Contribution of *PNPLA3* gene to the natural history of liver diseases

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Abstract

In 2008, a genome-wide association studies (GWAS) showed a strong association between a variant (rs738409 C>G p.I148M) in the *PNPLA3* and nonalcoholic fatty liver disease. Further replication studies have shown robust associations between *PNPLA3* and steatosis, fibrosis/cirrhosis, and hepatocellular carcinoma on a background of metabolic, alcoholic, and viral insults. The *PNPLA3* protein has lipase activity towards triglycerides in hepatocytes and retinyl esters in hepatic stellate cells. The I148M substitution leads to a loss of function promoting triglyceride accumulation in hepatocytes. Although *PNPLA3* function has been extensively studied, the molecular mechanisms leading to hepatic fibrosis and carcinogenesis remain unclear. This unsuspected association has highlighted the fact that liver fat metabolism may have a major impact on the pathophysiology of liver disease. Conversely, alone, this locus may have limited predictive value with regard to liver disease outcomes in clinical practice. Additional studies at the genome-wide level will be required to identify new variants associated with liver damage and cancer to explain a greater proportion of the heritability of these phenotypes. Thus, incorporating *PNPLA3* and other genetic variants in combination with clinical data will allow for the development of tailored predictive models. This attractive approach should be evaluated in prospective cohorts. (Acta gastroenterol. belg., **2017**, 80, **43-51**).

Key words : steatosis, triglycerides, fibrosis, hepatocellular carcinoma, genetic association studies, heritability.

Abbreviations : GWAS, genome-wide association study ; CHC, chronic hepatitis C ; SNP, single nucleotide polymorphism ; HCV, hepatitis C virus ; *PNPLA3*, *patatin-like phospholipase domain containing 3* ; NAFLD, nonalcoholic fatty liver disease ; ALT, alanine aminotransferase ; BMI, body mass index ; HCC, hepatocellular carcinoma ; NASH, nonalcoholic steatohepatitis ; ALD, alcoholic liver disease ; OR., odds ratio ; HIV, human immunodeficiency virus ; TG, triglycerides ; WT, wild type ; VLDL, very low density lipoproteins ; MAF, minor allele frequency ; NGS, next-generation sequencing ; ROC, receiver operator characteristic ; MZ, monozygotic ; DZ, dizygotic ; AUC, area under the curve.

Introduction

Liver diseases account for 2% of total deaths worldwide (1). Like other complex diseases, liver diseases are caused by a combination of environmental and genetic factors and their interactions (2). Twin studies have provided compelling evidence that all human traits are heritable (3). The proportion of phenotypic variation in a trait (e.g. height, liver diseases) that is due to underlying genetic variation is defined as the heritability (2). Albeit limited in number compared to other pathologies, genome-wide association studies (GWAS) in the field of liver diseases have significantly contributed to the field by revealing unsuspected associations between genetic variations and various phenotypes, and have generated new pathophysiological hypotheses (4). Thus, Romeo and colleagues performed a GWAS in 2008 and tested the association between 9,229 coding SNPs and liver steatosis evaluated by proton magnetic resonance spectroscopy in 2,111 unrelated adults of diverse ethnicities (5). They showed that a common non-synonymous variant rs738409 C>G (p.Ile148Met) located on chromosome 22 in the *patatin-like phospholipase domain containing 3 (PNPLA3)* gene (also known as *adiponutrin*) was associated with susceptibility to nonalcoholic fatty liver disease (NAFLD). This association was independent of body mass index (BMI), diabetes, alcohol consumption, and ancestry. In addition, they observed an association between the same

PNPLA3 variant and elevation of serum alanine aminotransferase (ALT) in Hispanics. This novel link between a gene coding for an enzyme with over 50% amino-acid identity within the patatin domain, the most abundant protein of potato tubers, and NAFLD generated strong enthusiasm in the field of liver diseases both at the genetic and biological levels.

