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REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY AND HEPATOLOGY

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Metabolic Phenotyping and Systems Biology Approaches to Understanding Metabolic Syndrome and Fatty Liver Disease

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Metabolic syndrome, a cluster of risk factors for type 2 diabetes mellitus and cardiovascular disease, is becoming an increasing global health concern. Insulin resistance is often associated with metabolic syndrome and also typical hepatic manifestations such as nonalcoholic fatty liver disease. Profiling of metabolic products (metabolic phenotyping or metabotyping) has provided new insights into metabolic syndrome and nonalcoholic fatty liver disease. Data from nuclear magnetic resonance spectroscopy and mass spectrometry combined with statistical modeling and top-down systems biology have allowed us to analyze and interpret metabolic signatures in terms of metabolic pathways and protein interaction networks and to identify the genomic and metagenomic determinants of metabolism. For example, metabolic phenotyping has shown that relationships between host cells and the microbiome affect development of the metabolic syndrome and fatty liver disease. We review recent developments in metabolic phenotyping and systems biology technologies and how these methodologies have provided insights into the mechanisms of metabolic syndrome and nonalcoholic fatty liver disease. We discuss emerging areas of research in this field and outline our vision for how metabolic phenotyping could be used to study metabolic syndrome and fatty liver disease.

Keywords: Metabonomics; Metabolic Syndrome; Nonalcoholic Fatty Liver Disease; Nonalcoholic Steatohepatitis.

Metabolic syndrome is a cluster of disorders that increase the risk of type 2 diabetes mellitus and cardiovascular disease as defined in a joint consensus statement from international cardiovascular and diabetes groups.[1] This syndrome is associated with obesity and leads to disease in numerous organ systems, so organ-specific features are unlikely to provide a definitive phenotype. The main hepatic phenotypic manifestation of insulin resistance is primary nonalcoholic fatty liver disease (NAFLD). Approximately 34% of Western adults older than 20 years of age have metabolic syndrome[2,3] and 33.7% of US adults are obese (body mass index >30 kg/m^2), with associated and increasing rates of type 2 diabetes.[4] Many genomic and environmental factors affect the risk of developing metabolic syndrome, including age, sex, ethnicity, socioeconomic status, smoking status, diabetes, and amount of exercise.[4] Also, the cardiovascular risk associated with metabolic syndrome varies based on the combination of the components of metabolic syndrome, whereas NAFLD remains an independent factor.[5,6]

The spectrum of fatty liver disease comprises NAFLD and nonalcoholic steatohepatitis (NASH) with and without fibrosis, cirrhosis, and hepatocellular carcinoma.[7] NAFLD is defined histologically, based on the presence of simple hepatic steatosis without hepatocyte injury (ballooning), whereas NASH is defined by the presence of steatosis and inflammation with ballooning, with or without fibrosis.[8] Metabolic syndrome is a strong predictor of the presence of steatohepatitis in patients with NAFLD,[9] although the exact prevalence of NAFLD varies with the method of diagnosis; for instance, the prevalence of NAFLD can reach 46% when it is diagnosed by ultrasonography or magnetic resonance imaging, with or without measurement of hepatic enzyme levels.[10,11] The true incidence of NASH is likely to be underreported[8]; in magnetic resonance spectroscopy studies without liver biopsies, the prevalence of NAFLD has been reported to be as high as 65% among obese patients.[12] Epidemiological studies have indicated that NAFLD[13–15] has varying effects on mortality, depending on the diagnostic approach (NAFLD fibrosis score[16,17] or disease severity). Irrespective of mortality, the morbidity and economic burden are significant and NAFLD is now the most common cause of liver disease in the West, accounting for an

Abbreviations used in this paper: **BCAA, branched-chain amino acid; FMO3, flavin-containing monooxygenase 3; GSH, glutathione; iMIM, integrated metabolome and interactome mapping; LC, liquid chromatography; mGWAS, metabolomic genome-wide association studies; mQTL, metabolomic quantitative trait locus; MS, mass spectrometry; MSEA, metabolite set enrichment analysis; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NMR, nuclear magnetic resonance; RYGB, Roux-en-Y gastric bypass; SNP, single nucleotide polymorphism; TMA, trimethylamine; TMAO, trimethylamine-*N*-oxide.**

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increasing proportion of patients who undergo liver transplantation (15%–20%).[18]

Considerable challenges remain in the diagnosis, staging, and prognosis of metabolic syndrome, NAFLD, and NASH.[19] For example, results from tests of liver biochemistry can be within normal ranges for patients with NAFLD and NASH, so these are not sufficiently sensitive for screening. Composite clinical scores that predict which patients will develop fibrosis, such as the NAFLD fibrosis score, lack specificity.[20] A definitive diagnosis requires liver biopsy analysis, which is expensive, is invasive, and has associated morbidities.[19]

A molecular definition of the syndrome would offer advantages over a phenotypic definition, because the disorder could be diagnosed at early stages, populations could be stratified based on risk, and therapeutic strategies could be selected for specific groups of people. This will likely require a multimodal "omics" approach that integrates multiple factors determining the metabolic phenotype in this complex and multifactorial disorder because obesity affects the cardiovascular system by causing insulin resistance,[21] although numerous pathways have been shown to be involved in the pathophysiology of metabolic syndrome.[22]

Just as we are able to analyze entire genomes and gene expression profiles, we are also able to characterize metabolic profiles of cells, tissues, and organisms via metabonomic and metabolomic analyses.[23–25] Metabolic profile analysis (metabolic phenotyping or metabotyping)[26] can be used to identify specific features of metabolic syndrome. The metabolic profile results from genomic and environmental features of a cell or organism. Preclinical and clinical studies have generated unique insights into the molecular mechanisms of disease progression. Metabolic phenotyping can be used to identify biomarkers and different phenotypes of metabolic syndrome. We review recent technical developments in high-throughput metabotyping and top-down systems biology approaches (ie, data-driven approaches, as opposed to bottom-up model- and annotation-driven approaches), along with what we have learned about metabolic syndrome, NAFLD, and NASH via metabolic analyses. We also discuss the clinical applications that can be derived by increasing our understanding of the metabolic processes involved in metabolic syndrome and fatty liver disease.

Metabolic Phenotyping and Systems Biology

Unlike transcripts and proteins, metabolites are not directly encoded by the genome (Table 1). The comprehensive measurement and analysis of metabolites and their variation in reaction to genetic or external stimuli is usually referred to as metabolomics[25] and metabonomics,[23] although both use the same experimental tools. Metabolic phenotyping in particular corresponds to the use of analytical chemistry methods in metabolomics to generate high-resolution metabolic observations about various disease and treatment conditions. Such characterization of molecular phenotypes in a biomedical environment can be extended to the term *phenome*. From an analytical point of view, a phenome is an integrated set of measureable physical and clinical features coupled to chemical, metabolic, and physiological properties that define biological subclasses. On a more philosophical level, it is the direct product of gene-environment (exposome) interactions on an individual or group operating throughout development and life: a dynamic property.

How Are Metabolic Phenotypes Detected?

The systematic study of metabolism and metabolic response to stimuli, or metabonomics, is usually achieved

Table 1. High-Throughput "Omics" Approaches in Integrative Biology

Technology	Molecule	Knowledge	Limits	Clinical applications
Genomics	DNA	Genetic polymorphisms, haplotypes, full genome sequence through next-generation sequencing	Restricted to genetic determinants, ignores the environment	Genome-wide association studies Personal genomics
Transcriptomics	RNA	Expression patterns	Expression does not necessarily match protein abundance	Blood and biopsy transcript signatures
Proteomics	Proteins	Targeted and untargeted protein abundance profiles	Protein abundance does not mean function Protein identification can be challenging	Numerous clinical applications for measurement of circulating endogenous protein markers
Metabonomics (understanding the response of living systems to stimuli[23]) and metabolomics (a comprehensive characterization of the metabolic complement of the cell[25])	Metabolites	Intermediary phenotypes related to metabolism in the absence of genetic information Observation of effects from host genetics, lifestyle, and environment (including microbiome)	Structural assignment can be challenging Trade-off between targeted and untargeted approaches (ie, precise knowledge of detected metabolites vs extensive coverage of the metabolome)	Metabolome-wide association studies and metabolomic GWAS Patient stratification through pharmacometabonomic approaches Perioperative applications

using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS)[23,24] and requires statistical analysis of the spectra (Figure 1 and Table 1).

