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Non-alcoholic fatty liver disease and risk of cardiovascular disease

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) has become the leading cause of chronic liver diseases worldwide, causing considerable liver-related mortality and morbidity. During the past decade, it has also become increasingly evident that NAFLD is a multisystem disease that affects many extra-hepatic organ systems, including the heart and the vascular system. In this updated clinical review, we discuss the rapidly expanding body of clinical and epidemiological evidence that supports a strong association of NAFLD with cardiovascular diseases (CVDs) and other functional and structural myocardial abnormalities. We also discuss some recently published data that correlate NAFLD due to specific genetic polymorphisms with the risk of CVDs. Finally, we briefly examine the assessment tools for estimating the global CVD risk in patients with NAFLD as well as the conventional and the more innovative pharmacological approaches for the treatment of CVD risk in this group of patients.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) describes the alcohol-like liver disease that occurs in the absence of alcohol abuse, and spans simple steatosis, non-alcoholic steatohepatitis (NASH) with/without cirrhosis and hepatocellular carcinoma (HCC). NAFLD is the most common cause of chronic liver diseases worldwide, causing considerable liver-related mortality and morbidity [1,2]. However, over the past decade, it has become increasingly clear that NAFLD is also strongly associated with an increased risk of cardiovascular disease (CVD) [2–7], which represents the leading cause of mortality among NAFLD patients [1–3]. While most NAFLD cases are strongly associated with obesity and other metabolic syndrome (MetS) traits, an as yet unknown proportion of NAFLD cases are associated with specific genetic polymorphisms among which the variant (G) allele at

Abbreviations: AF, atrial fibrillation; CVD, cardiovascular disease; FHBL, familial hypobetalipoproteinemia; FRS, Framingham risk score; GGT, gamma-glutamyltransferase; GWAS, genome wide association study; HCC, hepatocellular carcinoma; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PA, physical activity; PNPLA3, patatin-like phospholipase domain containing 3; TM6SF2, trans-membrane 6 superfamily member 2.

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rs738409 of the patatin-like phospholipase domain containing-3 (PNPLA3) gene is probably the most common and best characterized [2].

Despite emerging evidence which suggests that NAFLD is also linked to other chronic diseases [7–10], it is the adverse impact of NAFLD on CVD risk which deserves particular attention owing to the epidemics of obesity/MetS leading to a dramatic surge in the proportion of NAFLD patients in the general population.

Thus, in this clinical review we provide an update on the current evidence for a strong association of NAFLD with CVD and other structural and functional cardiac complications. As a novelty of this updated review that was not previously reviewed elsewhere, we also discuss in-depth the role of some genetic polymorphisms in the development and progression of both NAFLD and CVD. Indeed, it is uncertain whether genetic-related NAFLD (e.g., *PNPLA3*-related NAFLD) carries the same risk as NAFLD occurring with the MetS for the development of CVD/cardiac diseases. Finally, we also discuss how the CVD risk should be currently estimated and managed in patients with NAFLD.

2. NAFLD, CVD and Other Cardiac Diseases: Clinical Data

2.1. *NAFLD and CVD*

As shown in Fig. 1, patients with NAFLD have a myriad of traditional and non-traditional risk factors for CVD [2,3,11]. This finding is the basis for therapeutic actions in preventing the development of CVD events in patients with NAFLD.

Abundant data link NAFLD with subclinical CVD markers [2–4]. In their meta-analytic review involving 7 cross-sectional studies, Sookoian et al. have firstly shown that NAFLD was associated with increased carotid-artery intimal medial thickness and an increased prevalence of carotid atherosclerotic plaques [12]. These findings were recently corroborated in a larger meta-analysis involving 27 cross-sectional studies that clearly showed that NAFLD was associated with increased carotid-artery intimal medial thickness, impaired flow-mediated vasodilation, increased arterial stiffness and increased coronary artery calcification. All these associations were independent of traditional risk factors and MetS traits across a wide range of patient populations [13]. Recent results from the Framingham Heart study further confirmed this notion, showing that there was a significant association between NAFLD and subclinical CVD outcomes, independently of many metabolic diseases/traits [14].

Several hospital- and population-based studies have consistently shown that the prevalence of clinically manifest CVD is also remarkably increased in patients with NAFLD (as extensively reviewed elsewhere [2–5]). Again, in patients referred for clinically indicated coronary angiography, NAFLD was independently associated with a greater severity of coronary artery disease [15–17]. Worryingly, NAFLD was also associated with a higher prevalence of high-risk coronary atherosclerotic plaques, independently of the extent and severity of coronary atherosclerosis and traditional risk factors [18]. Similarly, NAFLD was associated with carotid artery inflammation (which may reflect atherosclerotic plaque vulnerability) in a large cohort of healthy individuals [19]. Collectively, these findings suggest that NAFLD patients, further to being

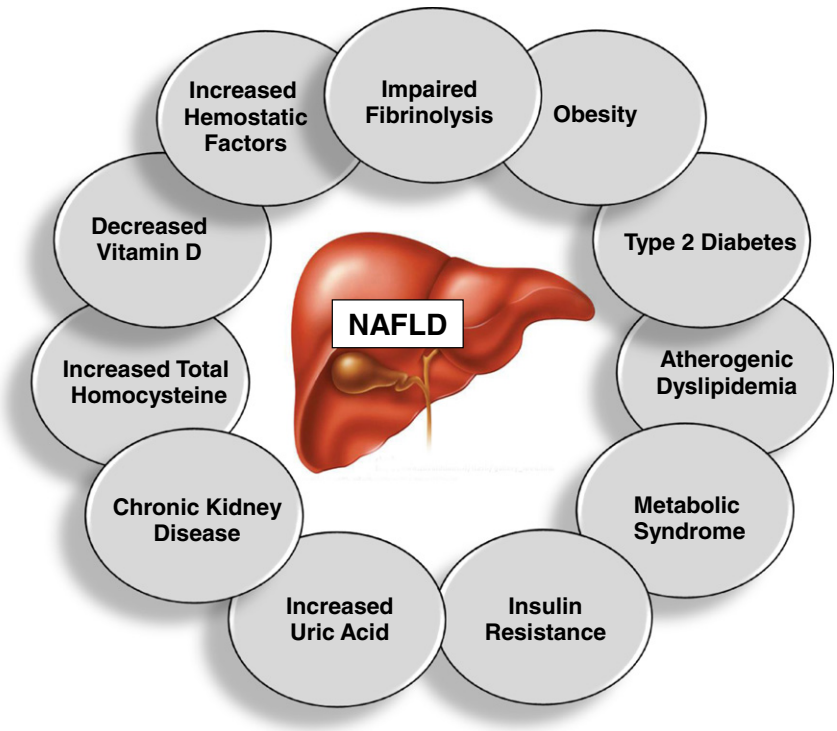

Fig. 1 – The myriad of CVD risk factors in patients with NAFLD.Patients with NAFLD have the typical traits of the metabolic syndrome and have multiple non-traditional risk factors and risk markers for CVD.