PNPLA3 and liver damage in major chronic liver diseases

Nonalcoholic fatty liver disease

Other authors replicated the association between the variant and steatosis and further highlighted the fact that rs738409[C] was also a risk factor for liver damage in NAFLD (6-9). These findings have been summarized in several meta-analyses (10-14) (Table 1). Since the physiopathology of NAFLD is tightly related to the metabolic syndrome, a link between rs738409[G] and the parameters that define the metabolic syndrome was a likely hypothesis. Indeed, some authors have observed an association between *PNPLA3* and insulin resistance (15), and lower fasting triglycerides in severely obese patients and in individuals from Asian descent (16, 17). However, the vast majority of studies have shown that *PNPLA3* was not associated with BMI, serum lipid, or glycemic traits (5,8,18).

Alcoholic liver disease

In 2009, Tian and colleagues performed a large candidate gene study in 1,221 Mestizo (mixed European and Native American ancestry) heavy drinkers at various stage of the natural history of alcoholic liver disease (ALD). The authors elegantly demonstrated an allele-dose effect of rs738409[G] from alcoholics without evidence of liver damage compared to ALD patients with elevated ALT without clinical, biological, or imaging signs of cirrhosis, and ultimately alcoholic cirrhotics (19). This association was subsequently replicated in several independent cohorts, mostly among individuals of European descent (20,21), and summarized in two meta-analyses (22,23) (Table 1). More recently, the first GWAS in ALD comparing 1,426 heavy drinkers without liver injury to 712 patients with alcoholic cirrhosis, all of European descent, reported that *PNPLA3*, *TM6SF2,* and *MBOAT7*, three independent loci all involved in lipid metabolisM., were significantly associated with a more severe phenotype (24). Among theM., variants in the *PNPLA3* gene had the strongest signal with GWAS-significance (p-value $< 5 \times 10^{-8}$).

Chronic viral hepatitis B and C

Variants in the *PNPLA3* region have also been tested for association with the natural history of viral hepatitis. In CHC, a significant association of rs738409[G] with steatosis (25-28), and/or fibrosis (25,27,28) was observed in several independent European cohorts following adjustment for other known environmental risk factors. The association with fibrosis has now been confirmed in a recent meta-analysis (29) (Table 1). Interestingly, the impact of the SNP seems to be lower compared to fatty liver diseases, as indicated by the lower odds-ratios (OR) observed in carriers of rs738409[G] with histological damage phenotypes in

this etiology. Thus, the association between rs738409[G] and liver damage appears to mainly concern patients who are homozygous for the mutant G allele following a recessive model of inheritance (i.e. GG vs. CC+CG genotypes) (25,27,28). In addition, the association with steatosis was found to be less pronounced (30), or even absent, in patients with HCV genotype-3 infection (26, 27). It is likely that the stronger effect of "environmental injurious exposure" (here HCV) downplayed the impact of *PNPLA3* on steatosis and fibrosis in CHC.

In chronic hepatitis B, the association between *PNPLA3* and liver damage seems to be limited to liver fat and inflammation but not fibrosis accumulation *per se* (31-35). This link does not seem to be modulated by hepatitis B virus genotypes (31,32). Overall, the impact of *PNPLA3* on liver damage is far more pronounced in fatty liver diseases (NAFLD and ALD) compared to viral hepatitis and seems to parallel the contribution of steatosis in the pathogenesis of chronic liver diseases (36).

PNPLA3 and hepatocellular carcinoma

A robust association with non-viral-induced liver cancer

As *PNPLA3* (rs738409 C>G) was initially reported to be associated with more pronounced steatosis, advanced fibrosis, and cirrhosis it was tempting to speculate that this gene might also promote liver carcinogenesis.

In alcoholic cirrhotic patients, the level of evidence is solid. All initial case-control studies conducted in this population reported significant associations with *PNPLA3* variants, with ORs indicating increased risk (37-40). Most studies in this field were conducted in Europe and included a homogeneous population of nearly 1,500 Caucasian patients with alcoholic cirrhosis, complicated or not by HCC. This led to an initial meta-analysis (41), which also had the advantage of using individual participant data (Table 1). This methodologically sound approach confirmed the potential influence of *PNPLA3* on liver carcinogenesis in alcoholic cirrhotic patients of European ancestry, in whom a two-fold HCC risk was observed for those bearing the rs738409[G] allele. Two subsequent meta-analyses confirmed this association (11,23) (Table 1).

