

# Opportunities and barriers in omics-based biomarker discovery for steatotic liver diseases

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## Summary

The rising prevalence of liver diseases related to obesity and excessive use of alcohol is fuelling an increasing demand for accurate biomarkers aimed at community screening, diagnosis of steatohepatitis and significant fibrosis, monitoring, prognostication and prediction of treatment efficacy. Breakthroughs in omics methodologies and the power of bioinformatics have created an excellent opportunity to apply technological advances to clinical needs, for instance in the development of precision biomarkers for personalised medicine. Via omics technologies, biological processes from the genes to circulating protein, as well as the microbiome – including bacteria, viruses and fungi, can be investigated on an axis. However, there are important barriers to omics-based biomarker discovery and validation, including the use of semi-quantitative measurements from untargeted platforms, which may exhibit high analytical, inter- and intra-individual variance. Standardising methods and the need to validate them across diverse populations presents a challenge, partly due to disease complexity and the dynamic nature of biomarker expression at different disease stages. Lack of validity causes lost opportunities when studies fail to provide the knowledge needed for regulatory approvals, all of which contributes to a delayed translation of these discoveries into clinical practice. While no omics-based biomarkers have matured to clinical implementation, the extent of data generated has enabled the hypothesis-free discovery of a plethora of candidate biomarkers that warrant further validation. To explore the many opportunities of omics technologies, hepatologists need detailed knowledge of commonalities and differences between the various omics layers, and both the barriers to and advantages of these approaches.

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## Introduction

More than one-third of the adult population have steatotic liver disease either metabolic dysfunction-associated steatotic liver disease (MASLD), alcohol-related liver disease (ALD) or a combination thereof (MetALD).<sup>1–3</sup> Patients with progressive disease experience high liver-related morbidity, extrahepatic complications and premature all-cause mortality.<sup>4,5</sup> There is consequently an urgent need for accurate risk stratification and effective treatments that modify the natural course of disease.<sup>6,7</sup> Progression of steatotic liver disease follows a profibrotic path, resulting in pivotal liver-related events that critically affect prognosis. It is consequently important to explore biomarkers that predict precursors of cirrhosis and portal hypertension in the form of significant and advanced fibrosis, as these disease stages predict later liver-related events, including

decompensation, acute-on-chronic liver failure, hepatocellular carcinoma, and death.<sup>8–10</sup>

The performance of existing and future biomarkers depends on their intended context of use and validation (Fig. 1, Table 1).<sup>11</sup> General practitioners and hepatologists managing ALD, MetALD and MASLD lack tests for the accurate diagnosis of significant fibrosis ( $\geq F2$ ) and steatohepatitis, for prognosis, monitoring and prediction, and for evaluating the efficacy of interventions.<sup>8,12</sup> Traditionally, the diagnostic accuracy of a biomarker is evaluated by the area under the receiver-operating characteristic curve, sensitivity, specificity and predictive values. However, these performance characteristics depend on disease prevalence in the studied population.<sup>13</sup> Consequently, future biomarkers need to be tailored to the intended population and tested in cohorts which reflect the appropriate disease prevalence.

Keywords: Non-invasive test; genetics; microbiome; metagenomics; metatranscriptomics; viromics; metabolomics; lipidomics; proteomics.

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1 See the section on The Taormina Workshop on Occult HBV Infection Faculty Members, near the end of the paper.

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## Key points

- There is an urgent need for accurate biomarkers in patients with steatotic liver disease, to stage and grade fibrosis and inflammation, for monitoring disease progression and for improving drug development and approval pipelines.
- The rapid development and decreased costs of high-throughput omics technologies in combination with excellent computational power has created a golden opportunity for new types of biomarkers which reflect biological disease processes and can be combined in multiplex systems. Multi-omics may thereby facilitate an era of accurate, personalised diagnostics.
- Heterogeneity in the development and progression of steatotic liver disease may be disentangled by studying the interplay between host genetics, transcriptomics, proteomics, metabolomics and lipidomics on the one hand, and gut microbial, viral and fungal metagenomics and meta-transcriptomics on the other hand.
- Hypothesis-free approaches have revealed the potential of omics technologies for the discovery of liver disease biomarkers and have proposed many more candidate biomarkers than the traditional hypothesis-driven studies. However, few of these omics-based biomarker candidates have been rigorously tested in independent cohorts, and none have been implemented in clinical practice.

This review will explore the advantages and limitations of omics technologies for biomarker discovery across the spectrum of steatotic liver disease. We highlight the state of the art of individual omics technologies: genetics, transcriptomics, proteomics, lipidomics, metabolomics, metagenomics, meta-transcriptomics, viromics and mycobiomics. These technologies have been selected from a wider list of currently available omics technologies as they are the most common and represent the promises and obstacles of omics-based biomarkers for clinical hepatology.

## Opportunities for omics technologies

Recent years have witnessed the beginning of a new era in biomarker development, thanks to high-throughput omics technologies combined with increasing computational power and the ability to apply artificial intelligence and machine learning methods with routine hardware and software. This major advance allows for hypothesis-free testing of thousands or even millions of analytes.<sup>14,15</sup> Multi-omics is thereby able to disentangle the complex molecular interplay between host genes, gene transcription, proteins, metabolites and lipids, in addition to interactions between the host and microbiome (consisting of bacteria, viruses and fungi) (Fig. 2), resulting in a multitude of candidate biomarkers.<sup>16–19</sup> In addition, to enable the accurate separation of patients with progressive liver disease from those with non-progressive disease, researchers have looked to understand disease heterogeneity and pathophysiology through the lens of host-gut-environment interactions.<sup>20</sup> Recent developments and promising biomarker targets from omics technology are highlighted in Table 2.

In the struggle to identify effective anti-fibrotic interventions for MASLD and ALD, omics-based biomarkers that reflect biological fibrotic processes may be used to identify future drug targets, thereby abating the frequent failures of phase III clinical trials.<sup>21</sup> There is a similar search for accurate biomarkers to reduce clinical trial screening failures.<sup>17</sup> Finally, non-invasive biomarkers to replace liver biopsy as the surrogate endpoint would effectively allow for shorter, less costly trials and reduced patient discomfort.<sup>22</sup>

The analysis costs of genetics, transcriptomics, proteomics, lipidomics, metabolomics, metagenomics and metatranscriptomics are decreasing thanks to technological development and an increase in the capacity of high-throughput omics platforms.<sup>23,24</sup> We therefore expect multi-omics approaches to

become increasingly accessible for the clinical management of patients with liver disease over the next decade.

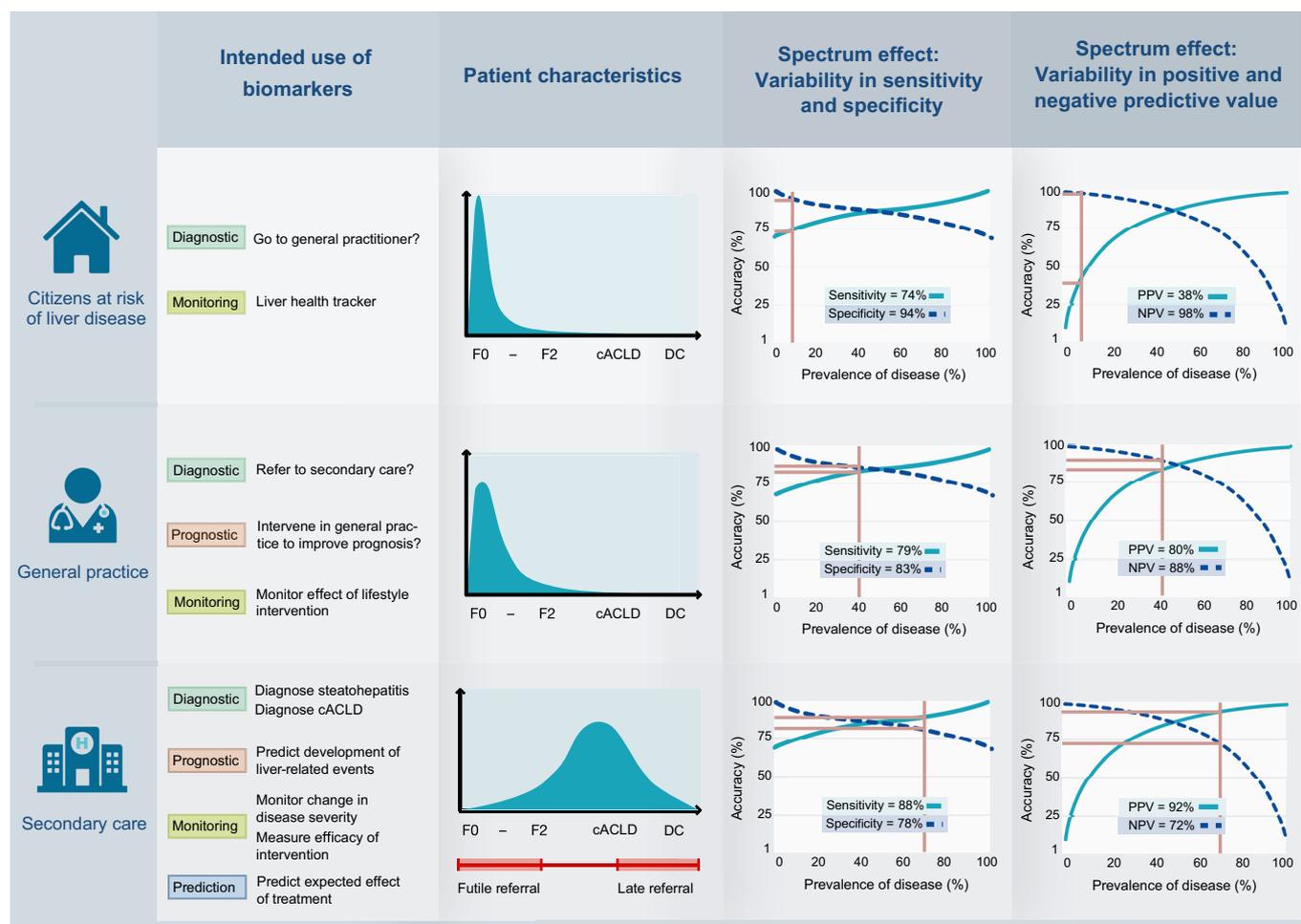
## Barriers to omics technologies

Omics-based biomarkers offer more opportunities for discovery than traditional biomarkers, which quantify a low number of analytes, often only one. However, no omics-based biomarker has penetrated from development to implementation. This shortcoming can be attributed to several barriers across different omics technologies, including 1) technological maturity, 2) cost, 3) analytical validity, 4) untargeted coverage and 5) semi-quantitative measurements, which are usually laboratory or instrument specific.

Except for genetics, omics technologies are in their infancy (Fig. 3). This immaturity results in several obvious limitations, most notably that the evidence base remains incomplete.

Technological development is moving rapidly from high cost and low throughput to low cost and high throughput.<sup>15,25</sup> However, finite budgets remain a challenge for the maturation of omics-biomarkers. Current cost pressures create a trade-off between analyte depth and abundance vs. sample throughput and sample size.<sup>18</sup> The limited ability to robustly detect low-abundance analytes generates 'technological bias'.<sup>26</sup> Omics studies typically aim for great depth to discover low-abundance biomarkers, but this means that investigators cannot afford as many samples, thus risking spurious findings. The high-dimensional nature of omics data also requires extensive computational protocols and processing power, further increasing time usage and costs.<sup>27</sup> However, increasingly higher demands for omics technologies within the healthcare system will lead to the development of routine protocols and market competition, driving costs downward.

Omics measurements can be divided into two analytical methods: non-targeted and targeted. Non-targeted omics takes a hypothesis-free approach to the semi-quantitative analysis of a very large number of molecules, often aided by machine learning and other advanced bioinformatics. Non-targeted omics is consequently highly suited for discovery of new biomarkers. However, this approach faces three major challenges: 1) semi-quantitative measurements are relative and, as such, study specific. Findings are therefore difficult to replicate in external validation. Candidate biomarkers detected by untargeted approaches must therefore be validated using a targeted platform, such as ELISA, for absolute concentrations.<sup>28</sup> 2) Non-targeted measurements are more prone to



**Fig. 1. Intended use of biomarkers and the spectrum bias.** Due to the spectrum effect, diagnostic accuracy for the same biomarker will change when tested in populations with different prevalence of disease. Discrete types of omics allow biomarkers to be tailored to the different contexts of use and different disease spectrums. Plots illustrate variability in sensitivity and specificity, as well as PPV and NPV, with disease prevalence in the studied cohort, derived from Usher-Smith *et al.*<sup>13</sup> cACLD, compensated advanced chronic liver disease; DC, decompensated cirrhosis; F0–F2, denotes liver fibrosis stage; NPV, negative predictive value; PPV, positive predictive value.

analytical biases such as batch effect and variations related to sample handling and processing.<sup>29</sup> 3) Non-targeted approaches usually require more complex and therefore less standardised bio-informatics pipelines.

The targeted approach uses quantitative assays to measure concentrations of predefined panels of up to a few hundred molecules.<sup>30,31</sup> Targeted omics can be done, for example, by using calibration curves and spike-in of internal standards to allow for absolute quantification and is well suited to either searching for high-abundance biomarkers or for hypothesis-driven biomarker evaluation. Discovery of novel targets and pathways is especially useful for drug discovery and investigations of disease aetiology; however, its application in routine analysis in the clinic is still being evaluated.

Different omics technologies each have their own set of specific advantages which hold great potential for personalised and precision medicine (Fig. 4; Table 2). Nevertheless, in order to bring omics-based biomarkers into the clinic, the current process involves transforming them into analytically reproducible assays that can be validated across laboratories and cohorts while also meeting regulatory requirements.<sup>32,33</sup> These requirements can be insurance against hurried, spurious

findings but can also limit the speed of discovery and development to validation.

The subsequent sections delineate the technical complexities and biomarker prospects across diverse omics disciplines.

