Perspective

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Personalized Medicine

Personalizing care for nonalcoholic fatty liver disease patients: what are the research priorities?

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Nonalcoholic fatty liver disease (NAFLD) is a common chronic liver disease whose prevalence has reached global epidemic proportions, not only in adults but also in children. From a clinical point of view, NAFLD stems a myriad of challenges to physicians, researchers and patients. In this study, we revise the current knowledge and recent insights on NAFLD pathogenesis and diagnosis in the context of a personalized perspective with special focus on the following issues: noninvasive biomarkers for the evaluation of disease severity and progression, lifestyle-related patients' recommendations, risk prediction of disease by genetic testing, management of NAFLD-associated comorbidities and patient-oriented therapeutic intervention strategies.

Keywords: ALT • AST • CAD • fatty liver • genetics • insulin resistance • metabolic syndrome • NAFLD • NASH • personalized medicine • PNPLA3 • prediction risk

Nonalcoholic fatty liver disease (NAFLD) is a common chronic liver disease whose prevalence has reached global epidemic proportions, both in adults and children [1].

By definition, NAFLD develops in the absence of significant alcohol consumption and other causes of secondary hepatic steatosis [2]. From a morphological point of view, NAFLD refers to the abnormal accumulation of triglycerides in the liver cells, which may progress from a benign histological disease stage characterized by plain fat accumulation (called as simple steatosis) to a more severe histological form characterized by liver cell injury, a mixed inflammatory lobular infiltrate and variable fibrosis named nonalcoholic steatohepatitis (NASH) [3]. The histological diagnosis is based exclusively on a liver biopsy, which is currently the gold standard for defining the disease diagnosis and prognosis accurately [2]. Unfortunately, this method is prone to patient complications, expensive, done in a special setting, and may be biased as a small portion of hepatic tissue is sampled.

Interestingly, NAFLD is not merely a liver disease but is now regarded as the hepatic manifestation of the metabolic syndrome (MetSyn) and has been associated with increased cardiovascular disease (CVD) risk [4–7].

Once diagnosed, treatment of NAFLD is complex and often requires pharmacological intervention to treat both the liver disease and its associated risk factors, such as insulin resistance (IR), abnormal circulating lipid profiles or arterial hypertension. Moreover, lifestyle intervention, including weight loss and physical activity/exercise, showed favorable effects on liver fat reduction and improvement in insulin sensitivity.

Hence, in this complex scenario, NAFLD stems a myriad of challenges to physicians, researchers and patients.

In this study, we revise the current knowledge and recent insights regarding NAFLD pathogenesis and diagnosis in the context of a perspective on personalized medicine. Box 1 shows a comprehensive list of research priorities identified as challenging problems in the management of NAFLD.

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Box 1. Challenging problems in the management of nonalcoholic fatty liver disease and future research priorities.

Disease diagnosis: biomarker discovery for noninvasive exploration of advanced disease
- The meaning of elevated/normal liver transaminases (ALT and AST)
- The role of noninvasive biomarkers for evaluation of disease progression
- The role of combined panels of multi-biomarkers to early disease diagnosis
- The timing of indicating a liver biopsy to patients with fatty liver

Physicians to NAFLD patients' recommendations
- Alcohol abstinence or tight restrictions on alcohol consumption opposed to moderate alcohol intake
- Coffee intake or restriction
- The best dietary indication: a balanced macronutrient composition
- Lifestyle intervention and physical activity

- **Risk prediction of disease by the use of genetic testing**
- The role of SNPs in the stratification of disease risk and progression
- The role of genetic markers in the prediction of liver cancer associated with the disease
- Interaction between gene variants and nongenetic risk factors

NAFLD and the systemic risk of comorbidities
- The role of NAFLD as surrogate of CVD risk
- Further assessment of associated CVD and insulin resistance
- Intermitent hypoxia and obstructive sleep apnea

Treatment approaches
- Pharmacogenetics and tailoring therapeutic strategies

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CVD: Cardiovascular disease; NAFLD: Nonalcoholic fatty liver disease.

NAFLD: noninvasive disease evaluation of histological disease progression

NAFLD has no definitive either biochemical marker or imaging method for predicting the degree of histological disease severity noninvasively. During earlier decades, NAFLD was diagnosed by the use of liver ultrasound and the measurement of serum levels of aminotransaminases, which if mildly elevated were regarded as a surrogate indicator of serious disease. Nevertheless, levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) considered in the 'normal' range can be observed in some patients with advanced stages of NAFLD [8]. Thus, liver enzymes are no longer considered as diagnostic criteria for indicating a liver biopsy to an NAFLD patient. In addition, this is not the only aspect that should be revised regarding the use of liver enzymes in the clinical setting. Recent explorations on changes in the liver metabolism during NASH development [9,10], along with remarkable findings from high-throughput circulating profiling of metabolic status in patients with MetSyn [11–13] prompted us to speculate that elevated levels of ALT and AST in patients with NAFLD are a consequence of a deregulated liver metabolism of amino acids, including glutamate and aromatic amino acid, rather than a mere biomarker of liver injury [14]. Indeed, we postulated that abnormal levels of liver enzymes might reflect high levels of hepatic transamination of amino acids in the liver tissue [14], and we are currently testing this hypothesis. Thereby, the biological meaning of elevated serum levels of ALT and AST should be explored in the frame of the global patients' metabolic profile to understand the specific process they are linked to.