more prone to develop coronary atherosclerosis, are also more likely to develop those atherosclerotic plaques, which are more susceptible to rupture.

Although the association between NAFLD and increased CVD prevalence is strong and consistent, the direct contribution of NAFLD to the development of CVD remains more controversial. Table 1 summarizes the retrospective and prospective studies that have assessed the relationship between NAFLD and the risk of CVD events and mortality [17,20–36]. Most of these studies support the assertion that CVD is the most common cause of mortality among patients with NAFLD. This table does not include those population-based studies that showed that mildly elevated serum liver enzyme levels (mainly gamma-glutamyltransferase [GGT]) independently predict the development of CVD events [2,9,37].

Regarding the biopsy-diagnosed NAFLD (Table 1) [20–25], some retrospective studies with a reasonably long duration of follow-up have shown that the all-cause mortality rate was significantly greater in patients with NAFLD than in matched control populations, and that this increase was largely due to CVD and liver-related causes. These studies have also shown that the histologic severity of liver fibrosis was the main determinant of all-cause and cause-specific mortality. However, it should be noted that the retrospective design and the relatively small sample size of these cohort studies do not permit the generalizability of the findings. It is argued whether simple steatosis or NASH accounts for excess CVD risk. The answer to this question has important implications for the management of NAFLD patients. However, further studies in larger cohorts of well-characterized patients with biopsy-proven NAFLD are needed to elucidate this question.

Regarding the imaging-diagnosed NAFLD [17,26–36], several retrospective and prospective cohort studies have shown that NAFLD was associated with an increased incidence of major CVD events, independently of traditional risk factors and MetS traits. In contrast, and surprisingly, using data from the Third National Health and Nutrition Examination Survey database, the investigators did not observe any significant association between ultrasonography-diagnosed NAFLD and the 14-year risk of all-cause and cause-specific mortality in United States adults [31,32]. However, the latest analyses of the same database reported that NAFLD with advanced hepatic fibrosis was associated with increased all-cause mortality, and that this increase in mortality was largely due to CVD causes [36]. A meta-analytic review confirmed that NAFLD patients had an approximately doubled risk of fatal and non-fatal CVD events compared to control populations [38].

2.2. NAFLD and Altered Cardiac Function and Structure

Plentiful data link NAFLD with functional and structural myocardial alterations both in adults and in children/adolescents with or without co-existing MetS traits [4]. For instance, in a community-based cohort of Korean adults, Kim et al. found that NAFLD was associated with left ventricular (LV) diastolic dysfunction, independently of traditional risk factors [39]. Another study assessing the effect of different ectopic fat depots on LV function in non-diabetic men with NAFLD reported that only intra-hepatic triglyceride content and visceral adipose tissue were independent predictors of LV diastolic dysfunction [40]. Some studies also suggest that there was a significant, graded relationship between LV diastolic dysfunction and the severity of NAFLD histology [41,42]. These findings suggest that the association between NAFLD and cardiac dysfunction may be because of toxic systemic effects. Again, a recent cross-sectional analysis of 2713 participants from the community-based Coronary Artery Risk Development in Young Adults study confirmed that NAFLD was independently associated with subclinical myocardial remodeling and LV systolic and diastolic dysfunction, and provided further insight into a possible link between NAFLD and heart failure [43]. Indeed, some evidence suggested that moderately elevated serum liver enzymes in the absence of excessive alcohol consumption are associated with an increased risk of new-onset heart failure in population-based cohort studies [44,45]. However, further prospective studies, using more accurate methods for diagnosing NAFLD, are needed to examine the prognostic role of NAFLD on the development of heart failure.

2.3. NAFLD, Cardiac Arrhythmias and Heart Valve Calcification

Atrial fibrillation (AF) is a major public health burden worldwide, and its prevalence is set to increase owing to widespread population aging. Two community-based cohort studies that have used serum liver enzymes, as proxy markers of NAFLD, have shown that this disease was independently associated with an increased risk of new-onset AF [46,47]. More recently, type 2 diabetic patients with ultrasonographic NAFLD had an increased prevalence of permanent or persistent AF [48], and were more likely to develop incident AF over 10 years of follow-up compared to their counterparts without NAFLD [49]. Interestingly, in both studies the association between NAFLD and AF risk remained significant even after adjustment for multiple AF risk factors [48,49].

Recent data also suggested that the presence and severity NAFLD on ultrasonography were independently associated with increased QTc interval duration (i.e., a powerful predictor of ventricular arrhythmias and sudden cardiac death), which might partly account for the increased CVD mortality associated with NAFLD [50].

Finally, the presence of aortic-valve sclerosis, a progressive heart valve disease that shares multiple pathogenic risk factors with atherosclerosis and is associated with an increased risk of CVD events [51], has also been linked with NAFLD, independently of traditional CVD risk factors, in both diabetic and non-diabetic individuals [52,53]. More recently, a cross-sectional study of type 2 diabetic patients with no previous history of chronic heart failure, heart valve diseases or known hepatic diseases, reported that NAFLD was a strong and independent predictor not only of aortic-valve sclerosis but also of mitral annulus calcification [54].

