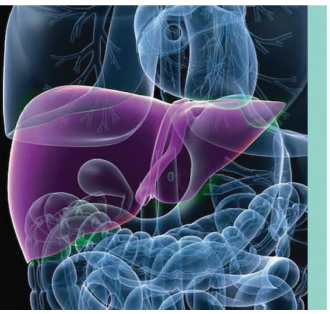

Clinical Phenotyping and the Application of Precision Medicine in MAFLD

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Metabolic associated fatty liver disease (MAFLD) encompasses a broad disease spectrum that impacts an estimated one billion people worldwide.[1] Initially described as non-alcoholic fatty liver disease (NAFLD) in 1980, there is now increasing recognition that the absence of excess alcohol use may be insufficient to characterize this disease process, and interaction between several metabolic risk factors leads to an array of dynamic phenotypes. Although this paradigm change could ultimately help guide new therapeutic strategies, multiple barriers continue to pose clinical care and discovery challenges, including disease heterogeneity, natural history variability, imperfect nomenclature, and suboptimal diagnostic and surveillance tools (Table 1). The application of precision medicine may hold promise for meaningful progress in the future.

MAFLD CLINICAL PHENOTYPES

Phenotypes for MAFLD occur in the context of multiple metabolic risk factors that affect hepatic lipid accumulation, inflammation, and fibrosis. They include demographic traits (age, sex, and ethnicity), lifestyle characteristics (diet, tobacco and alcohol use, and weight), medical comorbidities (glucose intolerance, diabetes mellitus, and other endocrinopathies), surgical interventions (cholecystectomy), intestinal microbiomic composition, genetics, epigenetics, and metabolomics.[1,2] These elements contribute to both established and evolving phenotypes.

Among the established MAFLD phenotypes, the traditional phenotype includes individuals with excess weight, a history of minimal-to-moderate alcohol use and, and comorbidities such as diabetes mellitus, hypertension, dyslipidemia, and vascular disease. It is particularly common among Caucasians and captures approximately 80% of patients with MAFLD.[3] Disease progression among patients with this phenotype is typically limited. Only a small fraction develops cirrhosis, and the average rate of progression is several years to decades between fibrosis stages.[2,4] Alternatively, lean MAFLD is observed more frequently in

Abbreviations: MAFLD, metabolic associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; PDFF, proton density fat fraction.

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TABLE 1. CHALLENGES IN DIAGNOSIS, PROGNOSTICATION, AND THERAPEUTICS DEVELOPMENT FOR MAFLD

Process	Challenges
Screening/Diagnosis	Imperfect/evolving nomenclature Disease heterogeneity Coexistance of alternative chronic liver diseases Lack of early and sensitive testing for at-risk individuals Cost and availability of testing modalities
Staging/surveillance	Lack of accurate modalities to detect steatohepatitis and early/intermediate fibrosis Inefficient surveillance tools to monitor therapeutic response
Treatment	Limited pharmacotherapy Lack of tools to predict therapeutic response Clinical trial enrollment and retention limitations
Prognostication	Cardiovascular and oncologic comorbidities Long latency period prior to the development of liver-related outcomes Variability in disease progression

the East Asian population, occurring in non-obese individuals with possible genetic predisposition, intestinal dysbiosis, and endocrinopathies; it portends an increased risk for advanced liver disease.[5] Finally, Hispanic patients suffer excess burden from MAFLD, with higher disease prevalence and relatively faster progression; although this phenotype is likely multifactorial in etiology, common genetic variants have been identified.[6,7] Overall, among patients with MAFLD, the risks of hepatic decompensation, cardiovascular events, and malignancy are well-described (Fig. 1).[8-12]

Although these phenotypes have become increasingly recognized in the medical community, they lack the necessary granularity for clinical practice and research. Through the use of precision medicine tools, diverse genetic, epigenetic, and metabolomic signatures can ultimately be used to identify specific and targetable phenotypes. The building blocks for this approach currently exist, but future studies are necessary to determine how different types of molecular data can be synthesized in ways that are applicable for clinicians and researchers. Before this can be accomplished, barriers in diagnosis and staging will likely need to be overcome.