## Genetics

Genetics is the most widely investigated omics technology, linking single nucleotide polymorphisms (SNPs) to cirrhosis, hepatocellular carcinoma and steatosis, particularly for MASLD and ALD.<sup>24,34,35</sup> From family and population-based studies, the heritability of MASLD ranges from 20–70% depending on ethnicity and how MASLD is diagnosed.<sup>36</sup> For the heritability of ALD, studies suggest alcohol use disorder heritability ranges from 30–50% and ALD-related cirrhosis ranges from 21–67%.<sup>37</sup> However, disagreement within the field exists on the proportion of the genetic variance for ALD that is independent of the genetic predisposition to alcohol dependence.<sup>37,38</sup>

Genotyping of individuals for genome-wide association studies (GWAS) is typically performed using microarrays to measure common variants, due to the higher cost of

**Table 1. Biomarker indications and clinical use.**

|                                    | Diagnostic  | Prognostic*   | Monitoring   | Prediction*  | Surrogate endpoint  |
|------------------------------------|---|---|--|--|---|
| Outcome of interest                | Disease present or not; disease staging   | Development of clinical events, mortality   | Change in disease severity   | Effect of treatment  | Substitute for one or more clinical outcomes  |
| Subclasses of biomarkers           | Screening   | Susceptibility/risk stratification  | Efficacy of intervention; pharmacodynamic response   | Safety (adverse events)  | Reasonably likely surrogate endpoint  |
| Measurement timing                 | Baseline  | Baseline  | Longitudinal   | Baseline, before intervention  | Start and end of intervention study   |
| Clinical characteristic            | Reflects true disease state   | Reflects patient or disease characteristics   | Biomarker changes correlate with changes in extent or status of disease                          | Reflects patient or disease characteristics  | Effect on the surrogate endpoint predicts a clinical benefit  |
| Statistics used                    | Discriminative accuracy, sensitivity, specificity, NPV, PPV, calibration curves, goodness of fit, information criterium, odds ratio | C-statistics, hazard ratio, time-dependent receiver operating characteristics curve, Aalen-Johansen or Kaplan-Meier estimator | Correlation coefficients: diagnostic and prognostic accuracy of $\Delta$ biomarker**             | Treatment effect in biomarker positive vs. biomarker negative patients if patient groups have the same prognosis | Correlation coefficients: diagnostic accuracy of $\Delta$ biomarker to detect change; prognostic accuracy of $\Delta$ biomarker |
| Examples of omics-based biomarkers | Proteomics for diagnosis of ALD fibrosis, inflammation and steatosis <sup>15</sup>  | Genetic risk polymorphisms for development of hepatocellular carcinoma in the population <sup>47</sup>                        | Changes in lysophosphocholines by lipidomics in MASLD during dietary intervention <sup>137</sup> | A polygenic score to predict weight loss in response to physical activity <sup>138</sup>                         | No omics markers approved as surrogate endpoints, but single molecules may arise from omics discovery                           |

ALD, alcohol-related liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic curve.

\*A prognostic biomarker is used to identify the likelihood of a clinical event in a patient, while predictive biomarkers identify patients who are more likely to experience beneficial or adverse effects of an intervention.

\*\* $\Delta$  means change from baseline.

next-generation sequencing (NGS). NGS methods encompass: 1) whole-exome sequencing, which targets coding regions with functional significance and 2) whole-genome sequencing, which captures nearly every genotype across the genome, both coding and non-coding, including rare variants. Whole-genome sequencing is expected to become the method of choice in the future for untargeted discovery as costs continue to decrease.<sup>39</sup> NGS methods can be effective tools for precision diagnostics in rare monogenic forms of liver disease. Patients who remain undiagnosed despite comprehensive clinical workups may benefit from genomic analysis to improve disease prognostication. Examples include *ABCB4*, *ABCB11* and *ATP8B1* to distinguish idiopathic cholestasis.<sup>40</sup>

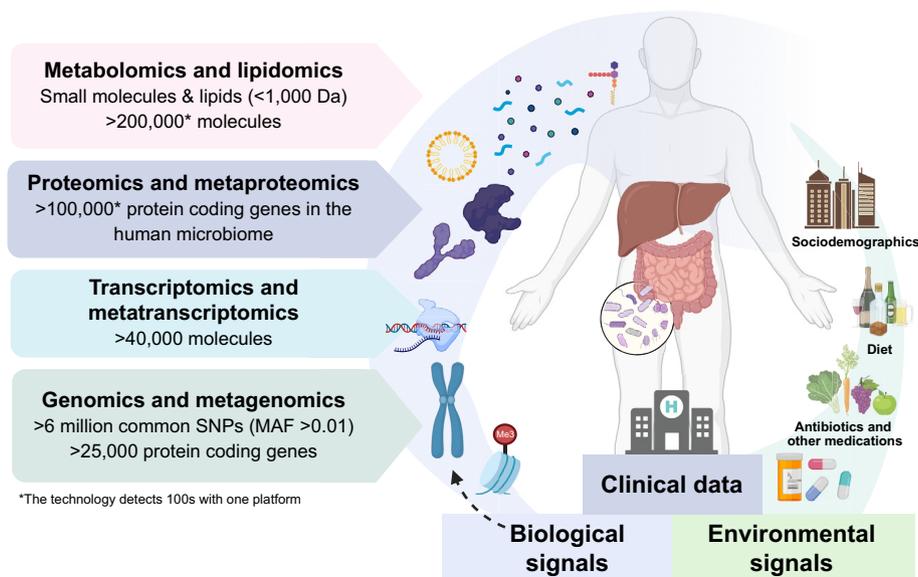
Large-scale GWAS and meta-analyses have elucidated the genetic architecture of steatosis, steatohepatitis, and fibrosis from ALD and MASLD, using liver biopsies, imaging, elastography, liver enzymes and electronic health records. These efforts have identified risk loci common to ALD and MASLD, including *PNPLA3*, *TM6SF2*, *GCKR*, *SERPINA1* and *MBOAT7*.<sup>41–45</sup> Novel protective loci include *HSD13B17*, *MTARC1*, *GPAM* and *PSD3*.<sup>35,45,46</sup>

Genetic risk scores (GRS) combining multiple SNPs with genome-wide significance ( $p < 5 \times 10^{-8}$ ) can be used for risk prediction and stratification. A higher GRS, including *PNPLA3*, *TM6SF2* and *HSD17B13*, was shown to confer a 12-fold increased risk of cirrhosis and a 29-fold increased risk of hepatocellular carcinoma in the European population.<sup>47</sup> Likewise, a higher GRS derived from *PNPLA3*, *TM6SF2*, *MBOAT7*, *GCKR* and *HSD13B17* was shown to amplify the effect of liver

steatosis on the risk of subsequent hepatic events.<sup>48</sup> Despite considerable interest, the predictive value of a given GRS over simple biochemical biomarkers has been marginal. Combining *PNPLA3*, *TM6SF2*, *HSD17B13* and *MBOAT7* with metabolic traits slightly increases the area under the curve for diagnosing advanced liver fibrosis, from 0.75 to 0.80 in patients with ALD.<sup>49</sup> Regarding the prediction of 10-year cirrhosis risk, the addition of a GRS to the APRI score (aspartate aminotransferase-to-platelet ratio index) provided little additional prognostic information and only marginally improved the C-index from 0.804 to 0.809 in the UK Biobank.<sup>50</sup> This limited impact is likely due to the fact that clinical features from 5 to 10 years before disease onset explain more variance than the few SNPs with small effect sizes identified so far.<sup>51</sup> Yet there is promise: a study based on UK Biobank data demonstrated that a GRS improves risk stratification and diagnostic accuracy, particularly in subgroups of individuals with diabetes, obesity or a fatty liver index above 60. This suggests that integrating a GRS with non-invasive clinical markers holds the potential to refine individual risk prediction for severe liver disease, especially in individuals at risk for MASLD.<sup>52</sup>

Polygenic scores have achieved greater predictive power than GRS for complex diseases by including hundreds to thousands of SNPs, rather than being restricted to only those that reach genome-wide significance ( $p < 5 \times 10^{-8}$ ).<sup>53</sup> Polygenic scores developed for liver diseases are still under development and require well-powered GWAS studies, validated in independent study populations of varying ancestries to ensure generalisability.

## THE OMICS POTENTIAL IN BIOMARKER DEVELOPMENT



**Fig. 2. The potential of omics-based biomarkers.** Illustrated by layers of biological signals and the complexity of biological molecules within the human body. The environmental signals introduce another layer of complexity as individual risk factors of disease. MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

### Transcriptomics

The transcriptome is the sum of all RNA transcripts of a tissue or blood sample, commonly used to examine gene expression. Circulating RNA species include several classes of shorter RNAs, with microRNAs (miRNA) being by far the most studied. miRNAs can be quantified by sequencing or reverse-transcription quantitative PCR (qPCR), using targeted or multiplexed panels. These methods are sensitive, often quantitative, and relatively low in cost. In contrast, sequencing all small RNAs is considerably more expensive but allows for measurement of other RNA types, such as PIWI-interacting RNAs, transfer-RNA fragments, ribosomal and nucleolar RNAs, each of which contains tens to thousands of different species.<sup>54,55</sup> Small RNAs in circulation constitute a novel source of MASLD-related biomarker candidates, e.g. miR-122, miR-34a, and miR-193a.<sup>56–58</sup> Once a promising RNA biomarker has been identified, the RNA can be detected with high sensitivity and accuracy based on targeted reverse-transcription qPCR or microfluidics-based nano-sensors.

The extracellular RNAs are an especially interesting subtype of circulating miRNAs.<sup>59</sup> They are enclosed in vesicles or are protein bound, which protects them from degradation and facilitates their transport, in turn allowing for cell-to-cell paracrine communication or long-distance signalling.<sup>60</sup> Liver-derived miRNAs, as extracellular RNA, appear to be important regulators of metabolic disease, particularly MASLD and steatohepatitis.<sup>56</sup> Recent studies show that levels of liver-derived miRNAs are modified by weight-loss or insulin-sensitising treatments.<sup>61,62</sup>

MiRNAs also show promise as biomarkers for ALD, MASLD and steatohepatitis, with miR-34a, which is part of the NIS2+ score, being a notable example.<sup>63,64</sup> In addition, both miR-193 and miR-122 plasma levels have been shown to be increased in patients with MASLD who have steatohepatitis and advanced

fibrosis.<sup>65,66</sup> Liver-specific miR-122 also predicts type 2 diabetes and decreases following weight loss.<sup>61,62</sup> Yet, low miR-122 is a marker of poor prognosis in patients with cirrhosis.<sup>67</sup> Therefore, it appears that the increase in hepatic miR-122 expression is temporary, from upregulation as steatohepatitis progresses, to a decline in patients with cirrhosis – a similar non-linear pattern is seen for changing body weight. While this naturally limits the potential use of miR-122 as a diagnostic biomarker, it points toward a possible role in causal pathways. It also illustrates the importance of consecutive recruitment and inclusion across the disease spectrum in biomarker research.

### Microbiome

The human body is home to a large number of microbes, on all skin and mucous surfaces.<sup>68</sup> The vast majority reside in the gut, which is home to ten trillion bacteria.<sup>69</sup> The gut microbiota exerts important effects on host physiology by producing diverse metabolites, modulating the immune system and preventing infection by pathogens.<sup>70</sup> The gut microbiota can profoundly affect the liver, as microbial products can enter the blood circulation and thereby encounter the liver as the very first organ.<sup>23,71,72</sup>

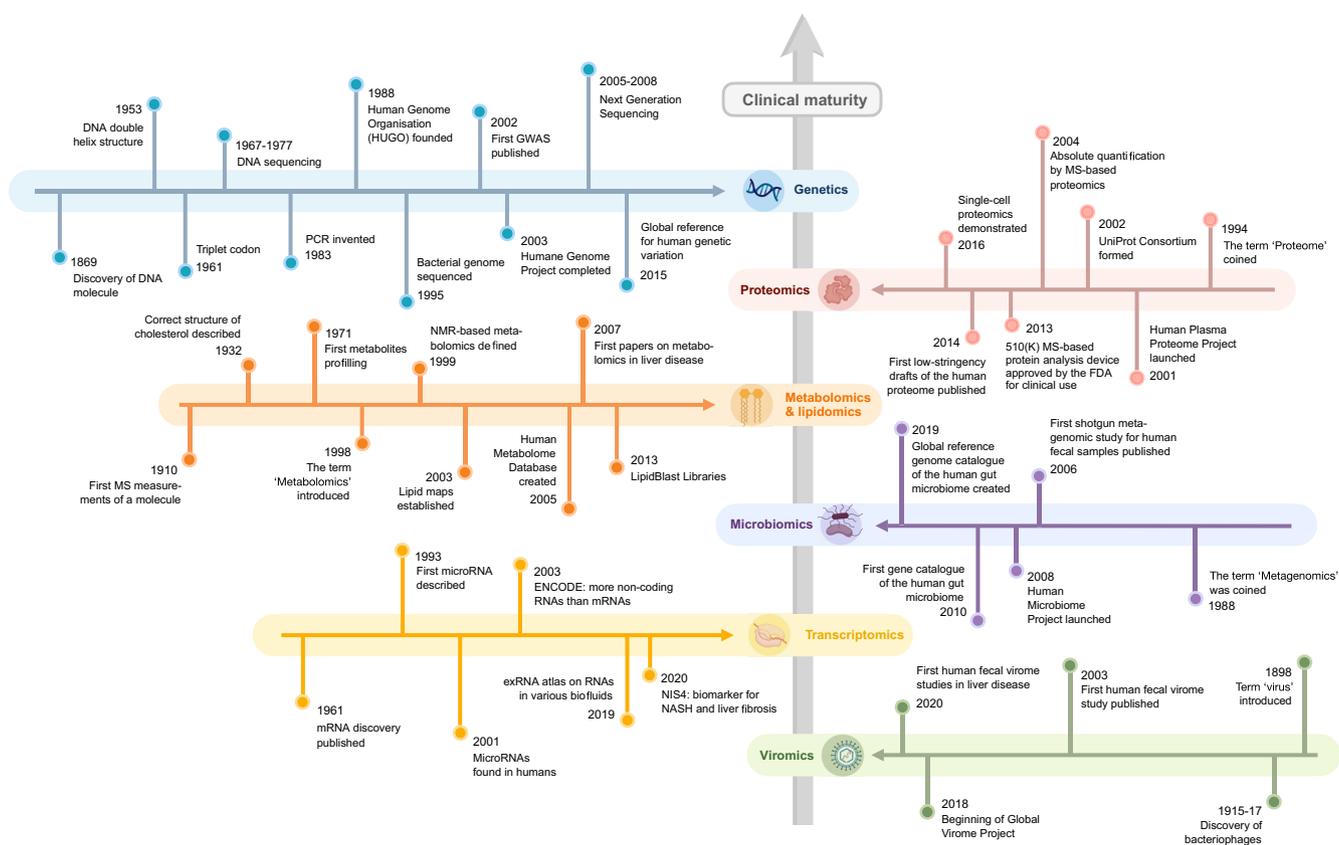
Shotgun metagenomic sequencing evaluates both the species-level taxonomic profile and the functional profile of the microbiome but requires resource-heavy sequencing equipment and advanced bioinformatics. The cheaper amplicon sequencing of the bacterial 16 S ribosomal RNA genes enables determination of a taxonomic profile without large computational resources, but with lower resolution, at the genus or family level. Metatranscriptomics quantifies microbial RNA to describe how gene transcriptional activity across bacterial species can change according to health or disease.<sup>73</sup>

