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## Atherosclerosis

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## Editorial

## “Not all forms of NAFLD were created equal”. Do metabolic syndrome-related NAFLD and *PNPLA3*-related NAFLD exert a variable impact on the risk of early carotid atherosclerosis?




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Nonalcoholic fatty liver disease (NAFLD), which defines alcohol-like liver histologic changes in nonalcoholic individuals who are free of any other competing etiologies of liver disease, is a common disease worldwide [1]. Over the past years, however, it has become increasingly evident that NAFLD is not merely a liver disease but also a multisystem condition [2], whose natural course may include cardiovascular, metabolic, neoplastic or liver-related complications [3]. Substantial insight has recently been gained into the role of genetic [4,5] and epidemiological risk modifiers [6], which may affect the development and progression of NAFLD. The genetic polymorphisms of the patatin-like phospholipase domain containing 3 (*PNPLA3*) and the trans-membrane 6 superfamily member 2 (*TM6SF2*) genes have been identified as the two most relevant genetic modifiers of the risk of developing liver-related or extrahepatic manifestations of NAFLD [4,5]. A key concept is that so inextricable is the bidirectional relationship between NAFLD and the metabolic syndrome (MetS) that NAFLD may occur as either a cause or a consequence of the MetS [4,7]. However, based on a current paradigm, individuals who are carriers of the G allele at rs738409 of the *PNPLA3* gene are at increased risk of NAFLD progression, but they are usually spared from insulin resistance and MetS traits [4]. If this is the case, it can reasonably be anticipated that, based on the *PNPLA3* genetic variants, at least two distinct forms of NAFLD do exist: one with and another without MetS and its inherent cardio-metabolic risk.

Against this complex scenario, the proof-of-concept study published in this issue of *Atherosclerosis* by Di Costanzo et al. [8] further supports the notion that NAFLD is a heterogeneous and complex disease and that, stated otherwise, “not all forms of NAFLD were created equal”, especially with regard to the NAFLD-related cardio-metabolic risk. In their case-control study, the authors have

compared carotid intima-media thickness (CIMT) measurements, a reliable marker of subclinical atherosclerosis [9], in three highly selected groups of individuals: 83 blood donors with the mutant *PNPLA3* GG genotype (group G; 42.2% of whom had imaging-diagnosed NAFLD), 100 patients with coexistent NAFLD and MetS, but with the wild-type *PNPLA3* CC genotype (group M), and 74 blood donors with the wild-type *PNPLA3* CC genotype (controls; 21.7% of whom had NAFLD). After adjustment for potential confounding factors (age, sex, smoking and steatosis severity), the authors found that the median CIMT in group M was significantly higher than that in group G, which was similar to that in the control group (median CIMT: 0.84 vs. 0.66 vs. 0.70 mm, respectively). Results remained unchanged even when NAFLD-positive participants from groups M and G were compared to controls without hepatic steatosis on ultrasonography [8]. Collectively, these findings are compatible with the view that the MetS-related NAFLD and the *PNPLA3*-related NAFLD may exert a differential impact on early carotid atherosclerosis. These data, therefore, support the possibility that NAFLD is associated with an increased burden of subclinical atherosclerosis only when it is linked to MetS traits rather than when it occurs owing to the *PNPLA3*rs738409 gene polymorphism.