On the other hand, there have been substantial efforts to find an ideal biomarker for predicting noninvasive liver fibrosis. The more replicated efforts were done by the use of: an imaging method named transient elastography (TE), which measures liver stiffness; and a biochemical marker that measures plasma levels of caspase-cleaved cytokeratin-18 fragments, also known as cytokeratin-18 fragments. Nevertheless, a recent meta-analysis of current studies showed that neither approach has enough sensitivity for early and accurate diagnosis of disease progression [15]. For instance, transient elastography showed a sensitivity and specificity for early liver fibrosis prediction of 79 and 75%, respectively, and the technique failed in obese patients [15]. Similarly, plasma levels of CK18 fragments showed a pooled sensitivity of 66% and specificity of 82% in diagnosing NASH [15].

More recently, the search for the ideal biomarker for NAFLD diagnosis was focused on small noncoding RNA molecules called miRNAs that are not only key regulators of gene transcription but also mirror the status of the tissue of which they are derived from accurately. In this regard, miRNA-122 has captured the focus of the research, as it is produced mostly in the liver tissue. Others [16] and we [17,18] observed that while miRNA-122 is as much sensitive and specific for predicting NASH as both CK18 and liver enzymes are, its performance is far from an ideal biomarker. Therefore, the search for the ideal biomarker continues.

A future research agenda should focus on the utility of composite panels [19,20] that may combine the utility of different novel biomarkers and canonical risk factors that help clinicians to score the risk of their patients individually to develop a more advanced disease. Eventually, a multiscoring panel might minimize the need for a liver biopsy for distinguishing between simple steatosis disease and NASH [19] if combined with anthropometric data and genetic information (Figure 1).

Physician–patient recommendations & challenges of personalized medicine

While NAFLD is defined by exclusion of alcohol consumption, a recent paradox has challenged clinicians who often recommend alcohol abstinence to NAFLD patients or apply tight restrictions on alcohol

consumption under the assumption that alcohol drinking might aggravate the liver injury. Surprisingly, epidemiological studies suggested that the prevalence and histological disease severity of NAFLD [21] are lower for people who drink modest amounts of alcohol than for abstainers. In our population, NAFLD and NASH prevalence and serum liver enzymes and systemic inflammatory markers were lower in subjects who took modest amounts of alcohol compared with abstainers [22]. In addition, quantitative evidence from a recent meta-analysis performed for our group showed a significant protective effect of about 31% on the risk of having NAFLD associated with modest alcohol consumption; the study included 43,175 adult individuals: 30,791 nondrinkers and 12,384 modest drinkers [22]. This beneficial effect seems to be independent of covariates, such as BMI, but was much influenced by sex (among females, the protective effect of modest alcohol consumption on NAFLD was higher, ~53% than in males, ~30%) [22]. In relation to the severity of the histological disease, modest alcohol consumption showed an average protective effect of about 50% on the risk of developing an advanced disease stage (NASH); data from 822 patients who were diagnosed by liver