NMR and MS

High-resolution NMR spectroscopy has been used to identify chemical structures for decades and is now used extensively in metabolic studies. The robustness and reproducibility of ^{1}H NMR spectroscopy data, combined with the capacity for widespread acquisition of reference spectra, make NMR spectroscopy a reliable technique for identification of structures in biofluids (cell media, urine, plasma, and so on), intact biopsy specimens, and tissues with minimal sample preparation. It is also possible to use 2-dimensional NMR spectroscopy, including heteronuclear ^{1}H-^{13}C NMR, to resolve complex ^{1}H NMR signals along a second ^{13}C dimension.[27] The main benefit of MS over NMR is the high level of sensitivity of MS; it can detect metabolites in very low concentrations. To improve spectral

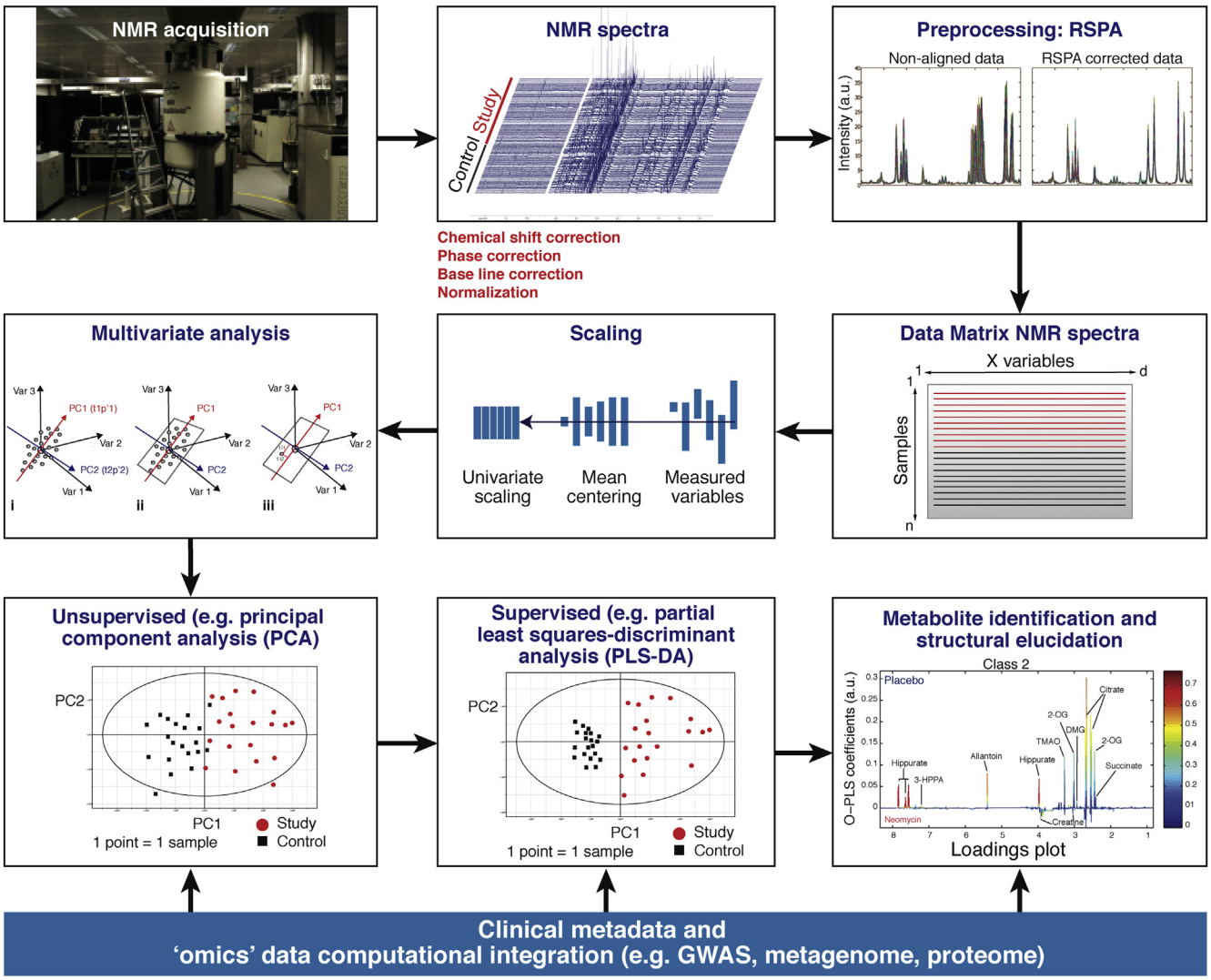

Figure 1. Workflow for metabolic phenotyping studies. Spectra are acquired (in this case by NMR) and preprocessed before analysis to account for experimental variations in the spectral data sets. Recursive segment-wise peak alignment (RSPA)[116] aligns small regions of the NMR spectrum requiring alignment, and clustering-based identification techniques have been developed that use a suitable reference spectrum for target alignment of other spectra in a data set.[117] Several approaches have also been introduced to minimize the influence of information redundancy in spectral data sets that use untargeted feature extraction. This was originally achieved by binning spectra in equal segments (bins of 0.04, 0.01, or 0.001 ppm for NMR spectra, for instance). More recently, a statistical recoupling of variables (SRV) was introduced for NMR[118,119]; instead of generating equal size bins, the SRV algorithm identifies the boundaries of each individual peak, delivering a data set reduction of a factor of approximately 100 with >98% signal recovery. A similar approach has also been developed for feature extraction of LC-MS data sets (XC-MS[120]). Data undergo a process of scaling, and to improve model quality, metabonomic data sets are commonly subjected to logarithmic transforms,[121] which provided better explanation of the model residuals and reduces the influence of multiplicative noise.[122] Finally, the data are analyzed by supervised and unsupervised multivariate techniques, which can be used to determine the presence of latent biomarkers that describe differences between sample classes or clinical states.

resolution, MS is usually coupled to a separation step that involves gas or liquid chromatography (LC). MS/MS systems such as triple quadrupole detectors are essential for multiple reaction monitoring to ensure accurate quantification of several compounds in parallel using labeled standards to provide large-scale absolute quantification.[23,24,28–31] The use of labeled standards also increases intralaboratory and interlaboratory reproducibility.[32]

Untargeted and Targeted Metabolic Profiling

There are 2 approaches to metabolic profiling: hypothesis-free untargeted profiling, which presents a high potential for discovery of new biomarkers, and targeted profiling, which focuses on accurate quantification of a subset of metabolites. The use of ^{1}H NMR for untargeted profiling can typically identify about 200 high-abundance metabolites in a particular data set, out of 1000 likely to be observed in biological samples, whereas targeted profiling can typically quantify about 80 metabolites per sample. Typically, more than 5000 uncharacterized metabolic features (corresponding to unassigned metabolites, potentially new metabolites) can be measured using ultra-performance LC coupled to quadrupole time-of-flight MS in the untargeted mode. However, most advanced targeted profiling assays claim to measure about 600 metabolites by matching standard LC-MS acquisitions with a pure compound reference database acquired under the same conditions.