CHALLENGES IN DIAGNOSIS AND STAGING

The diagnosis of MAFLD currently depends on a combination of clinical, laboratory, and radiographic assessments that include individuals' risk factor profiles, basic labs and liver chemistries, serological testing to exclude alternative forms of liver disease, and relevant imaging findings, including features of hepatic steatosis and/or fibrosis. In conjunction with the diagnostic evaluation, an early determination of the presence and severity of fibrosis is critical given its association with all-cause and cardiovascular mortality.[13]

Non-invasive tools, including clinical scoring systems, plasma biomarkers, and elastography, are currently used to identify those with MAFLD, MAFLD with steatohepatitis, and MAFLD with advanced fibrosis. In particular, transient elastography (FibroScan), shear wave elastography, and MR elastography are well-established tools that allow for non-invasive assessment of hepatic fibrosis.[14] Unfortunately, a number of these modalities lack the diagnostic discrimination for intermediate fibrosis stages, as well as identification of those with high steatohepatitis inflammatory activity. Thus, these diagnostic tests are likely not adequately sensitive to identify high-risk patients, particularly those seeking enrollment in clinical trials. However, recent multi-national validation of the FibroScan-AST is one example of a risk score developed to identify those individuals with MAFLD, high inflammatory activity, and moderate-to-advanced fibrosis for clinical trial enrollment.[15]

Finally, a lack of consistent nomenclature also continues to pose challenges, limiting the transition of phenotypic concepts to the clinical medicine. The term "MAFLD" is potentially more representative and inclusive than "NAFLD," highlighting the premise that metabolic stress dictates phenotypes (and not alcohol use) and emphasizes that MAFLD is not a diagnosis of exclusion. However, it remains imperfect largely due to lack of specificity. Replacing the phrase "non-alcoholic" with a general term such as "metabolic associated" can create ambiguity among providers and researchers since metabolic dysregulation plays a role in a multitude of disease processes that impact the liver. Frequent changes in nomenclature may also lead to confusion among non-hepatologists and lead to barriers in interdisciplinary practices.

Future clinical care and research in MAFLD, therefore, is heavily contingent on appropriate host identification,