Several studies have shown alterations in the gut microbiome of patients with cirrhosis or steatohepatitis related to

Table 2. Omics-based biomarkers in hepatology.

|                             | Specimen                                     | Outcomes of interest  | Technology (untargeted)   | Technology (targeted)   | Number of analytes (targeted tech.)  | Examples of biomarker candidates   |
|-----------------------------|--|---|---|---|--|--|
| Genetics                    | Whole blood, buffy coat                      | SNPs, candidate genes, GRS, polygenic scores  | Whole genome sequencing   | Microarray-based genotyping or whole exome sequencing   | >6*10 <sup>6</sup> common SNPs (MAF >0.01)   | <i>PNPLA3</i> , <i>TM6SF2</i> , <i>GCKR</i> , <i>MBOAT7</i> , <i>HSD17B13</i> , <i>SERPINA1</i> <sup>14,41,45,139</sup>  |
| Transcriptomics             | All tissue types, plasma, serum, whole blood | RNA sequences: non-coding RNA (miRNA, long noncoding RNA), coding mRNA, steady state RNA levels     | Reverse transcription-quantitative PCR or small RNA-sequencing  | Reverse transcription-quantitative PCR<br>Targeted sequencing panels  | 10 <sup>5</sup>  | miR-34a, <sup>140</sup> miR-122, miR-21  |
| Proteomics                  | All tissue types and body fluids             | Protein abundance   | Mass spectrometry, Proximity Extension Assay (commercialized by Olink Explore) and SomaScan Assay (commercialized by SomaLogic) | Mass spectrometry (parallel or multiple reaction monitoring). Proximity Extension Assay (used by Olink Target), ELISA | 1 -10 <sup>4</sup>   | TREM-2 was discovered by single-cell sequencing, subsequently developed into an ELISA assay. <sup>141–143</sup> Compliment component C7 identified as a fibrosis marker in two independent biomarker studies. <sup>15,113</sup>  |
| Metabolomics and lipidomics | Plasma, urine, stool, liver, adipose tissue  | Metabolite abundance. Lipid abundance w.r.t. lipid class, lipid saturation/unsaturation, lipid size | Gas or liquid chromatography coupled to mass spectrometry   | Triple-quadrupole mass spectrometry, NMR spectroscopy   | 10 <sup>2</sup> -10 <sup>3</sup>   | Glutamate and glutamine <sup>144</sup><br>Triglycerides, such as TG(48:0) <sup>145</sup> and TG(50:2); <sup>117</sup><br>Phosphatidylcholines, such as PC(36:4); <sup>146</sup> Sphingomyelins, such as SM(41:1) <sup>117,118</sup><br>The Metabolomics-Advanced steatohepatitis fibrosis score developed to detect at-risk MASH |
| Viromics                    | Stool, saliva, plasma, skin                  | Viral genomes (DNA or RNA) and their encoded genes  | Shotgun metagenomic sequencing  | Quantitative PCR  | Variable (depending on sequencing depth and sample diversity) with limited overlap between samples | None, but bacteriophages which target cytolytic <i>Enterococcus faecalis</i> could potentially be markers of resistance against alcohol-induced liver injury. <sup>139</sup>   |
| Microbiomics                | Stool, saliva, skin, mucosa                  | Bacteriomics  | Shotgun metagenomics or amplicon sequencing   | Quantitative PCR or antibody test   | 100-1,000 species per sample, with 10 <sup>5</sup> -10 <sup>6</sup> genes                          | Cytolytic <i>Enterococcus faecalis</i> <sup>139</sup>  |

GRS, genetic risk scores; MAF, minor allele frequency; MASH, metabolic dysfunction-associated steatohepatitis; SNP, single nucleotide polymorphisms; miRNA, microRNA; NMR, nuclear magnetic resonance.



**Fig. 3. Omics timeline with major scientific and technological breakthroughs, using genetics as reference.** The immaturity of most omics technologies result in a shortage of (a) high-quality diagnostic studies, (b) independent validation of novel biomarkers, (c) established cut-offs for clinical decision making, (d) analytical standardisation. GWAS, genome-wide association studies; miRNA, microRNA; exRNA, extracellular RNA; MS, mass spectrometry; NASH, non-alcoholic steatohepatitis; NMR, nuclear magnetic resonance.

ALD or MASLD, compared to healthy individuals.<sup>74–77</sup> The more severe stages of liver disease are associated with dysbiosis, decreased abundance of potentially beneficial families such as *Ruminococcaceae* and *Lachnospiraceae*, and an increase in potentially pathogenic families such as *Enterobacteriaceae* and *Bacteroidaceae*.<sup>23,78</sup> One metagenomic study in patients with decompensated cirrhosis found elevated levels of *Veillonella* and *Streptococcus* species, but reduced levels of butyrate-producing commensal bacteria, including *Faecalibacterium prausnitzii* and *Coprococcus comes*.<sup>77</sup> Other studies have demonstrated increased epithelial permeability in patients with liver disease, which would allow for translocation of bacterial components and metabolites, such as lipopolysaccharides, secondary bile acids and pathogen-associated molecular patterns, fuelling liver inflammation and fibrosis.<sup>79–82</sup> Consequently, microbial products can be important biomarkers of treatment effects, as in the RIFSYS trial, where circulating levels of the microbiome-generated metabolite trimethylamine-N-oxide remained stable in patients with cirrhosis treated with rifaximin- $\alpha$  but increased in placebo-treated patients.<sup>83</sup>

While accumulating evidence indicates that microbial disturbances play a role in the development and progression of liver diseases, the study of gut microbiota and their potential as biomarkers remains in its infancy.<sup>84,85</sup>

### Viromics and mycobionics

The virome and mycobionics, though considered premature omics fields, exhibit promise in light of advancing technologies, making them interesting for future exploration.

The gut virome mainly consists of bacteriophages (viruses infecting bacteria) and viruses infecting eukaryotic cells. Viruses are the most diverse genetic elements on earth, which poses several technical challenges for virome research.<sup>86</sup>

Due to the small genome size of viruses compared to prokaryotes and eukaryotes, the enrichment of faecal samples for viruses before DNA and RNA extraction is recommended. A reverse transcription step is also necessary to capture RNA viruses. As bacteriophages are highly diverse and highly individual specific, they are not sufficiently represented in databases. Hence, a *de novo* genome assembly approach and a viral identification method that is, at least partially, independent of databases is crucial to identify novel viruses from sequencing data.<sup>87</sup>

Recent developments in bioinformatics tools have allowed for improved identification (geNomad), taxonomic classification (vConTACT2), host prediction (iPHoP) and functional annotation (Genote-Taker2) of viral sequences, advancing the field to help identify associations between the virome and human

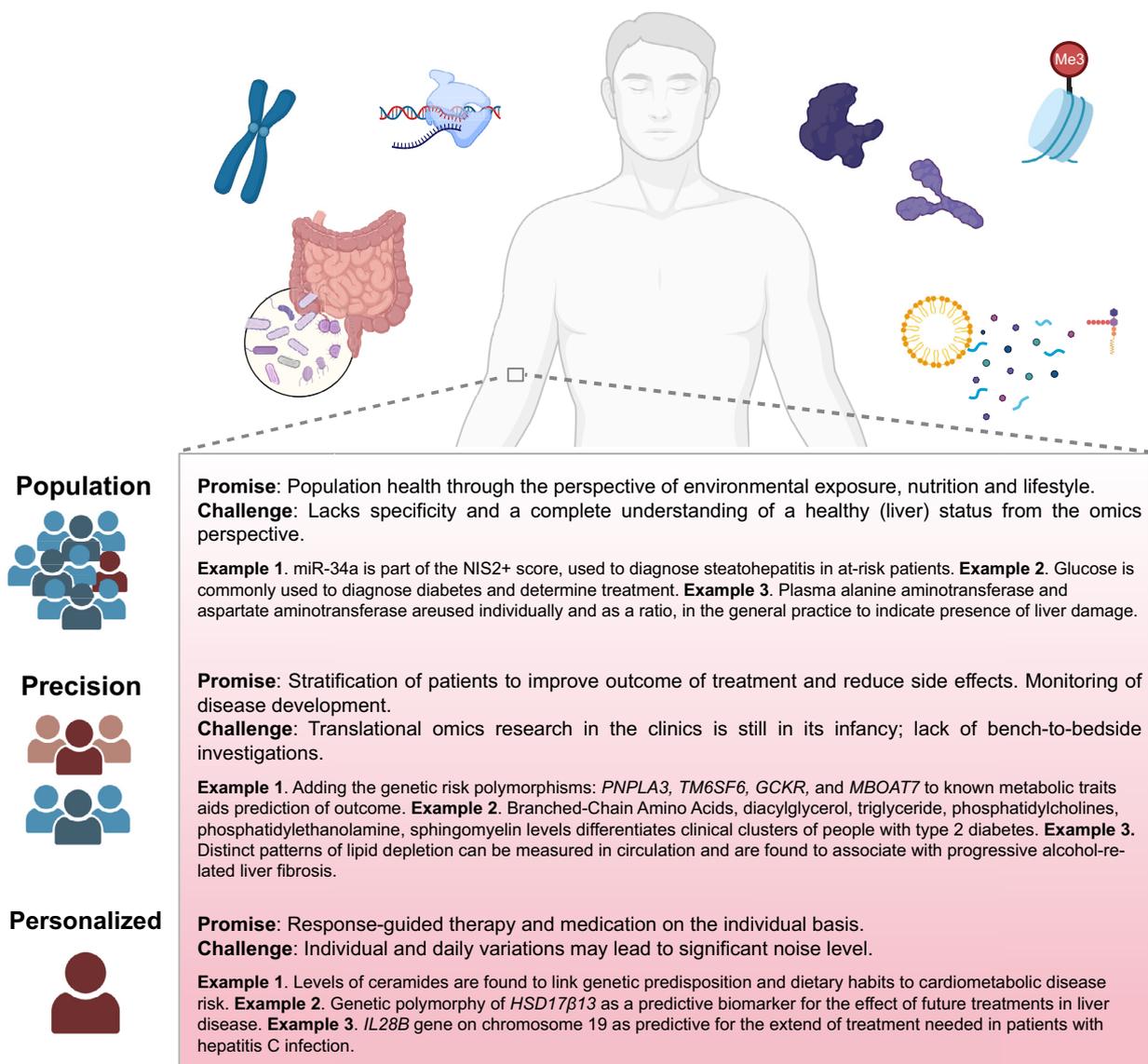


Fig. 4. Population-based vs. personalised omics biomarkers: Promises and challenges. miR, microRNA. Examples are based on references.<sup>64,117,147–150</sup>

health and disease.<sup>88–92</sup> Viruses can directly affect the human host by killing target cells such as hepatocytes or by modulating the immune system. The human host can also be indirectly affected by the gut virome through the effect of gut phages on the composition and function of the gut bacterial community.<sup>93</sup>

Changes in the gut virome have been linked to the presence and severity of liver diseases, such as MASLD, ALD, alcohol-related hepatitis and cirrhosis.<sup>94–97</sup> However, the high inter-individual variability of the human gut virome limits the identification of robust viral biomarkers.<sup>98</sup> Overall, viral diversity might be a better biomarker than a set of individual viruses, but viral diversity lacks disease specificity, as seen with dysbiosis.<sup>94,96</sup> Other approaches which overcome the low prevalence of individual viral genomes are to look for

virome biomarkers of higher taxonomical orders (e.g. families) or to group bacteriophages by their bacterial host, but these more diverse groups of viruses will be more difficult to detect using qPCR tests.<sup>99</sup> Finally, virally encoded genes might be less individual specific, for example, toxins or auxiliary metabolic genes, and hence better suited as biomarkers. These genes could be horizontally transferred to their bacterial hosts, thus altering the functional capacities of the targeted bacteria and thereby indirectly affecting the human host.

The fungal fraction of the microbiome, the mycobiome, is important in maintaining intestinal homeostasis and immunity. But although the field of mycobiome research has advanced, this omics technology is still in its infancy. Early studies have shown that *Candida* overgrowth can be linked to ALD and

cirrhosis, and that elevated levels of anti-*S. cerevisiae* antibodies, which cross-react with *Candida albicans*, are associated with increased mortality in ALD.<sup>100–102</sup>

## Proteomics

Proteins are the most prominent source of biomarkers and drug targets in human diseases. Routine laboratory testing is dominated by proteins (42% of all analytes) and, as of 2017, 75% of drugs approved by the US FDA target human proteins.<sup>28</sup> Aminotransferases, albumin, bilirubin and coagulation factors are examples of proteins that are routinely measured to assess liver function.