The issue for NAFLD patients is more difficult to address, as liver cancer may develop in individuals displaying features of metabolic syndrome without cirrhosis. In this setting, a rigorous methodology in patient selection must be applied in order to avoid false positive findings. Indeed, by only comparing genotype distribution between cases and controls without adjusting for the degree of fibrosis, it is impossible to conclude that *PNPLA3* is associated with HCC and not with the progression of NAFLD-related liver injury. A two-stage case-control study performed in NAFLD patients highlighted this issue (42). While comparing genotype distributions between HCC patients and individuals from the general population, this study reported an OR of a

large magnitude (12.19 95% confidence interval [6.89–21.58]). However, when controlling for the presence of cirrhosis, rs738409[G] conferred a much smaller increase in HCC risk (OR=2.26 95% confidence interval [1.23-4.14]). This latter observation underlines the importance of taking confounding factors into account. Taken together, and given the similarities between NAFLD- and ALD-related liver carcinogenesis, it seems reasonable to consider that carriage of rs738409[G] confers an HCC risk-effect in patients with NAFLD-related cirrhosis. Whether this *PNPLA3* variant acts as a direct modifier of hepatocarcinogenesis, regardless of fibrosis progression, deserves further clarification.

Results obtained in patients with HCV-related HCC remain controversial. Indeed, only two small-sample case-control studies from Italy (25,43) initially reported a positive association between rs738409[G] and HCC in HCV-infected patients, while at least 2 others did not observe this result (37, 38). Moreover, results obtained from meta-analyses of these studies did not provide firm conclusions (11,41). Finally, the American multi-centric HALT-C trial did not observe a higher risk of liver cancer occurrence in individuals carrying the rs738409[G] allele (44). Based on these recent data, it seems reasonable to consider that the association between rs738409[G] and HCC related to CHC in patients from European descent is not robust. More data are required in patients from other ethnicities.

Molecular mechanism of PNPLA3 *leading to the promotion of steatosis, fibrosis, and liver carcinogenesis*

PNPLA3 and steatosis/fibrosis accumulation

The aforementioned studies have used heterogeneous methods (e.g. liver biopsy, magnetic resonance imaging, liver stiffness) to assess the presence or severity of steatosis and fibrosis. The replication of associations between rs738409[G] and these phenotypes does not imply causality. Thus, in order to link *PNPLA3* variants to molecular functionality, various mechanistic experiments have been undertaken.

In humans, PNPLA3, a 481-residue protein, is observed in various tissues but is mostly expressed in the liver and the retina (45). In human liver cells, *PNPLA3* mRNA is more highly expressed in hepatic stellate cells than in hepatocytes (45). This gene is highly influenced by nutritional status (46). Thus, PNPLA3 is regulated at the transcriptional level by insulin through the induction of sterol regulatory element binding protein-1c (SREBP-1c) and carbohydrate response element-binding protein (ChREBP) (47-49). In human stellate cells, PNPLA3 is also down-regulated by intracellular retinol levels (45). At the cellular level, PNPLA3 is located in endoplasmic reticulum and lipid droplet membranes (49,50).

PNPLA3 exhibits predominantly hydrolase (lipase) activity *in vitro* against triglycerides (TG) and retinyl esters in hepatic stellate cells (49,51-53) (Fig. 1). Among triglycerides, PNPLA3 has a higher enzymatic activity against those containing monounsaturated and polyunsaturated fatty acids (51, 54).

