

## Review

# Multi-omics profiling: the way toward precision medicine in metabolic diseases

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**Metabolic diseases including type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), and metabolic syndrome (MetS) are alarming health burdens around the world, while therapies for these diseases are far from satisfying as their etiologies are not completely clear yet. T2DM, NAFLD, and MetS are all complex and multifactorial metabolic disorders based on the interactions between genetics and environment. Omics studies such as genetics, transcriptomics, epigenetics, proteomics, and metabolomics are all promising approaches in accurately characterizing these diseases. And the most effective treatments for individuals can be achieved via omics pathways, which is the theme of precision medicine. In this review, we summarized the multi-omics studies of T2DM, NAFLD, and MetS in recent years, provided a theoretical basis for their pathogenesis and the effective prevention and treatment, and highlighted the biomarkers and future strategies for precision medicine.**

**Keywords:** type 2 diabetes mellitus, non-alcoholic fatty liver disease, metabolic syndrome, biomarkers, multi-omics profiling, precision medicine

## Introduction

Abnormalities in energy metabolism can lead to conditions such as type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), and metabolic syndrome (MetS), which have become alarming health problems worldwide. T2DM, NAFLD, and MetS are three pathologic conditions that frequently coexist, while the incidence of NAFLD and MetS often parallels that of diabetes. T2DM is a complex disease characterized by chronic condition of hyperglycemia, insulin resistance, and insulin secretion defect. The causes of T2DM are not completely understood, but there are strong links of T2DM with overweight, obesity, and increasing age, as well as with ethnicity and heredity. NAFLD is a multifactorial disease with the biological basis of hepatocytic degeneration triggered by lipid metabolism disorder. Patients with NAFLD often have other metabolic disorders including obesity, T2DM, dyslipidemia, and insulin resistant, which is a key pathogenic trigger. Of note,

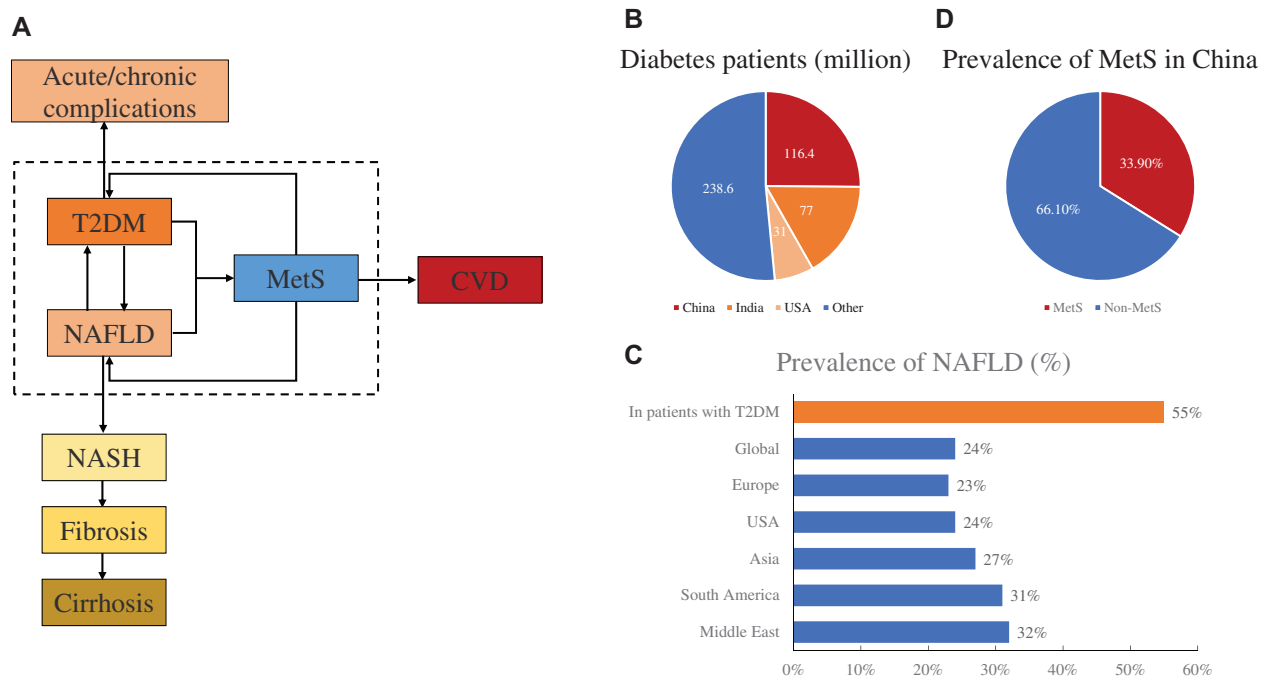
MetS is not a disease per se but rather a term that highlights traits in patients with an increased risk of cardiovascular disease (CVD) and T2DM. MetS refers to a group of clinical symptoms, including increased weight, insulin resistance, dyslipidemia, and hypertension, while NAFLD can be viewed as the hepatic manifestation of MetS (Figure 1).

Treatment for T2DM includes education, nutritional counseling, exercise, glucose monitoring, and anti-diabetic medications. Doctors choose different therapeutics based on the classification and clinical features of the patients (Kahn et al., 2014), following an algorithmic sequence according to relevant guidelines. However, not all choices are effective. Patient and clinical phenotypic characteristics such as sex, body mass index (BMI), age at diagnosis, baseline HbA1c, degree of  $\beta$ -cell dysfunction, insulin resistance, diabetes-associated antibodies, and specific mutations were associated with the response to specific anti-diabetic options (Jones et al., 2016; Dennis et al., 2018). Given this diversity, studies on modified therapies have either tried to stratify patients according to disease progression and risk of diabetic complications (based on phenotypic characteristics) (Ahlqvist et al., 2018) or used multivariable models containing these continuous clinical features to predict outcomes for individuals (Dennis et al., 2019). Both approaches represent new attempts toward precision medicine for diabetes. However, these

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**Figure 1** Internal relationships among T2DM, NAFLD, and MetS and their prevalence. **(A)** Relationships among T2DM, NAFLD, and MetS, and their internal relations. T2DM, NAFLD, and MetS are three pathologic conditions that frequently coexist, and they are all risk factors for CVD. **(B)** According to the 9th edition of the International Diabetes Federation Diabetes Atlas (<https://diabetesatlas.org/en/>), ~463 million people at the age of 20–79 years globally (i.e. 1 in 11 adults) had diabetes in 2019, with T2DM accounting for nearly 90% of diabetes cases worldwide. China maintains the largest number of adult diabetes patients with 116.4 million cases, followed by India with 77.0 million, and USA with 31.0 million cases. **(C)** The global prevalence of NAFLD is ~24%, with the Middle East (32%) and South America (31%) having the highest prevalence, followed by Asia (27%), USA (24%), and Europe (23%). The incidence of NAFLD among patients with T2DM is 55.5%, and patients with both diseases have a higher risk of NASH and liver fibrosis (Younossi et al., 2019). Evidence from a pooled population perspective study revealed that NAFLD increases the risk of T2DM and MetS by ~1.8 times (Ballestri et al., 2016). **(D)** Regarding MetS, the diagnostic criteria are not unified among different regions, the prevalence estimates vary, and no global data exist. According to previous researches, MetS is ~3 times more common than diabetes (Saklayen, 2018); therefore, the global prevalence can be estimated to be about one-quarter of the world population. According to a national survey covering 97098 adults over 31 provinces of mainland China in 2010, the prevalence of MetS in the general population is 33.9% (31.0% in males and 36.8% in females) (Lu et al., 2017).

strategies are non-etiological and highly dependent on the clinical variables included, which restricts their clinical utility.

NAFLD can progress from simple steatosis to non-alcoholic steatohepatitis (NASH) with variable degrees of fibrosis and cirrhosis. The management of patients with NAFLD should comprise treatment for the liver disease, as well as for the associated metabolic co-morbidities (Chalasani et al., 2012). Similarly, therapies for MetS targeting different metabolic abnormalities include lifestyle-based treatment, aiming to prevent CVD, T2DM, NAFLD, and other complications. As MetS is a group of metabolic abnormalities, there is no effective drug treatment to manage all of its components. Inflammation, gut microbiota, bile acid metabolism, microelements, and circadian rhythm have all been shown to play a role in NAFLD and MetS (Arrese et al., 2016; Handa et al., 2016; Arab et al., 2017; Chu et al., 2018; Moszak et al., 2020) and may represent novel targets for therapy.