Using standards labeled with isotopes (such as ^{13}C or ^{2}H), approximately 200 metabolites can be quantified from a single sample. As a result, targeted profiling is becoming increasingly appealing to clinicians and epidemiologists, because service companies offer reliable semiquantifications for 200 to 600 metabolites detectable by MS. However, associations discovered are limited to metabolites covered by the targeted assays. In contrast, untargeted profiling provides a comprehensive analysis of the metabolome with a high potential for discovery of novel associations, even though detailed structural assignment remains time consuming. The current trend leans toward the development of comprehensive profiling strategies, which include untargeted and targeted assays.

Statistical Analyses

Spectral data are very dense with thousands of signals, so mathematical modeling is necessary to highlight metabolites significantly affected by the experimental design. Many approaches have been introduced, from parallel univariate tests to multivariate statistics. One advantage of multivariate statistical analysis is that it provides information on multiple signals that can be mined to identify biomarkers of clinical use. Approaches to multivariate statistics of metabolic profiling data involve focusing on the variation within the spectral data only (ie, unsupervised) and investigating variations between the spectral data and other types of data, such as those associated with specific disease states (ie, supervised).[33] Most articles use principal component analysis as an unsupervised strategy, compressing the original data set into a set of principal components made of projections of individuals (scores) and model coefficients (loadings), and partial least squares regressions as a supervised approach. Partial least squares regression presents a series of advantages for a reliable and flexible analysis of NMR and MS data.[33] These statistical methods allow the identification of a set of significantly affected metabolites corresponding to the metabolic signature of the condition.

Metabolic signatures derived from NMR and MS are often complex and typically contain hundreds or thousands of metabolites. The interpretation of such signatures tends to be a difficult task that can be made objective through systems biology.

From Biomarkers to Metabolic Pathways

Identification of a metabolite of interest is a good starting point, but it is not systematic and does not take advantage of the full signature. It is possible to test whether the metabolites present in a given metabolic signature belong to a particular pathway (Figure 2). Metabolite set enrichment analysis (MSEA)[34–37] is the metabolomic counterpart of gene set enrichment analysis[38] and compares the molecular content of a metabolic signature with metabolites found in known metabolic pathway maps or databases. For example, if only one tricarboxylic acid cycle intermediate is significantly increased in a sample, it is unlikely that there is a coordinated change at the pathway level. However, if 5 tricarboxylic acid cycle intermediates are significantly increased, it is more likely that the tricarboxylic acid cycle has been significantly affected and that there is a coherent up-regulation at the pathway level. However, visualization of one pathway is not sufficient, because metabolites can belong to several pathways. To generalize this approach, metabolites present in a disease signature are systematically compared with exhaustive databases of metabolites and metabolic pathways, such as the Kyoto Encyclopedia for Genes and Genomes database.[39] Statistical tests can then be performed for metabolic pathway enrichment using recently developed MSEA.[34–37] A statistic describes the strength of the association between the two (usually χ^2 or hypergeometric analysis). In this way, a large list of potential target metabolites can be rapidly reduced into a handful of metabolic pathways, allowing for interpretation of the metabolic signature as a whole.

Identification of metabolic pathways associated with a specific disease or condition reveals the internal coherence of the metabolic signature at the level of the biochemical pathway. However, associations do not explain how specific metabolic patterns are generated; elucidation of these mechanisms requires further studies of signal transduction pathways.

Interactome Mapping of Signaling Pathways That Regulate Metabolism

Most biological processes are regulated by overlapping mechanisms, so metabolic profiles can identify which pathways are altered in specific diseases. For example, the

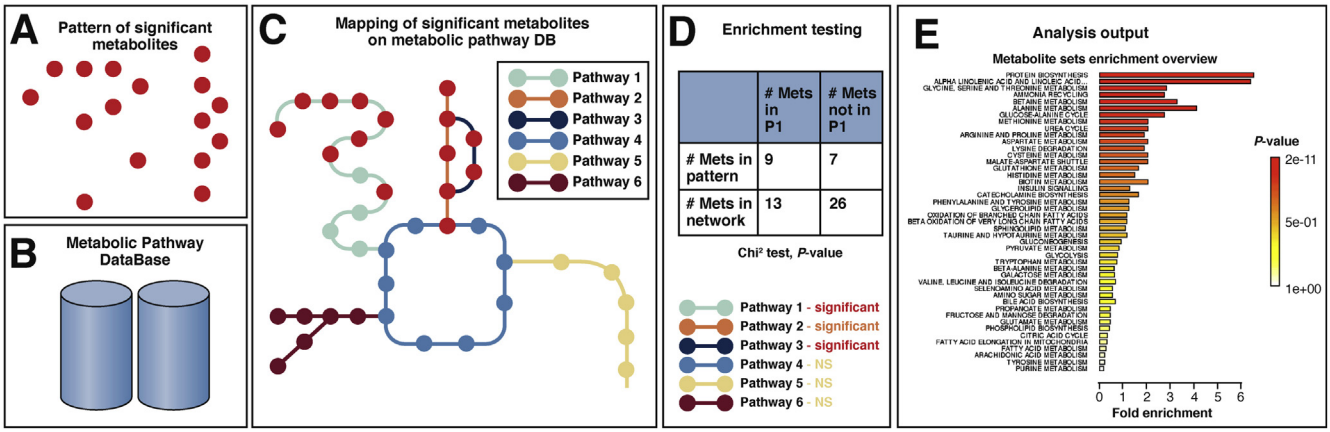

Figure 2. MSEA. Metabolites that change during development of disease form a complex metabolic pattern (*A*), which can be difficult to interpret. Using previous knowledge about metabolic pathways compiled in a database (*B*), the complex metabolic pattern is then mapped onto the metabolic pathways (*C*). The metabolic pattern is repeatedly tested for association with each pathway in the database (*D*) using enrichment tests such as χ^2 analysis in the case of a qualitative overrepresentation analysis or for a quantitative enrichment analysis.[35,36,123] The result of the analysis is finally displayed in a summary plot showing which pathways are significantly affected based on the set of metabolites actually observed and the set of metabolites potentially observable and mapped on the various pathways (*E*) as obtained using free online resources such as the MSEA webserver (www.msea.ca)[34,40] based on the compilation of annotated markers associated with NAFLD and NASH presented in Table 2.

metabolic spectral signature can be mapped onto protein-interaction networks using integrated metabolome and interactome mapping (iMIM) developed by Davidovic et al.[40] In iMIM, metabolites are connected with protein interaction networks based on activities of specific enzymes. iMIM provides an analysis of the topology of the resulting network; the shortest paths between genetic variants known to cause a specific disorder (eg, through deletion or silencing) and the metabolites that are altered by these changes are determined to identify the most connected proteins in the interaction network. This is achieved using network statistical analysis such as determination of "between-ness" (a measure of the centrality of a protein in a network), identification of factors connected by the shortest paths[41] that can therefore mediate the connection between a disease-associated genetic variant and resulting changes in the metabolic signature.

This approach relies on publicly available bioinformatic resources such as the Kyoto Encyclopedia for Genes and Genomes database, the human metabolome database, or the Consensus Pathway DataBase.[42] The key strength of the iMIM approach is that it not only uses a comprehensive compilation of protein interactions but also computes the shortest paths across the network for metabolic biomarkers of interest. This allows the identification of an interaction subnetwork and an efficient analysis of its topology (using network statistics such as between-ness) to identify which nodes (protein) are the busiest in the network. Although iMIM was initially developed for the study of fragile X syndrome, this network biology strategy can be used to study metabolic syndrome, NAFLD, or NASH.