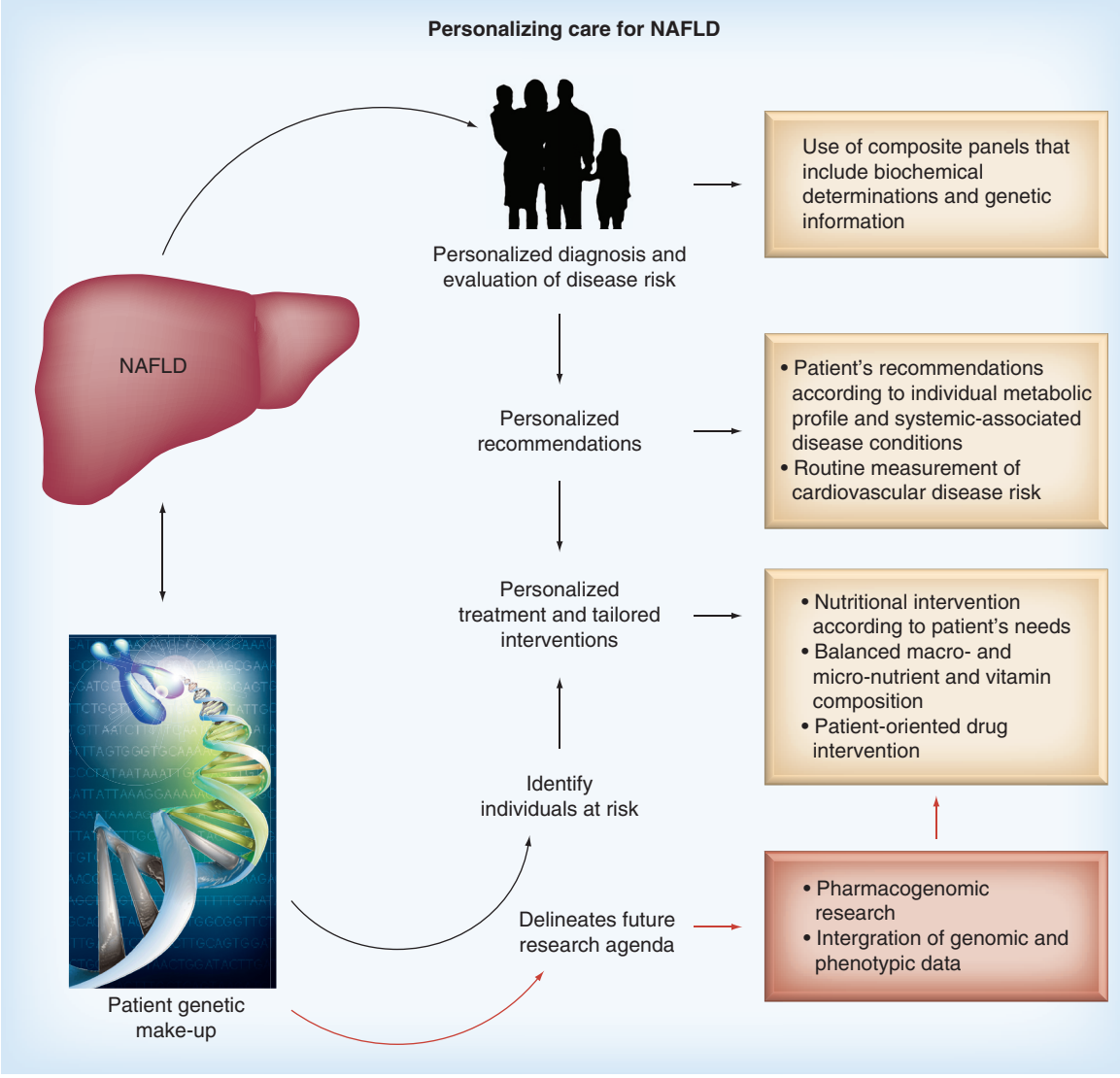

Figure 1. Nonalcoholic fatty liver disease: proposed challenges and future research agenda. An integration of histological information and complete patient health records with the individual genomic make-up would be the ideal frame for redirecting traditional clinical care into a personalized perspective. This integrative approach may orientate the research priorities to diagnostic strategies, patients' recommendations and pharmacological intervention. The maximum goal would be to move toward a personalized medicine that integrates electronic health records with patients' genomic, transcriptomic, proteomic and metabolomic information.
NAFLD: Nonalcoholic fatty liver disease.

biopsy (550 nondrinkers and 272 modest drinkers) [22]. The exact biological effect of modest alcohol drinking on the natural history of NAFLD is still unclear and more experimental and molecular studies are needed. A putative explanation of these observations might be related to either the systemic anti-inflammatory effect of low doses of alcohol, or the protective effects of polyphenols found in red wine, including resveratrol. For instance, alcohol is able to modulate adipocytokines [23], inflammasome activation [24] and the microbiome [25]. These findings are in line with other components of the MetSyn [26].

Furthermore, clinicians often restrict coffee intake to patients with CVD because of the putative deleterious effects on the cardiovascular system, as shown in a recent meta-analysis that concluded that in hypertensive individuals, caffeine intake produces an acute increase in blood pressure greater than 3 h [27]. Surprisingly, a second paradox was found regarding coffee intake and NAFLD histological outcomes because while coffee abstinence is associated with a lower hypertensive risk, recent epidemiological evidence demonstrated that coffee intake is inversely associated with advanced fibrosis [28]. Supporting this observation, Saab and coworkers showed that coffee consumption was associated with improved serum levels of liver enzymes, as well as with a decreased risk of progression to cirrhosis and lowered mortality rate in cirrhosis patients with NAFLD and chronic hepatitis [29].

Finally, recommendations should be oriented to modifications of patients' lifestyle behaviors. Timely lifestyle intervention programs are the first line of therapeutic approaches in patients with NAFLD [30]; diet and nutrition intervention showed substantial results specifically when decreased calorie consumption was advised to overweight or obese patients [31]. In fact, moderate weight reduction is currently the most replicated intervention in terms of beneficial effects on improving liver enzymes, reverting liver fat infiltration and histological outcomes [32].

Nevertheless, the ideal nutritional intervention is still under debate as to whether patients should be recommended either low-fat or low-carbohydrate diet, including restrictions of refined carbohydrates and saturated fats [33]. Surprisingly, a recently published experimental study in rodents showed that is not the amount of food but the macronutrient balance that dictates health consequences [34]. Of note, low-protein, high-carbohydrate diets were associated with increased lifespan and lessened cardiometabolic risk, and these beneficial diets could induce the unexpected effect by hepatic mTOR activation via branched-chain amino acids and mitochondrial function [34]. A note of caution should be added to these observations; first, the experiment was conducted in mice, which are distant from humans in terms of energy balance and regulation of liver/systemic metabolism; and second, there is no confirmation that this effect can be translated to diseased individuals. In addition, high-carbohydrate diets might be risky to implement in patients with NAFLD and Type 2 diabetes.