Pathogenesis and treatment of T2DM, NAFLD, and MetS are complex and multifactorial, involving genetic, transcriptomic, epigenetic, proteomic, and metabolomic approaches.

The underlying mechanisms of the three metabolic disorders overlap and interact, although their individual characteristics are different. It is critical to characterize the major mechanisms involved in these disorders, in order to implement targeted and effective treatments. This remains the challenge in current treatment strategies and is also the goal of precision medicine.

The concept of precision medicine was first put forward in 2008 and suggested that clinicians should make a diagnosis based on molecular detection instead of clinical experience (Katsnelson, 2013). In 2011, precision medicine was further proposed by the National Institutes of Health to prescribe personalized medical treatments tailored to the specific characteristics of each patient (National Research Council (US) Committee on A Framework for Developing a New Taxonomy of Disease, 2011). Beyond traditional phenotypes, precision medicine may characterize a patient's condition using genetic, epigenomic, transcriptomic, proteomic, and metabolic information obtained from various omics approaches. As the power of single-omics data is limited, combining multi-omics data may

allow a more thorough and comprehensive summary of individual characteristics (Ritchie et al., 2015). A recent study (Chen et al., 2020) reclassified six types of metabolic diseases into three molecularly and clinically different groups based on metabolomics, proteomics, peptidomics, and clinical information using a multi-omics-based framework, in an attempt to unveil intra-disease heterogeneity and inter-disease similarities. The results can be used as a reference for data analysis of multi-omics investigations and precision medicine.

Hopefully, precision medicine will enhance treatment tolerability and effectiveness in individuals with metabolic diseases. However, before it becomes common practice, there is still a long way to go in multi-omics profiling assays and analyses. In this review, we systematically summarize omics' development, biomarkers, and their applications in precision medicine for the most prevalent metabolic disorders, including T2DM, NAFLD, and MetS.

### Genomics in metabolic diseases

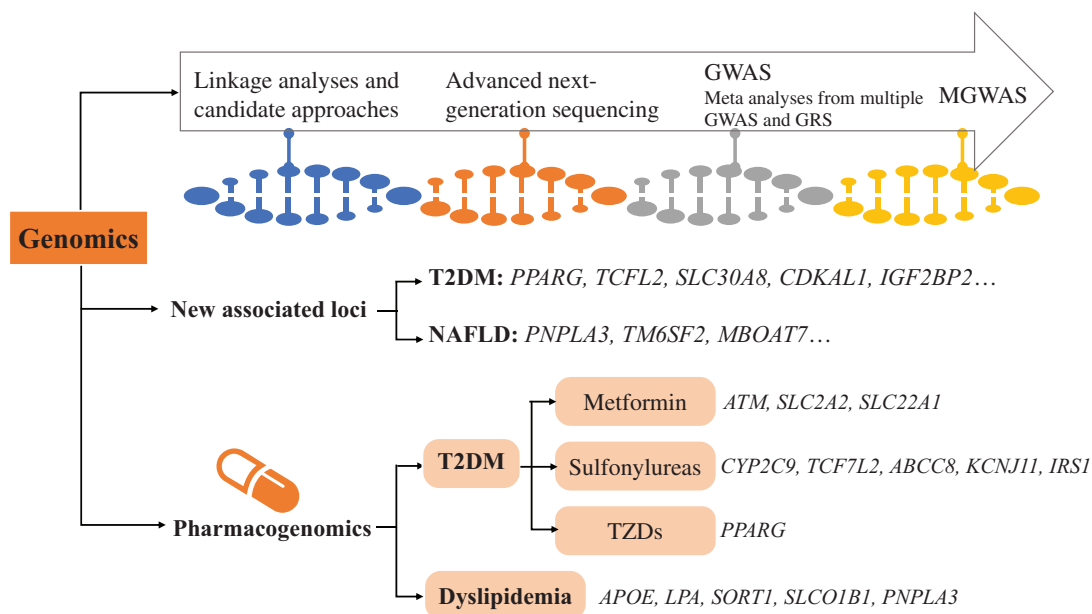
Genomics mainly studies the structure, evolution, mapping, editing, and function of an organism's whole genome (Figure 2).

#### Genetic biomarkers for T2DM

Familial aggregation (Meigs et al., 2000), ethnic differences (Kodama et al., 2013), and higher concordance rate of T2DM in monozygotic than in dizygotic twins (Poulsen et al., 1999) all indicate genetic contribution to T2DM. In the early 2000s,

peroxisome proliferator-activated receptor gamma (PPARG) (Altshuler et al., 2000) and transcription factor 7-like 2 (TCF7L2) (Grant et al., 2006) were confirmed to be associated with T2DM via linkage analyses and candidate approaches. With the development of advanced next-generation sequencing and extensive genome-wide association studies (GWAS), new T2DM-associated loci including solute carrier 30 A8 (SLC30A8), CDK5 regulatory subunit associated protein 1-like 1 (CDKAL1), and insulin-like growth factor 2 mRNA binding protein 2 (IGF2BP2) genes (Saxena et al., 2007; Scott et al., 2007; Wellcome Trust Case Control Consortium, 2007) were identified. The wave of GWAS was followed by meta-analyses combining data from multiple GWAS (Prasad and Groop, 2015), making these candidate loci more convincing. Apart from the organism's genome, metagenome-wide association studies (MGWAS) have linked gut microbiota dysbiosis with T2DM based on deep shotgun sequencing of the gut microbial DNA of 345 Chinese individuals (Qin et al., 2012). Thus, gut microbiota becomes a target in diabetes classification and therapy.

A single susceptible variant adds very little to the predictive power of T2DM risk (Poveda et al., 2016). Genetic risk score (GRS), the combined genetic information of multiple variants, can increase the predicting power. Researchers constructed three GRS containing different loci and explored the contribution of the GRS to the incidence of T2DM during a >9-year follow-up (Vaxillaire et al., 2014). Results showed that the two most inclusive GRS were significantly associated with increased fasting plasma glucose and increased incidence of impaired fasting



**Figure 2** Genomics of metabolic diseases. The linkage analyses and candidate approaches were first applied to diabetes study. With the development of advanced next-generation sequencing technologies and extensive GWAS, more new associated loci were identified. The wave of GWAS was then followed by meta-analyses combining data from multiple GWAS. And to improve the power in predicting the risk of metabolic diseases, genetic variants are aggregated into GRS. Meanwhile, MGWAS also help to characterize disease from the 'other genome', such as gut microbial. TZDs, thiazolidinediones.

glycemia and T2DM. By varying the number of single-nucleotide polymorphisms (SNPs) and their respective weights, various versions of GRS were computed and tested in the Estonian Biobank cohort. And the best-fitting GRS was chosen for the subsequent analysis of T2DM incident (386 cases) (Läll et al., 2017). The hazard for T2DM incident was 3.45 times higher in the highest GRS quintile compared with the lowest quintile. In addition, the proposed GRS would improve the accuracy of T2DM risk prediction by improving continuous net reclassification by 0.324 when added to the currently used set of predictors. Compared with conventional risk factor-based models (CRM), predictive performance of genetic variants was more powerful. A meta-analysis with 23 studies reported that the area under the curve (AUC) for T2DM increased with the addition of genetic information to CRM (median AUC was increased from 0.78 to 0.79) (Bao et al., 2013).