Genomic Determinants of Metabolism

Genetic factors determine metabolic phenotypes (Figure 3), so metabolic profiles can be mapped directly onto the genome to identify metabolomic quantitative trait loci (mQTL).[43–45] In these integrative genomics approaches, metabolic variations can be correlated with polymorphisms in a segregating population.[41,46] In animal models, these studies typically involve analyses of congenic and recombinant inbred animals.[43,44] In human studies, this approach is used in metabolomic genome-wide association studies (mGWAS).[28,47–49] This parallel high-throughput approach identifies associations between metabolites and genetic loci and can provide important information about mechanisms of metabolism.[50] For example, crossing a normoglycemic strain of Brown Norway rats with Goto–Kakizaki rats, which spontaneously develop diabetes, we identified a polymorphism (deletion) in the gene encoding UDP-glucuronosyl-transferase 2b (Ugt2b, which glucuronides benzoate) associated with the benzoate signal associated with the diabetic allele.[43] When insulin-sensitive BALB/c mice were crossed with insulin-resistant 129S6 mice, which develop NAFLD,[51] a polymorphism was identified in the gene encoding glycerate kinase that was associated with variation in glycerate excretion.[44]

One of the key conclusions from mQTL and mGWAS approaches is that metabotypes can be treated like any other phenotype in a genetic analysis framework, which means that variations of each metabotype arise from genetic and environmental factors. In fact, we have shown that the genetic and environmental contributions to metabotypes are stable.[52] Thus, genetic susceptibility to produce a specific metabolite, and the effects of lifestyle factors such as diet or exercise, are not mutually exclusive and can be modeled in parallel. The linearity and reproducibility of metabolomic approaches and the number of traits measured by such approaches make systematic identification of the genetic determinants of mammalian and human metabolism possible.

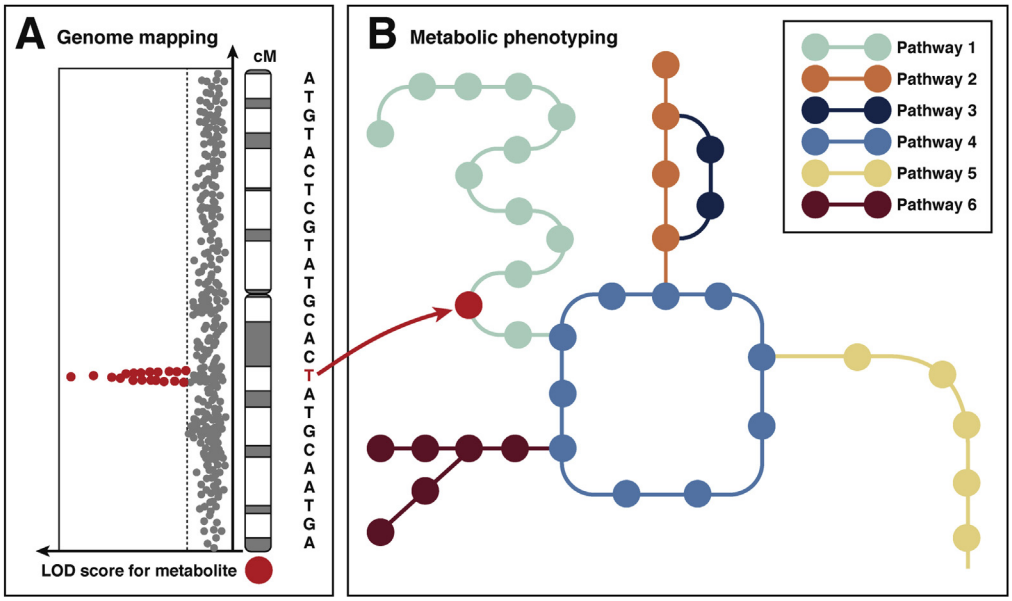

Figure 3. Genomic determinants of metabolism. The combination of (*A*) genetic polymorphism and (*B*) metabolic phenotype data can be used to map metabotypes onto the genome and identify genetic factors that regulate metabolism. Several frameworks are available to derive such associations, such as mQTL.[43,45] SNP data can be directly associated with metabolic phenotypes in mGWAS.[48] Each metabolomic trait is associated with genome polymorphisms, just like in a classic genome-wide association study. Multiple testing corrections are mandatory because there are tens of thousands of metabolites and hundreds of thousands of genomic markers.

Metabolic Markers of Metabolic Syndrome and NAFLD

Metabolomic strategies have been used to study the pathogenic mechanism of metabolic syndrome, NAFLD, and NASH. Several metabolites and their pathways derived from mammalian and gut microbial cometabolism have been associated with phenotypes of metabolic syndrome, including diabetes, hypertension, and obesity.

Biomarkers of Metabolic Syndrome in Humans

Using targeted LC-MS/MS lipidomics, Koves et al observed that high-fat feeding increases levels of post-prandial serum nonesterified fatty acids and acylcarnitines and that acylcarnitine profiles in skeletal muscle suggest excessive β-oxidation.[53] This study showed that diabetes, obesity, and a high-fat diet increase rates of β-oxidation, which is supported by the fact that deletion or inhibition of genes involved in mitochondrial lipid import protects against insulin resistance.[53] This study challenged the accepted view that insulin resistance decreases lipid uptake and oxidation.[54,55]

Metabolic profiling approaches have been used to determine if changes in serum lipid profiles are independent of genetic factors using LC-MS.[56] In a study of monozygotic twins, researchers found that obesity, independent of genetic factors, was related to increases in lysophosphatidylcholines (lipids found in inflammatory and atherogenic conditions) and to decreases in ether phospholipids, which have antioxidant properties. These lipid changes were associated with insulin resistance, a pathogenic characteristic of acquired obesity in these young adult twins.

Apart from biomarkers of disruption of fatty acid metabolism such as acylcarnitines and lysophosphatidylcholines, other biomarkers have been identified. Newgard et al profiled the plasma metabolome of 73 obese and 77 lean subjects and found that levels of branched-chain amino acids (BCAAs), such as valine, leucine, and isoleucine, were significantly increased in samples from obese patients.[57] BCAA supplementation in a high-fat diet in rats resulted in increased levels of phosphorylated mTOR, S6K1, and IRS1 (which are activated by BCAAs, leading to insulin resistance) in skeletal muscle compared with standard and high-fat diets. These findings indicated that BCAAs contribute to insulin resistance when consumed in a high-fat diet.[57]

The link between BCAA and metabolic syndrome has been further investigated. Wang et al monitored the blood metabolome of 2422 normoglycemic subjects for 12 years, and 201 developed diabetes.[58] Similarly to the study of Newgard et al, Wang et al associated levels of BCAAs (isoleucine, leucine, and valine) and aromatic amino acids (tyrosine and phenylalanine) with risk of type 2 diabetes.[58] More recently, the same groups profiled 1015 patients from the Framingham Heart Study and 746 patients from the Malmo Diet and Cancer Study to identify novel metabolites and pathways associated with cardiovascular risk.[59] They not only reproduced their previous results but also associated decreased levels of glutamine and increased levels of glutamate with diabetes and a high ratio of glutamine/glutamate with a lower risk of diabetes. These markers also have therapeutic value because glutamine supplementation in mice decreased their metabolic risk.

From these key studies of serum markers of metabolic syndrome, it emerges that the balance between essential amino acids (BCAAs, aromatic amino acids) and other amino acids is affected in metabolic syndrome as well as various phospholipids.

Biomarkers of NAFLD in Humans

A number of biomarkers have now been described for NAFLD and NASH (Table 2). Pathways covered by these markers were compiled and have been statistically tested using MSEA to illustrate the metabolic mechanisms involved in NAFLD (Figure 4).

Serum markers of NAFLD typically vary in fatty acid chain length, unsaturation pattern (monounsaturated fatty acids vs polyunsaturated fatty acids), and lipid oxidation patterns. In a comprehensive review, Meikle and Christopher[60] compiled several studies, including the work of Puri et al.[61] Essentially, the increased ratio of monounsaturated fatty acids/unsaturated fatty acid indicates increased activity of Δ9 desaturase. Furthermore, variations in polyunsaturated fatty acids indicate increased activity of Δ6 desaturase (18:2 n-6/18:3 n-3) and peroxisome dysfunction (22:5 n-3/22:6 n-3). These results show that NAFLD is associated with de novo lipogenesis.