Proteomics seeks to map all proteins in a biological sample, with existing platforms quantifying hundreds to tens of thousands of proteins, depending on the sample type. Several cell type-resolved human liver proteome maps have been published, establishing a robust reference for the abundance of over ten thousand proteins in human liver cells.<sup>103</sup> Mass spectrometry (MS)-based proteomics and affinity-based proteomics are commonly used technologies for the large-scale study of proteins. MS-based proteomics is the most comprehensive approach and the gold standard for the quantitative profiling of proteins, post-translational modifications and protein-protein interactions.<sup>104</sup> MS-based proteomics is an ideal approach for unbiased protein profiling across all organisms and sample types (Table 2). The untargeted approach, also known as discovery proteomics, offers a global view of the proteome and is often used to uncover novel biomarkers. However, the lack of standardisation as well as its semi-quantitative nature is a significant hurdle for discovery proteomics – values obtained in a specific study can typically only be compared horizontally to other samples acquired within the same study. In contrast, targeted MS-based proteomics focuses on specific proteins of interest, providing precise quantification, validation and clinical applications.

Recent technological advances in MS-based proteomics, including the automation of sample preparation, improvements in liquid chromatography, as well as the development of novel MS acquisition methods and sophisticated informatics solutions, have made it feasible to generate thousands of proteome profiles in a single clinical study.<sup>105</sup> This further translates into reproducible and robust results. At the same time, researchers have started to apply machine learning-based classification algorithms to demonstrate the predictive or discriminative power of proposed biomarkers in liver disease.

Affinity-based proteomics platforms, such as Olink and SomaScan, have been widely applied in human plasma and serum studies.<sup>45,106,107</sup> These platforms offer measurements for dozens and up to thousands of proteins, with standardised workflows allowing for value comparison across studies. However, studies comparing the two platforms have highlighted inconsistencies in quantification for a significant number of proteins.<sup>108</sup> Consequently, findings from these platforms often require validation by an orthogonal method, ideally mass spectrometry, which excels in its specificity of identification and quantification.<sup>109</sup> Other methods include ELISA and similar techniques, which measure the concentration of a single protein, making them better suited for biomarker validation and implementation.

The FDA-approved OVA1 test for ovarian cancer serves as an example of a biomarker identified by MS-based proteomics but which was ultimately developed using immunoassays. The test consists of a panel of five proteins, four of which were first published in 2004. Five years later the test received FDA clearance.<sup>110</sup>

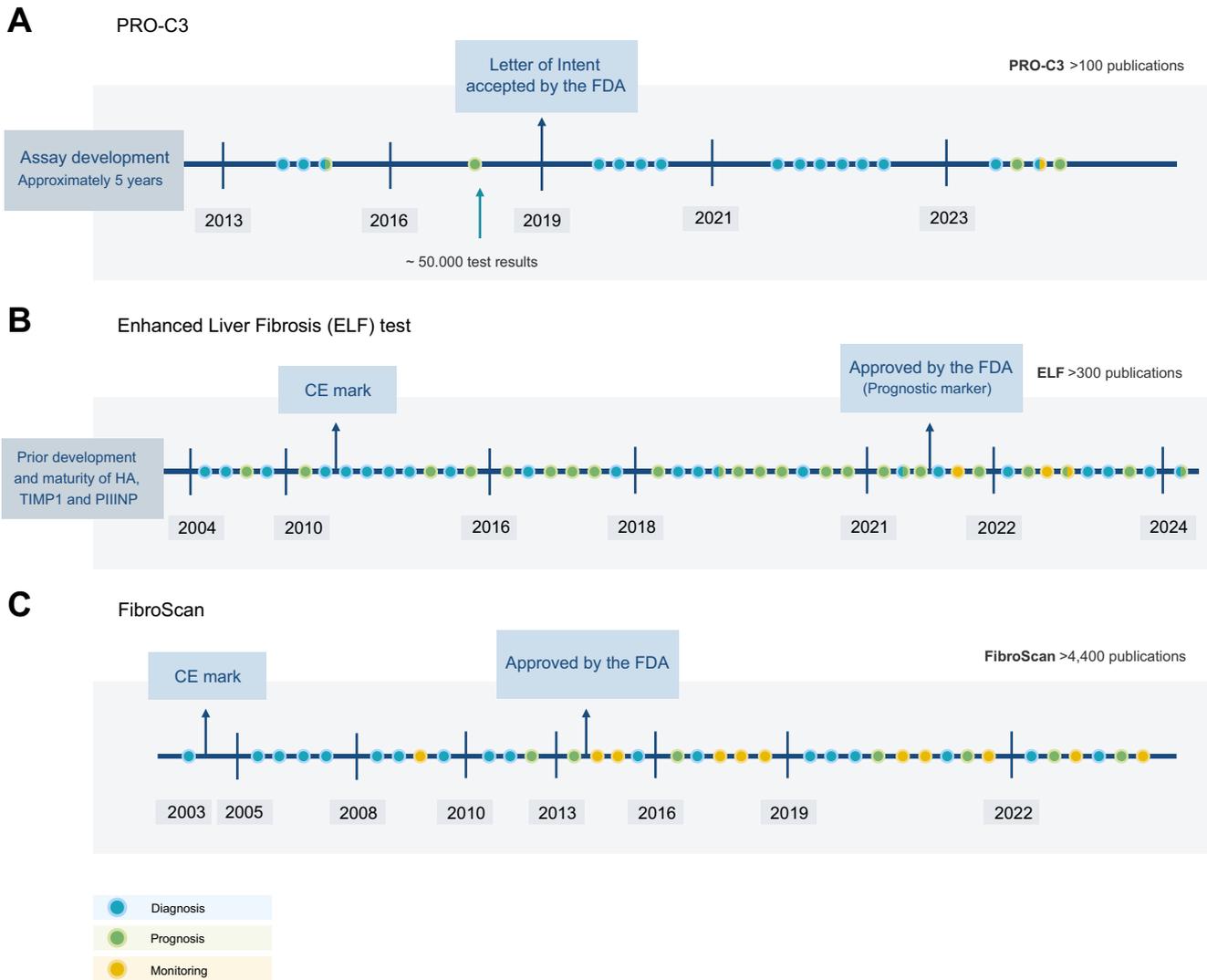
More than 200 candidate protein biomarkers have been reported for MASLD and 22 for ALD, although none have matured into clinical practice.<sup>15,111–113</sup> The two most recent proteomics biomarker studies were selected from 2,201 candidate proteins for MASLD fibrosis and 1,235 candidates for ALD fibrosis, resulting in eight- and nine-protein biomarker panels.<sup>15,113</sup> Complement component C7 was part of both panels, while the other proteins differed. Consequently, much work remains to be done in terms of evaluation of disease specificity and external validation of these signatures.

## Metabolomics and lipidomics

The metabolome comprises all small molecules in the human body, originating from both endogenous and environmental sources, and encompasses a biochemically diverse array of metabolites such as sugars, lipids, amino acids, fatty acids, alkaloids, and polyphenols.<sup>114</sup> One example of a lipid metabolite biomarker is phosphatidylethanol, used to detect alcohol consumption, derived from the trans-phosphatidylation of phosphatidylcholine in the presence of ethanol.<sup>115</sup>

Humans are thought to contain around 3,000 endogenous or common metabolites while the plant kingdom harbours around 200,000 metabolites, of which 90% are still unquantified or unidentified.<sup>114</sup> Metabolomics platforms are usually a combination of different chemical analyses using mass spectrometry. The platforms detect anything between 100 and 1,000 metabolites, and their quality is based on prior work identifying the metabolites with pure standards in in-house identification libraries. Public libraries are available to characterise molecular features but they only provide putative identifications as the certainty is insufficient to derive meaningful conclusions. In addition, machine learning approaches are used to identify the large number of new metabolites.<sup>116</sup> MS- and affinity-based metabolomics can detect several thousand human metabolites, although, as mentioned, the diverse nature of the metabolome necessitates the use of multiple analytical chemistry techniques (Table 2).<sup>114</sup>

Lipidomics is an especially promising metabolomics technique for biomarker discovery in steatotic liver disease. In a study of early ALD, the lipidomic signature of patients with ALD began to differ from matched healthy controls at the stage of minimal fibrosis.<sup>117</sup> The bioactive lipid classes sphingomyelins and phosphocholines were downregulated in both liver tissue and plasma with increasing fibrosis stages and were both diagnostic of significant fibrosis and predictive of liver-related outcomes. This finding was validated in an independent cohort of patients with advanced ALD cirrhosis.<sup>118</sup> Other studies suggest that lipid panels can predict advanced forms of MASLD: molecular lipids in blood have shown good diagnostic performance for MASLD and MASH (metabolic dysfunction-associated steatohepatitis) in well-powered studies, with elevated triglycerides and reduced lysophosphatidylcholines and phospholipids.<sup>119,120</sup> Interestingly, unsaturated triglycerides are increased with the presence of the *PNPLA3* risk



**Fig. 5. Regulatory pathways of three commercial biomarkers.** Illustrating the regulatory timeline of *nordic*PRO-C3<sup>TM</sup>, Enhanced Liver Fibrosis test (ELF) and FibroScan. Each timeline shows significant publications and regulatory milestones. Please refer to the supplementary materials for specific publications and milestones.

variant.<sup>121</sup> A 10-metabolite panel including eight eicosanoid molecules predicted advanced fibrosis with an area under the receiver-operating characteristic curve of 0.94.<sup>122</sup> Finally, recent data suggest that the liver lipidome of patients with ALD responds differently to acute alcohol intoxication than that of patients with MASLD.<sup>123</sup> This finding indicates that there are likely distinct molecular differences between the two diseases, which may explain the marked difference in disease progression and risk of liver-related complications.

The use of metabolomics and lipidomics in hepatology is challenged by specificity, as most known metabolites have common disease pathways.<sup>124</sup> Furthermore, while some metabolites are found to be stable, others, such as glucose and cholesterol, have been shown to exhibit a daily flux or be affected by diet.<sup>125</sup> Hence, the establishment of a baseline level is important, especially when measured longitudinally throughout liver disease progression or regression.

## Multi-omics

Clinical studies are increasingly generating multiple omics layers, allowing for integrated multi-omics investigations of liver disease.<sup>126,127</sup> Machine learning-based feature selection from several omics layers can help determine the diagnostic and prognostic weight of each omics layer, but more importantly, multi-omics integration can capture disease complexity by addressing biologically relevant interactions between genes, their expression and their products. Unfortunately, integrating multiple types of omics remains a computational barrier. Consequently, current multi-omics studies rarely integrate more than two omics layers, and often instead interpret the outputs in parallel.<sup>73,128</sup>

One study of multi-omics integration performed GWAS in 9,491 patients with MASLD and detected 20 gene variants predictive of steatosis and/or cirrhosis.<sup>45</sup> From this, the

researchers combined GWAS with transcriptomics and proteomics to derive expression quantitative trait loci and protein quantitative trait loci in the European population. This multi-omics integration resulted in 16 putative genes associated with 273 circulating proteins, enriched in order to enable multiple metabolic and catabolic processes, including the metabolism of hormones, lipids, alcohol, vitamins, steroids and monocarboxylic acid. This represents an integrative step forward in understanding disease mechanisms.

## The regulatory landscape from an omics perspective

The regulatory qualification of a biomarker requires thorough planning and patience.<sup>11</sup> For example, the Enhanced Liver Fibrosis test (Siemens Healthcare) obtained FDA approval in 2021, with the first core clinical study published in 2004 (Fig. 5).<sup>129,130</sup> For the *nordicPRO-C3*<sup>TM</sup> biomarker (pro-peptide of type III collagen, Nordic Bioscience and Roche Diagnostics), it took 5 years to complete assay development, minimising pre-analytical measurement uncertainty, followed by 6 years to create clinical evidence before having a Letter of Intent accepted by the FDA (Fig. 5).

Every year, thousands of papers on biomarkers are published, yet very few enter clinical practice.<sup>131</sup> This so-called *valley of death* is the consequence of the failed transition from academic studies to implementation and commercialisation.

There are many reasons for the transition to fail. First, understanding the biological, pre-analytical and analytical factors that contribute to measurement uncertainty is important.<sup>132</sup> Second, when validating a biomarker, the FDA mandates the establishment of a predefined hypothesis and statistical analysis plan. Hence, the distribution of the cohort needs to allow for sufficient statistical power to address the potential context of use, whether it is diagnostic, prognostic or predictive. The 2016 BEST (Biomarkers, EndpointS and other Tools) resource from the FDA and National Institutes of Health Biomarker Work Group provides a notable glossary of biomarker definitions.<sup>133</sup> These considerations are important in moving from discovery to the internal and external validation of a biomarker. Third, for a study to adhere to Good Clinical Practice, regulatory

standards, protocols and documents need to be in place, describing procedures for sample collection and handling, measurement techniques and quality assurance systems. Fourth, biomarker measurements need to be conducted within certified laboratories and the informed consent process should encompass the explicit acceptance of sample utilisation for research, as well as for registration and commercialisation. To make a real difference, a biomarker needs to be implemented on a worldwide platform, and while many biomarkers may be interesting in a research setting, very few qualify according to the Clinical and Standards Institute guidelines.