#### Genetic biomarkers for NAFLD and MetS

For NAFLD, there have been biochemical, imaging, genetic, and other omics biomarkers for its staging and progression (Wong et al., 2018). A strong heritability of NAFLD susceptibility has been identified in epidemiological, family, and twin studies (Dongiovanni and Valenti, 2016). The genetic component of NAFLD has emerged in GWAS recent years. Some genetic variants located in patatin-like phospholipase domain-containing protein 3 (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2), and membrane-bound O-acyltransferase 7-transmembrane channel like 4 (MBOAT7-TMC4) loci, respectively, have been associated with NAFLD susceptibility (Romeo et al., 2008; Kozlitina et al., 2014; Mancina et al., 2016), among which rs738409 in PNPLA3 is representative (Romeo et al., 2008). A meta-analysis showed that rs738409 exerts a strong influence on liver fat accumulation (Sookoian and Pirola, 2011). Additionally, rs738409 might influence the ability of weight loss to decrease liver fat and change insulin sensitivity after lifestyle intervention or bariatric surgery (Krawczyk et al., 2016). However, Kotronen et al. (2009) found that including the genetic variant rs738409 only improved the accuracy of NAFLD prediction by <1%. The missense SNP rs58542926 and the intronic SNP rs780094, located in TM6SF2 and GCKR, respectively, are both associated with a very modest risk, ~2-fold (Pirola and Sookoian, 2015) and 1.2-fold (Zain et al., 2015), respectively, for NAFLD progression. The loci uncovered by GWAS to date only explain a small fraction (<5%) of the total genetic heritability in NAFLD. Higher NAFLD-associated GRS was associated with increased liver fat accumulation in participants with lowest Mediterranean-style diet score or Alternative Healthy Eating Index scores, but not in those with stable or improved diet quality (Ma et al., 2018). NAFLD-associated GRS could also predict the development and prognosis of NAFLD. Three genetic variants including PNPLA3 were combined into a GRS in 110761 individuals from Copenhagen, Denmark, and 334691 individuals from the UK Biobank (Gellert-Kristensen et al., 2020). A higher GRS

was associated with increased plasma alanine aminotransferase, incidence of cirrhosis and hepatocellular carcinoma.

Genetic predisposition also contributes to MetS. Apart from T2DM susceptibility genes, genes encoding low-density lipoprotein (LDL), apolipoprotein E (ApoE), melanocortin 4 receptor, fat mass and obesity (FTO), and TCF7L2 have been associated with other components of MetS, including obesity, dyslipidemia, and hypertension (Taylor et al., 2013). However, GWAS have identified few genetic loci that contribute to all MetS traits (Abou Ziki and Mani, 2016), although the combined genetic contribution to obesity and abdominal body fat distribution may cause several MetS phenotypes (Emdin et al., 2017).

#### Pharmacogenomics of metabolic diseases

Pharmacogenomics is the branch of genetics that uses genetic variants to predict treatment responses, helping to build individualized therapies based on personal genetic characteristics.

##### T2DM

For T2DM, glycemic control varies among patients who receive similar anti-diabetic regimens. This variability may be attributed to biological and non-biological factors. Biological factors include genetic and non-genetic factors involved in pharmacokinetics and pharmacodynamics. Pharmacogenomics mainly concentrates on genetic polymorphisms that exert effects on pharmacodynamics and pharmacokinetics. Many studies have discovered key genetic polymorphisms responsible for the different efficacies of diabetic drugs, which is beneficial for precise medicine through identification of genetic markers. Genetic variants regulating genes encoding proteins involved in drug transport or metabolism are plausible candidates in pharmacogenomics of T2DM. The pharmacogenomics of different anti-diabetic drugs are summarized in Table 1.

##### NAFLD and MetS

Considering that, apart from extrinsic factors such as diet, environmental chemicals, alcohol, and drug-drug interactions, genetic factors also contribute greatly to the development of NAFLD and MetS (Dongiovanni and Valenti, 2016), pharmacogenomics studies for putative drugs for NAFLD and MetS are ongoing for personalized treatment. Currently, there is no approved therapy specific for NAFLD and MetS. Statins are candidate agents for lowering LDL-cholesterol levels, and dyslipidemia is a common comorbidity in NAFLD and a common trait in MetS. The pharmacogenomic studies of statins are also shown in Table 1.

Although pharmacogenomics studies aiming new perspectives on precision medicine are flourishing, they remain in early stages due to the complex etiology of metabolic diseases. Large cohorts with well-defined phenotypes and genomic data are essential to tailor the most appropriate treatment for metabolic diseases.

### Transcriptomics in metabolic diseases

Environmental and other factors influence the expression of genes, thus affecting an individual's phenotype and risk of metabolic disease. Current transcriptomics studies for metabolic disease have mostly focused on islets and peripheral tissues, including the liver, skeletal muscle, and adipose tissue.

#### T2DM

Oligonucleotide microarrays were among the first tools to study transcriptome changes in T2DM patients. And RNA-sequencing (RNA-seq) has greatly improved knowledge on gene expression in T2DM. Given the variability in islet cell type composition, single-cell RNA-seq has been an important breakthrough to detect cell type-specific transcriptomic features. Studies have identified transcriptional differences in islets, liver, muscle, adipose tissue, and peripheral blood using these techniques, which are shown in [Table 2](#).

#### NAFLD and MetS

To date, transcriptomics studies on NAFLD mainly focus on the liver ([Table 2](#)). A differential expression analysis in severe vs. non-severe NAFLD and normal liver ([Baselli et al., 2020](#)) showed 320 genes differentially expressed in severe NAFLD. Of these, 16 genes were deregulated in PNPLA3 rs738409 variant carriers. The authors also identified a higher expression of genes involved in hepatic fibrogenesis, among which interleukin 32 (IL-32) was the most robustly upregulated in severe NAFLD, together with suppressor of cytokine signaling 1 (SOCS1) and

aldo-keto reductase family 1 member B10 (AKR1B10). In another study ([Suppli et al., 2019](#)), liver transcriptome profiles of healthy normal-weight individuals and obese individuals cluster together and are clearly separated from NAFLD/NASH patients. Gene regulation in patients with NAFLD and NASH was found to be associated with stimulated synthesis of fatty acids and cholesterol, increased lipoprotein activity, impaired insulin function, increased farnesoid X receptor (FXR) signaling, modulation of monocyte differentiation and recruitment, inflammation signaling, proapoptotic activity, and stimulated collagen formation ([Suppli et al., 2019](#)). Although these transcriptomic studies on NAFLD are observational, they have offered some clues for the treatment of NAFLD, which is beneficial for precision medicine. As to MetS, most current studies have focused just on specific traits, while few study covers all the aspects of MetS.

### Epigenomics in metabolic diseases

Regulatory mechanisms of gene expression, such as epigenetics, may influence disease susceptibility more than genetics. Epigenetic regulation includes multiple layers, including DNA methylation, histone modifications, higher-order chromatin structure, and non-coding RNAs such as microRNAs (miRNAs), which can regulate cell differentiation, cell-specific gene expression, parental imprinting, X chromosome inactivation, as well as genomic stability and structure.

**Table 1** Pharmacogenomics of metabolic diseases.

Disease	Drugs	Genes	References
T2DM	Metformin	ATM, SLC2A2, SLC22A1, SLC22A2, SLC47A1	Becker et al. (2009a, b); DeGorter and Kim (2009); Zhou et al. (2011, 2016); Duong et al. (2013); Dujic et al. (2015); Mahrooz et al. (2015)
	Sulfonylureas/glinides	PSMD6, CYP2C9, TCF7L2, ABCC8, KCNJ11, IRS1, CYP2C8	Niemi et al. (2003); Holstein et al. (2005); Ragia et al. (2009); Chen et al. (2015); Song et al. (2017); Mannino et al. (2019)
	Thiazolidinediones	PSMD6, PPARG	Chen et al. (2015); Mannino et al. (2019)
	DPP-4 inhibitors/GLP-1 receptor agonists	GLP-1R	Mannino et al. (2019)
NAFLD and MetS	SGLT-2 inhibitors: empagliflozin	SLC5A2	Zimdahl et al. (2017)
	Statins	APOE, SLCO1B1, PNPLA3	Link et al. (2008); Postmus et al. (2014); Dongiovanni et al. (2015)

**Table 2** Transcriptomics in metabolic diseases.

Disease	Transcripts	Tissue	Change	References
T2DM	HNF4a, IRS2, AKT2, IGFBP2, FXYD2	Islets	Downregulated	Gunton et al. (2005); Marselli et al. (2010); Segerstolpe et al. (2016); Lawlor et al. (2017)
	DLK1, DGK $\beta$	Islets	Upregulated	Segerstolpe et al. (2016); Lawlor et al. (2017)
	ANK1	Skeletal muscle	Upregulated	Scott et al. (2016)
	ST6GAL1, THBS2	Adipose tissues	Upregulated	Saxena et al. (2019)
	IRS1, WFS1, KCNQ1	Peripheral blood mononuclear cells	Downregulated	Li et al. (2016)
NAFLD		Liver	Upregulated	Suppli et al. (2019); Baselli et al. (2020)