The cytosine (C) to guanine (G) transversion in rs738409 causes an isoleucine (I) to methionine (M) exchange at amino acid 148 (I148M). In mice, He et al. showed that the I148M substitution seems to abolish hydrolase activity by reducing substrate access to the enzyme's active site and so promotes TG accumulation suggesting a loss of function (49). Using recombinant human PNPLA3 and PNPLA3-I148M proteins, studies showed that fatty acid release increased linearly with increased TG and was reduced by the I148M substitution, indicating a loss of function (51, 55). Nevertheless, He et al. also observed that an overexpression of wild type (WT) PNPLA3 in mouse liver created no obvious
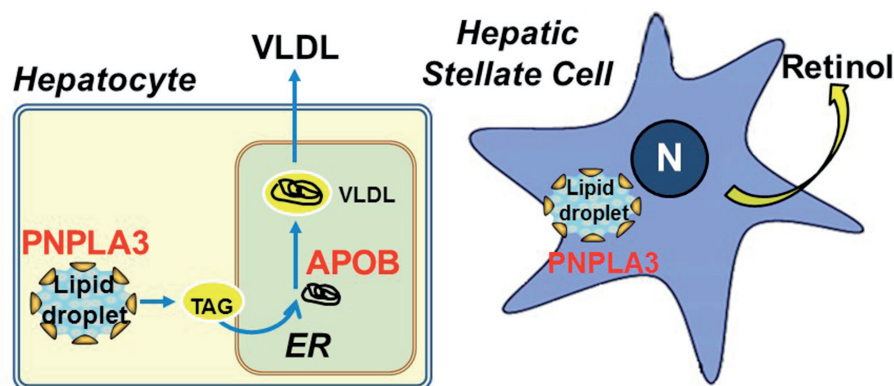

Fig. 1. — PNPLA3 is highly expressed in human hepatic stellate cells and hepatocytes. In both cells this protein is located in lipid droplets and it has hydrolase activity towards triglycerides and retinol esters in hepatocytes and hepatic stellate cells, respectively. The I148M mutation results in loss of function of the protein with liver fat retention in hepatocytes and retinol retention in hepatic stellate cells. Abbreviations: ER: endoplasmic reticulum; APOB: apolipoprotein B100; VLDL: very low density lipoprotein; N: nucleus; TAG: triglycerides.

phenotype and, more specifically, did not reduce hepatic TG content (49). This could suggest a gain of function and a lipogenic role for PNPLA3 that would be enhanced by the presence of the I148M mutation. In addition, other studies performed in PNPLA3 knockout mice did not advocate for a major role of PNPLA3 in steatosis accumulation (56, 57). Thus, PNPLA3⁻/⁻ mice did not significantly differ from WT, fed a specific regimen or not, for body weight, adipose mass/development, insulin sensitivity, or glucose tolerance. Nonetheless, more advanced mouse models were able to confirm that the PNPLA3 mutant protein resulted in a loss of function. Indeed, overexpression of mutant PNPLA3 in transgenic mice generated TG liver accumulation (54). Subsequently, using knockin mice expressing the I148M mutant protein, Smagris et al. observed marked increased liver fat levels with a high-sucrose diet and not with a normal chow diet (58). This finding confirmed the absence of hydrolase activity in the mice carrying the mutant protein and highlighted the interaction of this mutation with the environment.

In humans, the *PNPLA3* I148M mutation has been shown to influence not only intrahepatic remodeling but also reduces very low density lipoproteins (VLDL) secretion (59). VLDL secretion represents the efflux pathway of fat from the liver and, therefore, a reduction in this pathway can partially explain the increase in liver fat found in carriers of the *PNPLA3* mutation (59). Intrahepatic de novo lipogenesis is believed to be a driver of the increase in hepatic fat content (60). Recently, a study showed that carriers of the rs738409[G] allele have lower de novo lipogenesis as compared to non-carriers due to a reduction in liver SREBP1c mRNA levels (61). The reduction in hepatic de novo lipogenesis may be interpreted as a compensatory effect of hepatic fat increase.