A final concept should be introduced about the role of physical activity and exercise on improving the liver phenotype. A study that prospectively enrolled NAFLD patients proven by liver biopsy pre- and post-lifestyle intervention showed that moderate exercise was as effective as a moderate fat/low processed carbohydrate diet in improved liver histology [35]. Whether short or long-term aerobic or resistance training [36,37] is the best approach is still under debate.

Notably, we demonstrated that epigenetic changes could offer a mechanistic explanation on the importance of unstructured exercise in the modification of the histological picture of NAFLD [38]. We observed that regular exercise was significantly and inversely associated with the level of methylation of genes of the mitochondrial DNA, specifically a key mitochondrial gene involved in oxidative phosphorylation, such as the *MT-ND6*, an epigenetic modification associated with lowering the expression of protein and structural changes in mitochondria [38].

In conclusion, it is time to revise our current knowledge concerning the best lifestyle interventional program and to think about how to translate these results into tailored, patient-oriented advice. The recommendations we offer to NAFLD patients should be integrative in terms of suggested lifestyle changes, including behavioral support [39] and patients' preferences.

Risk prediction of disease susceptibility & the role of genetic testing

Although the pathogenesis of NAFLD is not understood fully, a growing body of evidence indicates that the disease develops from a complex process in which many factors, including genetic susceptibility and environmental insults, are involved. While candidate gene association studies identified several loci associated with the disease susceptibility and progression [40–44], data from the first genome-wide association study on NAFLD have significantly contributed to our knowledge of the genetic component of the disease [45]. The authors of the Dallas Heart Study observed an association between fatty liver and a nonsynonymous SNP of *PNPLA3* (also known as adiponutrin or calcium-independent phospholipase A2-epsilon), the rs738409 C/G variant, (encoding an amino acid substitution I148M) [45]. This finding was thereafter replicated widely around the world, confirming that the G allele

in the coding strand is significantly associated with an increased risk of hepatic triglyceride accumulation and fatty liver disease [46]. Interestingly, we replicated the initial finding in NAFLD patients proven through biopsy and demonstrated [47] that the rs738409 was also significantly associated with histological disease severity and disease progression (odds ratio 1.88 per G allele; 95% CI: 1.03–3.43; $p < 0.04$). Moreover, we confirmed that the risk effect of the rs738409 on developing fatty liver is perhaps one of the strongest ever reported for a common variant modifying the genetic susceptibility for complex diseases (5.3% of the total variance) [47].

Of note, annotation of nearby SNPs in LD with the rs738409 shows the *SAMM50* gene, which is a component of the sorting and assembly machinery complex of the outer mitochondrial membrane [48]. Interestingly, a nonsynonymous SNP (rs3761472) in *SAMM50* was associated with elevated liver enzymes [49], thus, *SAMM50* represents an attractive candidate gene for follow-up studies and for exploration of epistatic and gene–gene interaction effects [44].

Even though no doubt exists concerning the impact of the rs738409 on the natural history of NAFLD, many important questions remain unanswered, for example, whether or not the rs738409 has a place in individual risk assessment of NAFLD. Indeed, one may wonder whether the effect of the rs738409 not only on modifying disease susceptibility but also on disease progression can facilitate the implementation of personalized medicine, in which individual risk assessment can be tailored to an individual genetic profile.

Answers to these questions have been given partially for individual studies. For example, Kotronen and coworkers evaluated the performance of predicting NAFLD by combining routine clinical and laboratory data and the rs738409 genotypes, and observed a sensitivity of 86% and specificity of 71% in the estimation of increased liver fat content [50]. Surprisingly, addition of the genetic information to the score improved the accuracy of the prediction by less than 1%.

Even more challenging is the possibility of incorporating the genetic markers to a noninvasive test that discriminates between simple steatosis and NASH. In this regard, the incorporation of genetic tests is not promising owing to the relatively small effect associated with the risk of advanced disease [44]. Perhaps, as already mentioned, combining genetic, demographic and environmental factors may improve the performance of the test [51].

Although the performance of genetic testing in predicting disease progression is limited, some interesting results were reported in patients with cirrhosis and hepatocellular carcinoma in whom a higher risk of cancer was observed in subjects homozygous GG for the rs738409 variant [52]. While this observation suggests that *PNPLA3* might be involved in hepatocarcinogenesis, the final answer remains to be elucidated [53].

A different approach of the use of genetic testing based on the rs738409 is focused on the potential of interacting with either environmental variables or MetSyn phenotypes. For instance, it was shown in a small number of subjects that a genetic variation in *PNPLA3* might confer sensitivity to weight loss-induced decrease in liver fat in obese patients, as weight loss was more effective in decreasing liver fat in subjects who were homozygous for the rs738409 G allele [54].

Nevertheless, the best scenario to understand the interplay between genetic risk and environmental clues is the study of epigenetic marks, either DNA methylation or histone post-translational modifications. The dynamic nature of epigenetic modifications is not only an ideal explanation to explain how intermediate phenotypes associated with fatty liver are linked, for example, to IR [55,56], but is also an attractive target of therapeutic intervention [38,56].