Collectively, although the literature data are not all methodologically robust and most of the published studies lack a definite, histological diagnosis of NAFLD, we believe that the concept of NAFLD as an independent contributor to the development of CVD and other structural and functional cardiac abnormalities appears to be sufficiently substantiated

Table 1 – Principal prospective and retrospective studies of the risk of CVD mortality and morbidity in patients with NAFLD (defined by liver biopsy or imaging only). Adapted from Byrne et al. [5].

Study and year [reference]	Study design, Sample size, Population and Mean Follow up	Diagnosis of NAFLD	Main Findings
Matteoni CA et al. 1999 [20]	Retrospective cohort 132 US NAFLD patients, 18 years	Biopsy [a]	Patients with NASH had higher rates of all-cause and liver-related mortality than those without the disease. CVD mortality did not differ between the groups
Dam-Larsen S et al. 2004 [21]	Retrospective cohort 109 Danish NAFLD patients (without NASH at baseline), 16.7 years	Biopsy [a]	No significant difference in mortality rates between patients with simple steatosis and the general population
Ekstedt M et al. 2006 [22]	Retrospective cohort 129 Sweden NAFLD patients, 13.7 years	Biopsy [a]	Patients with NASH, but not those with simple steatosis, had higher rates of all-cause (~2-fold), CVD (~2-fold) and liver-related (~10-fold) mortality than the general population matched for age and sex
Rafiq N et al. 2009 [23]	Retrospective cohort 173 US NAFLD patients, 13 years	Biopsy [a]	CVD, cancer and liver-related complications were the most common causes of mortality in this cohort of NAFLD patients
Söderberg C et al. 2010 [24]	Retrospective cohort 118 Sweden NAFLD patients, 24 years	Biopsy [a]	Patients with NASH, but not those with simple steatosis, had higher rates of all-cause (~2-fold), CVD (~2-fold) and liver-related mortality than the general population matched for age and sex
Ekstedt M et al. 2015 [25]	Retrospective cohort 229 Sweden NAFLD patients, 26.4 years	Biopsy [a]	NAFLD patients had increased risk of death (HR 1.29, CI 1.04–1.59), with a high risk of death from CVD (HR 1.55, 95%CI 1.11–2.15) and liver-related disease (HR 3.2, 95%CI 1.05–9.81). NAFLD activity score (NAS) was not able to predict all-cause death, whereas fibrosis stage predicted all-cause, CVD and liver-related death
Jepsen P et al. 2003 [26]	Retrospective cohort 1804 Danish hospitalized patients with NAFLD, 6.2 years	Ultrasound [a]	Patients with NAFLD had higher rates of all-cause (2.6-fold), CVD (2.1-fold) and liver-related (19.7-fold) mortality than the general population
Adams LA et al. 2005 [27]	Retrospective cohort 420 US NAFLD patients, 7.6 years	Biopsy/imaging [a]	Patients with NAFLD (especially those with cirrhosis and NASH) had higher rates of all-cause, CVD and liver-related mortality than the age and sex-matched general population
Targher G et al. 2007 [28]	Prospective cohort 2103 Italian outpatients with type 2 diabetes without viral hepatitis and CVD at baseline, 6.5 years	Ultrasound [c]	NAFLD was associated with an increased risk of fatal and nonfatal CVD events (HR 1.87, 95% CI 1.2–2.6), independently of age, sex, body mass index, smoking, diabetes duration, hemoglobin A1c, LDL-cholesterol, metabolic syndrome features, medication use
Hamaguchi M et al. 2007 [29]	Community-based cohort 1637 Japanese individuals, 5 years	Ultrasound [b]	NAFLD was associated with an increased risk of nonfatal CVD events (HR 4.10, 95% CI 1.6–10.7), independently of age, sex, body mass index, alcohol intake, smoking history, LDL-cholesterol and metabolic syndrome features
Haring R et al. 2009 [30]	Population-based cohort 4160 German individuals, 7.3 years	Ultrasound [a]	NAFLD was associated with an increased risk of all-cause and CVD (HR 6.22, 95% CI 1.2–31.6) mortality in men, independently of age, waist circumference, alcohol consumption, physical activity, civil status, equalized income, functional comorbidity index, blood pressure, diabetes status
Wong VW et al. 2011 [17]	Prospective cohort 465 Chinese patients with coronary heart disease as diagnosed by coronary angiography, 1.8 years	Ultrasound [d]	NAFLD was independently associated with an increased prevalence of CVD at baseline but there was no significant association between NAFLD and risk of incident CVD events
Lazo M et al. 2011 [31] and Stepanova M et al. 2012 [32]	National-based cohort 11,371 US adults from the National Health and Nutrition Examination Survey 1988–94, 14.5 years	Ultrasound [a]	No significant association between NAFLD and all-cause and cause-specific (CVD, cancer and liver) mortality
Treeprasertsuk S et al. 2012 [33]	Retrospective community-based cohort 309 US NAFLD patients, 11.5 years	Ultrasound/computed tomography [e]	Framingham risk score accurately predicted the higher 10-year coronary heart disease risk in NAFLD patients and was the only

(continued on next page)

Table 1 (continued)

Study and year [reference]	Study design, Sample size, Population and Mean Follow up	Diagnosis of NAFLD	Main Findings
			variable significantly associated with the risk of developing new-onset CHD events in this patient cohort
Zhou YJ et al. 2012 [34]	Community-based cohort 3543 Chinese adult individuals, 4 years	Ultrasound [a]	Patients with NAFLD had ~3-fold higher rates of all-cause and CVD mortality than those without NAFLD
Dunn MA et al. 2013 [35]	Retrospective cohort 2343 US type 2 diabetics seen in the primary care and specialty clinics of a large integrated delivery network, 5 years	Computed tomography [c]	No significant association was found between NAFLD and risk of all-cause mortality and cause-specific (CVD, cancer and liver) mortality and morbidity. NAFLD patients (steatosis >30% on imaging) averaged 8 years younger than those without NAFLD
Kim D et al. 2013 [36]	National-based cohort study 11,154 US adults from the Third National Health and Nutrition Examination Survey, 14.5 years	Ultrasound and advanced fibrosis score systems [a]	NAFLD was not associated with increased all-cause mortality. However, NAFLD with advanced hepatic fibrosis (defined by NAFLD fibrosis score (NFS), APRI index or Fib-4) was independently associated with a 69% increased risk of all-cause mortality. Increase in mortality was almost entirely from CVD causes (for NFS: HR 3.46, 95%CI 1.91–6.25; for APRI: HR 2.53, 95%CI 1.33–4.83; for Fib-4: HR 2.68, 95%CI 1.44–4.99)

