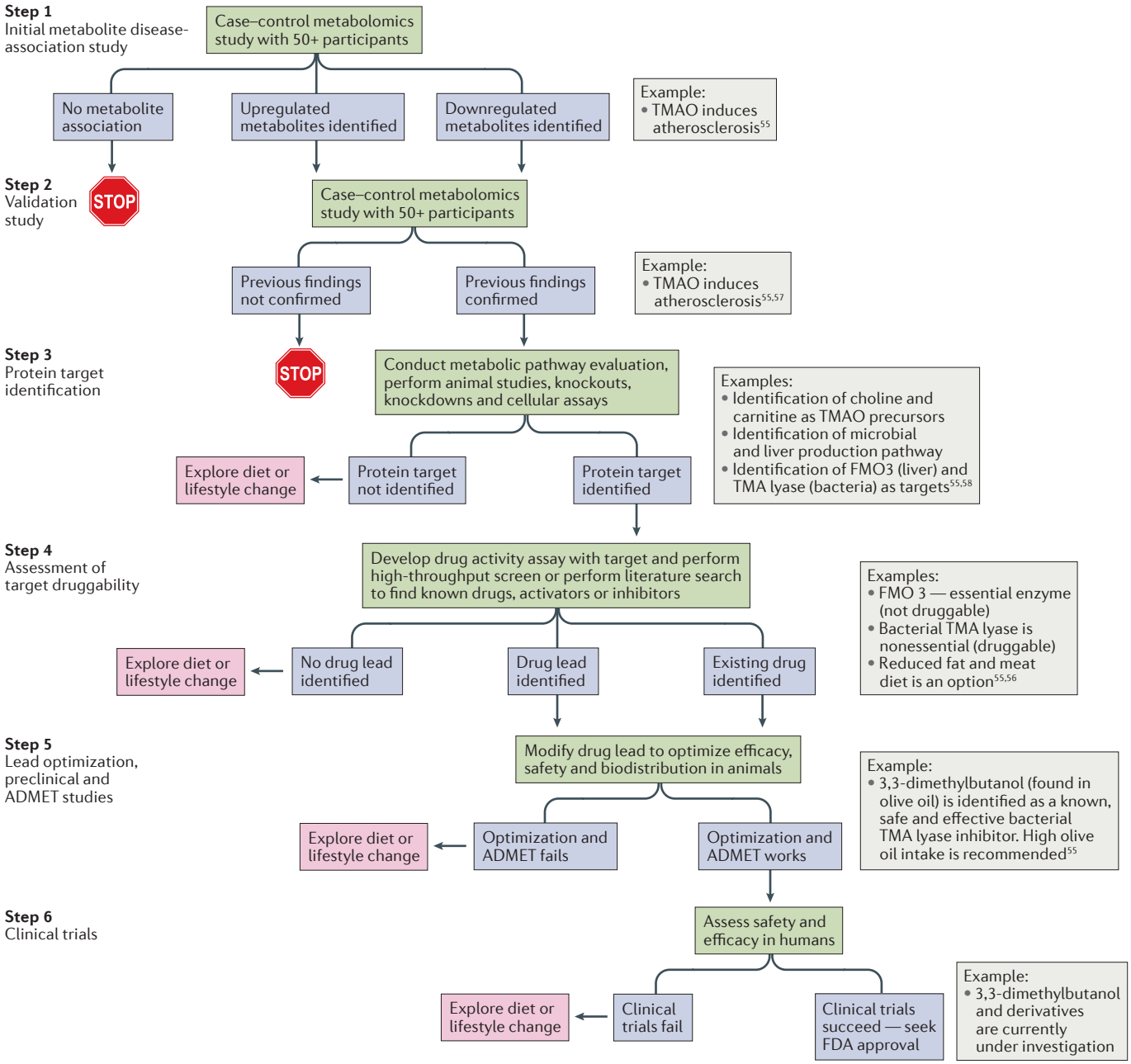

Figure 2 | **A decision tree for metabolite-based drug discovery and development using atherosclerosis as an example.** Drug development steps or processes are marked in green, outcomes in blue and alternative actions in red. ADMET, absorption, distribution, metabolism, excretion and toxicity; FDA, US Food and Drug Administration; FMO3, flavin monooxygenase 3; TMA, trimethylamine; TMAO, trimethylamine *N*-oxide.