A final consideration for the future research agenda is the exploration of rare variants in the pathogenesis of NAFLD. A recent multi-ethnic exome-wide association study of liver fat content showed that the rs738409 and rs2281135, both located in the *PNPLA3* locus, are still among 138,374 sequence variants most significantly associated with liver fat content [57]. Nevertheless, it is important to note that thousands of patients and controls are needed to reach valid conclusions. All these studies were based on the hypothesis 'common variants for common diseases'. With the advance of next-generation sequencing technologies, either for whole-genome or whole-exome sequencing, we can start testing the alternative hypothesis 'rare variants for common diseases' with the hope of finding rare variants but with much higher effects, such as those associated with Mendelian traits.

NAFLD & the systemic risk of associated comorbidities

As mentioned before, NAFLD is associated with a significant risk of CVD and an increased morbidity and mortality associated with cardiovascular events [4]. Indeed, the problem behind this association is that NAFLD is involved critically in the development of a CVD-proned profile. For example, we demonstrated that NAFLD is associated not only with abnormal circulating levels of molecular mediators of atherosclerosis (including soluble intercellular adhesion molecule, sICAM-1, plasminogen activator inhibitor, PAI-1 and soluble CD40 ligand, sCD40L), but is also involved

in its abnormal expression in the liver [6]. These findings are translated clinically into a more pronounced risk of atherogenesis [5]. In addition, when compared with simple steatosis, patients with NASH show significantly increased levels of liver expression of genes associated with endothelial damage, blood pressure regulation, inflammation and cytokine signaling, cell proliferation/growth, extracellular matrix remodeling and cell adhesion-cell-matrix [7].

Thus, patients with NAFLD may benefit from a personalized care that specifically includes, for example, routine measurement of atherosclerotic plaques in large arteries.

NAFLD: tailoring therapeutic strategies to individual patient's profiles

Patients with NAFLD are frequently offered therapeutic interventions that are focused specifically on improving the associated disease conditions, for example, IR or dyslipidemia [58]. The drug spectrum varies and the most explored pharmacological interventions are pioglitazone and vitamin E [59]. Nevertheless, the spectrum is being currently enriched by the inclusion of drugs that focus on molecular targets, for instance, a natural agonist of the farnesoid X receptor involved in the regulation of glucose and lipid metabolism [60]. In addition, to treat obesity complications, bariatric surgery is also advised to patients who have morbid obesity [58] and incretin action enhancers are an option [61].

Nevertheless, from the personalized perspective, treatment options should be integrated into a therapeutic model to include a holistic intervention that considers, for instance, treatment of cardiovascular complications and reduction of the risk of cardiovascular morbidity and mortality. For example, we demonstrated that the use of losartan – an angiotensin II type 1 receptor (AT1R) antagonist reduces hepatic expression of PAI-1 [62]. Moreover, we observed that patients with NAFLD treated with enalapril because of arterial hypertension showed significantly lower scores of liver fibrosis when compared with matched patients [7]. Consequently, treatment approaches should include global risk management and drugs should be targeted to this goal.

Finally, although we carefully select the best drug that fit the patient profile, challenges remain. For instance, we might target the pharmacological intervention by previous exploration of patient's genetic profile [44]. This kind of strategy based on pharmacogenetics, which was performed in other liver diseases [63], is still unexplored in the field of NAFLD but is promising. In fact, using systems biology, we suggested that vitamin E shows a chemical–protein interaction with CYP4F2 (cytochrome P450, family 4, subfamily F, polypeptide 2) and looking at genetic variants of CYP4F2, perhaps one may predict patients' treatment responses to vitamin E therapy [44,61].

A more complex scenario, yet poorly explored, might be the stratification of patients' response to medications or lifestyle intervention according to their genetic background. In addition, it might be interesting to explore the interaction between patients' genetic background and environmental risk factors (i.e., diet). For instance, a recent study showed that overweight and dietary sucrose might modify the association between rs738409 and fasting triglyceride levels [64]. Moreover, Santoro *et al.* showed that the interaction between genetic background (rs738409) and dietary dysbalance of omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids may contribute to liver damage in obese children [65].

Conclusion

NAFLD has captured the attention of physicians of different disciplines and is high among the causes of consulting medical practitioners around the world. In addition, NAFLD is a serious disease problem that clinicians need to deal with. Consequently, NAFLD challenges ordinary clinical practice and invites physicians to improve both conventional diagnostic procedures and therapeutic approaches. The knowledge of the human genome sequence and the availability of recent technologies to decipher individual patients' genetic make-up also invite a fully integrated genomic medicine. Nevertheless, integration of the 'omics' technologies and phenotypic data is still full of uncertainties. Hopefully, future research programs that integrate health records – ideally electronic ones – with patients' genomic, transcriptomic, proteomic and metabolomic information will change our ability to control or cure the disease eventually.

Future perspective

The field will evolve in the future by targeting an integrative approach of NAFLD diagnosis and therapeutics. For instance, the application in the clinical setting of a multi-scoring panel that not only minimizes the need for a liver biopsy for distinguishing between simple steatosis and NASH but also predicts a patient's global metabolic risk might be combined with a patient's genetic make-up and other data gathered from omics technologies to choose the right therapeutic program and to predict the individual chances of response (Figure 1). This personalized diagnosis will improve our capability to understand the disease biology and allow us to deal with a tailored medical intervention. Whether this strategy will evolve from whole-genome sequencing is not known. Nevertheless, practical implementation

of this kind of approach together with the limitations of these technologies remains an enormous challenge.