## T2DM

As a disease affecting multiple organ systems, the decline of pancreatic  $\beta$ -cell function and insulin resistance in insulin target organs, such as skeletal muscle, liver, and adipose tissue, are all important factors in T2DM development. The tissue-specific epigenetic changes are shown in Table 3. These studies identified epigenetic changes in T2DM patients, and the regions were also associated with differential expression of genes. Further studies are needed to identify causal epigenetic changes that were responsible for T2DM and related traits.

miRNAs have also been intensely investigated as potential biomarkers for T2DM. They are small RNA molecules ranging from 18 to 22 nucleotides in size; they regulate gene expression by binding to target mRNAs at 3' untranslated regions, targeting them for cleavage or translational repression (Bartel, 2004). In T2DM, the first study to reveal a plasma miRNA signature for T2DM was performed in a large population-based cohort involving 822 individuals from the Bruneck study (Zampetaki et al., 2010). The initial microarray screening and quantitative polymerase chain reaction assessment revealed lower plasma levels of miR-20b, miR-21, miR-24, miR-15a, miR-126, miR-191, miR-197, miR-223, miR-320, and miR-486 in prevalent diabetes, but a modest increase in miR-28-3p levels. Importantly, the observed reductions in miR-15a, miR-29b, miR-126, and miR-223 levels and elevated increases in miR-28-3p levels antedated the manifestation of the disease. Interestingly, 91/99 (92%) controls and 56/80 (70%) diabetes cases were correctly classified using expression profiles of the above five most significant miRNAs.

## NALFD

High-fat diet (HFD) can induce modifications in the chromatin structure, thereby contributing to metabolic disease (Leung et al., 2014). FAIRE-seq was performed in the livers of C57BL/

6J mice induced by HFD and control diet, which identified 28484 open chromatin sites in control and 28253 sites in high-fat livers. The regions of greatest variation are targeted by liver transcription factors, including HNF4 $\alpha$ , CCAAT/enhancer-binding protein  $\alpha$  (CEBP $\alpha$ ), and forkhead box A1 (FOXA1) (Leung et al., 2014). These altered chromatin accessibility factors further changed gene expression, including that of *Lpin1*, which contains transcriptional factor combining sites. HFD leads to chromatin remodeling in mouse liver tissue to change lipid metabolism, which elucidates regulatory mechanisms associated with metabolic disorders such as obesity and hepatic steatosis.

miR-122 is the most abundantly expressed miRNA in hepatocytes, representing 70% of the total miRNA content. Its down-regulation has been robustly validated in metabolic disorders, including liver steatosis and fibrosis, both in vivo and in vitro, and shown to be involved in the upregulation of fibrotic pathways by inducing hypoxia-inducible factor-1 $\alpha$  and mitogen-activated protein kinase 1 (Csak et al., 2015; Pirola et al., 2015). Increasing evidence suggests that miR-21 and miR-34a regulate hepatic lipogenesis, lipid secretion, and glucose metabolism deficits in the pathogenesis of NAFLD (Xu et al., 2015; Calo et al., 2016). These promising results indicate that miRNAs may be useful tools for early prediction of NAFLD. However, because of different study designs, insufficient sample size, and different miRNA measurements, the current findings show minimal replication.

## MetS

Certain epigenetic changes were identified in different tissues according to previous tissue-specific DNA methylation analyses. The related epigenetic modifications in MetS are also shown in Table 3. In addition, several other miRNAs, including miR-126, miR-24, miR-181b, and miR-150, have been associated

**Table 3 Epigenomics of metabolic diseases.**

Disease	Epigenomics	Cell or tissue type	Change	References
T2DM	INS, PDX1, PPARGC1A, ADCY5, FTO, HHEX, IRS1, KCNQ1, PPARG, TCF7L2, GLP1R	Pancreatic islets	DNA methylation	Ling et al. (2008); Yang et al. (2011, 2012); Hall et al. (2013); Dayeh et al. (2014)
	MALT1	Whole blood	DNA methylation	Yuan et al. (2014)
	PPARG, KCNQ1, TCF7L2, IRS1	Adipose tissue	DNA methylation	Nilsson et al. (2014)
	PDGFA	Liver	DNA methylation	Abderrahmani et al. (2018)
	PPARGC1A promoter	Skeletal muscle	DNA methylation	Brons et al. (2010)
NAFLD	H2K9me2 sites in the PTEN and IL-1A promoter region	Peripheral blood mononuclear cell	Histone modifications	Miao et al. (2007); Hou et al. (2011); Paneni et al. (2015)
	SREBF2, FASN, AGPAT3, ESR1, HNF4 $\alpha$ , CEBP $\alpha$ , FOXA1	Liver	DNA methylation Chromatin structure modifications	Bruce et al. (2009) Leung et al. (2014)
MetS	FTO, HIF3A, IRS1	Adipose tissue	DNA methylation	Almén et al. (2012, 2014); Aslibekyan et al. (2015)
	IL-18 and MECP2	Skeletal muscle	DNA methylation	Barrès et al. (2012); Ling and Rönn (2019)
	ABCG1, CD38, CPT1A	Blood	DNA methylation	Aslibekyan et al. (2015); Ling and Rönn (2019)
	PPARG promoter region	Adipose tissue	Chromatin structure modifications	Huang et al. (2018)

with obesity and metabolic disorders (Sun et al., 2016; Ying et al., 2016). MetS is considered to be a heterogeneous disease, and obesity is a central component of the disease. Current research supports a role of BMI in epigenetic changes and disease pathogenesis. Further studies are required to illustrate the biological meaning of epigenetic variability.

Epigenetic modification is an important mechanism linking the environment with gene expression changes for metabolic diseases. However, their targets and underlying mechanisms are still unclear, and further exploration is needed to link these epigenetic changes to precision medicine in T2DM, NAFLD, and MetS.

### Proteomics of metabolic diseases

Over the past decades, technical advances in proteomics and improved tools in bioinformatic analysis have driven remarkable progress in proteomics science.

#### T2DM

Proteomics can be applied to disease biomarker discovery and the exploration of disease pathogenesis. T2DM is usually diagnosed by fasting glucose, 2-h glucose, or HbA1c concentrations. In addition, serum insulin concentrations are used to calculate the homeostasis model assessment index to evaluate insulin resistance. However, there are still limitations in the assessment of the occurrence, development, and prognosis of diabetes, especially of the process from pre-diabetes to diabetes. There are several proteins associated with incidence and progression of T2DM, which are shown in Table 4. The approach of constructing a model comprising multiple serum biomarker seemed to be promising and critical for the detection, diagnosis, and prognosis of T2DM (Kolberg et al., 2009); however, relevant findings have not been routinely used in clinical laboratory tests. Moreover, it is challenging to characterize the broad and dynamic spectrum of serum proteins, especially in the case of low-abundance proteins. Thus, more large-scale prospective follow-up studies are needed to explore and verify the sensitivity, validity, reliability, and reproducibility of T2DM biomarkers.

#### NAFLD

In the case of NAFLD, the first study on serum protein profiles used surface-enhanced laser desorption/ionization time of flight mass spectrometry on 98 obese patients (91 were diagnosed with NAFLD; seven obese participants without NAFLD served as study controls), revealing 12 significantly different protein peaks (Younossi et al., 2005). ApoE and lymphocyte cytosolic protein 1 (LCP1) were significantly upregulated, while IGFBP3 and vitamin D-binding protein were downregulated in patients with NASH compared with healthy subjects (Miller et al., 2014). In addition, growing evidence indicates that mitochondrial abnormalities may be involved in the pathogenesis of NAFLD (Rector et al., 2010). Carbamoyl phosphate synthetase 1, as a specific mitochondrial enzyme, regulates the urea cycle; it is deacetylated by sirtuin 5 in the mitochondrial matrix during caloric restriction (Nakagawa et al., 2009). This mechanism could potentially explain why patients with NAFLD have higher serum uric acid concentrations (Sirota et al., 2013).