and kidney toxicity of lead compounds. Unfortunately, despite the clear benefits and potential cost savings, these approaches were not widely adopted by the pharmaceutical industry. This reluctance may be due to the fact that the NMR-based chemometrics methods originally used were not sufficiently sensitive, quantitative or platform-independent to permit routine or widespread implementation[94]. With the recent shift in metabolomics towards cheaper, more-automated, more-sensitive and/or more-quantitative methods, it may be worthwhile for the pharmaceutical industry to revisit this approach.

In addition to animal toxicity studies, metabolomics can also be used in clinical trials or other settings to: measure drug metabolites and metabolism[96,97]; monitor patient compliance or adherence (detecting drug levels or drug intake)[98]; assess non-compliance (detection of alcohol or illicit drugs)[99,100]; track dietary compliance[101]; measure therapeutic response (through drug- or disease-associated biomarkers)[102]; monitor adverse responses (through toxicity biomarkers)[103]; determine optimal drug doses (through drug-level measurements)[104,105]; stratify patients (for treatment selection)[106];

Chemometrics
A field of information science that extracts useful information from chemical data using statistical or data-driven techniques.

identify optimal responders (for patient selection)[107,108]; and distinguish fast-metabolizing drugs from slow-metabolizing drugs [3,96]. An indication of the magnitude of the problem of non-compliance or non-adherence can be gleaned from a recent metabolomic study that used LC-tandem MS (LC-MS/MS) to screen for over 40 commonly prescribed antihypertensive drugs or drug metabolites in spot urines from 200 patients with hypertension[98]. Astonishingly, it was found that 25% of the patients were non-compliant, and patient blood pressure levels directly correlated with the degree of medication non-adherence. Given the considerable cost associated with late-stage drug failures or drug withdrawals[79,80], and the potential for metabolomics to detect 'outlier' or non-complaint patients (who can contribute to these failures), it is surprising that metabolomics is not used more routinely in clinical trial monitoring. Indeed, metabolomics can be (and has been) used in nearly every phase of the drug development process — from target discovery to ADMET testing, patient trials, efficacy monitoring and dose optimization[3].

Metabolomics and precision medicine

The goal of precision or personalized medicine is to use advanced omics testing to customize an individual's medical treatment according to their specific biomarker profiles. Perhaps the most successful example of precision medicine to date has been the application of metabolomic methods towards newborn screening[26–28]. Not only does MS-based newborn screening help in diagnosing or even predicting disease, but the same techniques can also be used to determine the optimal therapy (for example enzyme replacement or diet restriction) and monitor or customize the therapeutic dose. Although newborn screening technically began well before the dawn of the metabolomics era, the concepts and instrumentation it uses (that is, the characterization of dozens of molecules simultaneously using LC-MS or GC-MS methods) are essentially identical to targeted metabolomics[28]. One of the main reasons for the success and widespread adoption of high-throughput MS-based screening has been the very low sample costs. Substantial cost reductions in gene sequencing, SNP profiling and even proteomics have opened the door to the integration of these omics technologies into precision or personalized medicine. As a result, a number of personalized multi-omics initiatives have begun to emerge. Of particular interest are the efforts led by Mike Snyder on integrated personal omics profiling (iPOP)[109] and those of Leroy Hood on predictive, preventive, personalized and participatory medicine (P4 medicine)[110]. Both initiatives are longitudinal studies in which multi-omics data (including genomic, transcriptomic, proteomic, metagenomic and metabolomic data) from hundreds to thousands of volunteers is regularly collected over a period of years. The data are used to assess each individual's health status, identify illnesses and guide medical treatment[109,110]. The long-term goal of these projects is primarily to help improve our understanding of disease, and the near-term goals are to assess the feasibility and utility of personalized and/or precision medicine. Both initiatives were initially genocentric; however, they have since expanded to include broader phenotypic or environmental measures such as metabolomics (metabolic phenotyping), exposomics and microbiomics[109–111]. Interestingly, some of the most compelling findings and most actionable results have arisen from metabolomics or clinical chemistry assays. These include the identification and treatment of pre-diabetes (using glucose levels), haemochromatosis (using iron levels) and dietary deficiencies (using vitamins and other micronutrients) in several individuals[109,111].