Using thin-layer chromatography, gas chromatography/MS, and LC-MS/MS, Puri et al[61] showed that eicosanoids and products of oxidation of the inflammatory arachidonic acid by lipoxygenase are particularly affected during progression to NAFLD and then NASH. The investigators showed that, among eicosanoids, an increase in 5-lipoxygenase, 8-lipoxygenase, and 15-lipoxygenase products (hydroxyeicosatetraenoic acids: 5-HETE, 8-HETE, and 15-HETE) contributed to inflammation and that 11-HETE was specific to NASH.[61] This study indicated that lipogenesis associated with NAFLD and NASH was directly responsible for the formation of free fatty acids with lipotoxic properties, which goes hand in hand with synthesis of inflammatory lipids (eicosanoids).

Using capillary electrophoresis coupled to MS, Soga et al identified circulating γ-glutamyl dipeptides as serum markers of NAFLD. The presence of γ-glutamyl dipeptides indicates increased glutathione (GSH) synthesis as part of an oxidative stress response.[62] GSH is a tripeptide derived from glutamate, cysteine, and glycine. GSH is the predominant antioxidant in hepatocytes and reacts with electrophilic chemical intermediates through glutathione-*S*-transferase activity, which involves a nucleophilic attach of GS^- to electrophilic carbons of reactive oxygen species. GSH synthesis is catalyzed by γ-glutamylcysteine synthetase, leading to the formation of γ-glutamylcysteine and then GSH by glutathione synthetase. Under reducing conditions, the GSH bioavailable pool down-regulates GCS and the first step of the pathway. However, under oxidizing conditions, the GSH pool is lower; as a result, there is increased γ-glutamylcysteine synthetase activity, leading to γ-glutamylcysteine. Excess γ-glutamylcysteine is handled by γ-glutamyl transpeptidase, catalyzing the substitution of cysteine by other transaminable amino acids, and the synthesis of various γ-glutamyl dipeptides, which are then released into the bloodstream. The findings of Soga et al are important because serum levels of aspartate transaminase, alanine transaminase, and γ-glutamyl transpeptidase are routinely measured to assess liver function (typically for alcohol-associated liver disease but also for NAFLD[14]); this metabolomic study extends the assessment of liver function to the circulating products of γ-glutamyl transpeptidase.[62]

It emerges that reprocessing of fatty acids and in particular desaturation and elongation is a key process involved in the metabolic signature of NAFLD, coupled with lipotoxicity and synthesis of inflammatory lipids belonging to the eicosanoids family, and finally oxidative stress. However, over the past decade, another class of NAFLD and metabolic syndrome markers associated with gut microbial metabolism has been identified.

Gut Microbial Markers of Metabolic Syndrome and Fatty Liver Disease

Over the past decade, various studies of metabolic syndrome and fatty liver disease have highlighted the increasing role of gut bacteria in our understanding of these pathologies. The collective genome of the intestinal microbiome (conservatively estimated to exceed 4.4 million genes)[63] dwarfs that of the human and has an essential role in mammalian energy production and lipid metabolism.[64] The intestinal microbiome varies greatly among individuals; at a strain level, we may share as little as 1% of the same microbiota. However, at a phyla level, this large metabolic "organ" undergoes age-dependent shifts and varies among geographically distinct populations. Moreover, its architecture is highly sensitive to dietary and social circumstances, so researchers have proposed that the microbiome has an important role in the maintenance of health, particularly in elderly people.[65]

Changes in the composition of the intestinal microbiome have been associated with conditions ranging from obesity and diabetes to autoimmune diseases and neuropsychiatric disorders.[64] Turnbaugh et al associated obesity with significant fluctuations in the structure of the intestinal microbiota, particularly in changes in the ratio between the bacterial phyla *Bacteroidetes* and *Firmicutes*.[66] The gene richness of the gut microbiome correlates with metabolic syndrome[67] and can be modulated by dietary intervention,[68] suggesting that a diverse gut ecology is critical for health.

The gut microbiome has a strong effect on host metabolic phenotypes,[69] which are relayed by complex signaling pathways that connect organs such as the liver, brain, and immune systems.[64,70] The advance of systems biology technologies for interactome mapping of metabolic profiles[40] has led to the concept of a signaling metabolome, the subset of metabolites that can act as ligands for receptors and modulate transduction pathways, from a purely pharmacological angle.[71]

Methylamines (trimethylamine [TMA], trimethylamine-*N*-oxide [TMAO], and dimethylamine) were identified as markers of NAFLD in 129S6 mice, which are models of dietary-induced fatty liver disease.[51,72] Methylamines are a

Table 2. Metabolic Markers of Fatty Liver Disease

Study	Metabolites associated with fatty liver disease	Sense of variation	Significantly enriched metabolic pathways
Soga et al[62] Serum capillary electrophoresis (CE)-MS study of 237 patients with 9 types of liver diseases; identified γ-glutamyl dipeptides as biomarkers for hepatocellular carcinoma and NAFLD	Glucosamine, methionine sulfoxide, γ-L-glutamyl-L-taurine, γ-L-glutamyl-L-alanine, γ-L-glutamyl-L-leucine, γ-L-glutamyl-L-valine, γ-L-glutamyl-L-glutamate, γ-L-glutamyl-L-glycine, γ-L-glutamyl-L-lysine, γ-L-glutamyl-L-arginine, γ-L-glutamyl-L-serine, γ-L-glutamyl-L-threonine, γ-L-glutamyl-L-histidine, γ-L-glutamyl-L-phenylalanine, γ-L-glutamyl-L-methionine, γ-L-glutamyl-L-glutamine, γ-L-glutamyl-L-citrulline	Up	Glutathione metabolism NS
	5-Methoxyindoleacetate, *N*-acetylornithine, glycylglycine L-asparagine, homoarginine, L-taurine, L-aspartate, creatine, glycerophosphorylcholine, L-glycine, L-hypoxanthine, L-glutamate, 3-methylhistidine, betaine, 5-oxoproline, γ-butyrobetaine, α-aminoadipate, creatinine, L-proline, guanidinoacetate, L-threonine, L-phenylalanine, N-γ-ethylglutamine, citrulline, L-lysine, L-serine, L-alanine, L-histidine, L-valine, L-leucine, L-tryptophan, indole-3-acetamide, L-methionine, pipecolic acid, kynurenine, trimethylamine *N*-oxide, sarcosine, L-tyrosine, *N*-*N*-dimethylglycine, γ-L-glutamyl-L-tyrosine, γ-L-glutamyl-L-tryptophan	Down	Aminoacyl-tRNA biosynthesis; glycine, serine, and threonine metabolism; arginine and proline metabolism; nitrogen metabolism; alanine, aspartate, and glutamate metabolism
Barr et al[112] LC-MS profiling of mouse and human serum metabolites associated with progression of NAFLD	Arachidonic acid, PC (20:4/0:0), PC (18:1/0:0), PC (16:0/20:4), PC (14:0/20:4), PC (16:0/20:3), PC (18:1/0:0), PC (P-18:0/20:4), PC (18:2/0:0), PC (20:0/0:0), PC (18:2/18:2), PC (P-24:0/0:0), PC (P-22:0/0:0), PC (O-20:0/0:0)		Not available for lipids
Barr et al[113] LC-MS profiling of 467 subjects with NAFLD	Diacylglycerophosphoinositol, monoetherglycerophosphoethanolamine, monoacylglycerophosphoethanolamine, ether-acyl-glycerophosphoethanolamine, diacyl-glycerophosphoethanolamine, monoetherglycerophosphocholine, monoacylglycerophosphocholine, ether-acyl-glycerophosphocholine, diacylglycerophosphocholine, sphingoid, sphingomyelin		Not available for lipids
Dumas et al[51] ^{1}H NMR profiling of plasma and urine from BALB/c and 129S6 mice fed a high-fat diet leading to NAFLD (in 129S6)	Choline, TMA, TMAO, dimethylamine, methylamine	Up	Methane metabolism
	Creatine, glycerate, isovalerate, pyruvate	Down	Glycine, serine, and threonine metabolism; pentose phosphate pathway