#### MetS

In the exploration of the mechanism underlying MetS, proteomics has enabled significant advances. Evidence indicates that hyperglycemia induces metabolic changes in  $\beta$  cells that markedly reduce mitochondrial metabolism and adenosine triphosphate (ATP) synthesis (Haythorne et al., 2019). A study using phospho-proteomics revealed the glycogen synthase kinase 3-pancreatic and duodenal homeobox 1 axis as a key pathogenic signaling node in insulin secretion (Sacco et al., 2019). Insulin resistance is the main pathophysiological mechanism of MetS as well as diabetes. Another study showed that the increased abundance in protein heat shock protein A5, HSP90AB1, and collagen type VI  $\alpha$ 1 chain was indicative of increased cellular stress, while the downregulation of ATP synthase-subunit and creatine kinase B pointed toward perturbations in ATP synthesis and mitochondrial metabolism in T2DM (Højlund et al., 2003). This is consistent with studies on mitochondrial oxidation dysfunction in the skeletal muscle of obese individuals and patients with T2DM (Mootha et al., 2003; Giebelstein et al., 2012). Hittel et al. (2005) proposed that increased protein and enzymatic activity of adenylate kinase 1 (AK1) is representative of a

**Table 4** Proteomics of metabolic diseases.

Disease	Proteomics	Cell or tissue type	Change	References
T2DM	MASP	Plasma	Elevated	von Toerne et al. (2016); Krogh et al. (2017); Huth et al. (2019)
	Leptin, t-PA, Renin, 1L-1ra, HGF, Cathepsin D, FABP4	Plasma	Elevated	Nowak et al. (2016)
	Fetuin-A	Plasma	Elevated	Ix et al. (2008); Sujana et al. (2018)
NAFLD	ApoE, LCP1	Serum	Upregulated	Miller et al. (2014)
	IGFBP3, vitamin D-binding protein	Serum	Downregulated	Miller et al. (2014)
MetS	HMGCoA synthase, HMGCoA reductase, DHCR7	Pancreatic islets	Upregulated	Haythorne et al. (2019)
	ATP synthase-subunit, creatine kinase, brain isoform, MRLC2-A	Skeletal muscle	Downregulated	Højlund et al. (2003)
	Phosphoglucomutase-1, HSP90 $\beta$ , GRP78, MRLC2-B, $\alpha$ 1(VI) collagen	Skeletal muscle	Upregulated	Højlund et al. (2003)

compensatory glycolytic drift to counteract reduced mitochondrial function. Furthermore, studies have discovered potential phosphorylation sites indicative of abnormalities in mitochondrial oxidative metabolism and reduced AK1 content in obesity and T2DM (Højlund et al., 2003, 2010). Hepatic tissue proteomics analysis using various animal models, including the db/db mice (Guzmán-Flores et al., 2018), T2DM rhesus macaque (Du et al., 2017), insulin receptor-knockout mice (Capuani et al., 2015), and insulin-resistant Akt1<sup>+/-</sup>/Akt2<sup>-/-</sup> mice (Pedersen et al., 2015), have revealed differentially expressed proteins involved in biological processes such as glucose metabolism (glycolysis/gluconeogenesis), lipid metabolism (fatty acid metabolism), mitochondrial function, and oxidative stress in various abnormal metabolic statuses.

The adipose tissue proteins identified in proteomic studies addressing diabetes and insulin resistance mainly participate in energy and metabolism, immune response/inflammation, oxidative stress, cytoskeleton, and apoptosis/cell cycle (Murri et al., 2013, 2014; Kim et al., 2014; Gómez-Serrano et al., 2016; Alfadda et al., 2017). Changes in mitochondrial protein expression during adipogenesis also indicate that mitochondrial biogenesis and remodeling are key events in white adipocyte differentiation (Wilson-Fritch et al., 2003). However, due to the high lipid content in complex adipose tissue cell lysates, appropriate separation techniques prior to analysis are needed to avoid masking the detection of low-abundance proteins. Since the discovery of leptin, adipocyte-secreted proteins are of particular interest when examining adipocyte dysfunction. DPP-4 was identified as a novel adipokine via comprehensive proteomic profiling of the primary human adipocyte proteome (Lamers et al., 2011). Neu-related lipocalin, a novel adipokine identified by high-throughput proteomics (Chen et al., 2005), has been demonstrated to participate in energy metabolism, glucose and lipid homeostasis, and insulin resistance (Law et al., 2010). These proteomics-based studies on adipose tissue or adipocytes provide important insight on the link between adipose dysfunction with obesity and MetS.

With the development of mass spectrometers and the improvement of information technology, including tools and databases for data availability, the field of proteomics has greatly expanded. Thus, translation medicine combined with metabolic characteristics and protein analysis in tissue biopsies will help make substantial progress in understanding the mechanisms underlying the pathogenesis and progression of metabolic diseases.

#### Pharmacoproteomics of metabolic diseases

Pharmacoproteomics is the application of proteomics to pharmacological issues, which is useful in characterizing drug mode of action, side effects, toxicity, and resistance. In an early pharmacoproteomic study, the liver tissue from obese diabetic mice (ob/ob) was used to examine the effects of the well-characterized highly selective PPAR $\alpha$  agonist—WY14 643 (Edvardsson et al., 1999). And 14 proteins affecting the peroxisomal fatty acid synthesis were identified (Edvardsson et al., 2003). Proteomics studies using

the liver, white and brown adipose tissue, and muscle showed that rosiglitazone affected protein expression involved in fatty acid and carbohydrate metabolism (Sanchez et al., 2003). Rosiglitazone has been found to bind to and activate PPAR- $\gamma$ 1 in adipocytes and PPAR- $\gamma$ 2 in hepatocytes of lep/lep mice and 11 polypeptides were significantly modulated by rosiglitazone treatment of the obese mice (Sanchez et al., 2003). A differential analysis of secreted proteins released from rat adipocytes in the conditioned medium treated with and without insulin revealed the changes that occur in adipokines (Chen et al., 2005). These studies focused on the changes in protein secretion on drug therapy, providing early insights into the pharmacoproteomics approaches on metabolic disorders. Besides, bioinformatic solutions for proteomic data management are also urging.

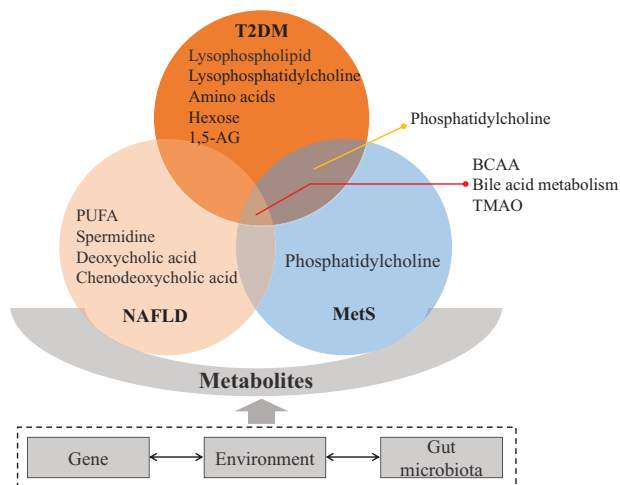
#### Metabolomics in metabolic diseases

Located on the downstream of other omics, metabolomics provides an integrated profile of pathophysiological status and a complement to other omics analyses. Metabolomics has been used to evaluate metabolite changes in humans, animals, plants, and other systems to assess their status and search for biomarkers for pathogenesis, therapeutic responses, and prognosis of diseases. Metabolic diseases including T2DM, NAFLD, and MetS comprise a series of metabolic disturbance in carbohydrates, lipids, and proteins; therefore, metabolomics is quite feasible for studying these disorders (Figure 3).