A recent study on primary human hepatic stellate cells has shown that *PNPLA3* is highly expressed and synthesized in hepatic stellate cells and that PNPLA3 catalyzes the hydrolysis of retinyl esters (45). Consistently, obese and patients at risk for NASH with the PNPLA3 I148M allele have lower circulating concentrations of free retinol and retinol binding protein 4 (62). Finally, a study examining differences in intrahepatic retinol from human liver samples showed higher concentrations of retinol in liver specimens from I148M mutation homozygotes (63). Together, in vitro, human genetics, and ex vivo data suggest that carriers of the I148M mutation have intracellular retinol retention in hepatic stellate cells. In response to chronic inflammation, hepatic stellate cells change their phenotype from quiescent retinol-storing cells to activated myofibroblast-like cells secreting the collagen responsible for liver fibrosis (64). During this phenotypic change, hepatic stellate cells extracellularly release their retinol content. It is unknown whether retinol loss contributes to hepatic stellate cell activation and liver damage. Thus, the overall role of PNPLA3 in this activation process remains to be clarified. Overall,

although several studies have established the role of PNPLA3 in intrahepatic fat accumulation, the molecular mechanisms connecting the enzyme to inflammation and fibrogenesis remain unclear.

Hypothetical mechanisms for liver cancer promotion

Whether the rs738409[G] variant promotes liver carcinogenesis by creating a favorable microenvironment constituted by steatosis, inflammation, and fibrosis, or by specifically triggering the carcinogenic process, is still unknown. In particular, these complex entities composing the tissue environment might interact and thereby drive cancer/host cross-talk, but the details of this interplay are unclear. Mechanistic studies should unravel specific impaired biological pathways explaining the frequently reported associations between rs738409[G] and HCC in patients with ALD or NAFLD. Such results are currently emerging. Thus, the 148M mutation has been shown to favor retinoid release in hepatic stellate cells resulting in intracellular retention (45). These liposoluble micronutrients are known to influence cell proliferation and differentiation (65), and have been shown to be effective in liver cancer prevention trials (66).

The contribution of *PNPLA3* to liver damage risk and its predictive value in liver diseases

The contribution of PNPLA3 to the risk of liver damage

GWAS have uncovered thousands of robust associations between common genetic variants (i.e. with a minor allele frequency [MAF] ≥ 5%) and complex (liver) diseases (67). In the early 2000s, the GWAS design was considered the pre-eminent tool for discovering genes that influence disease susceptibility following the "common disease–common variant hypothesis" (68). This model argues that the major source of genetic variance for complex disease susceptibility is largely attributable to a moderate number of common variants, each of which explains a small fraction of the risk in a population (69). With a global MAF of 26.2% in the 1000 Genomes database (70), *PNPLA3* (rs738409 C>G) lies in that category. Nevertheless, the vast majority of common variants captured by GWAS are associated with small ORs (often less than 1.3) and even taken together, only explain a very limited proportion of the disease heritability (i.e. the proportion of the phenotypic variation in a population that this due to genotypic variation) (69, 71). As an illustration in a pathology that has been much more studied at the genetic level, 163 loci have been associated with Crohn's disease, accounting for 13.6% of the phenotype variability (72). Since Crohn's disease has an estimated 50% heritability (73), approximately 27.2% of it has been explained to date. Heritability can be estimated by comparing the resemblance (e.g. correlation) among monozygotic (MZ) twins and dizygotic (DZ) twins who generally

Table 1. — **Summary of meta-analyses evaluating the association between *PNPLA3* (rs738409[G]) and main chronic liver diseases.**