Table 2. Continued

Study	Metabolites associated with fatty liver disease	Sense of variation	Significantly enriched metabolic pathways
Li et al[114] ^{1}H NMR profiling of serum from C57Bl/6 mice fed a methionine-choline deficient diet leading to NAFLD	Lactate, glutamine, glutamate, creatine, methionine, alanine, acetate, lysine, arginine	Up	Aminoacyl-tRNA biosynthesis; D-glutamine and D-glutamate metabolism
	Very-low-density lipoprotein, low-density lipoprotein, glucose, pyruvate, *N*-acetyl-glycine, leucine, phosphorylcholine, choline[a], TMAO[a], betaine[a]	Down	Glycine, serine, and threonine metabolism
Cobbold et al[72] Kalhan et al[115] LC/gas chromatography–MS profiling of plasma from patients with hepatic steatosis, NASH, and NAFLD	Glycocholic acid, taurocholic acid, glycochenodeoxycholic acid, glutamylvaline, linolenate, undecanoic acid, carnitine, butyrylcarnitine, propionylcarnitine, 2-methylbutyrylcarnitine, glucose, pyruvate, mannose, lactate, phenylalanine, leucine, isoleucine, valine, glutamate, aspartate, tyrosine, lysine	Up	Aminoacyl-tRNA biosynthesis; valine, leucine, and isoleucine biosynthesis; pantothenate and CoA biosynthesis; glycolysis or gluconeogenesis; nitrogen metabolism; valine, leucine, and isoleucine degradation
Metabolic patterns related to control vs NASH	Cysteine-glutathione-disulfide, eicosapentaenoic acid, docosahexaenoic acid, 10-undecenoic acid, capric acid, glycerophosphocholine, 1-oleoylglycerophosphocholine, 1-lineoylglycerophosphocholine, 1-arachidonoylglycerophosphocholine	Down	NS
Puri et al[61] Thin-layer chromatography and LC-MS profiling of plasma lipids; 50 controls, 25 patients with NAFLD	Myristic acid, palmitic acid, myristoleic acid, palmitoleic acid, vaccenic acid, oleic acid, γ-linolenic acid, homo-γ-linolenic acid, stearidonic acid, docosapentaenoic acid	Up	Fatty acid biosynthesis
	Increase in lipoxygenase metabolites or arachidonic acid (NS); 5-HETE, 8-HETE, and 15-HETE characterized progression from normal to NAFLD to NASH; 11-HETE is specific to NASH		
Meikle and Christopher[60] quoting Puri et al[61]	Increased monounsaturated/unsaturated fatty acid ratio suggests increased activity of Δ9 steraroyl-coA desaturase (SCD) (adaptive but insufficient response)	Up	Fatty acid biosynthesis
	Changes in polyunsaturated fatty acids suggest increased activity of Δ6 desaturase (18:2 n-6/18:3 n-3) and peroxisome dysfunction (22:5 n-3/22:6 n-3)		
	Increases in 5-lipoxygenase, 8-lipoxygenase, and 15-lipoxygenase products (5-HETE, 8-HETE, and 15-HETE) contribute to the proinflammatory state		

NOTE. Significantly enriched metabolic pathways were identified by the MSEA webserver (www.msea.ca)[34] using default parameters.
NS, not significant; HETE, hydroxyeicosatetraenoic acid.
[a]A decrease in these metabolites is directly due to the absence of choline in the diet.

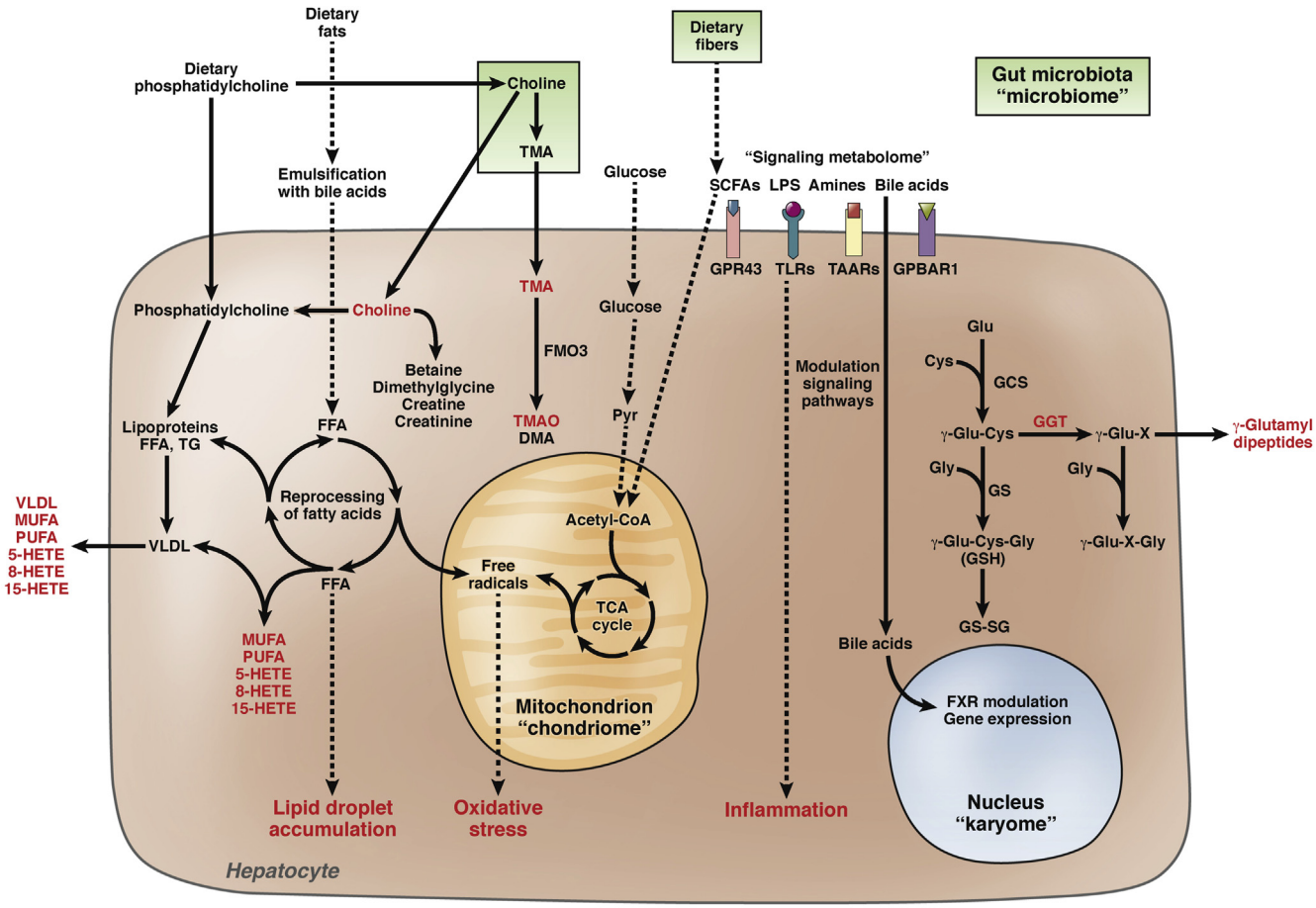

Figure 4. Metabolic markers of fatty liver disease. A metabolic chart was constructed from the markers presented in Table 2. DMA, dimethylamine; FFA, free fatty acid; FXR, farnesoid X receptor; GCS, γ-glutamylcysteine synthetase; GGT, γ-glutamyltransferase; GPBAR1, G protein–coupled bile acid receptor 1; GPR43, G protein–coupled receptor 43; GS, glutathione synthase; GS-SG, oxidized glutathione; HETE, hydroxyeicosatetraenoic acid; LPS, lipopolysaccharide; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SCFAs, short-chain fatty acids; TAARs, trace amine-associated receptors; TCA, tricarboxylic acid cycle; TG, triglyceride; TLRs, Toll-like receptors; VLDL, very-low-density lipoprotein.