#### T2DM

Circulating metabolite patterns, including inhibited lysophospholipids, altered composition of the bile acid pool, and reduced branched-chain amino acid (BCAA) concentration, are predictive for T2DM, according to a prospective cohort study (Zeng et al., 2019). These metabolite patterns can monitor T2DM risk >10 years prior to disease onset. Several other studies have also revealed that the altered metabolism of amino acids, lipids, bile acids, and carbohydrates is associated with the incidence of T2DM (Fall et al., 2016; Qiu et al., 2016). Among these metabolites, circulating BCAA concentrations can be elevated up to 1.5-fold in patients with T2DM than in healthy subjects (Guasch-Ferré et al., 2016), and thus have been used as markers for the development of insulin resistance (Würtz et al., 2013). Furthermore, the causal role of BCAA metabolism in T2DM and insulin resistance has been verified via Mendelian randomization analysis (Mahendran et al., 2017). All these studies prompt that BCAA may lie on the pathway from insulin resistance to T2DM. In isolated rat  $\beta$  cells, lysophosphatidylcholine promotes insulin secretion via an orphan G protein-coupled receptor (Soga et al., 2005), prompting that lysophospholipid metabolism may be associated with insulin secretion. Both FXR and the G protein-coupled bile acid receptor 1, also known as TGR5, are prominent signaling molecules mediating bile acid signaling (Chiang, 2017). In mouse models, activation of FXR represses the expression of gluconeogenic genes, decreases serum glucose,





**Figure 3 Metabolomics of metabolic diseases. Metabolites are the interactions of genes, environment, and gut microbiota, and they can enable a better characterization of individuals beyond traditional classification, which is beneficial for precision medicine. Many of these metabolites are tightly connected with T2DM, NAFLD, and MetS, which are associated with insulin resistance, bile acid, and lipid metabolism. Among these metabolites, BCAA, bile acid metabolism, and TMAO are associated with T2DM, NAFLD, and MetS. Phosphatidylcholine is associated with T2DM and MetS.**

and improves insulin sensitivity (Zhang et al., 2006). Activation of TGR5 stimulates GLP-1 release from enteroendocrine L cells (Thomas et al., 2009). These functional studies indicate that bile acids are important metabolic regulators of glucose metabolism, therefore suggesting that both FXR and TGR5 may be targets for diabetes therapy. Carbohydrate metabolite alterations are mainly due to the dysregulation of glucose, as well as glycolipid and glycoprotein biosynthesis and degradation. Metabolites such as 1,5-anhydroglucitol (1,5-AG), the 1-deoxy form of glucose in circulation, are currently used as a monitor of short-term glycemic control in patients with diabetes (McGill et al., 2004). Metabolomic profiles, including amino acids, phosphatidylcholine, and hexose, are associated with HbA1c levels in T2DM (Yun et al., 2019).

#### NAFLD and MetS

Besides T2DM, many of the identified metabolites associated with insulin resistance, lipids, and bile acid metabolism are also tightly connected with NAFLD and MetS. Fasting plasma BCAA levels correlate with NAFLD severity in women according to a cohort study (Grzych et al., 2020). Polyunsaturated fatty acid metabolites are distinct between NAFLD and NASH, thus offering potential biomarkers for the non-invasive diagnosis of NASH (Loomba et al., 2015). Gut microbiota profiling is also associated with NAFLD (Del Chierico et al., 2017). Multiple metabolites were found to be associated with the histological severity of NAFLD in a study using a multiplatform

metabolomics approach (Ioannou et al., 2020); among them, spermidine levels were 2-fold lower in advanced than in early fibrosis, supporting spermidine's protective role against NAFLD progression. Another study showed that the primary to secondary bile acid ratio is higher in patients with NASH than in healthy controls (Mouzaki et al., 2016). Jiao et al. (2018) reported an elevation in bile acid production in patients with NAFLD, consistently supported by the hepatic gene expression pattern and gut microbiome composition in these patients. Moreover, the levels of deoxycholic acid, an antagonist of FXR, which is a key molecule in bile acid metabolism, were shown to be increased in NAFLD, whereas those of the FXR agonist chenodeoxycholic acid were decreased. These results suggest that FXR signaling and the gut microbiome are promising targets for NAFLD interventions.

MetS is characterized by several metabolite changes in the plasma, reflecting abnormalities in several metabolic pathways. Multiple investigations have revealed changes in amines, amino acids, and lipids in the setting of MetS. Gut-derived metabolite trimethylamine-N-oxide (TMAO) was found to be positively associated with BMI, visceral adiposity index, insulin resistance, and MetS (Barrea et al., 2018). BCAAs, including isoleucine, leucine, and valine, were shown to play an important role in the development of metabolic disease (Newgard, 2012). Moreover, tyrosine and isoleucine levels were significantly elevated in patients with nascent MetS without T2DM and CVD, indicating that they might be early biomarkers for MetS (Reddy et al., 2018). Phosphatidylcholine 34:2 was also shown to be significantly elevated in nascent MetS and correlated with waist circumference, plasma glucose, serum lipids, and pro-inflammatory markers, suggesting that it may participate in MetS via the inflammatory pathway (Ramakrishnan et al., 2018). Collectively, these findings suggest that metabolic changes are tightly connected with MetS, and such metabolites may be used as potential biomarkers or therapeutic targets in MetS.

#### Pharmacometabolomics of metabolic diseases

Metabolomics offer a better characterization of individuals beyond traditional classification, which is beneficial for the personalized treatment of metabolic disorders (Jacob et al., 2019). Metabolic phenotypes, as the direct reflection of the effects of environmental factors (such as nutritional status, gut bacteria, age, concomitant disease, and drug use) on the organism, are key determinants of individual pharmacokinetics (Navarro et al., 2016), drug metabolism (Clayton et al., 2009), efficacy (Trupp et al., 2012), and adverse responses (Weng et al., 2016). The application of metabolomics in pharmacology gave rise to a new field called 'pharmacometabolomics', the basic aim of which is to determine the effects of drug treatment on the body's metabolic scenario. It is also used to identify specific metabolic pathways responsible for drug-mediated outcomes and for developing new drugs (Kaddurah-Daouk and Weinshilboum, 2014). A cohort study of 22 patients with T2DM showed urine

metabolic differences between metformin responders and non-responders, with three metabolites, citric acid, myoinositol, and hippuric acid, identified as predictive of metformin response (Park et al., 2018). Another post hoc analysis found that the levels of metabolites such as valine, tyrosine, carnitine, and leucine/isoleucine are associated with metformin treatment but not predictive of the glucose-lowering effect of metformin (Safai et al., 2018).

Based on the fact that NAFLD and MetS are affected by gut microbiota (Ji et al., 2019; Moszak et al., 2020), administration of prebiotics or probiotics is beneficial for metabolic disorders via increasing the gut microbiota (Santos-Marcos et al., 2019). As endotoxin-induced cytokines play a role in NAFLD, administration of rifaximin, a non-absorbable antibiotic for Gram-negative bacteria, appears to be effective in NAFLD treatment (Gangarapu et al., 2015). However, insulin resistance and NAFLD were more severe in mice that received lifelong sub-therapeutic antibiotic treatment, possibly due to microbiome perturbation caused by the antibiotics (Mahana et al., 2016). Therefore, the role of different microbiota in body metabolism should be further investigated. Besides microbiota, it is also revealed that bile acids and their derivatives are useful in MetS treatment (anić et al., 2018). As FXR signaling plays a role in metabolic diseases, an FXR agonist was found to ameliorate insulin resistance and metabolic abnormalities in a rabbit model of MetS (Maneschi et al., 2013); however, its role in human MetS remains unknown.

Metabolomics will undoubtedly play a determinant role in accelerating the understanding of pathogenesis, effective prevention, treatment of T2DM, NAFLD, and MetS; however, most metabolic studies are in the discovery stage, and the role of the identified metabolites in pathogenesis needs further certification. More mature technologies, new analytical methods, and the integration of different omics are also indispensable.

### Integrating multi-omics

Interpretation of omics studies at a multi-omics level is essential for the comprehensive analysis of metabolic diseases, important for prediction, diagnosis, and treatment (Figure 4). Rapidly evolving technologies have offered unparalleled opportunities to assess and integrate individual omics data, which has helped capture biological variation to facilitate specific clinical treatment.