[a] Study outcome was all-cause and cause-specific mortality.
[b] Study outcome was nonfatal coronary heart disease and stroke.
[c] Study outcome was a combined endpoint of CVD mortality and nonfatal myocardial infarction, ischemic stroke and coronary revascularization procedures.
[d] Study outcome was a combined endpoint of CVD mortality and nonfatal myocardial infarction and coronary revascularization procedures.
[e] Study outcome was a combined endpoint of CVD mortality and nonfatal congestive heart failure, angina, myocardial infarction and coronary revascularization procedures.

by the current evidence as to integrate it into the clinical approach for both the patient with NAFLD and the patient with CVD.

3. Genetic-related NAFLD and CVD Risk

3.1. *PNPLA-3 and TM6SF2 Genetic Variants*

The genetic risk of NAFLD is strongly influenced by the nonsynonymous 738409 variant encoding an amino acid substitution p.Ile148Met and located in the *PNPLA3* gene [55,56]. After the first description of the association by a genome wide association study (GWAS) on NAFLD [55], this missense variant was associated not only with increased intra-hepatic triglyceride content but also with the histological severity and progression of disease [56,57]. Overall, the *PNPLA3*-rs738409 was responsible for about 5% of the total genetic variance attributed to NAFLD [56], one of the largest effects described for a common variant for a complex disease reported ever.

A recent effort to refine known loci or find new genes associated with NAFLD led to the discovery of another nonsynonymous variant (rs58542926) encoding an amino acid substitution p.Glu167Lys and located in trans-membrane 6 superfamily member 2 (*TM6SF2*) gene that was associated with an increased intra-hepatic triglyceride content [58]. Notably, simultaneous efforts to refine known loci or find new genes associated with plasma lipid phenotypes have also led to the conclusion that the *TM6SF2*-rs58542926 is a genetic modifier of lipidemia, including serum total cholesterol, low-density lipoprotein cholesterol and triglyceride levels, but with the allelic effect in the opposite direction [59,60]. However, the role of this genetic variant in the pathogenesis and NAFLD-disease severity is conflicting, as while the large majority of subsequent studies have replicated the association between the minor *TM6SF2*-rs58542926-T allele and fatty liver or intra-hepatic triglyceride content [61–64], the association with liver fibrosis remains still unclear. For instance, while one study, including a large sample of patients with NAFLD from Europe, observed a moderate effect on the development of liver fibrosis [65], another large study showed a significant association with advanced liver fibrosis [61] that did not remain significant after adjusting for potential confounders [66]. Nevertheless, it was demonstrated that this genetic variant might affect the histological degree of hepatic steatosis thereby accounting for the association with NASH and the fibrosing outcome of disease [61,62]. Interestingly, it was also demonstrated that patients with NASH had decreased liver protein levels and also that Lys167 was associated with a decreased liver protein content compared to Glu167-allele [62], a result consistent with those obtained from animal models [58].

As summarized in Table 2 [56,58–62,67–74], the two above-mentioned missense genetic variants may play important roles in the regulation of hepatic lipid metabolism. Specifically, the *PNPLA3*-rs738409 modulates basic aspects of the physiology of liver lipid droplets, including lipid partitioning [69,71] and,

Table 2 – Missense variants in *PNPLA3* and *TM6SF2* associated with NAFLD: differences and similarities on the regulation of lipid traits.

Features	rs738408 C/G	rs58542926 C/T
Gene name	*PNPLA3*	*TM6SF2*
Chromosome localization	Chromosome 22:43928847 (forward strand)	Chromosome 19:19268740 (forward strand)
Residue change	p.Ile148Met	p.Glu167Lys
MAF	0.28 (G)	0.06 (T)
Variant effect on gene expression	Loss of function [67]	The T allele is associated with decreased gene and protein expression in the liver of patients with NAFLD [62]. In vitro and experimental models showed that the variant is associated with reduction in TM6SF2 function [58,68]
Variant effect on lipid droplet physiology	Regulates the morphology and physiology of liver lipid droplets [69]	Affects the secretion of triglyceride-rich lipoproteins [58,68]
Association of the variant with metabolic syndrome	Lack of association with insulin resistance or body mass index as shown in large meta-analysis [56]	The C (Glu167) ancestral allele is positively associated with total cholesterol, LDL-cholesterol and triglycerides as oppose to the negative association with NAFLD [58–62] A variant in *CILP2*[a] locus (rs10401969) was associated with the risk of type 2 diabetes [70]
Evidence of sexual dymorphism	There is a negative association between the effect of rs738409 on liver fat content and male sex [56]	Unknown
Gene function	Triacylglycerol lipase that mediates triacylglycerol hydrolysis [71]; plays a role in the hydrolysis of glycerolipids [67]	Unknown
Enzyme activity	Acylglycerol O-acyltransferase	Unknown
Subcellular localization	Plasma membrane	Endoplasmic reticulum (ER) and the ER–Golgi intermediate compartment of human liver cells [68]. Highly expressed in the cytoplasm of human hepatocytes [62]
Gene regulation	Highly regulated by changes in energy balance and glucose [72–74]	Unknown

CILP2: Cartilage Intermediate Layer Protein 2;
[a] *CILP2* is mapped in a multi-locus region (*NCAN/TM6SF2/CILP2/PBX4*) which some SNPs are in moderate or high linkage disequilibrium.

putatively, the release of both arachidonic acid and prostaglandin E2 [75,76]. By contrast, the *TM6SF2*-rs58542926 is apparently required for normal very-low-density lipoprotein secretion [58,68].