The current failure of omics to transition from academic research to implementation and commercialisation may be partly due to the untargeted nature of most omics analyses, rendering them best suited for discovery. But the field also remains hampered by study designs dominated by retrospective studies that do not adhere to regulatory requirements.<sup>134</sup> However, the burden is not only on biomarker research and development units, but also on regulatory agencies such as the FDA and the EMA, which have been slow to adapt their approval procedures to the large data generated by omics on novel measurement platforms, using advanced biostatistical methods. The Head of Medicines Agencies report on Big Data was only issued in 2019, along with a subgroup report on Bioanalytical Omics.<sup>135,136</sup>

## Conclusion

Omics technologies offer several advantages. They can identify associations between biomolecules and diseases, uncover underlying mechanisms and identify new biomarkers with untargeted hypothesis-free or targeted hypothesis-driven approaches. Despite the growing enthusiasm, we currently find ourselves in an exploratory phase where there is a lack of sufficient high-quality studies to provide the conclusive evidence of analytical validity, discovery, development and validation that would meet the requirements of regulatory authorities. The next 5 to 10 years should see crucial improvements in the evidence base and maturity of multi-omics, allowing for the first omics-based biomarkers to enter clinical practice as precision tools for personalised medicine.

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## Abbreviations

ALD, alcohol-related liver disease; GCKR, glucokinase regulator; GPAM, glycerol-3-phosphate acyltransferase, mitochondrial; GRS, genetic risk scores; GWAS, genome-wide association studies; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; MASLD, metabolic dysfunction-associated steatotic liver disease; MAF, minor allele frequency; MBOAT7, membrane bound O-acyltransferase domain-containing 7; MetALD, MASLD with increased alcohol intake; miRNA, microRNA; MS, mass spectrometry; MTARC1, mitochondrial amidoxime reducing component 1; PNPLA3, patatin-like phospholipase domain-containing protein 3; PSD3, pleckstrin and Sec7 domain-containing 3; qPCR, quantitative

PCR; SERPINA1, serpin family A member 1; SNPs, single nucleotide polymorphisms; TM6SF2, transmembrane 6 superfamily member 2.

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### Conflicts of interest

MT: Speaker's fee from Echosens, Siemens Healthcare, Norgine, Tillotts Pharma, and advisory fee from GE Healthcare. DJL and MK: Fulltime employee and stockholder of Nordic Bioscience. AK: Speaker for Novo Nordisk, Norgine, Siemens and Nordic Bioscience and participated in advisory boards for Norgine, Siemens, Resalis Therapeutics, Boehringer Ingelheim and Novo Nordisk, all outside the submitted work. Research support; Norgine, Siemens, Nordic Bioscience, Astra, Echosens. Consulting Takeda, Resalis Therapeutics, Zealand Pharma, Novo Nordisk, Boehringer Ingelheim. Board member and co-founder Evido. JT: Speaker and/or consulting fees from Versantis, Gore, Boehringer-Ingelheim, Falk, Grifols, Genfit and CSL Behring. LJJ: Speaker's fee from Boehringer Ingelheim Fonds.

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### Supplementary data

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### References

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- [1] Rehm J, Shield KD. Global burden of alcohol use disorders and alcohol liver disease. *Biomedicine* 2019;7. <https://doi.org/10.3390/biomedicines7040099>.
- [2] Riazi K, Azhari H, Charette JH, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2022;7:851–861. [https://doi.org/10.1016/s2468-1253\(22\)00165-0](https://doi.org/10.1016/s2468-1253(22)00165-0).
- [3] Rinella ME, Lazarus JV, Ratzliff V, et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. *J Hepatol* 2023;79: 1542–1556. <https://doi.org/10.1016/j.jhep.2023.06.003>.

- [4] Hagström H, Thiele M, Roelstraete B, Söderling J, Ludvigsson JF. Mortality in biopsy-proven alcohol-related liver disease: a population-based nationwide cohort study of 3453 patients. *Gut* 2021;70:170–179. <https://doi.org/10.1136/gutjnl-2019-320446>.
- [5] Sanyal AJ, Van Natta ML, Clark J, et al. Prospective study of outcomes in adults with nonalcoholic fatty liver disease. *New Engl J Med* 2021;385:1559–1569. <https://doi.org/10.1056/NEJMoa2029349>.
- [6] Crabb DW, Im GY, Szabo G, Mellinger JL, Lucey MR. Diagnosis and treatment of alcohol-associated liver diseases: 2019 practice guidance from the American association for the study of liver diseases. *Hepatology* 2020;71:306–333. <https://doi.org/10.1002/hep.30866>.
- [7] Harrison SA, Allen AM, Dubourg J, Noureddin M, Alkhoury N. Challenges and opportunities in NASH drug development. *Nat Med* 2023;29:562–573. <https://doi.org/10.1038/s41591-023-02242-6>.
- [8] Anstee QM, Castera L, Loomba R. Impact of non-invasive biomarkers on hepatology practice: past, present and future. *J Hepatol* 2022;76:1362–1378. <https://doi.org/10.1016/j.jhep.2022.03026>.
- [9] Israelsen M, Torp N, Johansen S, et al. Validation of the new nomenclature of steatotic liver disease in patients with a history of excessive alcohol intake: an analysis of data from a prospective cohort study. *Lancet Gastroenterol Hepatol* 2024;9:218–228. [https://doi.org/10.1016/s2468-1253\(23\)00443-0](https://doi.org/10.1016/s2468-1253(23)00443-0).
- [10] Thiele M, Johansen S, Israelsen M, et al. Noninvasive assessment of hepatic decompensation. *Hepatology* 2023. <https://doi.org/10.1097/hep.0000000000000618>.
- [11] Rasmussen DGK, Anstee QM, Torstenson R, et al. NAFLD and NASH biomarker qualification in the LITMUS consortium - lessons learned. *J Hepatol* 2023;78:852–865. <https://doi.org/10.1016/j.jhep.2022.11.028>.
- [12] European Association for the Study of the Liver. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis – 2021 update. *J Hepatol* 2021;75:659–689. <https://doi.org/10.1016/j.jhep.2021.05.025>.
- [13] Usher-Smith JA, Sharp Stephen J, Griffin SJ. The spectrum effect in tests for risk prediction, screening, and diagnosis. *BMJ* 2016;353. <https://doi.org/10.1136/bmj.i3139>.
- [14] Ferkingstad E, Sulem P, Atlason BA, et al. Large-scale integration of the plasma proteome with genetics and disease. *Nat Genet* 2021;53:1712–1721. <https://doi.org/10.1038/s41588-021-00978-w>.
- [15] Niu L, Thiele M, Geyer PE, et al. Noninvasive proteomic biomarkers for alcohol-related liver disease. *Nat Med* 2022;28:1277–1287. <https://doi.org/10.1038/s41591-022-01850-y>.
- [16] Masoodi M, Gastaldelli A, Hyötyläinen T, et al. Metabolomics and lipidomics in NAFLD: biomarkers and non-invasive diagnostic tests. *Nat Rev Gastroenterol Hepatol* 2021;18:835–856. <https://doi.org/10.1038/s41575-021-00502-9>.
- [17] Angelini G, Panunzi S, Castagneto-Gissey L, et al. Accurate liquid biopsy for the diagnosis of non-alcoholic steatohepatitis and liver fibrosis. *Gut* 2022. <https://doi.org/10.1136/gutjnl-2022-327498>.
- [18] Niu L, Sulek K, Vasilopoulou CG, et al. Defining NASH from a multi-omics systems biology perspective. *J Clin Med* 2021;10:4673. <https://doi.org/10.3390/jcm10204673>.
- [19] Niu L, Geyer PE, Gupta R, et al. Dynamic human liver proteome atlas reveals functional insights into disease pathways. *Mol Syst Biol* 2022;18: e10947. <https://doi.org/10.15252/msb.202210947>.
- [20] **Qian T, Fujiwara N, Koneru B, et al.** Molecular signature predictive of long-term liver fibrosis progression to inform antifibrotic drug development. *Gastroenterology* 2022;162:1210–1225. <https://doi.org/10.1053/j.gastro.2021.12.250>.
- [21] Wouters OJ, McKee M, Luyten J. Estimated research and development investment needed to bring a new medicine to market, 2009–2018. *JAMA* 2020;323:844–853. <https://doi.org/10.1001/JAMA.2020.1166>.
- [22] Wong VW, Adams LA, de Lédinghen V, Wong GL, Sookoian S. Noninvasive biomarkers in NAFLD and NASH - current progress and future promise. *Nat Rev Gastroenterol Hepatol* 2018;15:461–478. <https://doi.org/10.1038/s41575-018-0014-9>.
- [23] Trebicka J, Bork P, Krag A, Arumugam M. Utilizing the gut microbiome in decompensated cirrhosis and acute-on-chronic liver failure. *Nat Rev Gastroenterol Hepatol* 2021;18:167–180. <https://doi.org/10.1038/s41575-020-00376-3>.
- [24] Whitfield JB, Schwantes-An T-H, Darlay R, et al. A genetic risk score and diabetes predict development of alcohol-related cirrhosis in drinkers. *J Hepatol* 2021. <https://doi.org/10.1016/j.jhep.2021.10.005>.