#### T2DM

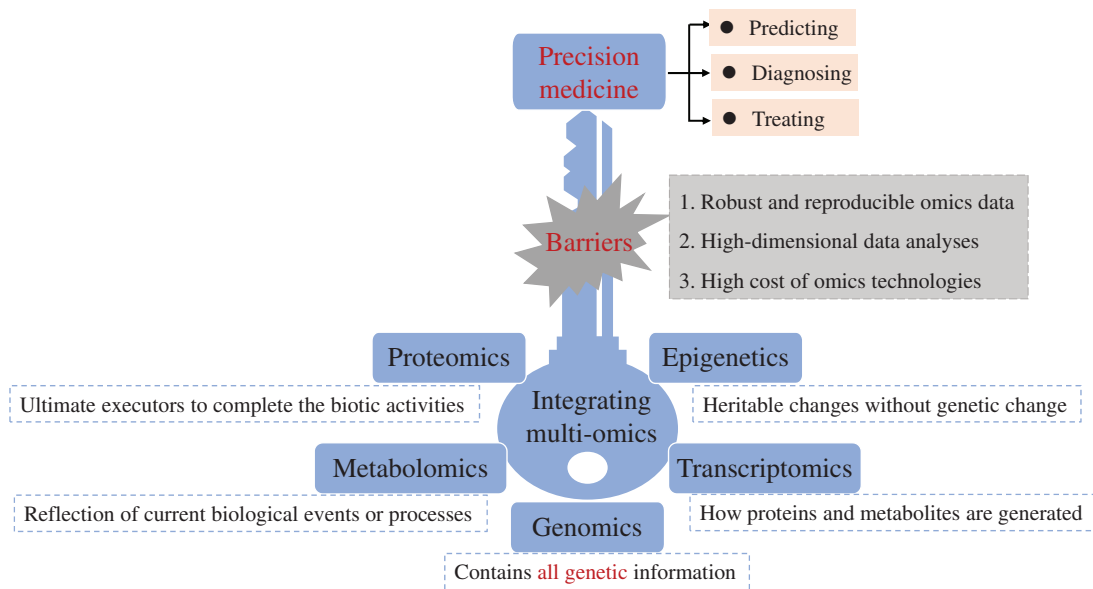
T2DM has been the focus of most multi-omics studies. In an analysis of 1622 non-diabetic participants, the combination of genetics, metabolomics, and clinical factors improved the prediction of future T2DM (Walford et al., 2014). Specifically, a 62-variant GRS showed an AUC of 64%; the addition of metabolites increased the AUC to 82%, while the combination

of genetics, metabolomics, and clinical factors achieved an AUC of 88%.

Besides using multi-omics for T2DM prediction, recent studies have also combined multiple data sources with treatment responses, paving the way for future precision medicine in T2DM and other metabolic diseases. Clinical management of T2DM mainly focuses on reducing plasma glucose level and lowering the risk of diabetic complications. However, significant variability exists in responses to even the same intervention. Thus, a better understanding of the underlying causes of different pharmacological responses is necessary to catalyze the development and implementation of the most accurate intervention strategy based on a patient's unique characteristics. This is the foundation of integrated multi-omics data that allows for implementing precision medicine for T2DM. For example, researchers have combined information collected by genomics, metabolomics, proteomics, and microbiome analyses in an integrated framework to develop personalized dietary interventions for T2DM (Price et al., 2017). Moreover, studies have integrated data on dietary intake, biomarkers, physical activity, sleep, anthropometric variables, and gut microbiota using an appointed algorithm, reporting that nutritional interventions based on this algorithm are more effective than traditional dietary advice in reducing postprandial blood glucose (Zeevi et al., 2015). GWAS have also been integrated with high-throughput metabolomic profiling to provide biological insights into how genetic variation influences metabolism and how such metabolic differences in plasma can help to identify relevant genes within genomic regions associated with T2DM (Shin et al., 2014). Besides, deep learning methods, which can identify highly complex patterns in large datasets, have been shown to be useful in disease predictive models and biological mechanism prediction (Zou et al., 2019). Taken together, these findings suggest that a multi-omics approach provides complementary information for the prediction and clinical treatment of T2DM. In the near future, deep learning methods can also be applied in multi-omics studies on T2DM.

#### NAFLD

In the case of NAFLD, the combination of genetic and metabolic parameters has been reported to improve the accuracy of diagnosis without requiring the implementation of liver biopsy. Moreover, the combination of the extended fatty liver index, calculated based on the oral glucose tolerance test-derived fold change in plasma triglycerides, along with 2 h blood glucose and the rs738409 C>G SNP in PNPLA3 was shown to improve the predictive power in NAFLD diagnosis (Kantartzis et al., 2017). Additionally, Perakakis et al. (2019) designed a non-invasive model consisting of lipids, glycans, and hormones that could diagnose the presence of NAFLD with very high accuracy (>90%). Pirola and Sookoian (2018) performed an integrative analysis by selecting a list of genes associated with NAFLD and metabolites known to be altered in NAFLD and NASH. The



**Figure 4 Integrating multi-omics. Genomics contains all genetic information. Proteins are the ultimate executors to complete biotic activities and the determinants of different biological statuses. Metabolomics is a reflection of current biological events or processes, while epigenetics and transcriptomics suggest how these changes (proteins and metabolites) are generated. Therefore, interpretation at multi-omics levels is beneficial for a comprehensive analysis of metabolic diseases, which is important in predicting, diagnosing, and treating.**

authors identified two pathways involved in NAFLD pathophysiology: ABCC and SLC transporters pathways.

#### Challenges in integrating multi-omics data into precision medicine

Despite these advances, integrating multi-omics in metabolic diseases is still in its infancy, and more efforts are needed before multi-omics can be used for precision medicine in clinical practice. First, there is a lack of robust and reproducible omics data. Cutting-edge omics technologies have not yet delivered reliable and stable biomarkers for predicting metabolic diseases nor have they captured enough biological variation to enable the construction of sensible and discrete categories and to facilitate specific clinical treatment. For example, when biomarkers identified by GWAS and metabolomics studies were added to a risk prediction model of traditional risk factors, the model showed only a modest improvement in predicting the risk of T2DM (Walford et al., 2014). Second, data by themselves are not useful unless they are analyzed, interpreted, and acted on. Therefore, attention has to be allocated to high-dimensional data analyses. Third, larger sample sizes are also needed when joint analysis of multi-omics data is performed. Currently, there are several common analysis methods, including matrix factorization (Zhang et al., 2012), correlation-based analysis (Chen and Zhang, 2016), multiple kernel learning, and multi-step analysis (Ritchie et al., 2015). New bioinformatics tools for data analysis are imperative given the large volume and complexity of available data. Last but not least, the high cost of omics technologies is probably a barrier in the application of multi-omics in precision medicine.

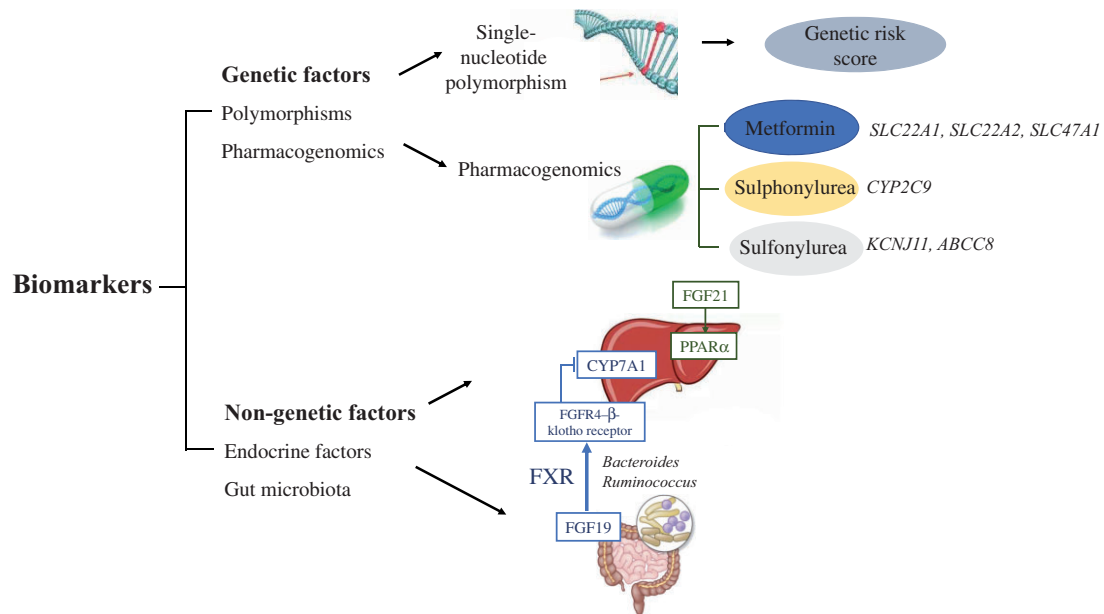
Therefore, to achieve multi-omics integration and application to precision medicine in metabolic diseases, it is important to address the current challenges by establishing a solid evidence base. This can be accomplished through more rigorous study designs, integration of high-dimensional data from various sources, development of computational approaches to large amounts of data, and reduction in cost of omics analyses.