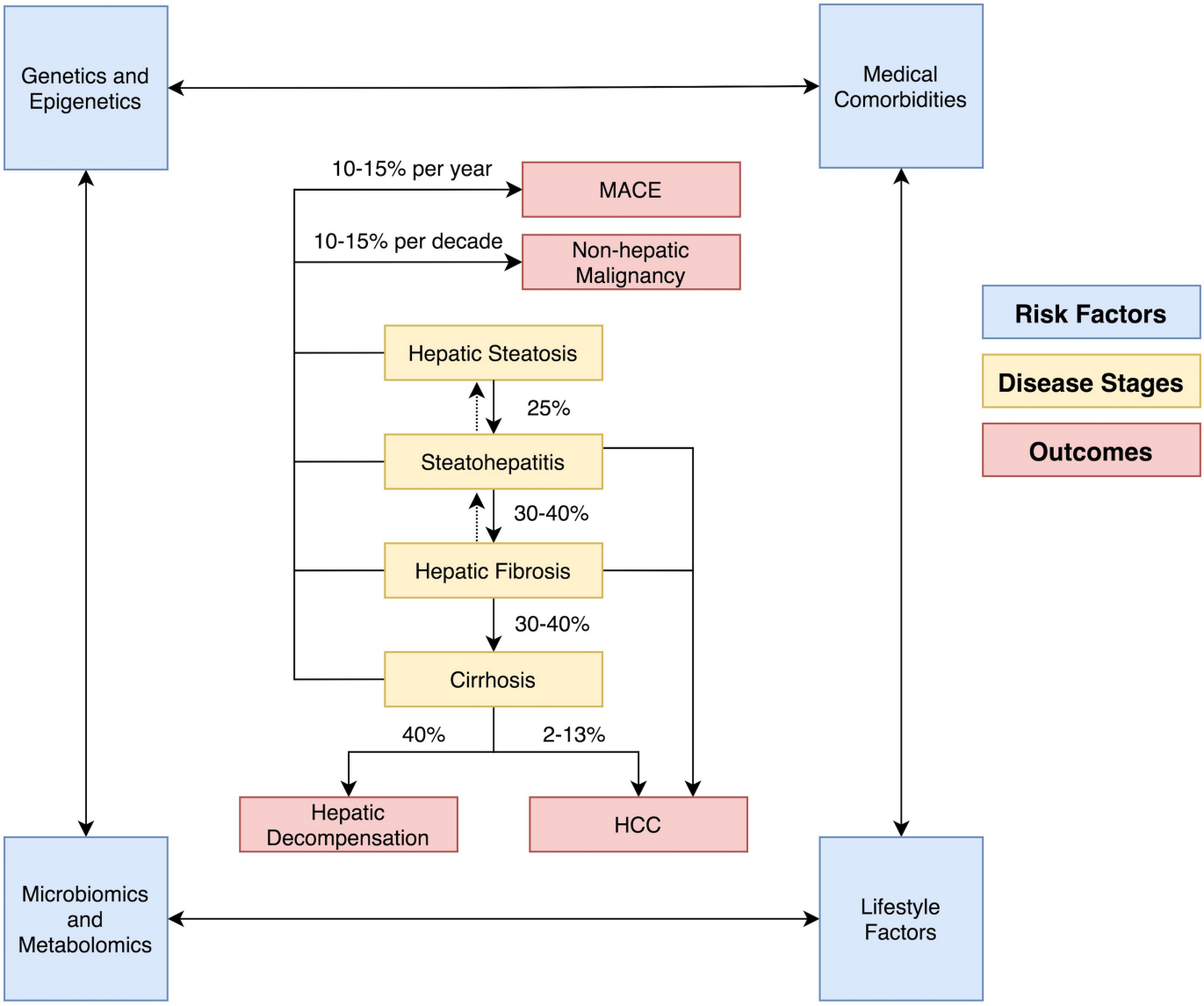

FIG 1 Natural history of MAFLD. Abbreviation: MACE, major adverse cardiovascular events.

specific and consistent nomenclature, and the application of non-invasive, widely available, effective, and dynamic diagnostic and staging tools that incorporate precision medicine techniques.

EMERGING PHENOTYPING TOOLS

Genetic and Epigenetic Biomarkers

Genome- and phenome-wide association studies in obese and non-obese patients of different ethnic backgrounds have identified single nucleotide polymorphisms in candidate genes that impact processes such as lipid remodeling, lipid metabolism, glycogen storage, and/or lipophagy (Table 2).[16-18] The effects of *PNPLA3* polymorphisms have been evaluated in multiple epidemiologic studies, and findings suggest that specific variants can impact disease severity, progression, and responses to intervention. In particular, the *PNPLA3* G risk allele is associated with an earlier age of diagnosis, especially among Hispanic patients, and M-variants have been associated with an increased risk for adverse outcomes, including hepatic decompensation, hepatocellular carcinoma, and death.[6,7] Patients with the I148M polymorphism have been shown to have limited responses to statin use and may derive significant benefits from dietary modification.[19]