- [25] Wetterstrand KA. The cost of sequencing a human genome. *Natl Hum Genome Res Inst* 2021. <https://www.genome.gov/about-genomics/factsheets/Sequencing-Human-Genome-cost>. [Accessed 1 August 2023]. Published November 1.
- [26] Schöhlz C, Lyon D, Refsgaard JC, et al. Avoiding abundance bias in the functional annotation of post-translationally modified proteins. *Nat Methods* 2015;12:1003–1004. <https://doi.org/10.1038/nmeth.3621>.
- [27] Mato JM, Martínez-Chantar ML, Lu SC. Systems biology for hepatologists. *Hepatology* 2014;60:736–743. <https://doi.org/10.1002/hep.27023>.
- [28] Geyer PE, Holdt LM, Teupser D, Mann M. Revisiting biomarker discovery by plasma proteomics. *Mol Syst Biol* 2017;13:942. <https://doi.org/10.15252/msb.20156297>.
- [29] Geyer PE, Voytik E, Treit PV, et al. Plasma Proteome Profiling to detect and avoid sample-related biases in biomarker studies. *EMBO Mol Med* 2019;11:e10427. <https://doi.org/10.15252/emmm.201910427>.
- [30] Feldman A, Eder SK, Felder TK, et al. Clinical and metabolic characterization of lean caucasian subjects with non-alcoholic fatty liver. *Am J Gastroenterol* 2017;112:102–110. <https://doi.org/10.1038/ajg.2016.318>.
- [31] López-Vicario C, Checa A, Urdangarin A, et al. Targeted lipidomics reveals extensive changes in circulating lipid mediators in patients with acutely decompensated cirrhosis. *J Hepatol* 2020. <https://doi.org/10.1016/j.jhep.2020.03.046>.
- [32] Marchand CR, Farshidfar F, Rattner J, Bathe OF. A framework for development of useful metabolomic biomarkers and their effective knowledge translation. *Metabolites* 2018;8:59. <https://doi.org/10.3390/metabo8040059>.
- [33] de Gonzalo-Calvo D, Marchese M, Hellemans J, et al. Consensus guidelines for the validation of qRT-PCR assays in clinical research by the CardioRNA consortium. *Mol Ther Methods Clin Dev* 2022;24:171–180. <https://doi.org/10.1016/j.omtm.2021.12.007>.
- [34] Buch S, Stickele F, Trepo E, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. *Nat Genet* 2015;47:1443–1448. <https://doi.org/10.1038/ng.3417>.
- [35] Anstee QM, Darlay R, Cockell S, et al. Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically-characterised cohort. *J Hepatol* 2020;73:505–515. <https://doi.org/10.1016/j.jhep.2020.04.003>.
- [36] Sookoian S, Pirola CJ. Genetic predisposition in nonalcoholic fatty liver disease. *Clin Mol Hepatol* 2017;23:1–12. <https://doi.org/10.3350/cmh.2016.0109>.
- [37] Stickele F, Moreno C, Hampe J, Morgan MY. The genetics of alcohol dependence and alcohol-related liver disease. *J Hepatol* 2017;66:195–211. <https://doi.org/10.1016/j.jhep.2016.08.011>.
- [38] Kranzler HR, Zhou H, Kember RL, et al. Genome-wide association study of alcohol consumption and use disorder in 274,424 individuals from multiple populations. *Nat Commun* 2019;10:1499. <https://doi.org/10.1038/s41467-019-09480-8>.
- [39] Uffelmann E, Huang QQ, Munung NS, et al. Genome-wide association studies. *Nat Rev Methods Primers* 2021;1:59. <https://doi.org/10.1038/s43586-021-00056-9>.
- [40] Zheng M, Hakim A, Konkwo C, et al. Advancing diagnosis and management of liver disease in adults through exome sequencing. *EBioMedicine* 2023;95:104747. <https://doi.org/10.1016/j.ebiom.2023.104747>.
- [41] Emdin CA, Haas M, Ajmera V, et al. Association of genetic variation with cirrhosis: a multi-trait genome-wide association and gene-environment interaction study. *Gastroenterology* 2021;160:1620–1633. <https://doi.org/10.1053/j.gastro.2020.12.011>.
- [42] Haas ME, Pirruccello JP, Friedman SN, et al. Machine learning enables new insights into genetic contributions to liver fat accumulation. *Cell Genom* 2021;1. <https://doi.org/10.1016/j.xgen.2021.100066>.
- [43] Parisinos CA, Wilman HR, Thomas EL, et al. Genome-wide and Mendelian randomisation studies of liver MRI yield insights into the pathogenesis of steatohepatitis. *J Hepatol* 2020;73:241–251. <https://doi.org/10.1016/j.jhep.2020.03.032>.
- [44] Vujkovic M, Ramdas S, Lorenz KM, et al. A multi-ancestry genome-wide association study of unexplained chronic ALT elevation as a proxy for nonalcoholic fatty liver disease with histological and radiological validation. *Nat Genet* 2022;54:761–771. <https://doi.org/10.1038/s41588-022-01078-z>.
- [45] Sveinbjornsson G, Ulfarsson MO, Thorolfsson RB, et al. Multiomics study of nonalcoholic fatty liver disease. *Nat Genet* 2022;54:1652–1663. <https://doi.org/10.1038/s41588-022-01199-5>.
- [46] Mancina RM, Sasidharan K, Lindblom A, et al. PSD3 downregulation confers protection against fatty liver disease. *Nat Metab* 2022;4:60–75. <https://doi.org/10.1038/s42255-021-00518-0>.
- [47] Gellert-Kristensen H, Richardson TG, Davey Smith G, et al. Combined effect of PNPLA3, TM6SF2, and HSD17B13 variants on risk of cirrhosis and hepatocellular carcinoma in the general population. *Hepatology* 2020;72:845–856. <https://doi.org/10.1002/hep.31238>.
- [48] Liu Z, Suo C, Shi O, et al. The health impact of MAFLD, a novel disease cluster of NAFLD, is amplified by the integrated effect of fatty liver disease-related genetic variants. *Clin Gastroenterol Hepatol* 2022;20:e855–e875. <https://doi.org/10.1016/j.cgh.2020.12.033>.
- [49] Israelsen M, Juel HB, Detlefsen S, et al. Metabolic and genetic risk factors are the strongest predictors of severity of alcohol-related liver fibrosis. *Clin Gastroenterol Hepatol* 2022;20:1784–1794.e17. <https://doi.org/10.1016/j.cgh.2020.11.038>.
- [50] Innes H, Morling JR, Buch S, et al. Performance of routine risk scores for predicting cirrhosis-related morbidity in the community. *J Hepatol* 2022;77:365–376. <https://doi.org/10.1016/j.jhep.2022.02.022>.
- [51] Johansen S, Thiele M, Juel HB, Hansen T, Krag A. External validation of a genetic risk score that predicts development of alcohol-related cirrhosis. *J Hepatol* 2022;77:1720–1721. <https://doi.org/10.1016/j.jhep.2022.06.006>.
- [52] De Vincentis A, Tavaglione F, Jamialahmadi O, et al. A polygenic risk score to refine risk stratification and prediction for severe liver disease by clinical fibrosis scores. *Clin Gastroenterol Hepatol* 2022;20:658–673. <https://doi.org/10.1016/j.cgh.2021.05.056>.
- [53] Choi SW, Mak TS-H, O'Reilly PF. Tutorial: a guide to performing polygenic risk score analyses. *Nat Protoc* 2020;15:2759–2772. <https://doi.org/10.1038/s41596-020-0353-1>.
- [54] Ozata DM, Gainetdinov I, Zoch A, O'Carroll D, Zamore PD. PIWI-interacting RNAs: small RNAs with big functions. *Nat Rev Genet* 2019;20:89–108. <https://doi.org/10.1038/s41576-018-0073-3>.
- [55] Pandey KK, Madhry D, Ravi Kumar YS, et al. Regulatory roles of tRNA-derived RNA fragments in human pathophysiology. *Mol Ther Nucleic Acids* 2021;26:161–173. <https://doi.org/10.1016/j.omtn.2021.06.023>.
- [56] Atic AI, Thiele M, Munk A, Dalgaard LT. Circulating miRNAs associated with nonalcoholic fatty liver disease. *Am J Physiology-Cell Physiol* 2023;324:C588–C602. <https://doi.org/10.1152/ajpcell.00253.2022>.
- [57] Sletten AC, Davidson JW, Yagabasan B, et al. Loss of SNORA73 reprograms cellular metabolism and protects against steatohepatitis. *Nat Commun* 2021;12:5214. <https://doi.org/10.1038/s41467-021-25457-y>.
- [58] Huang P, Tu B, Liao H-J, et al. Elevation of plasma tRNA fragments as a promising biomarker for liver fibrosis in nonalcoholic fatty liver disease. *Scientific Rep* 2021;11:5886. <https://doi.org/10.1038/s41598-021-85421-0>.
- [59] Srinivasan S, Yeri A, Cheah PS, et al. Small RNA sequencing across diverse biofluids identifies optimal methods for exRNA isolation. *Cell* 2019;177:446–462.e41. <https://doi.org/10.1016/j.cell.2019.03.024>.
- [60] Murillo OD, Thistlethwaite W, Rozowsky J, et al. exRNA atlas analysis reveals distinct extracellular RNA cargo types and their carriers present across human biofluids. *Cell* 2019;177:463–477.e415. <https://doi.org/10.1016/j.cell.2019.02.018>.
- [61] Hess AL, Larsen LH, Udesen PB, et al. Levels of circulating miR-122 are associated with weight loss and metabolic syndrome. *Obesity (Silver Spring)* 2020;28:493–501. <https://doi.org/10.1002/oby.22704>.
- [62] Willeit P, Skroblin P, Moschen AR, et al. Circulating MicroRNA-122 is associated with the risk of new-onset metabolic syndrome and type 2 diabetes. *Diabetes* 2017;66:347–357. <https://doi.org/10.2337/db16-0731>.
- [63] Hendy OM, Rabie H, El Fouly A, et al. The circulating micro-RNAs (-122, -34a and -99a) as predictive biomarkers for non-alcoholic fatty liver diseases. *Diabetes Metab Syndr Obes* 2019;12:2715–2723. <https://doi.org/10.2147/dms0.S231321>.
- [64] Harrison SA, Ratzliff V, Magnanensi J, et al. NIS2+™, an optimisation of the blood-based biomarker NIS4® technology for the detection of at-risk NASH: a prospective derivation and validation study. *J Hepatol* 2023;79:758–767. <https://doi.org/10.1016/j.jhep.2023.04.031>.
- [65] Zhang X, Mens MMJ, Abozaid YJ, et al. Circulatory microRNAs as potential biomarkers for fatty liver disease: the Rotterdam study. *Aliment Pharmacol Ther* 2021;53:432–442. <https://doi.org/10.1111/apt.16177>.
- [66] Johnson K, Leary PJ, Govaere O, et al. Increased serum miR-193a-5p during non-alcoholic fatty liver disease progression: diagnostic and mechanistic relevance. *JHEP Rep* 2022;4:100409. <https://doi.org/10.1016/j.jhepr.2021.100409>.

- [67] Waidmann O, Köberle V, Brunner F, et al. Serum microRNA-122 predicts survival in patients with liver cirrhosis. *PLoS One* 2012;7:e45652. <https://doi.org/10.1371/journal.pone.0045652>.
- [68] **Huttenhower C, Gevers D, Knight R, et al.** Structure, function and diversity of the healthy human microbiome. *Nature* 2012;486:207–214. <https://doi.org/10.1038/nature11234>.
- [69] Velasquez-Manoff M. Gut microbiome: the peacekeepers. *Nature* 2015;518:S3–S11. <https://doi.org/10.1038/518S3a>.
- [70] Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. *New Engl J Med* 2016;375:2369–2379. <https://doi.org/10.1056/NEJMra1600266>.
- [71] Lang S, Schnabl B. Microbiota and fatty liver disease—the known, the unknown, and the future. *Cell Host Microbe* 2020;28:233–244. <https://doi.org/10.1016/j.chom.2020.07.007>.
- [72] Tripathi A, Debelius J, Brenner DA, et al. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018;15:397–411. <https://doi.org/10.1038/s41575-018-0011-z>.
- [73] Lloyd-Price J, Arze C, Ananthakrishnan AN, et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 2019;569:655–662. <https://doi.org/10.1038/s41586-019-1237-9>.
- [74] Loomba R, Seguritan V, Li W, et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metab* 2017;25:1054–1062.e10. <https://doi.org/10.1016/j.cmet.2017.04.001>.
- [75] Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* 2013;57:601–609. <https://doi.org/10.1002/hep.26093>.
- [76] Boursier J, Mueller O, Barret M, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016;63:764–775. <https://doi.org/10.1002/hep.28356>.
- [77] **Qin N, Yang F, Li A, et al.** Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014;513:59–64. <https://doi.org/10.1038/nature13568>.
- [78] Trebicka J, Macnaughtan J, Schnabl B, Shawcross DL, Bajaj JS. The microbiota in cirrhosis and its role in hepatic decompensation. *J Hepatol* 2021;75:S67–S81. <https://doi.org/10.1016/j.jhep.2020.11.013>.
- [79] Parlesak A, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *J Hepatol* 2000;32:742–747. [https://doi.org/10.1016/s0168-8278\(00\)80242-1](https://doi.org/10.1016/s0168-8278(00)80242-1).
- [80] Miele L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009;49:1877–1887. <https://doi.org/10.1002/hep.22848>.
- [81] Bajaj JS, Reddy KR, O’Leary JG, et al. Serum levels of metabolites produced by intestinal microbes and lipid moieties independently associated with acute-on-chronic liver failure and death in patients with cirrhosis. *Gastroenterology* 2020;159:1715–1730.e1712. <https://doi.org/10.1053/j.gastro.2020.07.019>.
- [82] Seki E, De Minicis S, Osterreicher CH, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007;13:1324–1332. <https://doi.org/10.1038/nm1663>.
- [83] Patel VC, Lee S, McPhail MJW, et al. Rifaximin- $\alpha$  reduces gut-derived inflammation and mucin degradation in cirrhosis and encephalopathy: RIFSYS randomised controlled trial. *J Hepatol* 2022;76:332–342. <https://doi.org/10.1016/j.jhep.2021.09.010>.
- [84] Wirbel J, Pyl PT, Kartal E, et al. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. *Nat Med* 2019;25:679–689. <https://doi.org/10.1038/s41591-019-0406-6>.
- [85] **Kartal E, Schmidt TSB, Molina-Montes E, et al.** A faecal microbiota signature with high specificity for pancreatic cancer. *Gut* 2022;71:1359–1372. <https://doi.org/10.1136/gutjnl-2021-324755>.
- [86] **Garmaeva S, Sinha T, Kurilshikov A, et al.** Studying the gut virome in the metagenomic era: challenges and perspectives. *BMC Biol* 2019;17:84. <https://doi.org/10.1186/s12915-019-0704-y>.
- [87] **Espen LV, Bak E, Beller L, et al.** A previously undescribed highly prevalent phage identified in a Danish enteric virome catalog. *mSystems* 2021;6:e0038221. <https://doi.org/10.1128/mSystems.00382-21>.
- [88] Townsend EM, Kelly L, Muscatt G, et al. The human gut phageome: origins and roles in the human gut microbiome. *Front Cel Infect Microbiol* 2021;11:643214. <https://doi.org/10.3389/fcimb.2021.643214>.
- [89] **Bin Jang H, Bolduc B, Zablocki O, et al.** Taxonomic assignment of uncultivated prokaryotic virus genomes is enabled by gene-sharing networks. *Nat Biotechnol* 2019;37:632–639. <https://doi.org/10.1038/s41587-019-0100-8>.
- [90] Roux S, Camargo AP, Coutinho FH, et al. iPHoP: an integrated machine learning framework to maximize host prediction for metagenome-derived viruses of archaea and bacteria. *Plos Biol* 2023;21:e3002083. <https://doi.org/10.1371/journal.pbio.3002083>.
- [91] Tisza MJ, Belford AK, Dominguez-Huerta G, Bolduc B, Buck CB. Cenote-Taker 2 democratizes virus discovery and sequence annotation. *Virus Evol* 2021;7:veaa100. <https://doi.org/10.1093/ve/veaa100>.
- [92] Camargo AP, Roux S, Schulz F, et al. Identification of mobile genetic elements with geNomad. *Nat Biotechnol* 2023. <https://doi.org/10.1038/s41587-023-01953-y>.
- [93] Virgin HW. The virome in mammalian physiology and disease. *Cell* 2014;157:142–150. <https://doi.org/10.1016/j.cell.2014.02.032>.
- [94] **Lang S, Demir M, Martin A, et al.** Intestinal virome signature associated with severity of nonalcoholic fatty liver disease. *Gastroenterology* 2020;159:1839–1852. <https://doi.org/10.1053/j.gastro.2020.07.005>.
- [95] Hsu CL, Zhang X, Jiang L, et al. Intestinal virome in patients with alcohol use disorder and after abstinence. *Hepatol Commun* 2022. <https://doi.org/10.1002/hep4.1947>.
- [96] **Jiang L, Lang S, Duan Y, et al.** Intestinal virome in patients with alcoholic hepatitis. *Hepatology* 2020;72:2182–2196. <https://doi.org/10.1002/hep.31459>.
- [97] Bajaj JS, Sikaroodi M, Shamsadini A, et al. Interaction of bacterial metagenome and virome in patients with cirrhosis and hepatic encephalopathy. *Gut* 2021;70:1162–1173. <https://doi.org/10.1136/gutjnl-2020-322470>.
- [98] Shkoporov AN, Clooney AG, Sutton TDS, et al. The human gut virome is highly diverse, stable, and individual specific. *Cell Host Microbe* 2019;26:527–541.e525. <https://doi.org/10.1016/j.chom.2019.09.009>.
- [99] **de Jonge PA, Wortelboer K, Scheithauer TPM, et al.** Gut virome profiling identifies a widespread bacteriophage family associated with metabolic syndrome. *Nat Commun* 2022;13:3594. <https://doi.org/10.1038/s41467-022-31390-5>.
- [100] Lang S, Duan Y, Liu J, et al. Intestinal fungal dysbiosis and systemic immune response to fungi in patients with alcoholic hepatitis. *Hepatology* 2020;71:522–538. <https://doi.org/10.1002/hep.30832>.
- [101] **Yang AM, Inamine T, Hochrath K, et al.** Intestinal fungi contribute to development of alcoholic liver disease. *J Clin Invest* 2017;127:2829–2841. <https://doi.org/10.1172/jci90562>.
- [102] Bajaj JS, Liu EJ, Kheradman R, et al. Fungal dysbiosis in cirrhosis. *Gut* 2018;67:1146–1154. <https://doi.org/10.1136/gutjnl-2016-313170>.
- [103] Sun A, Jiang Y, Wang X, et al. Liverbase: a comprehensive view of human liver biology. *J Proteome Res* 2010;9:50–58. <https://doi.org/10.1021/pr900191p>.
- [104] Sinha A, Mann M. A beginner’s guide to mass spectrometry-based proteomics. *The Biochemist* 2020;42:64–69. <https://doi.org/10.1042/bio20200057>.
- [105] Geyer PE, Kulak NA, Pichler G, et al. Plasma proteome profiling to assess human health and disease. *Cell Syst* 2016;2:185–195. <https://doi.org/10.1016/j.cels.2016.02.015>.
- [106] Luo Y, Wadhawan S, Greenfield A, et al. SOMAscan proteomics identifies serum biomarkers associated with liver fibrosis in patients with NASH. *Hepatol Commun* 2021;5:760–773. <https://doi.org/10.1002/hep4.1670>.
- [107] Fourman LT, Stanley TL, Ockene MW, et al. Proteomic analysis of hepatic fibrosis in human immunodeficiency virus-associated nonalcoholic fatty liver disease demonstrates up-regulation of immune response and tissue repair pathways. *J Infect Dis* 2022;227:565–576. <https://doi.org/10.1093/infdis/jiac475>.
- [108] Katz DH, Robbins JM, Deng S, et al. Proteomic profiling platforms head to head: leveraging genetics and clinical traits to compare aptamer- and antibody-based methods. *Sci Adv* 2022;8:eabm5164. <https://doi.org/10.1126/sciadv.abm5164>.
- [109] Bader JM, Albrecht V, Mann M. MS-based proteomics of body fluids: the end of the beginning. *Mol Cel Proteomics* 2023;22:100577. <https://doi.org/10.1016/j.mcpro.2023.100577>.
- [110] Fung ET. A recipe for proteomics diagnostic test development: the OVA1 test, from biomarker discovery to FDA clearance. *Clin Chem* 2010;56:327–329. <https://doi.org/10.1373/clinchem.2009.140855>.
- [111] Niu L, Geyer PE, Wewer Albrechtsen NJ, et al. Plasma proteome profiling discovers novel proteins associated with non-alcoholic fatty liver disease. *Mol Syst Biol* 2019;15:e8793. <https://doi.org/10.1525/msb.20188793>.
- [112] Govaere O, Hasoon M, Alexander L, et al. A proteo-transcriptomic map of non-alcoholic fatty liver disease signatures. *Nat Metab* 2023. <https://doi.org/10.1038/s42255-023-00775-1>.