Meta-analysis	Cause of liver disease	Number of studies, and individuals	Ethnicity	Main findings	Comments
Sookoain et al. 2011 (10)	NAFLD	n=16, 12,677 individuals	EUR, AFR, AMR, EAS	Comparison between GG and CC genotypes. - NAFLD, OR=3.26 [2.73-3.90] - NASH, OR=3.26 [2.15-4.95] - Necroinflammation, OR= 3.25 95% CI [2.84-3.72]	- Adult and pediatric populations. - A meta-regression showed a negative correlation between male sex and the effect of rs738409 on steatosis.
Singal et al. 2014 (11)	NAFLD, ALD, CHC, CHB, HFE	n=24, 9,915 individuals	EUR, AFR, AMR, EAS, SAS, OTH	Using a recessive genetic model. • Advanced fibrosis - overall, OR= 1.32 95% CI [1.20-1.45] - NAFLD and ALD, OR=1.23 95% CI [1.10-1.37] - Other etiologies, OR=1.44 95% CI [1.28-1.61] • HCC (overall), OR = 1.40, 95% CI [1.12-1.75]	- The association explored is limited to advanced fibrosis. - Many studies evaluating the association with HCC are lacking.
Shen et al. 2015 (12)	NAFLD, ALD, CHC	n=7, 2,023 individuals	EUR, SAS, OTH	Comparison between GG and CC genotypes. Cirrhosis (overall), OR= 1.86 95% CI [1.64-2.12]	- The association explored is limited to cirrhosis.
Zhang et al. 2015 (13)	NAFLD	n=12, 11,926 individuals	EAS, SAS	Using a dominant genetic model. - NAFLD, OR= 1.92 95% CI [1.54-2.39] - Higher ALT levels, SMD= 7.03 95% CI [2.47-11.60] - Higher Fibrosis score, SMD= 0.39 [0.18-0.60]	- Limited to patients of Asian descent. - Only two studies were used to calculate pooled OR for fibrosis.
Xu et al. 2015 (14)	NAFLD	n= 23, 16,437 individuals	EUR, AFR, AMR, EAS	Comparison between GG and CC genotypes. - NAFLD, OR=3.41, [2.57-4.52] - Necroinflammation, OR= 3.13, 95% CI [2.76-3.56] - Fibrosis, OR=3.11, 95% CI [2.66-3.65] - NASH, OR=4.44, 95% CI [3.39-5.82]	- Adult and pediatric populations.
Chamorro et al. 2014 (22)	ALD	n=11, 8,533 individuals	EUR, EAS, OTH	Comparison between GG and CC genotypes. - ALD, OR=3.68, 95% CI (2.56-5.29) - Cirrhosis, OR= 4.30, 95% CI [1.25-5.69]	- Analyses were stratified with three types of controls (healthy non-drinkers and heavy drinkers with/without liver disease)
Salameh et al. 2015 (23)	ALD	n=10, 4,112 individuals	EUR, OTH	Using a dominant genetic model. - Steatosis, OR=0.74, 95% CI [0.54-1.03] - Fibrosis, OR= 1.45, 95% CI [1.24-1.69] - Cirrhosis, OR=2.09, 95% CI [1.79-2.44] - HCC, OR = 1.43, 95% CI [0.76–2.72]	- The association with steatosis included only two cohorts from a single study (20) - A subgroup analysis included 5 studies with individual participant data. - Several studies evaluating the association with HCC are lacking.
Fan et al. 2015 (29)	CHC	n=5, 2,037 individuals	EUR, EAS	Using a recessive genetic model. - Advanced fibrosis, OR=2.19 , 95% CI [1.59-3.02] - Steatosis, OR=4.33, 95% CI [2.59-7.22]	- Only one study included patients that were not from European descent. - Analyses stratified by HCV-genotype are difficult to interpret due to the limited number of studies and the absence of individual participant data.
Trépo et al. 2014 (41)	ALD, CHC	n=7 2,503 individuals	EUR	Using an additive model - HCC (overall), OR= 1.77, 95% CI [1.42-2.19] - HCC (ALD), OR=2.20, 95% CI [1.80-2.67] - HCC (CHC), OR=1.55, 95% CI [1.03 -2.34]	- All patients were cirrhotics - individual participant data

ALD: alcoholic liver disease, CHC: chronic hepatitis C, CHB: chronic hepatitis B, CI: confidence interval, HCC: hepatocellular carcinoma, HFE: hemochromatosis, NAFLD: nonalcoholic fatty liver disease, OR: odds ratio, SMD: standard mean difference, EUR: European, AFR: African/African American, AMR: Latino, EAS: East Asian, SAS: South Asian OTH: other

share 100% and 50% of their genes, respectively. This also assumes that common environmental factors in MZ and DZ twins are equal in magnitude (74). Using this classical twin design, Loomba et al. recently studied the heritability of NAFLD-associated steatosis and fibrosis in 60 twin pairs (42 MZ and 18 DZ) from Southern California (75). After adjustment for age, sex, and ethnicity, heritability was estimated at 0.52 for steatosis and 0.5 for fibrosis. Inclusion of rs738409 C>G in these multivariable models showed that this variant was not