class of metabolites produced by the intestinal microbiota[29,30] via bacterial degradation of choline into TMA in the distal intestine, where it is absorbed into the enterohepatic system.[73,74] TMA is then metabolized into TMAO by flavin-containing monooxygenase 3 (FMO3) and potentially other isoforms. In fact, a missense mutation in the *FMO3* gene[75] has been shown to cause fish odor syndrome, characterized by a lack of detoxification of TMA into TMAO and trimethylaminuria.[73] Al-Waiz et al showed the bacterial origin of TMA in the mouse,[74] and FMO3 was found to metabolize TMA into TMAO and, in lower quantities, FMO1.[76] Expression of FMO3 is induced by bile acids via a farnesoid X receptor–related mechanism. Methylamines have also been associated with other cardiovascular diseases; it has been claimed that TMAO contributes to atherosclerosis in $ApoE^{-/-}$ mice and patients.[77] Phosphatidylcholine was proposed to be involved in choline metabolism in the intestine and with TMAO and atherosclerosis. Despite previously reported health benefits, L-carnitine, which is associated with meat consumption and is a substrate for TMA and TMAO formation, has also been associated with risk of cardiovascular disease in one study.[78] Increased excretion of TMA, TMAO, and L-carnitine was observed in people who consume large amounts of meat, compared with those who eat less or no meat, by Stella et al.[79] Although TMA is produced by bacteria,[51,74,77] conventional intestinal microbiomes of most humans have this metabolic function. Urinary excretion of TMA is determined by genetic factors; a single nucleotide polymorphism (SNP) can alter excretion of TMA by 64% (rs7072216 on chromosome 10).[45] This means that essentially every person has the microbial ability to produce TMA. Thus, excretion of TMA is predominantly influenced by the function of the host's detoxification system, which is mostly determined by the host's genetics. The exact mechanisms by which TMA and TMAO contribute to NAFLD, metabolic syndrome, atherosclerosis, and health in general have yet to be identified.[80] Studies of germ-free animals (without any contaminating commensal or pathological organisms) have provided insights into how the intestinal microbiome affects liver physiology and metabolism.[81,82]

Interactome mapping of metabolic profiles could help assess the role of gut microbial signaling metabolites that bind to mammalian receptors. For example, one microbial-mammalian metabolic axis involves signaling from bile acids, which are synthesized by the host and heavily metabolized by the microbiota, binding to nuclear (farnesoid X receptor) and G protein–coupled receptors (TGR5) in the liver that eventually affect host gene expression profiles.[83] It was recently shown that a bile acid, deoxycholic acid, induces a senescence-associated secretory phenotype, leading to the development of hepatocellular carcinoma in mice.[84] Short-chain fatty acids, which are derived from colonic fermentation of polysaccharides and oligosaccharides, provide energy for colon cells (butyrate), butyrate for histone deacetylation inhibitors, and ligands for GPR43 and GPR41 (butyrate, propionate, and acetate).[71] TMA acts as a pheromone that binds trace amine-associated receptor (TAAR5) in the mouse olfactory epithelium.[85] The human *TAAR5* gene was recently shown to be activated by TMA.[86] It is anticipated that these microbial metabolites could be developed as lead therapeutics for metabolic syndrome and NAFLD.

Clinical Applications

Epidemiological Applications

Several developments in molecular epidemiology and metabolic phenotype analyses have enabled large-scale profiling of clinical samples from large cohorts. The development of metabolome-wide association studies,[87] which compute associations between NMR- or MS-derived metabotypes and disease phenotypes or their risk factors, has allowed for deep analysis of the metabolic patterns associated with disease states in the absence of genetic markers. The International Study on Macronutrients and Artery Pressure (INTERMAP) profiled the metabolome of 2 urine specimens from each of 4630 participants in 4 countries and associated levels of formate with blood pressure.

When genetic markers are available, the coanalysis of genome polymorphisms and metabolic phenotypes is powerful, as exemplified in mQTL[43,44] studies and mGWAS.[28,45,47–49] Future analyses of statistical associations between the microbiome and the metabolome will lead to identification of new metagenomic determinants of mammalian and human metabolism.[82,88]

In a study of ^{1}H NMR blood profiles from 7098 subjects in Finland, Würtz et al associated levels of BCAAs (leucine, isoleucine, and valine), aromatic amino acids (tyrosine, phenylalanine), glucogenesis intermediates (alanine, glutamine, pyruvate, and lactate), and ketone bodies (acetoacetate and 3-hydroxybutyrate) with homeostasis model of assessment–insulin resistance scores.[89] Only the gene encoding the glucokinase regulatory protein had a variant significantly associated with homeostasis model of assessment–insulin resistance and 12 metabolites (including arachidonic acid). In a targeted metabolomics study that measured 163 metabolites in 965 subjects and 180 metabolites in 890 subjects, Jourdan et al associated serum levels of BCAAs, ratios of total BCAAs/glucogenic amino acid, and levels of free carnitine with fat-free mass index scores (a marker of muscle mass).[90] Muscle accounts for one-third to one-half of total protein content in the body, and serum levels of BCAA increase during fasting due to muscle breakdown; BCAAs are used for gluconeogenesis. Using a similar metabolomic approach, the same group characterized 140 metabolites during the progression of 4297 subjects from normoglycemia to impaired glucose tolerance and type 2 diabetes.[91] In this study, glycine, acetylcarnitine, and lysophosphatidylcholine 18:2 were associated with impaired glucose tolerance. Glycine and lysophosphatidylcholine 18:2 were also associated with 2-hour fasting glycemia levels in glucose tolerance tests and with risk of type 2 diabetes after 7 years. The Potsdam cohort of the European Prospective Investigation Into Cancer and Nutrition was also characterized using the same approach.[92]

Metabonomic technologies have been used to study type 2 diabetes for approximately 30 years.[93,94] For instance, it was shown in 1984 that increases in serum levels of alanine and reduction in levels of BCAAs (corresponding to decreased amino acid gluconeogenesis and increased ketogenesis) after insulin withdrawal from patients with type 2 diabetes, as well as modification of plasma lipid and lipoprotein profiles, could be used to determine response to therapy.[93]

Many metabotypic traits are genetically determined in animals[43,44] and in humans.[49] The Molecular Phenotyping to Accelerate Genomic Epidemiology (MolPAGE) Consortium reported that metabotypes of human urine and plasma samples are stably controlled by genetic and environmental factors, which are not mutually exclusive.[52] In an mQTL study, the MolPAGE Consortium associated a polymorphism in a liver drug metabolizing enzyme, *N*-acetyl transferase 2, with the excretion of *N*-acetylated metabolites in urine.[45] The control of human metabolomic traits by genetic factors has been studied in mGWAS of the Cooperative Research in the region of Augsburg (KORA) cohort.[28,47–49] Findings from these studies led to development of the concept of human metabolic individuality[48,95] based on Garrod's chemical individuality. Likewise, an extensive analysis of 4 Finnish cohorts, comprising 2637 patients with metabolic syndrome and 7927 controls, both free of diabetes, was performed using the top loci in an independent sample with transcriptome and NMR-based metabonomic data.[96] This study associated an SNP in the *APOA1/C3/A4/A5* gene cluster (rs964184) with metabolic syndrome. The association was supported by analysis of serum metabolites, which associated rs964184 with very-low-density lipoprotein, triglyceride, and high-density lipoprotein metabolites. The study associated 22 previously identified susceptibility loci with individual metabolic syndrome component traits, most of which were lipid phenotypes, and no loci with 2 or more uncorrelated metabolic syndrome components. The genetic risk score, calculated based on the number of risk alleles in loci associated with individual metabolic syndrome traits, was strongly associated with metabolic syndrome status. The study concluded that genes that regulate lipid metabolism are involved in metabolic syndrome but

found little evidence for pleiotropy linking dyslipidemia and obesity to the other metabolic syndrome component traits, such as hypertension and glucose intolerance.