Hence, in this complex scenario, it is reasonable to speculate that both genetic variants may mediate the link between NAFLD and the CVD risk. Nevertheless, while compelling evidence demonstrated that the minor T allele of the *TM6SF2*-rs58542926 has a dual and paradoxical role in protecting against CVD and in increasing the risk of fatty liver [61,62] as recently shown in two human studies on NAFLD, the putative role of the *PNPLA3*-rs738409 on the risk of NAFLD-associated CVD remains arguable. Indeed, the effect of the *TM6SF2*-rs58542926-C risk allele on CVD risk was demonstrated by a large GWAS on myocardial infarction, which found that the T-allele (that was associated with reduced levels of plasma lipids) also conferred a reduced risk of myocardial infarction [59]. Conversely, the possible adverse effect of the *PNPLA3*-rs738409 on the development of CVD, expressed as the severity of carotid atherosclerosis, was reported in an observational study of 162 patients with NAFLD, in which the authors showed that patients carrying the homozygous GG genotype had increased carotid-artery intima–media thickness [77]; surprisingly, however, this observation was restricted only to the subjects at younger age. Thus, the putative role of *PNPLA3* locus on CVD development has questionable evidence that needs to be further confirmed. For example, against the hypothesis of the *PNPLA3*-rs738409 is involved in CVD development, the results of two large association studies, such as the Swedish Obese Subjects study and the Go-DARTS study, that showed that carriers of the *PNPLA3*-rs738409-G risk allele had lower serum triglyceride levels [78].

However, Pirazzi et al. postulated that PNPLA3 may affect the secretion of apolipoprotein-B containing lipoproteins and the 148M isoform is the product of a loss-of-function mutation [79]. Moreover, a GWAS on the putative genetic regulation of soluble intercellular adhesion molecule-1 (ICAM-1) levels found a significant association with the *PNPLA3*-rs738409 [80]. ICAM-1, which mediates the adhesion and transmigration of leucocytes to the vascular endothelial wall, was shown to be involved in liver damage and inflammation in patients with NAFLD [81].

Collectively, to date, there is no ultimate evidence on the role of the *PNPLA3*-rs738409 in modulating plasma lipid phenotypes or the development of arterial atherosclerotic plaques. It is reasonable to suggest that the association between *PNPLA3*-rs738409 and development of carotid plaques might simply result from NAFLD and CVD being commonly associated [3,12]. Thereby, patients homozygous for the risk allele, who are also at higher risk of NAFLD, are those who have carotid thickening as patients with NAFLD are shown to carry an increase of 13% of carotid intima–media thickness [12]. However, future prospective studies are needed to elucidate whether *PNPLA3*-related

NAFLD or *TM6SF2*-related NAFLD carries the same risk as NAFLD occurring with the MetS for the development of CVD events.

Finally, it is also worth mentioning that while the involvement of *PNPLA3* and *TM6SF2* loci in the biology of NAFLD is irrefutable, the magnitude of the genetic effect of both *PNPLA3*-rs738409 and *TM6SF2*-rs58542926 is highly dissimilar. For instance, a large meta-analysis on the effect of the *PNPLA3*-rs738409 on NAFLD showed that GG homozygous had an approximately three-fold higher risk of liver inflammation and fibrosis when compared with CC homozygous [56]. By contrast, two case–control association studies on NAFLD showed that the risk of NASH on carriers of the *TM6SF2*-rs58542926-T risk allele was around 1.84 and 1.66, respectively [61,62]. In addition, one study reported no significant association with NASH in the replication stage but only with liver fibrosis [65].

In addition, while the *PNPLA3*-rs738409 is a common genetic variant (Table 2), the *TM6SF2*-rs58542926-T risk allele has an overall very low frequency around 0.06 that seems to be higher in South Asian (T:11%) and Chinese (T:16%) populations and lower in African population (T:5%); information extracted from the 1000 Genomes browser (http://browser.1000genomes.org/).

Remarkably, there would not seem to be any interaction between the effect of the *PNPLA3*-738409 and *TM6SF2*-rs58542926 variants on the risk of NAFLD [58,62,65]; hence, despite the *TM6SF2*-rs58542926 partly accounting for the missing heritability of the disease, so far the *PNPLA3*-rs738409 remains the most important contributor to the genetic risk of NAFLD.

3.2. *Hypobetalipoproteinemia*

Familial hypobetalipoproteinemia (FHBL) is an autosomal dominant disease that is typically characterized by abnormally low levels of apolipoprotein-B containing lipoproteins and by the presence of NAFLD that results from an impaired capacity to export lipids from the hepatocytes into the bloodstream in the absence of insulin resistance [82–87]. Thus, FHBL might be regarded as a unique model useful in evaluating the contribution of NAFLD to atherosclerosis in absence of hypercholesterolemia and insulin resistance. Some studies suggested that compared with matched controls, FHBL patients had slightly decreased arterial stiffness but similar carotid intima–media thickness measurements [88]. This finding suggests that the atherogenic impact of NAFLD could have partly decreased the positive effect of hypocholesterolemia in FHBL patients. However, prospective studies are needed to evaluate the risk of incident CVD in this group of patients.

4. Estimation and Management of CVD Risk in NAFLD

4.1. *Estimation of CVD Risk in NAFLD*

Presently, estimation of the global CVD risk in patients with NAFLD should be performed using the same methods used for individuals without NAFLD in primary prevention of CVD [2–5]. The Framingham risk score (FRS) is currently the most practical tool to calculate the 10-year risk of developing a CVD event in adults aged ≥20 years, who do not have a prior history of CVD or diabetes. This risk score accurately predicted the CVD risk in a cohort of United States patients with NAFLD, who were followed for 10 years [33]. However, the FRS is more accurate in populations of Anglo-Saxon descent and may over-estimate the CVD risk in south-European populations [89]. In addition, it is possible to assume that the FRS and other available risk scoring systems (such as the QRISK2) may underestimate the CVD risk in NAFLD patients, given that the subclinical inflammatory, insulin-resistant and hypertriglyceridemic states of NAFLD are not considered in any of these CVD risk scoring systems. However, with regard to the QRISK2, it is possible to assume that this risk assessment tool might also perform better than the FRS in predicting the CVD risk in patients with NAFLD because the QRISK2 also includes the presence of obesity and diabetes as risk factors into the risk equation [90]. At present, however, no studies have specifically tested the validity of the QRISK2 in predicting CVD risk among NAFLD patients.