The utility of metabolomics in these precision-medicine initiatives is not surprising. As noted above, genes and genetic risk scores can be used to indicate what might happen in terms of biochemical or cellular functions, whereas metabolic profiling and metabolic phenotyping indicate what is happening at a biochemical level. With more than 190 clinically approved metabolite biomarkers, metabolomics (through clinical chemistry) actually offers as many biomarker options as all other omics techniques combined[112]. The success of metabolomics in biomarker translation, at least relative to other omics techniques, seems to lie in the fact that the underlying instrumentation (the tandem mass spectrometer) is robust, highly quantitative, easily adapted to new assays and already located in many clinical testing laboratories[112,113]. Furthermore, many clinical chemistry laboratories have the appropriately qualified individuals, the necessary regulatory approvals and sufficient knowledge to perform proper chemical biomarker validation and translation. This pre-existing 'chemical biomarker infrastructure' and a favourable cost–benefit ratio has helped metabolomics to overcome many of the traditional challenges facing proteomics[114] and transcriptomics[115]. With the continued developments in metabolomic automation and quantification[29–32], along with software and protocol improvements to biomarker selection and validation[112], it is likely that the intrinsic advantages of metabolomics will continue to be recognized, with many more metabolite disease biomarkers receiving clinical approval in the near future.

Another area in which metabolomics is affecting precision medicine is drug response and monitoring. Drug responses are often highly variable and are greatly affected by an individual's capacity to metabolize or utilize the drug. These responses are sometimes determined by variations in certain cytochrome P450 (CYP) genes or drug target genes[116]. Although the field of pharmacogenomics, with its focus on genotyping, is now well established, the newly emerging field of pharmacometabolomics is showing how metabolomics can be used to complement this genomic information[117]. Because drug metabolism and utilization involves many different enzymes, multiple organs, several compartments and even the microbiome, it is not always possible to screen for all possible genetic or tissue variants. Furthermore, because drug metabolism varies with ethnicity, age, gender, weight, height and diet — as well as other physiological variables — it can be particularly challenging to predict how an individual will respond to a drug based on their genotype alone[117,118].

Microbiomics
A branch of omics science that involves the study of the microbiome.

Pharmacometabolomics
A branch of metabolomics that involves the metabolomic analysis of both pharmaceutical compounds and endogenous metabolites after the administration of a pharmaceutical compound.

However, because metabolomics allows one to measure the sum of all these genotypic, environmental and physiological effects, it can be used to directly monitor drug responses and customize drug dosing[117].

Precision medicine and pharmacometabolomics. Pharmacometabolomics is already yielding some exciting and medically useful results with regard to drug dosing and response measurement. For instance, in the field of organ transplantation, rapid MS-based monitoring of immunosuppressant drugs and their metabolites is now being used to individually optimize dosing for patients[119]. More specifically, in the field of kidney transplantation, quantitative LC-MS-based monitoring of the immunosuppressants cyclosporine A, mycophenolic acid, sirolimus, everolimus and tacrolimus is already being used in the clinic to modify or optimize patient dosing on a near-daily basis[119–122]. The speed, sensitivity and robustness of MS-based assays are also proving to be somewhat better than traditional immunoassays[119,123]. Close monitoring of immunosuppressive drugs in people with kidney transplants is needed because of their narrow therapeutic window and their potentially severe adverse consequences such as leukopenia, thrombosis, anaemia and diabetes-like symptoms[119]. Similar approaches are also emerging for therapeutic drug monitoring for people with Alzheimer disease, in whom dosage monitoring and drug efficacy are often difficult to assess[124].