- [113] Sanyal AJ, Williams SA, Lavine JE, et al. Defining the serum proteomic signature of hepatic steatosis, inflammation, ballooning and fibrosis in non-alcoholic fatty liver disease. *J Hepatol* 2023;78:693–703. <https://doi.org/10.1016/j.jhep.2022.11.029>.
- [114] Wishart DS, Guo A, Oler E, et al. Hmdb 5.0: the human metabolome database for 2022. *Nucleic Acids Res* 2022;50:D622–D631. <https://doi.org/10.1093/nar/gkab1062>.
- [115] Cabezas J, Lucey MR, Bataller R. Biomarkers for monitoring alcohol use. *Clin Liver Dis (Hoboken)* 2016;8:59–63. <https://doi.org/10.1002/cld.571>.
- [116] Guijas C, Montenegro-Burke JR, Domingo-Almenara X, et al. METLIN: a technology platform for identifying knowns and unknowns. *Anal Chem* 2018;90:3156–3164. <https://doi.org/10.1021/acs.analchem.7b04424>.
- [117] Thiele M, Suvitaival T, Trošt K, et al. Sphingolipids are depleted in alcohol-related liver fibrosis. *Gastroenterology* 2023;164:1248–1260. <https://doi.org/10.1053/j.gastro.2023.02.023>.
- [118] Kronborg TM, Gao Q, Trošt K, et al. Low sphingolipid levels predict poor survival in patients with alcohol-related liver disease. *JHEP Rep* 2024;6:100953. <https://doi.org/10.1016/j.jhepr.2023.100953>.
- [119] Luukkonen PK, Zhou Y, Sädevirta S, et al. Hepatic ceramides dissociate steatosis and insulin resistance in patients with non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1167–1175. <https://doi.org/10.1016/j.jhep.2016.01.002>.
- [120] Mayo R, Crespo J, Martínez-Arranz I, et al. Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: results from discovery and validation cohorts. *Hepatol Commun* 2018;2:807–820. <https://doi.org/10.1002/hep4.1188>.
- [121] Orešič M, Hyötyläinen T, Kotronen A, et al. Prediction of non-alcoholic fatty-liver disease and liver fat content by serum molecular lipids. *Diabetologia* 2013;56:2266–2274. <https://doi.org/10.1007/s00125-013-2981-2>.
- [122] Caussy C, Ajmera VH, Puri P, et al. Serum metabolites detect the presence of advanced fibrosis in derivation and validation cohorts of patients with non-alcoholic fatty liver disease. *Gut* 2019;68:1884–1892. <https://doi.org/10.1136/gutjnl-2018-317584>.
- [123] Israelsen M, Kim M, Suvitaival T, et al. Comprehensive lipidomics reveals phenotypic differences in hepatic lipid turnover in ALD and NAFLD during alcohol intoxication. *JHEP Rep* 2021;3:100325. <https://doi.org/10.1016/j.jhepr.2021.100325>.
- [124] Pietzner M, Stewart ID, Raffler J, et al. Plasma metabolites to profile pathways in noncommunicable disease multimorbidity. *Nat Med* 2021;27:471–479. <https://doi.org/10.1038/s41591-021-01266-0>.
- [125] Kervezee L, Cermakian N, Boivin DB. Individual metabolomic signatures of circadian misalignment during simulated night shifts in humans. *Plos Biol* 2019;17:e3000303. <https://doi.org/10.1371/journal.pbio.3000303>.
- [126] Atabaki-Pasdar N, Ohlsson M, Viñuela A, et al. Predicting and elucidating the etiology of fatty liver disease: a machine learning modeling and validation study in the IMI DIRECT cohorts. *Plos Med* 2020;17:e1003149. <https://doi.org/10.1371/journal.pmed.1003149>.
- [127] Wood GC, Chu X, Argyropoulos G, et al. A multi-component classifier for nonalcoholic fatty liver disease (NAFLD) based on genomic, proteomic, and phenomic data domains. *Sci Rep* 2017;7:43238. <https://doi.org/10.1038/srep43238>.
- [128] Tarazona S, Arzalluz-Luque A, Conesa A. Undisclosed, unmet and neglected challenges in multi-omics studies. *Nat Comput Sci* 2021;1:395–402. <https://doi.org/10.1038/s43588-021-00086-z>.
- [129] Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004;127:1704–1713. <https://doi.org/10.1053/j.gastro.2004.08.052>.
- [130] Gee Matthew SHDI. In: Kelm KB, editor. Prognostic test for assessment of liver related disease progression; 2021. [www.accessdata.fda.gov](http://www.accessdata.fda.gov).
- [131] Ioannidis JPA, Bossuyt PMM. Waste, leaks, and failures in the biomarker pipeline. *Clin Chem* 2017;63:963–972. <https://doi.org/10.1373/clinchem.2016.254649>.
- [132] Selby PJ, Banks RE, Gregory W, et al. Methods for the evaluation of biomarkers in patients with kidney and liver diseases: multicentre research programme including ELUCIDATE RCT. Programme Grants Appl Res 2018;6. <https://doi.org/10.3310/pgfar06030>.
- [133] Group F-NBW. BEST (biomarkers, Endpoints, and other tools) national Institutes of health (US), Bethesda (MD), 2016. <https://www.ncbi.nlm.nih.gov/books/NBK326791/>; 2016.
- [134] McShane LM, Cavenagh MM, Lively TG, et al. Criteria for the use of omics-based predictors in clinical trials: explanation and elaboration. *BMC Med* 2013;11:220. <https://doi.org/10.1186/1741-7015-11-220>.
- [135] König R, Cave A, Goldammer M, Meulendijks D. Bioanalytical omics subgroup report, 2019. [www.ema.europa.eu](http://www.ema.europa.eu); 2019.
- [136] Agency EM. HMA-EMA joint big data taskforce. Heads of Medicines Agencies 2019. [www.hma.eu/about-hma/working-groups/hma/ema-joint-big-data-steering-group/hma/ema-joint-big-data-steering-group.html](http://www.hma.eu/about-hma/working-groups/hma/ema-joint-big-data-steering-group/hma/ema-joint-big-data-steering-group.html).
- [137] Lehmann R, Franken H, Dammeyer S, et al. Circulating lysophosphatidylcholines are markers of a metabolically benign nonalcoholic fatty liver. *Diabetes Care* 2013;36:2331–2338. <https://doi.org/10.2337/dc12-1760>.
- [138] Borisevich D, Schnurr TM, Engelbrechtsen L, et al. Non-linear interaction between physical activity and polygenic risk score of body mass index in Danish and Russian populations. *PLOS ONE* 2021;16:e0258748. <https://doi.org/10.1371/journal.pone.0258748>.
- [139] Duan Y, Llorente C, Lang S, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature* 2019;575:505–511. <https://doi.org/10.1038/s41586-019-1742-x>.
- [140] Harrison SA, Ratzliff V, Boursier J, et al. A blood-based biomarker panel (NIS4) for non-invasive diagnosis of non-alcoholic steatohepatitis and liver fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol* 2020;5:970–985. [https://doi.org/10.1016/s2468-1253\(20\)30252-1](https://doi.org/10.1016/s2468-1253(20)30252-1).
- [141] Ramachandran P, Dobie R, Wilson-Kanamori JR, et al. Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature* 2019;575:512–518. <https://doi.org/10.1038/s41586-019-1631-3>.
- [142] Indira Chandran V, Wernberg CW, Lauridsen MM, et al. Circulating TREM2 as a noninvasive diagnostic biomarker for NASH in patients with elevated liver stiffness. *Hepatology* 2023;77:558–572. <https://doi.org/10.1002/hep.32620>.
- [143] Kothari V, Savard C, Tang J, et al. sTREM2 is a plasma biomarker for human NASH and promotes hepatocyte lipid accumulation. *Hepatol Commun* 2023;7. <https://doi.org/10.1097/hc9.0000000000000265>.
- [144] Gaggini M, Carli F, Rosso C, et al. Altered amino acid concentrations in NAFLD: impact of obesity and insulin resistance. *Hepatology* 2018;67:145–158. <https://doi.org/10.1002/hep.29465>.
- [145] Zhou Y, Llauro G, Orešič M, et al. Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. *J Hepatol* 2015;62:657–663. <https://doi.org/10.1016/j.jhep.2014.10.010>.
- [146] Muta K, Saito K, Kemmochi Y, et al. Phosphatidylcholine (18:0/20:4), a potential biomarker to predict ethionamide-induced hepatic steatosis in rats. *J Appl Toxicol* 2022;42:1533–1547. <https://doi.org/10.1002/jat.4324>.
- [147] Sliker RC, Donnelly LA, Fitipaldi H, et al. Distinct molecular signatures of clinical clusters in people with type 2 diabetes: an IMI-RHAPSODY study. *Diabetes* 2021;70:2683–2693. <https://doi.org/10.2337/db20-1281>.
- [148] Wittenbecher C, Cuadrat R, Johnston L, et al. Dihydroceramide- and ceramide-profiling provides insights into human cardiometabolic disease etiology. *Nat Commun* 2022;13:936. <https://doi.org/10.1038/s41467-022-28496-1>.
- [149] Collins JM, Isaacs C. Management of breast cancer risk in BRCA1/2 mutation carriers who are unaffected with cancer. *Breast J* 2020;26:1520–1527. <https://doi.org/10.1111/tbj.13970>.
- [150] Nashed AL, Rao KW, Gullely ML. Clinical applications of BCR-ABL molecular testing in acute leukemia. *J Mol Diagn* 2003;5:63–72. [https://doi.org/10.1016/s1525-1578\(10\)60454-0](https://doi.org/10.1016/s1525-1578(10)60454-0).

**Supplemental information**

**Opportunities and barriers in omics-based biomarker discovery for steatotic liver diseases**

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## nordicPRO-C3™

### Regulatory milestone

| Year | Description  |
|------|--|
| 2019 | A letter of intent accepted by the FDA for <i>nordicPRO-C3™</i> as a diagnostic marker |

### References

| Year | Context of use               | Aetiology            | Reference   |
|------|------------------------------|----------------------|---|
| 2013 | Diagnostic                   | ALD                  | Leeming et al.<br>Alimentary Pharmacology & Therapeutics<br>DOI: 10.1111/apt.12484            |
| 2014 | Diagnostic                   | HCV/HIV co-infection | Jansen et al.<br>PLoS One<br>DOI: 10.1371/journal.pone.0108544                                |
| 2015 | Diagnostic/Prognostic        | HCV                  | Nielsen et al.<br>Liver Int.<br>DOI: 10.1111/liv.12700  |
| 2018 | Prognostic                   | PSC                  | Nielsen et al.<br>Alimentary Pharmacology & Therapeutics<br>DOI: 10.1111/apt.14806            |
| 2019 | Diagnostic                   | T2D                  | F. Bril et al.<br>Diabetes Care<br>DOI: 10.2337/dc18-2578                                     |
| 2019 | Secondary endpoint           | MASH                 | S.A. Harrison et al.<br>Lancet<br>DOI: 10.1016/S0140-6736(19)32517-6                          |
| 2019 | Diagnostic                   | MASLD                | S. J. Daniels et al.<br>Hepatology<br>DOI: 10.1002/oby.22344                                  |
| 2019 | Diagnostic                   | MASLD                | M. Boyle et al<br>JHEP Reports<br>DOI: 10.1016/j.jhepr.2019.06.004                            |
| 2021 | Diagnostic                   | ALD                  | B. S. Madsen et al.<br>AP&T<br>DOI: 10.1111/apt.16513   |
| 2021 | Diagnostic                   | MASLD/MASH           | P.B. Lassen et al.<br>J of Clinical Endocrinology & Metabolism<br>DOI: 10.1210/clinem/dgab897 |
| 2021 | Diagnosis (systemic review)  | MASLD                | Mak et al.<br>Biomedicines<br>DOI: 10.3390/biomedicines9121920                                |
| 2021 | Trial inclusion (diagnostic) | MASH                 | M.J. Nielsen et al.<br>Journal of Hepatology<br>DOI: 10.1016/j.jhep.2021.08.016               |
| 2022 | Diagnostic                   | MASLD                | L Tang et al.<br>Hepatology International<br>DOI: 10.1007/s12072-022-10420-w                  |
| 2022 | Diagnostic                   | MASLD                | L Tang et al.<br>Metabolism Clinical and Experimental<br>DOI: 10.1016/j.metabol.2021.154958.  |
| 2023 | Screening (diagnostic)       | NAFLD/NASH           | Y. Vali et al. (LITMUS)<br>Lancet Gastro Hep<br>DOI: 10.1016/S2468-1253(23)00017-1            |

|      |                           |                        |  |
|------|---------------------------|------------------------|--|
| 2023 | Prognostic                | ALD                    | S. Johansen et al.<br>Liver International<br>DOI: 10.1111/liv.15595      |
| 2023 | Diagnostic and monitoring | MASLD                  | A. Armandi et al.<br>J of Clinical Medicine<br>DOI: 10.3390/jcm12020650  |
| 2023 | Prognostic                | HCV and a mixed cohort | M. J. Nielsen et al.<br>JHEP reports<br>DOI: 10.1016/j.jhepr.2023.100743 |