significantly associated with either phenotype and that the percentage of variation explained by this SNP was negligible. This study strongly suggests that *PNPLA3* does not make a large contribution to the heritability of steatosis and fibrosis in NAFLD. Nevertheless, most of this twin population was not obese or exposed to another well-identified risk factor for liver disease. Thus, this study did not take into consideration the interaction between *PNPLA3* and the environment and potentially underestimated its impact. New GWAS-identified variants may fill in some of this *missing heritability* as sample size increases, Thus, recent studies including larger sample sizes and using more recent chip arrays have also shown that other loci are associated with steatosis in NAFLD such as *TM6SF2* (8, 76). Overall, it is likely that as more studies at the genome-wide level are conducted, additional variants robustly associated with liver damage will be identified and will allow a

greater proportion of the heritability of this phenotype to be explained (77).

PNPLA3 as a predictor of liver diseases in clinical practice

Following the enthusiasm generated by the strong and reproducible association between rs738409[G] and liver damage/cancer in fatty liver diseases, several studies hypothesized that this variant could serve as a predictor in clinical practice. This statement was based on *p* values and ORs that were often greater than 2 per risk allele (Table 1) which is of remarkable magnitude for a common variant (67). However, extreme statistical significance and large ORs do not necessarily assure that the variant(s) will be clinically relevant (78). In other words, these metrics do not guarantee effective discrimination between patients with (cases) and without (controls) a disease which is better assessed
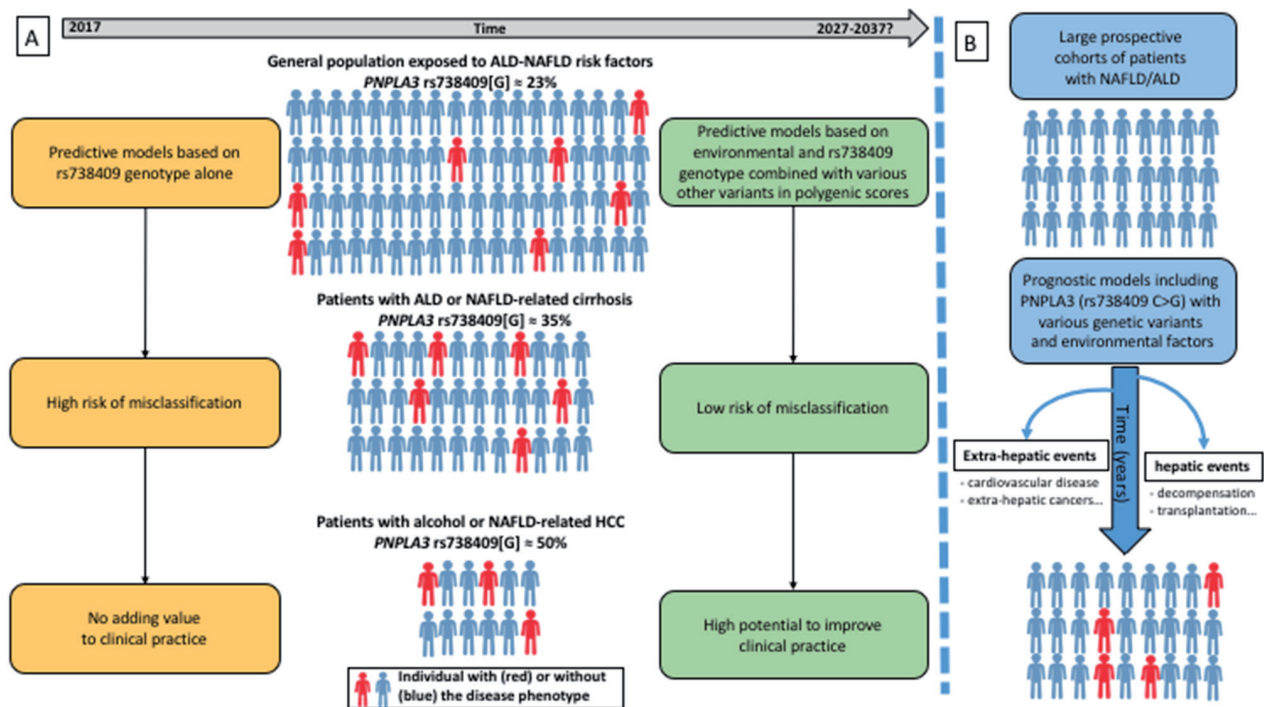