In generic drug-dosage determination, metabolomic methods can be used to measure metabolites from the 'Pittsburgh cocktail' (a collection of harmless drugs or drug proxies that have specific CYP targets), thereby allowing one to phenotype an individual's probable drug response before drug dosing[125]. Specifically, it has recently been shown that one simple, dilute mixture consisting of caffeine, chlorzoxazone, dapsone, debrisoquine, flurbiprofen and mephenytoin, along with the subsequent monitoring of these compounds and their metabolites in human urine (using LC-MS/MS), allows the simultaneous activity assessment of six common drug-metabolizing enzymes: CYP1A2, CYP2E1, *N*-acetyltransferase 2, CYP2D6, CYP2C9 and CYP2C19 (REF. 126). This simple metabolomic assay avoids the time-consuming and sometimes dangerous trial-and-error practice used by most clinicians in individual dose determination for drugs with a narrow therapeutic window, such as warfarin. It has also proven useful for determining the optimal dosing regimens for patients with existing liver conditions[126].

Pharmacometabolomics is yielding important insights into the underlying reasons behind the highly variable patient responses to statins[127], anti-depressants[128], aspirin[129,130] and several other drugs. In the case of statins, some of these variations seem to arise from individual differences in the microbiome[128]. More specifically, plasma values of several microbial metabolites, including the bile acids lithocholic acid, taurolithocholic acid and glycolithocholic acid, as well as microbially-derived 2-hydroxyvaleric acid, were able to predict 'good' statin responders from 'poor' statin responders. This suggests that certain gut microbial populations, or perhaps the response of certain classes of gut microorganisms to statins, may pre-dispose individuals towards treatment outcomes. In contrast to statins, variable patient responses to anti-depressants and aspirin seem to be due to individual variations in endogenous metabolism associated with specific neurotransmitter pathways[128,129], or individual perturbations in purine metabolism[130]. For instance, with aspirin therapy targeted towards preventing cardiovascular disease, higher plasma serotonin levels in patients pre- and post-aspirin correlated with high, post-aspirin platelet activity and platelet aggregation[129]. This finding was also confirmed *ex vivo*, where it was shown that serotonin increased platelet reactivity by 20% after co-incubation with aspirin[129]. Many of these discoveries could not have been made using genotyping alone. Furthermore, the data suggest that metabolomics could be used to predict good responders from poor responders for a variety of common drugs simply by measuring serum levels of a few key metabolites[127–130]. These data also suggest that metabolomics could be used to adjust dosing levels to optimize an individual's therapeutic response[119–126].

More recently, pharmacometabolomics has begun to reap benefits from several large-scale GWAS and metabolome-wide association studies (MWAS). These efforts are exploring SNP-induced variations in 'resting' metabolite levels in urine and serum among healthy individuals[131,132]. Such population-wide studies are not only helping to explain individual variations in metabolite levels, they are also highlighting how endogenous metabolites, in combination with SNP typing, may be used to predict or better understand an individual's response to a drug or therapeutic intervention[132]. One particularly interesting example involved the exploration of variations in patient responses to the antihypertensive drug atenolol[133]. Patients exhibiting greater response in terms of plasma fatty acid reduction after 9 weeks on the drug were found to have a specific intronic SNP (rs9652472) in their hepatic lipase gene, whereas those with a reduced response had a specific intronic SNP (rs7250148) in their phospholipase A2 gene. The search for and ultimate identification of the responsible SNPs were informed by the metabolomic data, which have subsequently been used to rationalize known individual and racial differences in atenolol response[133].

Precision medicine, metabolomics and cancer. Personalized or precision medicine is perhaps most highly developed in the field of cancer therapy. Tumour genomic profiling is now routinely used to classify tumour types, identify driver or germline mutations, perform prognostic assessments and make therapeutic decisions[134]. However, the heterogeneity of cancer genomes and cancer tissues can make it difficult to determine the underlying causes or ascertain the optimal treatment. Furthermore, the sheer number of mutations and the manifold combinations of tumour suppressors and oncogenes sometimes make individualized tumour classification or customized therapy almost impossible[135]. Recent developments in metabolomics, particularly in metabolite imaging, have the potential to substantially improve current cancer treatment. As noted earlier, many cancers exhibit a relatively small number

Intronic SNP
A single nucleotide polymorphism (SNP) found in an intron or a non-coding region of a gene.