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Executive summary

Background

- The relevance of 4P medicine (predictive, personalized, participatory and preventive) in a multifactorial disease such as nonalcoholic fatty liver disease (NAFLD) is highlighted below:

Noninvasive disease evaluation of histological disease progression

- The biological meaning of routine biochemical test, like serum aminotransferases should be revised in the context of NAFLD-associated systemic metabolic disorders.

Physician–patient recommendations & challenges of personalized medicine

- A patient-oriented portfolio of recommendations, including lifestyle and dietary restrictions/permissions, like alcohol or coffee intake in moderate amounts is suggested in patients with NAFLD to balance the narrow window between maximum protection and harm, which is not the same in men and women.
- Patients with NAFLD may benefit from a personalized care that includes specifically, for example, routine measurement of cardiovascular disease risk.
- The ideal nutritional intervention should be revised to advise patients to the most suitable diet composition to fit their metabolic profile better.

Role of epigenetic changes in disease biology

- Epigenetic changes, specifically mitochondrial epigenetics, suggest a mechanistic explanation regarding the importance of lifestyle intervention and the outcome of the histological picture of NAFLD.

Tailoring therapeutic strategies to individual patient's profiles

- Pharmacological intervention should include a deep knowledge of the biological effects of the selected drug and a patient-oriented interventional approach. Pharmacogenetic studies are needed to understand the causes of patients' nonresponsiveness or adverse effects to used drugs.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat. Rev. Gastroenterol. Hepatol.* 10(11), 686–690 (2013).

2 Chalasani N, Younossi Z, Lavine JE *et al.* The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 142(7), 1592–1609 (2012).

3 Brunt EM. Pathology of fatty liver disease. *Mod. Pathol.* 20(Suppl. 1), S40–S48 (2007).

4 Lonardo A, Sookoian S, Chonchol M, Loria P, Targher G. Cardiovascular and systemic risk in nonalcoholic fatty liver disease - atherosclerosis as a major player in the natural course of NAFLD. *Curr. Pharm. Des.* 19(29), 5177–5192 (2013).

5 Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. *J. Hepatol.* 49(4), 600–607 (2008).

• **The association between nonalcoholic fatty liver disease (NAFLD) and atherosclerosis was firmly established by means of a meta-analysis.**

6 Sookoian S, Castano GO, Burgueno AL *et al.* Circulating levels and hepatic expression of molecular mediators of atherosclerosis in nonalcoholic fatty liver disease. *Atherosclerosis* 209(2), 585–591 (2010).

7 Sookoian S, Gianotti TF, Rosselli MS *et al.* Liver transcriptional profile of atherosclerosis-related genes in human nonalcoholic fatty liver disease. *Atherosclerosis* 218(2), 378–385 (2011).

8 Mofrad P, Contos MJ, Haque M *et al.* Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 37(6), 1286–1292 (2003).

9 Mardinoglu A, Agren R, Kampf C *et al.* Genome-scale metabolic modelling of hepatocytes reveals serine deficiency in patients with non-alcoholic fatty liver disease. *Nat. Commun.* 5(2), 3083–3093 (2014).

10 Sookoian S, Pirola CJ. NAFLD: metabolic make-up of NASH: from fat and sugar to amino acids. *Nat. Rev. Gastroenterol. Hepatol.* 11(4), 205–207 (2014).

11 Cheng J, Joyce A, Yates K, Aouizerat B, Sanyal AJ. Metabolomic profiling to identify predictors of response to vitamin E for non-alcoholic steatohepatitis (NASH). *PLoS ONE* 7(9), e44106 (2012).

12 Newgard CB, An J, Bain JR *et al.* A branched-chain amino acid-related metabolic signature that differentiates obese

and lean humans and contributes to insulin resistance. *Cell Metab.* 9(4), 311–326 (2009).

•• **A dysregulation in amino acids metabolism, in particular the ratio glutamate/glutamine may be a characteristic signature of the metabolic syndrome.**

13 Wang TJ, Larson MG, Vasan RS *et al.* Metabolite profiles and the risk of developing diabetes. *Nat. Med.* 17(4), 448–453 (2011).

14 Sookoian S, Pirola CJ. Alanine and aspartate aminotransferase and glutamine-cycling pathway: their roles in pathogenesis of metabolic syndrome. *World J. Gastroenterol.* 18(29), 3775–3781 (2012).

15 Kwok R, Tse YK, Wong GL *et al.* Systematic review with meta-analysis: non-invasive assessment of non-alcoholic fatty liver disease--the role of transient elastography and plasma cytokeratin-18 fragments. *Aliment Pharmacol. Ther.* 39(3), 254–269 (2014).

16 Cermelli S, Ruggieri A, Marrero JA, Ioannou GN, Beretta L. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. *PLoS ONE* 6(8), e23937 (2011).

17 Pirola CJ, Gianotti TF, Castano GO, Sookoian S. Circulating microRNA-122 signature in nonalcoholic fatty liver disease and cardiovascular disease: a new endocrine system in metabolic syndrome. *Hepatology* 57(6), 2545–2547 (2013).