## Enhanced Liver Fibrosis (ELF) test

### Regulatory milestones

| Year | Description  |
|------|--|
| 2004 | Publication in Gastro with ELF measured on Immuno 1 platform                                     |
| 2011 | CE mark. The first time ELF was commercially available for sale as an in vitro diagnostic device |
| 2021 | Approved by the FDA as a prognostic marker   |

### References

| Year | Context of use            | Aetiology | Reference   |
|------|---------------------------|-----------|---|
| 2004 | Diagnostic                | Mixed     | Rosenberg et al.<br>Gastroenterology<br>DOI: 10.1053/j.gastro.2004.08.052   |
| 2008 | Diagnostic                | MASLD     | Guha et al.<br>Hepatology<br>DOI: 10.1002/hep.21984                         |
| 2008 | Prognostic                | PBC       | Mayo et al.<br>Hepatology<br>DOI: 10.1002/hep.22517                         |
| 2009 | Diagnostic                | MASLD     | Nobili et al.<br>Gastroenterology<br>DOI: 10.1053/j.gastro.2008.09.013      |
| 2010 | Diagnostic                | Mixed     | Friedrich-Rust et al.<br>BMC Gastroenterol<br>DOI: 10.1186/1471-230X-10-103 |
| 2010 | Prognostic                | Mixed     | Parkes et al.<br>Gut<br>DOI: 10.1136/gut.2009.203166                        |
| 2011 | Diagnostic                | HCV       | Parkes et al.<br>J Viral Hepat<br>DOI: 10.1111/j.1365-2893.2009.01263.x     |
| 2012 | Diagnostic                | HBV       | Kim et al.<br>PLoS One<br>DOI: 10.1371/journal.pone.0041964                 |
| 2013 | Diagnostic / Normal Range | HCV       | Lichtinghagen et al.<br>J Hepatol<br>DOI: 10.1016/j.jhep.2013.03.016        |
| 2013 | Normal Range              | ---       | Yoo et al.<br>Liver Int.<br>DOI: 10.1111/liv.12136                          |
| 2014 | Prognostic                | HBV       | Kim et al.<br>Hepatology  |

|      |                       |         |  |
|------|-----------------------|---------|--|
|      |                       |         | DOI: 10.1002/hep.27389   |
| 2015 | Diagnostic            | Mixed   | Fagan et al.<br>Liver Int.<br>DOI: 10.1111/liv.12760   |
| 2015 | Prognostic            | PSC     | Vesterhus et al.<br>Hepatology<br>DOI: 10.1002/hep.27825   |
| 2016 | Prognostic            | Mixed   | Irvine et al.<br>Liver Int.<br>DOI: 10.1111/liv.12896  |
| 2016 | Diagnostic            | MASLD   | NICE guideline NG49<br><a href="https://www.nice.org.uk/guidance/ng49">https://www.nice.org.uk/guidance/ng49</a> |
| 2016 | Prognostic            | HIV/HCV | Peters et al.<br>AIDS<br>DOI: 10.1097/QAD.0000000000000975   |
| 2016 | Prognostic            | HCV     | Puigvehí et al.<br>PLoS One<br>DOI: 10.1371/journal.pone.0164883   |
| 2017 | Prognostic            | PSC     | de Vries et al.<br>Liver Int.<br>DOI: 10.1111/liv.13402  |
| 2017 | Diagnostic            | MASLD   | Miele et al.<br>Int J Biol Markers<br>DOI: 10.5301/ijbm.5000292  |
| 2018 | Prognostic            | Mixed   | Loo et al.<br>Clin Chem<br>DOI: 10.1373/clinchem.2018.289108   |
| 2018 | Diagnostic            | ALD     | Thiele et al.<br>Gastroenterology<br>DOI: 10.1053/j.gastro.2018.01.005   |
| 2019 | Diagnostic            | MASLD   | Anstee et al.<br>Hepatology<br>DOI: 10.1002/hep.30842  |
| 2019 | Diagnostic/Prognostic | Mixed   | Day et al.<br>J Appl Lab Med<br>DOI: 10.1373/jalm.2018.027359  |
| 2019 | Prognostic            | HBV     | Liang et al.<br>Aliment Pharmacol Ther.<br>DOI: 10.1111/apt.15269  |
| 2019 | Prognostic            | PSC     | Muir et al.<br>Hepatology<br>DOI: 10.1002/hep.30237  |
| 2019 | Prognostic            | MASLD   | Sanyal et al.<br>Hepatology<br>DOI: 10.1002/hep.30664  |
| 2019 | Diagnostic            | MASLD   | Srivastava et al.<br>J Hepatol.<br>DOI: 10.1016/j.jhep.2019.03033  |
| 2019 | Prognostic            | MASLD   | Younossi et al.<br>Clin Gastroenterol Hepatol.<br>DOI: 10.1016/j.cgh.2019.02.024                                 |
| 2020 | Prognostic            | Mixed   | Trembling et al.<br>BMC Gastroenterol.<br>DOI: 10.1186/s12876-020-01251-w  |
| 2021 | Prognostic            | MASLD   | Are et al.<br>Clin Gastroenterol Hepatol.<br>DOI: 10.1016/j.cgh.2020.06.070                                      |

|      |                              |       |   |
|------|------------------------------|-------|---|
| 2021 | Diagnostic/Prognostic        | ALD   | Connoley et al.<br>BMC Gastroenterol.<br>DOI: 10.1186/s12876-021-01795-5        |
| 2021 | Prognostic                   | ALD   | Rasmussen et al.<br>J Hepatol.<br>DOI: 10.1016/j.jhep.2021.05.037               |
| 2021 | Screening                    | ALD   | Rhodes et al.<br>BMC Gastroenterol.<br>DOI: 10.1186/s12876-021-01728-2          |
| 2021 | Monitoring                   | PSC   | Trivedi et al.<br>Clin Gastroenterol Hepatol.<br>DOI: 10.1016/j.cgh.2020.07.032 |
| 2021 | Prognostic                   | MASLD | Younossi et al.<br>Gastroenterology.<br>DOI: 10.1053/j.gastro.2020.12.003       |
| 2022 | Screening                    | Mixed | Åberg et al.<br>United European Gastroenterol J.<br>DOI: 10.1002/ueg2.12323     |
| 2022 | Prognostic                   | MASLD | Johnson et al.<br>Hepatol Commun.<br>DOI: 10.1002/hep4.1852                     |
| 2022 | Monitoring                   | MASLD | Rinella et al.<br>J Hepatol.<br>DOI: 10.1016/j.jhep.2021.10.029                 |
| 2022 | Prognostic/Monitoring        | MASLD | Sanyal et al.<br>Hepatology.<br>DOI: 10.1002/hep.32204                          |
| 2022 | Diagnostic                   | MASLD | Younossi et al.<br>Aliment Pharmacol Ther.<br>DOI: 10.1111/apt.17346            |
| 2023 | Normal Range<br>(Diagnostic) | ---   | Palladino et al.<br>Clin Chim Acta.<br>DOI: 10.1016/j.cca.2023.117461           |
| 2023 | Prognostic                   | Mixed | Saarinen et al.<br>JHEP Rep.<br>DOI: 10.1016/j.jhepr.2023.100765                |
| 2023 | Diagnostic                   | MASLD | Sanyal et al.<br>Nat Med.<br>DOI: 10.1038/s41591-023-02539-6                    |
| 2024 | Diagnostic/Prognostic        | MASLD | Rinella et al.<br>Hepatology<br>DOI: 10.1097/HEP.0000000000000323               |

## FibroScan

### Regulatory milestones

| Year | Description   |
|------|---|
| 2003 | CE mark as a class IIa medical device                   |
| 2013 | First 510(k) FDA clearance for FibroScan medical device |

### References

| Year | Context of use | Aetiology | Reference  |
|------|----------------|-----------|--|
| 2003 | Diagnostic     | HCV       | Sandrin et al.<br>Ultrasound in Medicine & Biology |

|      |            |       |  |
|------|------------|-------|--|
|      |            |       | DOI: 10.1016/j.ultrasmedbio.2003.07.001  |
| 2006 | Diagnostic | Mixed | Foucher et al.<br>Gut<br>DOI: 10.1136/gut.2005.069153                                      |
| 2007 | Diagnostic | Mixed | Talwalkar et al.<br>Clin Gastroenterol Hepatol<br>DOI: 10.1016/j.cgh.2007.07.020           |
| 2008 | Diagnostic | Mixed | Friedrich-Rust et al.<br>Gastroenterology<br>DOI: 10.1053/j.gastro.2008.01.034             |
| 2009 | Monitoring | HCV   | Vergniol et al.<br>J Viral Hepat<br>DOI: 10.1111/j.1365-2893.2008.01055.x                  |
| 2010 | Diagnostic | MASLD | Wong et al.<br>Hepatology<br>DOI: 10.1002/hep.23312  |
| 2011 | Prognostic | HCV   | Vergniol et al.<br>Gastroenterology<br>DOI: 10.1053/j.gastro.2011.02.058                   |
| 2013 | Prognostic | HBV   | Ledinghen et al.<br>Aliment Pharmacol Ther<br>DOI: 10.1111/apt.12307                       |
| 2014 | Monitoring | HCV   | Vergniol et al.<br>Hepatology<br>DOI: 10.1002/hep.27069                                    |
| 2014 | Monitoring | HBV   | Jang et al.<br>Clin Res Hepatol Gastroenterol<br>DOI: 10.1016/j.clinre.2013.10.003         |
| 2016 | Prognostic | MASLD | Boursier et al.<br>Journal of Hepatology<br>DOI: 10.1016/j.jhep.2016.04.023                |
| 2018 | Monitoring | HBV   | Wu et al.<br>Liver International<br>DOI: 10.1111/liv.13623                                 |
| 2018 | Monitoring | HBV   | Liang et al.<br>J Viral Hepat<br>DOI: 10.1111/jvh.12814                                    |
| 2018 | Monitoring | HBV   | Li et al.<br>Clinical and Experimental Medicine<br>DOI: 10.1007/s10238-018-0486-5          |
| 2018 | Diagnostic | ALD   | Nguyen-Khac et al.<br>Lancet Gastroenterol & Hepatol<br>DOI: 10.1016/S2468-1253(18)30124-9 |
| 2019 | Diagnostic | MASLD | Siddiqui et al.<br>Clin Gastroenterol Hepatol<br>DOI: 10.1016/j.cgh.2018.04.043            |
| 2019 | Diagnostic | MASLD | Eddowes et al.<br>Gastroenterology<br>DOI: 10.1053/j.gastro.2019.01.042                    |
| 2020 | Diagnostic | Mixed | Papatheodoridi et al.<br>Journal of Hepatology<br>DOI: 10.1016/j.jhep.2020.11.050          |
| 2020 | Prognostic | MASLD | Petta et al.<br>Clin Gastroenterol Hepatol<br>DOI: 10.1016/j.cgh.2020.06.045               |
| 2020 | Monitoring | MASH  | Harrison et al.<br>Journal of Hepatology   |

|      |            |       |  |
|------|------------|-------|--|
|      |            |       | DOI: 10.1016/j.jhep.2020.02.027  |
| 2020 | Monitoring | MASH  | Newsome et al.<br>New England Journal of Medicine<br>DOI: 10.1056/NEJMoa2028395      |
| 2021 | Monitoring | MASH  | Rinella et al.<br>Journal of Hepatology<br>DOI: 10.1016/j.jhep.2021.10.029           |
| 2021 | Prognostic | MASH  | Sanyal et al.<br>Hepatology<br>DOI: 10.1002/hep.32204                                |
| 2021 | Diagnostic | MASLD | Mözes et al.<br>Gut<br>DOI: 10.1136/gutjnl-2021-324243                               |
| 2022 | Diagnostic | MASLD | Sanyal et al.<br>Journal of Hepatology<br>DOI: 10.1016/j.jhep.2022.10.034            |
| 2022 | Prognostic | MASH  | Loomba et al.<br>Gut<br>DOI: 10.1136/gutjnl-2022-327777                              |
| 2022 | Monitoring | MASH  | Alkhoury et al.<br>Journal of Hepatology<br>DOI: 10.1016/j.jhep.2022.04.003          |
| 2023 | Monitoring | MASH  | Sanyal et al.<br>Journal of Hepatology<br>DOI: 10.1016/j.jhep.2023.07.014            |
| 2023 | Prognostic | MASLD | Mözes et al.<br>Lancet Gastroenterol & Hepatol<br>DOI: 10.1016/S2468-1253(23)00141-3 |
| 2023 | Diagnostic | MASLD | Vali et al.<br>Lancet Gastroenterol & Hepatol<br>DOI: 10.1016/S2468-1253(23)00017-1  |