Fig. 2. — Genetic predisposition studies and limitations for application to clinical practice: the example of PNPLA3 (rs738409[G]) in alcoholic or non-alcoholic fatty liver diseases. Panel A. The association between *PNPLA3* (rs738409 C>G) and cirrhosis and hepatocellular carcinoma (HCC) development in fatty liver disease is one of the most robust discoveries in the field of genetic predisposition to chronic liver diseases. Therefore, its use as a genetic marker of disease progression could be advocated. However, the ability of *PNPLA3* (rs738409 C>G) testing alone to select individuals, who should be subjected to specific screening policies, is hampered by both the prevalence of the at-risk genotypes and by the complexity of intricate factors (genetic or not) affecting the course of chronic liver diseases. For example, by only considering genotype results, one would indeed miss most cases of cirrhosis and HCC that would develop in a given population exposed to excessive alcohol intake or the metabolic syndrome. Furthermore, a significant proportion of individuals harboring the at-risk rs738409[G] allele would undergo futile and costly surveillance. Panel B. Large well-phenotyped prospective cohorts of NAFLD and ALD patients are warranted to assess how *PNPLA3* (rs738409 C>G) could be combined with other genetic variants and environmental factors to predict the occurrence of various outcomes such as cirrhosis or HCC. Scoring systems derived from these predictive models will enable the stratification of these populations into appropriate risk classes. The prospective cohort study design will allow better accounting for gene–environment interactions, avoid bias related to incomplete follow-up, and perform competing risk analyses of outcomes in order to take into account both liver-related and extra-hepatic complications.

by classification-based methods (79). Thus, other parameters such as sensitivity, specificity, and positive and negative predictive values are more appropriate when genetic variant(s) are tested for risk prediction (78). The receiver operator characteristic (ROC) curve and the related area under curve (AUC) are more appropriate to describe the ability of a given factor to classify patients between cases and controls (79). Even for complex diseases with high heritability such as age-related macular degeneration, genetic variants identified to date typically explain too little of the variability in the disease (or outcome) occurrence to be of much clinical predictive value (79, 80). Although we are not aware of studies that have extensively assessed the clinical validity of rs738409[G], it is unlikely that this SNP alone will allow meaningful discrimination between patients with and without liver disease (77) (Fig. 2). Yet, it can be anticipated that as more risk variants are identified, the predictive value of cumulative genotype scores will increase (79). Therefore, a more rational approach will be the development of tailored predictive models integrating both clinical and genetic data that should be evaluated in prospective cohorts (80, 81) (Fig. 2).

Conclusion

In summary, *PNPLA3* is the first locus to be reproducibly and strongly associated with steatosis, fibrosis/cirrhosis in various liver diseases with different etiologies (NAFLD, ALD, and CHC) and even HCC in fatty liver diseases. These unsuspected but robust genetic associations have highlighted the fact that liver fat metabolism may have a major impact on the pathophysiology of liver damage. The fact that steatosis may potentially lead to inflammation, cirrhosis, and HCC has already been reported but whether changes in lipid turnover *per se* or another pathway leading to liver damage is disturbed or activated by the I148M mutation remains a key open question (40, 82). Undoubtedly, further experiments are required and will also help clarify these questions.

This discovery should also pave the way for the performance of further genetic association studies in the field of hepatology including much larger sample sizes and using NGS methods (83). In addition, rather than considering *PNPLA3* alone as a biomarker for disease and outcome prediction in patients with liver disease, large well-phenotyped cohorts with prospective follow-up should be created. Although they require greater resources in term of cost and time, these studies are less biased than case-control studies to assess exposure and risk factors and account for gene-environment interactions (84). Creation of predictive models integrating genetic with environmental/clinical data will facilitate a better translation of biological information into clinical practice to advance disease prevention, intervention, and treatment in chronic liver diseases.

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