18 Pirola CJ, Fernandez Gianotti T, Burgueno AL, Castano G, Sookoian S. Circulating micro-RNA profile in nonalcoholic fatty liver disease: potential biomarkers and its role in the modulation of the metabolic syndrome? *Hepatology* 58, 247A (2013).

19 Sookoian S, Castano G, Burgueno AL *et al.* A diagnostic model to differentiate simple steatosis from nonalcoholic steatohepatitis based on the likelihood ratio form of Bayes theorem. *Clin. Biochem.* 42(7–8), 624–629 (2009).

20 Grandison GA, Angulo P. Can NASH be diagnosed, graded, and staged noninvasively? *Clin. Liver Dis.* 16(3), 567–585 (2012).

21 Dunn W, Sanyal AJ, Brunt EM *et al.* Modest alcohol consumption is associated with decreased prevalence of steatohepatitis in patients with non-alcoholic fatty liver disease (NAFLD). *J. Hepatol.* 57(2), 384–391 (2012).

22 Sookoian S, Castano GO, Pirola CJ. Modest alcohol consumption decreases the risk of non-alcoholic fatty liver disease: a meta-analysis of 43175 individuals. *Gut* 63(3), 530–532 (2014).

• **This meta-analysis indicates that moderate alcohol consumption may be beneficial for NAFLD patients.**

23 Seth D, Haber PS, Syn WK, Diehl AM, Day CP. Pathogenesis of alcohol-induced liver disease: classical concepts and recent advances. *J. Gastroenterol. Hepatol.* 26(7), 1089–1105 (2011).

24 Petrasek J, Bala S, Csak T *et al.* IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic steatohepatitis in mice. *J. Clin. Invest.* 122(10), 3476–3489 (2012).

25 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* 57(2), 601–609 (2013).

26 Alkerwi A, Boutsen M, Vaillant M *et al.* Alcohol consumption and the prevalence of metabolic syndrome: a meta-analysis of observational studies. *Atherosclerosis* 204(2), 624–635 (2009).

27 Mesas AE, Leon-Munoz LM, Rodriguez-Artalejo F, Lopez-Garcia E. The effect of coffee on blood pressure and cardiovascular disease in hypertensive individuals: a systematic review and meta-analysis. *Am. J. Clin. Nutr.* 94(4), 1113–1126 (2011).

28 Bambha K, Wilson LA, Unalp A *et al.* Coffee consumption in NAFLD patients with lower insulin resistance is associated with lower risk of severe fibrosis. *Liver Int.* 34(8), 1250–1258 (2013).

29 Saab S, Mallam D, Cox Ii GA, Tong MJ. Impact of coffee on liver diseases: a systematic review. *Liver Int.* 34(4), 495–504 (2013).

30 Barrera F, George J. The role of diet and nutritional intervention for the management of patients with NAFLD. *Clin. Liver Dis.* 18(1), 91–112 (2014).

31 Neuschwander-Tetri BA. Lifestyle modification as the primary treatment of NASH. *Clin. Liver Dis.* 13(4), 649–665 (2009).

32 Promrat K, Kleiner DE, Niemeier HM *et al.* Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 51(1), 121–129 (2010).

33 Kechagias S, Ernersson A, Dahlqvist O *et al.* Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. *Gut* 57(5), 649–654 (2008).

34 Solon-Biet SM, McMahon AC, Ballard JW *et al.* The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell Metab.* 19(3), 418–430 (2014).

35 Eckard C, Cole R, Lockwood J *et al.* Prospective histopathologic evaluation of lifestyle modification in nonalcoholic fatty liver disease: a randomized trial. *Therap. Adv. Gastroenterol.* 6(4), 249–259 (2013).

36 Haus JM, Solomon TP, Kelly KR *et al.* Improved hepatic lipid composition following short-term exercise in nonalcoholic fatty liver disease. *J. Clin. Endocrinol. Metab.* 98(7), E1181–E1188 (2013).

37 Bacchi E, Negri C, Targher G *et al.* Both resistance training and aerobic training reduce hepatic fat content in type 2 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 Randomized Trial). *Hepatology* 58(4), 1287–1295 (2013).

38 Pirola CJ, Gianotti TF, Burgueno AL *et al.* Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease. *Gut* 62(9), 1356–1363 (2013).

•• **Describes for the first time the role of mitochondrial epigenetics in common human diseases.**

39 Stewart K, Haller D, Sargeant C *et al.* Readiness for behavior change in non-alcoholic fatty liver disease: implications for multidisciplinary care models. *Liver Int.* doi:10.1111/liv.12483 (2014) (Epub ahead of print).

40 Sookoian S, Castano G, Gemma C, Gianotti TF, Pirola CJ. Common genetic variations in CLOCK transcription factor are associated with nonalcoholic fatty liver disease. *World J. Gastroenterol.* 13(31), 4242–4248 (2007).

41 Sookoian S, Castano G, Gianotti TF *et al.* Genetic variants in STAT3 are associated with nonalcoholic fatty liver disease. *Cytokine* 44(1), 201–206 (2008).