TABLE 2. GENETIC, EPIGENETIC, METABOLOMIC, AND MICROBIOMIC MARKERS IN MAFLD

Genetic Polymorphisms	Risk Modification	Outcomes
PNPLA3 (G allele, M variants)	↑	Steatosis, steatohepatitis, fibrosis, decompensation, hepatocellular cancer, death
GCRK (P446L)	↑	Steatosis, steatohepatitis, fibrosis, hepatocellular cancer; synergistic effect with *PNPLA3* I148M
HSD17B13 (inactivating variants)	↓	Steatohepatitis, fibrosis, hepatocellular cancer; mitigates risk in patients with PNPLA3 I148M
TM6SF2 (E167K)	↑	Steatosis, steatohepatitis, fibrosis, hepatocellular cancer
MBOAT7 (rs641738)	↑	Steatosis, steatohepatitis, fibrosis, hepatocellular cancer
Genes with epigenetic changes		
AQP1 (overexpression)	↑	Fibrosis
FGFR2 (overexpression)	↑	Fibrosis
MicroRNAs		
miR-34a (overexpression)	↑	Steatosis, steatohepatitis
miR-122 (underexpression)	↑	Steatosis, steatohepatitis; in human studies (potentially differing effects in mice)
Metabolites		
Branched-chain amino acids	↑ or ↓ (depending on disease stage)	Steatosis, steatohepatitis, fibrosis
Lipids (triglycerides and fatty acids)	↑ or ↓ (depending on molecular subtype)	Steatosis, steatohepatitis, fibrosis
Carbohydrates (glycolytic products)	↑	Steatosis, steatohepatitis
Bile acids (total)	↑ (predominantly)	Steatosis, steatohepatitis, fibrosis; some bile acids reduce risk for steatosis and steatohepatitis
Gut microbiome		
Proteobacteria, Enterobacteria	↑	Steatosis, steatohepatitis, fibrosis
Firmicutes	↑ or ↓ (depending on bacterial species and disease stage)	Steatosis, steatohepatitis, fibrosis; Firmicutes concentrations may decrease with fibrosis progression
Bacteroidetes	↑ or ↓ (depending on bacterial species and disease stage)	Steatosis, steatohepatitis, fibrosis

Factors that impact gene regulation such as differential DNA methylation and miRNA expression have also been implicated in the pathogenesis and progression of MAFLD. Epigenome-wide association studies and microarrays have identified a subset of genes and miRNA sequences that impact lipid metabolism and inflammation in MAFLD (Table 2).[20,21]

Metabolomics

The addition of metabolomics to genetic and epigenetic data, microbiomics, and additional surrogate markers may enable targeted clinical research in MAFLD. Studies have already highlighted its potential impact by demonstrating that metabolic profiles incorporating lipid, carbohydrate, amino acid, bacterial, and/or bile acid markers from plasma, urine, or stool samples can be used to identify disease subtypes, monitor for disease progression, assess the risk for outcomes such as cardiovascular disease and cancer, and even discriminate between MAFLD fibrosis stages, in some cases outperforming standard scoring systems for the detection of advanced fibrosis (Table 2).[22-30] In particular, changes in the levels of particular amino acids (branched chain and glutathione metabolites) and alterations in fatty acid and bile salt composition have been studied. Increased levels of branched-chain amino acids, increased frequency of fatty acids with low carbon number and double bonds, and a preferential increase in primary bile acids may signify metabolically active MAFLD, whereas with disease progression, one may expect decreased levels of branched-chain amino acids and significant reductions in glutathione precursors.[22] Biomarker panels, which can be used to rapidly measure multiple metabolite levels using spectroscopy or chromatography, offer clinicians, patients, and researchers the possibility of trending disease activity and monitoring therapeutic responses in a much more robust manner, overcoming many of the limitations posed by conventional tools.

Radiographic Biomarkers

Finally, a number of MR-based biomarkers and methods, including proton density fat fraction (PDFF), spectroscopy, T1 mapping, gadoxetate, and multiparametric imaging, have been studied in the detection of steatosis, steatohepatitis, fibrosis, and hepatocyte function in MAFLD.[31] Unfortunately, the application of some of these techniques are limited by technological constraints (availability