Recently, as summarized in Fig. 2, we proposed a pragmatic algorithm for the diagnosis and management of CVD risk in NAFLD patients without established CVD [91]. We believe that current guidance to treat individuals with a 10-year CVD risk >20% based on the FRS may be too high for patients with NAFLD, and devising separate thresholds for close monitoring or treatment, such as >15%, may promote earlier intervention and decrease CVD events. On the basis of this algorithm, we believe that lifestyle modification is the foundation of treatment for all NAFLD patients, irrespective of their absolute CVD risk, but that those with established diabetes or with a 10-year CVD risk >15% should be promptly treated with a high-intensity statin therapy based on their absolute CVD risk, and not on their LDL-cholesterol concentration (*unless* LDL-cholesterol is <2.5 mmol/l prior to treatment) [91]. If we estimate the level of risk in NAFLD patients using the QRISK2 risk assessment tool, it is likely that in accord with the recent National Institute for Health and Care Excellence guidelines [90], a high-intensity statin treatment should be considered for primary prevention of CVD in those with a 10-year CVD risk >10%. However, it is important to remark that the use and the accuracy of all risk assessment tools in predicting CVD risk among patients with NAFLD need to be further validated by prospective studies in larger cohorts of well-characterized NAFLD patients of various ethnic backgrounds.

4.2. *Putative Mechanisms Linking NAFLD with CVD and Cardiac Diseases*

A clear understanding of the pathophysiological pathways that link NAFLD to the development of cardiovascular, cardiac and arrhythmic complications remains lacking, because of the complex and intertwined interconnections among NAFLD, visceral obesity and insulin resistance [2–5,91]. One or more among the subsequent pathogenic mechanisms might account for the relationship linking NAFLD with excess CVD risk.

Firstly, the risk of accelerated atherosclerosis may be associated with hepatic histology changes. In agreement with this notion, the severity of NAFLD histology was strongly associated with a more atherogenic lipid profile [92] and increased arterial stiffness [93]. Consistently, increased hepatic stiffness was associated with greater coronary-artery calcium scores in NAFLD patients, independently of coexisting cardiometabolic risk factors [94].

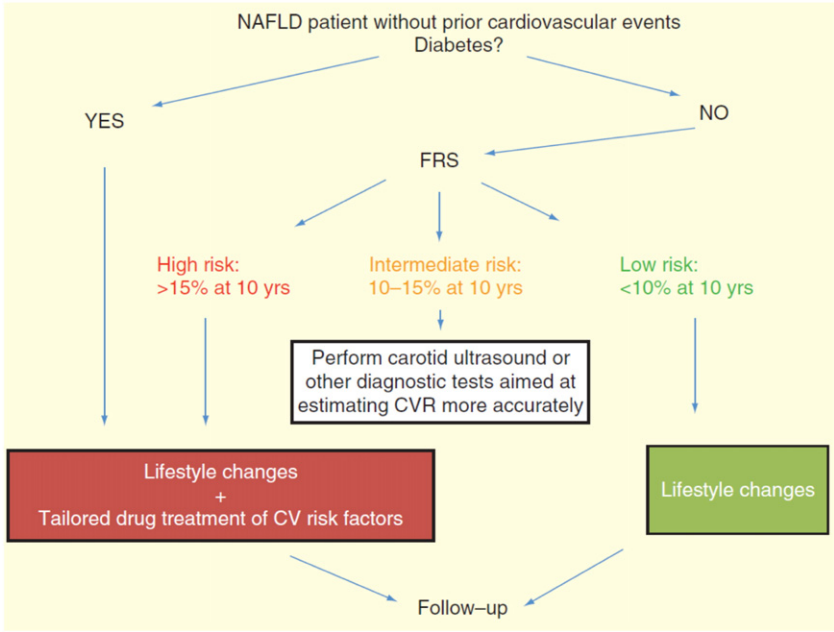

Fig. 2 – Tailored diagnosis and treatment of CVD risk as estimated by the Framingham risk score (FRS) in NAFLD patients in primary prevention for CVD. Reproduced, with permission from Ref [91]. In a patient with NAFLD at low CVD risk (i.e., FRS <10% at 10 years) lifestyle changes without drug treatment are proposed as the first-line therapeutic option. In a patient with NAFLD at intermediate CVD risk (i.e., FRS between 10% and 15% at 10 years), drug treatment is suggested whenever the CVD risk profile is not controlled following intensive lifestyle changes. At this level of CVD risk, the use of selected clinical and non-invasive CVD screening tests may be suggested in order to better identify those individuals who, in fact, belong to the category at higher CVD risk and, therefore, need to be treated more aggressively.In a patient with NAFLD at high CVD risk (i.e., FRS >15% at 10 years) an early, intensive, drug treatment should be conducted in order to correct all the coexisting cardio-metabolic risk factors.

Secondly, it may be that the more the hepatic fat content, the higher the CVD risk. Consistent with this notion, a progressive worsening in atherogenic dyslipidemia occurs across increasing levels of hepatic fat content even after adjusting for potential confounders [95]. Accordingly, there is a significant association between increased hepatic fat content in NAFLD patients and increased rates of MetS traits, independent of biopsy-detected NASH [96]. Again, increased hepatic fat content predicts the risk of CVD after adjustment for traditional risk factors, although the significance of this association was lost after further adjustment for insulin resistance [97], also suggesting that this metabolic disorder rather than increased hepatic fat content might account for excess CVD risk. These findings partly confirm that liver biopsy is not necessary in estimating CVD risk in NAFLD patients [91] and imply that non-invasive techniques, such as magnetic resonance imaging, which estimates hepatic fat content very accurately [98], might prove more useful in assessing CVD risk.

Thirdly, it may be that the coexisting increased oxidative stress, rather than NAFLD *per se*, is associated with an increased CVD risk. In agreement with this notion, CVD risk was more strongly associated with elevated serum GGT levels (a marker of increased oxidative stress) rather than with NAFLD in elderly type 2 diabetic patients [99].