42 Sookoian S, Castano G, Gianotti TF, Gemma C, Pirola CJ. Polymorphisms of MRP2 (ABCC2) are associated with susceptibility to nonalcoholic fatty liver disease. *J. Nutr. Biochem.* 20(10), 765–770 (2009).

43 Sookoian S, Castano GO, Burgueno AL *et al.* The nuclear receptor PXR gene variants are associated with liver injury in nonalcoholic fatty liver disease. *Pharmacogenet. Genomics* 20(1), 1–8 (2010).

44 Sookoian S, Pirola CJ. The genetic epidemiology of nonalcoholic fatty liver disease: toward a personalized medicine. *Clin. Liver Dis.* 16(3), 467–485 (2012).

45 Romeo S, Kozlitina J, Xing C *et al.* Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat. Genet.* 40(12), 1461–1465 (2008).

46 Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 53(6), 1883–1894 (2011).

•• **This meta-analysis firmly established the association of the PNPLA3 variant and NAFLD, liver transaminases and disease severity and estimated the magnitude of the effect and the corresponding genetic model.**

47 Sookoian S, Castano GO, Burgueno AL *et al.* A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *J. Lipid Res.* 50(10), 2111–2116 (2009).

48 Sookoian S, Pirola CJ. PNPLA3, the triacylglycerol synthesis/hydrolysis/storage dilemma, and nonalcoholic fatty liver disease. *World J. Gastroenterol.* 18(42), 6018–6026 (2012).

49 Yuan X, Waterworth D, Perry JR *et al.* Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am. J. Hum. Genet.* 83(4), 520–528 (2008).

50 Kotronen A, Peltonen M, Hakkarainen A *et al.* Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology* 137(3), 865–872 (2009).

51 Guichelaar MM, Gawrieh S, Olivier M *et al.* Interactions of allelic variance of PNPLA3 with nongenetic factors in predicting nonalcoholic steatohepatitis and nonhepatic complications of severe obesity. *Obesity (Silver Spring)* 21(9), 1935–1941 (2013).

52 Hassan MM, Kaseb A, Etzel CJ *et al.* Genetic variation in the PNPLA3 gene and hepatocellular carcinoma in USA: risk and prognosis prediction. *Mol. Carcinog.* 52(Suppl. 1), E139–E147 (2013).

53 Sookoian S, Pirola CJ. PNPLA3, the history of an orphan gene of the potate tuber PROTEIN family that found an organ: the liver. *Hepatology* 59(6), 2068–2071 (2013).

54 Sevastianova K, Kotronen A, Gastaldelli A *et al.* Genetic variation in PNPLA3 (adiponutrin) confers sensitivity to weight loss-induced decrease in liver fat in humans. *Am. J. Clin. Nutr.* 94(1), 104–111 (2011).

55 Sookoian S, Rosselli MS, Gemma C *et al.* Epigenetic regulation of insulin resistance in nonalcoholic fatty liver disease: impact of liver methylation of the peroxisome proliferator-activated receptor gamma coactivator 1alpha promoter. *Hepatology* 52(6), 1992–2000 (2010).

• **Describes for the first time the role of epigenetic changes in human liver in the modulation of NAFLD phenotype.**

56 Sookoian S, Pirola CJ. Epigenetics of insulin resistance: an emerging field in translational medicine. *Curr. Diab. Rep.* 13(2), 229–237 (2013).

57 Kozlitina J, Smagris E, Stender S *et al.* Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat. Genet.* 46(4), 352–356 (2014).

58 Chalasani N, Younossi Z, Lavine JE *et al.* The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Am. J. Gastroenterol.* 107(6), 811–826 (2012).

59 Sanyal AJ, Chalasani N, Kowdley KV *et al.* Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N. Engl. J. Med.* 362(18), 1675–1685 (2010).

• **Tested the effect of pioglitazone versus vitamin E in comparison to placebo for the treatment of nonalcoholic steatohepatitis.**

60 Mudaliar S, Henry RR, Sanyal AJ *et al.* Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology* 145(3), 574–582 (2013).

61 Samson SL, Bajaj M. Potential of incretin-based therapies for non-alcoholic fatty liver disease. *J. Diab. Complicat.* 27(4), 401–406 (2013).

62 Rosselli MS, Burgueno AL, Carabelli J *et al.* Losartan reduces liver expression of plasminogen activator inhibitor-1 (PAI-1) in a high fat-induced rat nonalcoholic fatty liver disease model. *Atherosclerosis* 206(1), 119–126 (2009).

63 Sookoian S, Castano G, Garcia SI *et al.* A1166C angiotensin II type 1 receptor gene polymorphism may predict hemodynamic response to losartan in patients with cirrhosis and portal hypertension. *Am. J. Gastroenterol.* 100(3), 636–642 (2005).

64 Stojkovic IA, Ericson U, Rukh G *et al.* The PNPLA3 Ile148Met interacts with overweight and dietary intakes on fasting triglyceride levels. *Genes Nutr.* 9(2), 388 (2014).

65 Santoro N, Savoye M, Kim G *et al.* Hepatic fat accumulation is modulated by the interaction between the rs738409 variant in the PNPLA3 gene and the dietary omega6/omega3 PUFA intake. *PLoS ONE* 7(5), e37827 (2012).