Metabotypes
The metabolic equivalent of phenotypes. A metabotype is a metabolic profile that defines or classifies an individual's biochemical state at a given point in time.

of distinct metabolic phenotypes[61,62]. Some tumours seem to prefer aerobic glycolysis (the Warburg effect)[136], others depend more on glutaminolysis[137], and others still are dependent on one-carbon metabolism (which uses choline or folate)[138]. Certain tumours may use a combination of two or more of these metabolic pathways[61,62,137]. Using non-invasive methods to identify which of the seven different 'metabotypes' a given tumour might belong to, or which oncometabolites it is accumulating, would enable better customization or informed adjustment of cancer therapies[64]. A number of impressive examples have already been reported demonstrating the use of MRS to chemically phenotype gliomas[136,139] and the use of metabolite-informed PET scanning (alone or in combination with MRS) to metabolically phenotype a variety of other cancers[36,37,136]. More specifically, MRS is being used to detect the concentrations of the oncometabolite 2-hydroxyglutarate in and around gliomas as a method to grade the severity of and determine the probable cause of the tumour[139], whereas the metabolite-informed PET studies are being used to assess the differing metabolic activity or substrate preferences of different tumour tissues[36,37,136].

The use of MALDI or DESI imaging to metabolically phenotype fixed tumour samples[40] and the use of iKnife technologies to characterize tumours in real time and *in situ*[38] represent equally compelling examples of the potential of metabolomics for precision medicine in cancer. Indeed, the iKnife has already made its transition into the operating room as a real-time diagnostic tool after having been extensively validated against over 80 postoperative histological assessments (in which the match between the intra-operative iKnife diagnosis and the postoperative histological diagnosis was 100%)[38].

In summary, personalized genomics and metabolomics could be used in each of the major phases of precision medicine, from personal monitoring, to disease diagnosis, to selecting the optimal therapy and adjusting doses, and finally to tracking outcomes.

Conclusions

Relative to other omics fields, metabolomics is still a relatively young discipline. Consequently, it is experiencing many of the growing pains and false-starts that genomics, transcriptomics and proteomics all experienced at one time or another. However, over the past decade, important advances in many key metabolomics technologies and methods have occurred[17–19]. Instruments and methods are faster, more sensitive, more reliable and more automated than ever before. Substantial informatics resources have been developed which are permitting larger numbers of metabolites to be identified and quantified with greater reliability[27,140,141]. Likewise, software tools and protocols for data processing, data interpretation, sample handling and quality control are becoming far more standardized, comprehensive and widespread[27,142,143]. As it is uniquely able to perform detailed phenotypic measurements, and because it allows scientists to explore the nexus of gene–environment interactions, metabolomics is also gaining increased traction through its ability to facilitate biomarker discovery[2,4,112]. It is through these biomarker discoveries and the corresponding biological insights that metabolomics is changing our understanding of many chronic diseases. These insights, coupled with metabolomics' unique ability to measure chemical phenotypes, are also changing how we need to think about drug discovery and development. Last, it is through the multitude of metabolomics applications originally developed for the drug industry that we are able to realize the benefits that metabolomics can bring to precision medicine.

Metabolomics is changing biomedical research. The question is: can it change health delivery? As a community, metabolomics researchers will need to become much more translational in their thinking, focusing on how to convert a discovery into a device, a drug or a clinical test. In particular, simple metabolite associations will need to be proven and converted to causal frameworks. Biomarkers will need to be validated and moved to the clinic. Researchers must work more closely with clinicians, and drug developers will need to work more closely with academics. To increase their accessibility and popularity, metabolomics methods must also become far more 'kit' oriented and metabolomics instruments will have to become far simpler and cheaper. Can this happen? Interestingly, this kind of thinking was relatively routine in the 1950s and 1960s, especially in the fields of analytical and clinical chemistry. Perhaps, with a little industrial or governmental encouragement, it could happen again.

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Acknowledgements
The author wishes to thank Genome Canada, the Canadian Institutes for Health Research (CIHR) and Alberta Innovates for financial support.

Competing interest statement
The author declares no competing financial interests