Finally, it was shown that NASH, but not simple steatosis, may increase CVD risk through the local over-expression of multiple atherogenic mediators, endothelial damage factors, blood pressure regulators, platelet count and abundance of transforming growth factor-beta mRNA levels [81]. Of note, NAFLD has also a distinguishing circulating microRNA profile that is associated with both a dysmetabolic disease state and increased CVD risk [100].

Collectively, given that the more the hepatic fat content the more the risk of NASH [101] and given that elevated serum GGT levels may be not only a marker of increased oxidative stress but also a marker of NAFLD/NASH [102], the above-mentioned mechanistic models should not be considered mutually exclusive.

In any case, whatever the role played by the steatotic and inflamed liver, accumulating evidence indicates that NAFLD, especially NASH, exacerbates hepatic/peripheral insulin resistance, induces atherogenic dyslipidemia and releases a myriad of pro-inflammatory factors, vasoactive factors and thrombogenic molecules that promote the development and progression of CVD and other structural and functional cardiac alterations [2–5,91]. Although all these mechanisms plausibly link NAFLD to CVD, no studies to date have proven a cause–effect relationship and further research is needed to gain mechanistic insights into the pathophysiology linking NAFLD to CVD.

4.3. *Treatment of CVD Risk in NAFLD*

There is no approved pharmacological treatment for NAFLD. Although it can be hypothesized that improving NAFLD may reduce the risk of CVD, there are limited data on changes in CVD risk in relation to the success of NAFLD treatment.

Therefore, no evidence-based recommendations can be formulated at present.

Strong evidence suggests that intensive lifestyle counseling improves dietary and physical activity (PA) habits, and reduces cardio-metabolic risk factors to a seemingly modest extent that is nevertheless meaningful in reducing CVD events [103]. Achievement of well-defined thresholds in body weight reduction has been mechanistically linked with improvements in NAFLD histology [91]. However, whether such thresholds in weight reduction are also associated with a decreased CVD risk is uncertain.

PA improves cardiovascular health and reduces insulin resistance, independent of weight loss. An inverse relationship exists between hepatic steatosis and degree of PA: ≥150 to ≥250 min/week moderate to vigorous PA may protect from hepatic steatosis, possibly via reduced inflammation and oxidative stress levels and improved fatty acid metabolism [104,105]. PA shows a dose–response association with NAFLD, independent of coexisting obesity or MetS, also suggesting that it will benefit everyone irrespective of their CVD risk [106]. Accordingly, PA improves cutaneous microvascular function and reverses endothelial dysfunction in NAFLD patients [107,108]. Notably, exercise may decrease hepatic fat content even in the absence of significant reductions in total body fat and visceral adipose tissue and at levels of exercising below the thresholds recommended for the management of obesity [109].

Taken singularly, both aerobic exercise and resistance exercise are similarly effective in reducing hepatic fat content and intra-abdominal fat depots in patients with type 2 diabetes and NAFLD [110]; however, resistance exercise seems to be more strongly associated with improved insulin sensitivity [111]. While exercise improves hepatic steatosis and underlying metabolic abnormalities in NAFLD, more studies are needed to define the most beneficial form and duration of exercise treatment [112,113]. Exercise should be promoted in motivated individuals, particularly in lean NAFLD patients where large weight loss cannot be pursued [114]. An experimental study in mice has also shown that regular exercise may protect from HCC development and this could be a further rationale for promoting regular PA in human NASH [115].

Finally, MT-ND6 methylation, a member of the mitochondrial OXPHOS-chain, was higher in the livers of patients with NASH than in those with simple steatosis, and a higher MT-ND6 methylated DNA/unmethylated DNA ratio was significantly associated with increased NAFLD activity score. Conversely, hepatic MT-ND6 mRNA expression was decreased in NASH patients and the protein level was also diminished. The status of hepatic MT-ND6 methylation in NASH was inversely associated with the level of regular PA [116].

4.3.1. *Drug Treatment*

4.3.1.1. *Conventional approach.* For NAFLD patients who are at high CVD risk (Fig. 2), a tailored drug treatment of all coexisting cardio-metabolic risk factors needs to be promptly started [91].

Evidence of efficacy in patients with NASH is available for vitamin E and pioglitazone, whose effectiveness has been shown mainly in non-diabetic adults, although the long-term safety and efficacy of both drugs have not yet been clearly established [117]. In addition, it is important to note that the effect size of these drugs is relatively modest and none is approved by the US Food and Drug Administration.

Various drug classes are potentially indicated for the treatment of coexisting cardio-metabolic factors in patients with NAFLD: statins, ezetimibe, fibrates, high-dose omega-3 fatty acids, vitamin D, vitamin E, sartans, insulin, metformin, pioglitazone and incretin-mimetics. Pros and cons of each individual drug class have been extensively discussed elsewhere [1,91,118].

Among these drugs, statins and metformin promise to reduce CVD risk without worsening and, possibly, also reducing the most important liver-related complications of NAFLD, notably including HCC. Statins are the mainstay of treatment to be safely conducted to reduce CVD events and mortality [119,120]. In patients at high CVD risk, who are intolerant or unresponsive to statins, the prescription of a lower-intensity statin therapy combined with ezetimibe may be suggested [121]. In the past, statin use in patients with NAFLD may possibly have been hampered owing to concerns of liver toxicity, although such concerns are not justified based on the currently available data [1,91,122–128]. Recent post-hoc analyses of randomized controlled trials suggested that the cardio-protective effect of statins is more pronounced among CVD patients with mild-to-moderate baseline elevations in serum transaminase levels [129,130].

Although there are few and controversial data on the effect of statins on liver histology in NAFLD patients, a recent large case–control study has shown that statin use is associated with a reduced risk of simple steatosis, NASH and advanced fibrosis [66]. Consistent with this view, increasing evidence suggests that statin use might also exert a beneficial effect on HCC development [131,132].