However, as reported in Table 1 [8,10–12], when specific reference is made to *PNPLA3* gene polymorphisms, data are conflicting, which weakens this general paradigm. The dogma that certain (genetic) forms of NAFLD are typically disconnected from insulin resistance and the MetS should, therefore, be currently accepted more as an important research question than as a piece of acquired knowledge. Indeed, growing evidence supports the existence of a strong association between NAFLD and increased risk of cardio-metabolic diseases. Several prospective studies have shown that NAFLD is strongly associated with an increased incidence of type 2 diabetes and MetS [1,2,6,7]. A number of studies have consistently demonstrated that patients with NAFLD have an excess risk of both subclinical and clinically manifest cardiovascular disease (CVD) [2,3,9,13]. In particular, convincing evidence now substantiates a link between NAFLD and various markers of sub-clinical atherosclerosis (notably including increased CIMT and elevated coronary artery calcification score [CAC]), independently of conventional risk factors and MetS traits, in a wide range of patient populations [14–16]. Some studies have also shown that NAFLD is associated with a higher prevalence of carotid atherosclerotic plaques and greater severity of coronary artery stenosis, independently of multiple cardio-metabolic risk factors [14]. Interestingly, in a large cohort study of adults with no history of CVD, NAFLD was associated with the development of CAC, independent of cardiovascular

**Table 1**  
Principal studies assessing the relationship between the patatin-like phospholipase-3 (*PNPLA3*) rs738409 gene polymorphism and the risk of atherosclerosis.

Authors, year [ref.]	Study characteristics	NAFLD diagnosis	Study outcome(s)	Main findings
Petta et al., 2013 [10]	South Italy cohort: 162 consecutive patients with NAFLD; Northern Italy cohort: 267 patients with NAFLD	Biopsy	Carotid intima-media thickness and plaque on ultrasound	In both study cohorts, the <i>PNPLA3</i> GG genotype was independently associated with greater severity of carotid atherosclerosis in younger patients with NAFLD (patients <50 years). In a subgroup of 63 patients, who underwent ultrasonographic follow-up of carotid assessment, the <i>PNPLA3</i> GG genotype was also independently associated with carotid intima-media thickness progression over time.
Posadas-Sánchez et al., 2016 [11]	The Genetics of Atherosclerotic Disease study of 1013 Mexican patients with premature ischemic heart disease and 1469 healthy controls	Computed tomography	Premature ischemic heart disease (defined as history of myocardial infarction, coronary revascularizations or stenosis >50% on coronary angiography, diagnosed before age 55 years in men and before age 65 years in women). Coronary calcium score on computed tomography	The I148M/ <i>PNPLA3</i> polymorphism was not significantly associated with premature ischemic heart disease in the entire group of participants. However, when patients and controls were divided into those with and without type 2 diabetes, under additive model, the I148M/ <i>PNPLA3</i> polymorphism was significantly associated with the presence of premature ischemic heart disease in patients with type 2 diabetes. Moreover, in the control group, the I148M/ <i>PNPLA3</i> polymorphism was independently associated with coronary artery calcification score $\geq 10$ .
Xia et al., 2016 [12]	Community-based cohort of 4300 middle-aged and elderly Chinese individuals	Liver fat content determined by a quantitative ultrasound method	Carotid intima-media thickness on ultrasound	Compared to the <i>PNPLA3</i> CC homozygotes, the GG homozygotes had higher liver fat and liver fibrosis scores. An increase in liver fat was accompanied by a significant increase in the average and maximum carotid intima-media thickness in subjects with the <i>PNPLA3</i> CC genotype, but not in those with the GG genotype.
Di Costanzo et al., 2016 [8]	Three selected groups of middle-aged Italian individuals: 74 blood donors with <i>PNPLA3</i> CC genotype (21.7% of whom had NAFLD), 83 blood donors with <i>PNPLA3</i> GG genotype (group G; 42.2% had NAFLD) and 100 patients with coexistent metabolic syndrome and NAFLD but with the <i>PNPLA3</i> CC genotype (group M)	Ultrasound and magnetic resonance (in a subgroup of 157 subjects)	Carotid intima-media thickness on ultrasound	Patients with NAFLD and <i>PNPLA3</i> CC genotype (group M) had significantly greater carotid intima-media thickness compared to the other two groups of individuals. In contrast, carotid intima-media thickness was comparable between blood donors with <i>PNPLA3</i> GG genotype and those with <i>PNPLA3</i> CC genotype. Results remained unchanged even when NAFLD-positive participants from groups M and G were compared to controls without hepatic steatosis on ultrasound.