#### Biomarkers and their application in metabolic diseases

Regardless of whether a single- or multi-omics approach is used, the ultimate goal is to accurately evaluate physiological processes and body states and identify better biomarkers that can serve in metabolic disease prevention, mechanistic studies, and precise treatment. A biomarker is defined as ‘a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to therapeutic intervention’ (Biomarkers Definitions Working Group, 2001). As shown in Figure 5, biomarkers may be genetic factors, which have been covered in previous sections; they can also be non-genetic factors such as metabolites, lipids, proteins, and chemicals applied in disease diagnosis, progression, therapy, and outcomes.

#### Non-genetic biomarkers for T2DM

Non-genetic factors such as endocrine factors, metabolic factors, and gut microbiota also are promising as biomarkers in the diagnosis and treatment of metabolic disorders. The fibroblast growth factor (FGF) family comprises 22 polypeptides involved in many biological functions, including cell growth and



**Figure 5 Biomarkers for metabolic diseases. Biomarkers include genetic and non-genetic factors applied in disease diagnosis, progression, therapy, and outcomes. Genetic biomarkers include risk variants, GRS, and pharmacogenomics. Non-genetic biomarkers include endocrine factors and gut microbiota.**

differentiation, angiogenesis, embryonic development, wound healing and repair, as well as metabolic regulation (Beenken and Mohammadi, 2009). Endocrine FGFs (FGF19 and FGF21) differ from other FGFs, acting as circulating hormones (Itoh, 2010), participating in metabolic homeostasis, and regulating bile acid, glucose, and lipid metabolism (Coskun et al., 2008; Song et al., 2009; Xu et al., 2009). Important insights into the potential roles of FGF21 in human disease are rapidly emerging. The ability of FGF21 to regulate glucose homeostasis has been widely verified in both gain- and loss-of-function studies in animals and humans (Gaich et al., 2013). Further studies have indicated that FGF21 ameliorates insulin and leptin resistance, enhances fat oxidation, and suppresses de novo lipogenesis in the liver, as well as activates futile cycling in adipose tissue (Coskun et al., 2008), thus providing novel insights regarding treatments for T2DM, obesity, and fatty liver disease. LY2405319, a variant of FGF21 identified in drug discovery studies, was the first FGF21 analog to reach a phase I clinical trial, achieving comparable reductions in plasma glucose, insulin, and body weight in patients with obesity and T2DM (Gaich et al., 2013; Degirolamo et al., 2016). Moreover, LY2405319 was found to significantly reduce LDL-cholesterol and increase high-density lipoprotein-cholesterol concentrations in obese patients and those with T2DM, suggesting the potential of FGF21-based therapies to prevent recurrent cardiovascular events in patients with metabolic disorders (Gaich et al., 2013).

The gut microbiome can affect host metabolism, aid in digestion, and contribute to normal immune function. It has been revealed that three major fuel sources (carbohydrates, lipids, and proteins) were all associated with T2DM (Ferrannini et al., 2013). Among these metabolites, 3-methyl-2-oxovalerate, the

degradation product of BCAA, is the most predictive biomarker of T2DM according to a population-based cohort (Menni et al., 2013).

#### Non-genetic markers for NALFD

Another FGF family member, FGF19, binds to the FGFR4- $\beta$ -klotho receptor complex, thus repressing the activation of cholesterol-7 $\alpha$ -hydroxylase, sterol regulatory element-binding protein 1C, and cAMP-response element-binding protein, when induced by bile acids or FXR agonists to suppress the synthesis of bile acids, triglycerides, and glucose, respectively (Song et al., 2009; Potthoff et al., 2011; Degirolamo et al., 2016). An engineered analog of FGF19 (NGM282), devoid of tumorigenesis activity from FGFR4 but fully reserving bile acid regulatory function, was used in clinical trials (Modica et al., 2012). Moreover, in animal studies, NGM282 was shown to protect from liver injury caused by intrahepatic and extrahepatic cholestasis. In healthy subjects, NGM282 decreased bile acid synthesis (Luo et al., 2014; Degirolamo et al., 2015). NGM282 is now investigated in patients with diabetes and primary biliary cirrhosis (Luo et al., 2014; Degirolamo et al., 2015). As promising therapeutic approaches for the treatment of a variety of chronic diseases, FGFs-based therapies have been endorsed for glucose and bile acid metabolism.

In a double-blinded study of patients with different stages of NAFLD, a panel of 20 plasma metabolites such as glycerophospholipids, sphingolipids, sterols are associated with NAFLD progression based on the liver biopsy, which can be used to as potential differential biomarkers between NASH and steatosis (Gorden et al., 2015). Apart from lipid metabolites,



inflammatory markers and mediators, such as C-reactive protein, tumor necrosis factor, IL-6 and IL-8, IL-1 receptor antagonist protein, and CXC-chemokine 10 (CXCL10), are also non-invasive diagnostic markers for NASH (Ajmera et al., 2017). Serum ferritin is also an independent predictor of advanced hepatic fibrosis among patients with NAFLD (Kowdley et al., 2012).

#### Biomarkers for MetS

Based on the mechanism of MetS, metabolites associated with central obesity, insulin resistance, hypertension, and lipid and glucose metabolism are all its promising biomarkers. Among these biomarkers, the high molecular weight (HMW) adiponectin may be the most reliable biomarker for MetS (Falahi et al., 2015), although the role of HMW adiponectin needs further certification. As we mentioned earlier, microbiota can affect host metabolism, alter gut microbiota profile, and contribute to the progression of T2DM, NAFLD, and MetS through influencing lipid and bile acid metabolism (Anand et al., 2016). *Bacteroides* was found to be independently associated with NASH, while *Ruminococcus* was associated with significant fibrosis in NAFLD progression (Boursier et al., 2016). Furthermore, gut-derived metabolite TMAO is an early biomarker of adipose dysfunction, NAFLD, and MetS (Barrea et al., 2018).

#### Summary

By integrating numerous biological measurements, data analysis strategies could offer novel insights for the integrative physiology of metabolic disorders, caused by an interplay of multiple genetic variants, lifestyle, and environmental factors. More and more genetic and non-genetic biomarkers have been identified and used in clinical practice; however, there is still a long distance to cover between the discovery of biomarkers and precise treatment. Nevertheless, efforts should be continued to translate research on biomarkers to clinical applications, to improve treatment capabilities for patients.

#### Conclusions

Facing the existing severe burden of metabolic disorders, numerous studies are trying to find most effective treatments; therefore, precision medicine is urgently needed. For polygenic diseases such as T2DM, NAFLD, and MetS, genetics is the foundation of phenotypes, though environmental factors, age, sex, disease subtypes, and gut microorganisms also exert a significant influence. The future direction of precision medicine relies on the combination of multi-omics technologies and corresponding analyses. While how to combine and analyze the data from multi-omics technologies is the key knowledge gap yet filled, it is also the main challenge of precision medicine in identification and implementation of these multi-omics data based on the clinical practice. The answer to this question will depend, to some extent, on the interactions between the clinical

characteristics and the underlying biology of the disease. We can define etiological subgroups of these diseases based on the physiological features characterized by multi-omics technologies, and then analyze the subgroup features with the clinical characteristics based on the laboratory parameters and imaging data from computed tomography/magnetic resonance imaging. Machine learning might be useful in analyzing these interactions. Precision medicine is bound to face these challenges, and the way scientists deal with these challenges determines the future direction of precision medicine.

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