Metformin is considered as a first-line agent to treat people with type 2 diabetes and may exert favorable effects on the vasculature of this patient population [133]. However, although metformin treatment is associated with HbA1c reduction and body weight reduction/neutrality, insufficient evidence supports the view that its use carries a substantially decreased risk of all-cause and CVD mortality [134]. Moreover, metformin may also have some gastrointestinal side effects [134]. To date, it remains uncertain whether metformin exerts a beneficial effect on NAFLD liver histology [135], indicating that insulin resistance may trigger the development of hepatic steatosis but is unlikely to sustain its progression to NASH [136]. Despite some uncertainties surrounding its hepatic and CVD end-points, increasing evidence suggests that metformin-treated diabetic patients (rather than those treated with insulin) might also be protected from HCC development [91].

Sartans could be eligible drugs for reducing hepatic fat content in hypertensive patients with NAFLD as it was also shown that these drugs improve some molecular mediators of CVD risk. For instance, in an experimental model of high-fat induced NAFLD, losartan significantly decreased the hepatic expression of plasminogen activator inhibitor-1 [137]. Likewise, results from some small clinical studies showed that ACE-inhibitors and sartans improved both CVD outcomes and liver fibrosis [81,138].

4.3.1.2. *Innovative Strategies.* Emerging therapies for NASH include obeticholic acid, a synthetic FXR agonist bile acid; GFT505, an insulin sensitizer liver-targeted dual PPAR-

alpha/delta agonist; cenicriviroc, a CCR2-CCR5 antagonist; aramchol, a fatty acid-bile acid conjugate; simtuzumab, a humanized, anti-lysyl-oxidase 2 monoclonal antibody; and RO5093151, an inhibitor of 11β-hydroxysteroid-dehydrogenase type 1 [116,139,140]. However, the potential for these drugs in reducing CVD risk is presently unclear. Finally, there are few and controversial data about the use of prebiotics/probiotics in NAFLD treatment and their possible effects on CVD risk [141–143].

5. Conclusions

In this updated review of the published studies assessing the association between NAFLD and the risk of CVD and other functional and structural heart diseases, we provide further support to the assertion that there is a clear association of NAFLD with CVD and structural/functional cardiac abnormalities, though causality remains to be proven in well-controlled prospective and intervention studies. In the meantime, from the perspective of clinical practice, the notion that NAFLD is a powerful trigger and amplifier of adverse CVD outcomes can justify targeting NAFLD in an attempt to reduce the risk of incident CVD (as schematically summarized in Fig. 3) [2–5,66,91,144–148].

However, uncertainty still surrounds the prognostic role of NAFLD in CVD risk stratification. Large, well-designed prospective studies are needed to assess whether the addition of NAFLD to the currently used risk assessment tools will improve CVD risk prediction. Statins are safe and may also reduce CVD events and mortality in NAFLD patients. Ongoing and future studies will clarify whether statins might also have a beneficial role in NAFLD treatment [149].

In this updated review we also discussed the role of some genetic polymorphisms in the development of NAFLD and CVD. However, further research is needed to better elucidate whether MetS-related NAFLD and genetic-related NAFLD carry the same risk of developing CVD and other cardiac complications.

Author Contributions

All authors researched the data, contributed to the discussion and wrote the manuscript.

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Disclosure Statement

The authors have no potential conflicts of interest to disclose.

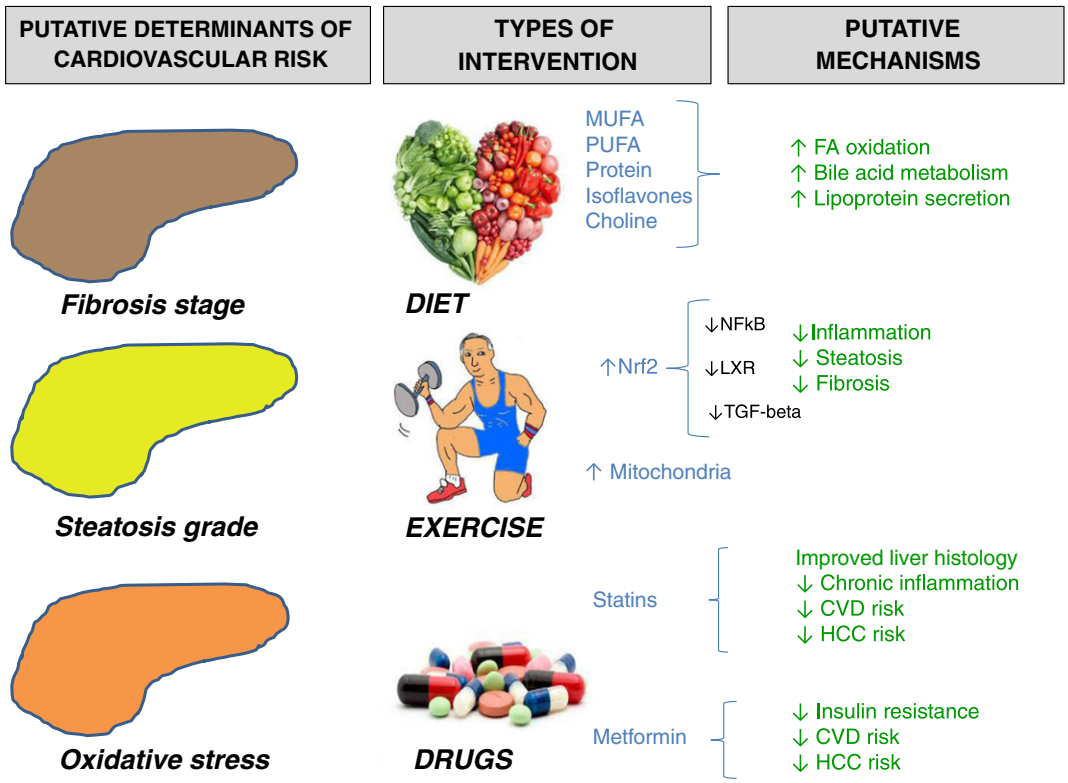

Fig. 3 – NAFLD treatment can reduce CVD risk. Based on data presented in this manuscript and on further published evidence [2–5,91,143–147], this cartoon summarizes how lifestyle and drug interventions might mechanistically reduce CVD risk by targeting various aspects of NAFLD pathogenesis and histology.

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