and metabolic risk factors [17]. Finally, a recent systematic review and meta-analysis of 16 observational studies, involving approximately 34,000 individuals (36.3% of whom with NAFLD as detected by imaging or histology) followed-up over a median period of 6.9 years, has confirmed that patients with NAFLD had a higher risk of fatal and non-fatal CVD events than those without NAFLD (random effect odds ratio [OR] 1.64, 95% confidence intervals [CI] 1.3–2.1). Patients with more severe NAFLD were more likely to develop fatal and non-fatal CVD events (OR 2.58; 95%CI 1.8–3.7) [18].

On this background of evidence, the results of the study by Di Costanzo et al. [8] provide further support to the view that the aetiology of NAFLD is multifactorial and this disease may be caused by common genetic variants. One of these, the *PNPLA3* variant, is associated with higher liver fat content and increased risk of NASH, but is not systematically associated with insulin resistance and MetS traits [4,5]. This study adds a further critical piece of information by suggesting that the MetS-related NAFLD and the *PNPLA3*-related NAFLD may differentially affect the risk of subclinical atherosclerosis and perhaps of clinical CVD [8]. However, it does not detract from the notion that NAFLD, especially NASH with varying degrees of fibrosis, may directly contribute to the development and progression of CVD [2,3,9], because genetic NAFLD is a subtly different disease and less than 15% of European

patients with NAFLD have the *PNPLA3* GG genotype [4,5]. Furthermore, as previously mentioned, the few observational studies that have assessed the association between the *PNPLA3* rs738409 gene polymorphism and risk of atherosclerosis have provided conflicting results (as summarized in Table 1) [8,10–12].

That said, the study by Di Costanzo et al. [8] has some important drawbacks that should be mentioned. Firstly, this study was limited by its single-center and cross-sectional (case-control) design, which does not allow drawing any firm conclusion about the temporality or causality of the observed associations. In addition, the sample size of the study was relatively small and, although the multivariate regression models were sufficiently adjusted (including for age that markedly differed among the three groups of participants), unmeasured confounding factors might potentially explain the observed associations. Furthermore, the diagnosis of NAFLD was based on either ultrasonography or magnetic resonance imaging/spectroscopy (only in a subgroup of individuals), but was not confirmed by liver biopsy, which is considered the reference standard for diagnosing and staging NAFLD. Finally, although the *PNPLA3* GG genotype is the strongest genetic determinant of NAFLD, the authors did not genotype their study participants for other genetic variants associated with NAFLD (e.g., the *TM6SF2* gene).

Notwithstanding these limitations, the findings of the study by

Di Costanzo et al. appear to be biologically plausible. Indeed, other examples of dissociation of NAFLD due to molecularly characterized genetic polymorphisms from cardio-metabolic risk have been previously described in humans [19]. The intriguing piece of translational research by Di Costanzo et al. [8] should, accordingly, be best confirmed in large and well-designed prospective studies. These studies should take into account the severity of NAFLD histology, which is a powerful contributor to the increased cardio-metabolic risk observed in patients with NAFLD [18]. Furthermore, all patients with NAFLD should be better genotyped in order to elucidate the possible differential impact of the *PNPLA3* gene and other genetic variants on liver disease progression and risk of CVD and other extra-hepatic complications, such as chronic kidney disease. For instance, preliminary evidence suggests that carriers of the *PNPLA3* GG genotype with a normal body weight have impaired kidney function (i.e., an established risk factor for CVD) compared to those carrying the CC genotype [20].

It is hoped that the results of these future large prospective studies with sufficiently long follow-up will provide a more tailored approach to the diagnosis and treatment of patients with NAFLD based on their individual profile of cardio-metabolic risk [9]. Additionally, it also hoped that the results of these studies will help to better elucidate the biological mechanisms dissociating increased liver fat content from cardio-metabolic complications in selected patients with NAFLD carrying specific genetic polymorphisms.

#### Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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