Monitoring Patients After Bariatric Surgery

Bariatric surgery is an effective treatment not only for morbid obesity but also for metabolic complications of obesity such as type 2 diabetes.[97] Gastric bypass causes a complex, systems-level response to coordinate changes in satiety hormones, bile acid signaling via nuclear receptors, and the intestinal microbiota to alter bile acid metabolites. In rats, bariatric surgery causes substantial shifts of the main gut phyla toward a higher proportion of *Proteobacteria* (52-fold increase, specifically in *Enterobacter hormaechei*) and a lower proportion of *Firmicutes* (4.5-fold decrease) and *Bacteroidetes* (2-fold decrease) compared with rats that underwent sham operations.[98] The changes in intestinal bacteria composition were mirrored by changes in fecal bile acids and cometabolic products of bacterial proteolysis and host detoxification, such as *p*-cresol sulfate, and were associated with changes in excretion of energy metabolites. Unintended consequences of bariatric surgery included a cytotoxic environment in the lumen of the distal bowel.[99]

After bariatric surgery, alteration of bile flow, reduced gastric size, anatomic gut rearrangement, altered nutrient flow, vagal manipulation, and enteric gut hormone modulation[100] all contribute to the resolution of obesity, type 2 diabetes mellitus, and therefore, by definition, metabolic syndrome.[31] Changes in the composition of the intestinal microbiota observed after bariatric surgery are likely to mediate many of these changes. Transplantation of gut microbiota from mice that underwent the Roux-en-Y gastric bypass (RYGB) procedure to those that did not undergo the surgery resulted in weight loss and reduced adiposity.[101] It will be important to define the metabolic basis for the resolution of diabetes in 80% of patients who receive RYGB.

Mutch et al profiled the serum metabolome of 14 women who underwent RYGB before and 3 and 6 months after surgery using gas chromatography–MS and LC-MS/MS.[102] Approximately 48% of the measured metabolites responded to RYGB, including BCAAs, unsaturated fatty acids, and sphingosines. The researchers identified a panel of 8 metabolites that could discriminate between obese and obese diabetic patients: lysophosphatidylcholine (C18:2), nervonate (C24:1), *p*-cresol sulfate, asparagine, lactate, lycopene, mannose, and glucose. *P*-cresol sulfate is a mammalian phase 2 detoxification product of *p*-cresol, a metabolite produced by the intestinal microbiota, providing further evidence for how the microbiome could mediate the effects of RYGB.[103] Because this was a small study that included patients with only positive outcomes from RYGB, it was not possible to distinguish the effects of surgery on type 2 diabetes from those of weight loss.

A study by Laferrère et al compared changes in circulating levels of amino acids and acylcarnitine between obese patients who lost weight after RYGB and those who received a dietary intervention.[104] The investigators found that RYGB, but not the diet intervention (which produced equivalent weight loss), reduced serum levels of total amino acids and BCAAs.

Assessment of Prognosis and Population Stratification

There is great potential for the use of metabonomic analyses in the clinic. Metabolic profiles of patients could provide information on their health status that can be tracked in real time or longitudinally.[105] For example, metabonomic data might be used in the management of metabolic syndrome, which has large geographical, interindividual, and intraindividual variations in phenotype. Disease prevention and therapeutic strategies could also be designed based on metabolic data; this pharmacometabonomic approach could be used to predict the toxicity or efficacy of specific drugs in different patients.[106]

The potential of pharmacometabonomics was shown in a study of 148 subjects assessed before and 6 weeks after treatment with 40 mg/day simvastatin.[107] The metabolic signatures of drug exposure among responders included essential amino acids, lauric acid, and α-tocopherol. Using pathway enrichment analysis, the investigators found that the metabolites of drug exposure were enriched for the pathway related to amino acid degradation. Reduction in low-density lipoprotein levels after treatment with simvastatin correlated with changed levels of cysteine, urea cycle intermediates, and the dibasic amino acids ornithine, citrulline, and lysine. These dibasic amino acids share plasma membrane transporters with arginine, the rate-limiting substrate for nitric oxide synthase, which mediates cardiovascular health. Levels of xanthine, 2-hydroxyvaleric acid, succinic acid, stearic acid, and fructose could be used to distinguish responders from nonresponders. Findings from this study indicate that clusters of metabolites involved in pathways not directly connected with cholesterol metabolism are associated with a response to simvastatin. Based on these metabotypes, it might be possible to identify patients most likely to benefit from this therapy.

Bao et al studied metabolic variations in 74 patients newly diagnosed with type 2 diabetes who were treated for 48 weeks with repaglinide, metformin, or rosiglitazone.[108] The investigators identified significantly altered metabolites in serum samples from diabetic subjects, including increased levels of valine, maltose, glutamate, urate, butanoate, and long-chain fatty acids (C16:0, C18:1, C18:0, octadecanoate, and arachidonate) and decreased levels of glucuronolactone, lysine, and lactate. The 3 diabetes drugs down-regulated levels of glutamate, but rosiglitazone was more effective in reducing levels of valine, lysine, glucuronolactone, C16:0, C18:1, urate, and octadecanoate.

The same approach has been applied to dietary intervention studies. Administration of either 15 mg vitamin D_3 or placebo each day[109] had no effect on measured markers of metabolic syndrome. K-means cluster analysis, based on 13 biochemical markers of metabolic syndrome and 25(OH)D concentrations, revealed 5 discrete biomarker clusters. One of these, characterized by lower serum levels of 25(OH)D and higher levels of adipokines, showed significant responses in insulin levels (15% decrease), homeostatic model

assessment scores (19% decrease), and C-reactive protein levels (54% decrease). Metabonomic analysis showed that the most discriminating metabolites included lactate, very-low-density lipoprotein, low-density lipoprotein, and choline. Other researchers studied the effects of different nut-containing diets in patients with metabolic syndrome. The urinary metabolome revealed 20 potential markers of nut intake, including fatty acid conjugated metabolites, phase II and microbial-derived phenolic metabolites, and serotonin metabolites.[109]

Finally, Spencer et al investigated the effects of depleting choline from the diet on microbiome composition and liver fat.[110] Interestingly, proportions of *Gammaproteobacteria* and *Erysipelotrichi* correlated with the level of liver fat in each subject during depletion of choline. These bacteria, liver fat content, and an SNP in the gene encoding phosphatidylethanolamine *N*-methyltransferase,[111] which catalyzes phosphatidylcholine synthesis, were used to create a model that accurately predicted the degree to which subjects developed fatty liver on choline-deficient diets. This study was one of the first to combine genomic, metagenomic, and metabolic markers to predict disease outcome. Altogether, these studies highlight the roles of the microbiome and microbial metabolism[71] on human physiology and pathology.

Future Directions

There have been many recent key technological advances in metabolic phenotyping and systems biology. Going forward, it will be important to:

- Determine the individual contributions of genetic and environmental factors, along with those of diseases (such as metabolic syndrome, obesity, and diabetes), to the metabolic phenotype
- Define how disease susceptibility genes affect the metabotype
- Determine how the microbiome affects, or is affected by, metabolic syndrome
- Translate metabotypes of cultured cells to in vivo systems and human disease
- Identify metabolomic markers of risk, development, or progression of metabolic syndrome
- Use metabolic markers to identify patients most likely to respond to bariatric surgery or other therapies.

Metabolic profiling technologies and the other top-down systems biology approaches are well suited for studies of metabolic syndrome and fatty liver disease. They will be used not only to identify markers of disease or predict the efficacy of treatments but also in hypothesis generation and identification of pathogenic mechanisms. Integration of metabolomic markers with genomic and metagenomic markers could increase our understanding of metabolic syndrome and fatty liver disease as well as responses to surgical procedures such as RYGB.

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