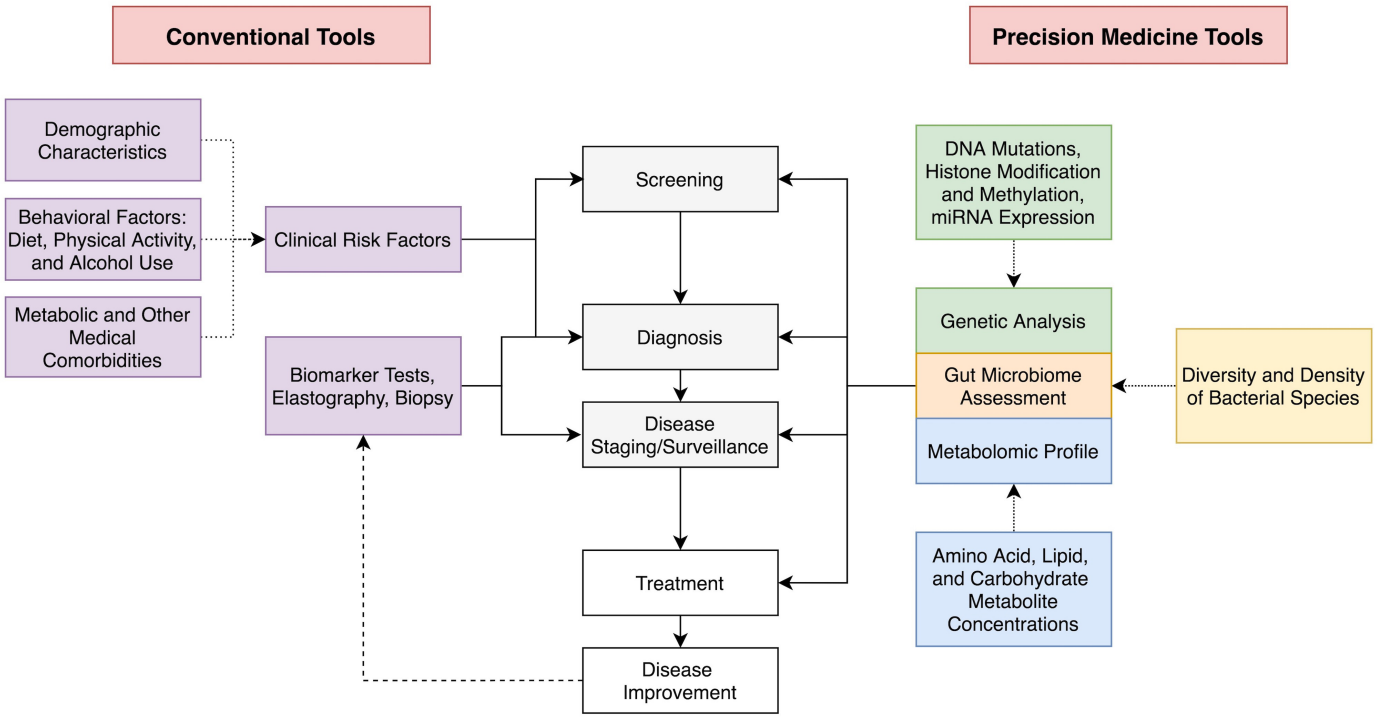

FIG 2 Precision medicine tools in diagnosis and management of MAFLD.

of specialized scanners). However, PDFF is readily available and has been applied longitudinally to track disease activity. Studies have demonstrated that changes in liver fat content measured via PDFF correlate with changes histologic disease activity, including fibrosis.[32,33]

FUTURE DIRECTIONS: THE PATH TO PRECISION MEDICINE

MAFLD is a heterogeneous disease with diverse phenotypes that incorporate a variety of risk factors. The spectrum of disease activity is vast, and outcomes can differ markedly among patients. However, our current diagnostic and therapeutic approaches are homogenous and rely largely on insensitive tools that are insufficient to identify the varied phenotypes in MAFLD. The rise of precision medicine in the form of genetic, epigenetic, metabolomic, and microbiomic techniques will help overcome these challenges as the burden of MAFLD continues to increase globally.

In the future, it may become possible to screen high-risk patients with a combination of genetic testing and metabolomic assays that augment conventional modalities, such as elastography and serological studies. The results of these assays can be used to phenotype patients using specific terminology, readily monitor the impact of conventional and experimental treatments, serve as the basis for new highly targeted molecular therapies, and inform prognosis (Fig. 2). The application of machine learning can potentially further improve the efficacy and efficiency of these precision medicine tools.

Although additional research will be required to understand how different types of data can be synthesized to develop more holistic diagnostic and treatment models, precision medicine will ultimately change the landscape of MAFLD. Initiatives, such as the Liver Investigation: Testing Marker Utility in Steatohepatitis (LITMUS) project, which aim to accomplish this goal, have been established. Armed with new tools, researchers and clinicians will soon be able to apply molecular techniques to accurately identify and monitor patients and tailor therapies based on personalized molecular signatures.

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