**Editorial: Is a fatty liver (always or ever) bad for the heart?**

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Non-alcoholic fatty liver disease (NAFLD) - a condition associated with the metabolic syndrome, has become common in an era of poor diet and reduced physical activity, affecting up to one in four adults[1]. Patients exhibit variability in the rate of liver disease progression but only a relative minority progress to cirrhosis and liver-related death. However, NAFLD is also associated with increased morbidity and mortality from coronary heart disease (CHD), with emerging evidence implicating hepatic fibrosis (a progressive feature of NAFLD) in other vascular disorders such as ischaemic stroke [2,3]. Therefore, an important research question in the past decade has been to tease apart whether NAFLD independently increases the risk of CHD.

Patients with NAFLD typically have multiple risk factors for CHD, including insulin resistance, dyslipidaemia, high blood pressure, and being overweight. Hepatocytes infiltrated with fat produce inflammatory cytokines, pro-thrombotic factors and adhesion molecules which have been associated with a further increase in cardiovascular disease risk[4]. Emerging cross-sectional and longitudinal evidence further identify associations between NAFLD and subclinical cardiovascular disease[5].

Regardless of causation, the association of NAFLD and CHD might have implications for cardiovascular risk prediction. However, to our knowledge the presence of NAFLD has yet to be evaluated for inclusion in risk prediction tools (e.g. QRISK2 and SCORE), some of which now include other non-cardiovascular co-morbidities such as rheumatoid arthritis[6,7].

However, a more challenging question is whether NAFLD itself plays a causal role in the development of CHD. Lauridsen and colleagues[8] set out to answer this, replicating the graded observational associations between liver fat content and CHD. However, the observational association of NAFLD and CHD might occur because both share common risk factors rather than one causing the other. Mendelian randomization (MR) (Figure 1A) is an established epidemiological approach that can provide insight on causality. MR uses genetic variants associated with an exposure to assess its causal effect on an outcome of interest. In the classic MR paradigm, genetic associations are free from confounding since they are assigned randomly at conception (according to Mendel's second law) and reverse causation is precluded since the sequence of the germline is not modifiable by disease. MR

can be thought of as analogous to a randomized controlled trial (RCT) that uses naturally randomized genetic variation rather than randomized allocation to a treatment, as the 'intervention' (Figure 1B). A causal explanation for the observational association between liver fat and CHD detected by the authors was not supported by an MR analysis using a known NAFLD associated genetic variant (I148M in *PNPLA3*) as an instrumental variable.

The current negative study does not come as a complete surprise. Some previous studies have suggested that only patients with the more severe, inflammatory liver phenotype (non-alcoholic steatohepatitis) rather than those with simple steatosis have an increased CHD mortality compared with a matched population[9]. Other robustly associated NAFLD variants in known genes (*TM6SF2*) have, perhaps counter intuitively, been found to be cardioprotective[10]. For example, a recent exome-wide association study of plasma lipids in >300,000 individuals demonstrated that the two most robustly associated NAFLD variants (I148M in *PNPLA3,* the variant studied here, and E167K in *TM6SF2*) tracked with higher liver fat, higher risk for type 2 diabetes, but with lower serum triglycerides, lower serum low density lipoprotein (LDL) cholesterol and lower risk for CHD[11].

This apparent paradox might be explained if the variants chosen to index liver fat act by reducing very low density lipoprotein (VLDL) secretion from the liver. This would cause an accumulation of liver fat but a reduction in circulating triglycerides and LDL cholesterol, providing a potential explanation for the observed inverse genetic association of such variants with CHD[12]. Such an effect was also observed in treatment trials of the microsomal triglyceride transfer protein (MTP) inhibitor, lomitapide, which works by preventing liver secretion of VLDL, the precursor to LDL. Lomitapide, evaluated as treatment for patients with homozygous familial hypercholesterolaemia, reduces LDL cholesterol and triglycerides, but causes an elevation in serum transaminases and accumulation of hepatic fat[13]. MR analyses of NAFLD and CHD that utilise alternative genetic variants that index consequences of increased hepatic lipid influx (rather than reduced hepatic lipid efflux) would therefore be of interest. These might provide a closer biological proxy for the highly prevalent form of NAFLD that arises from an adverse diet and lifestyle.

The authors rightly address this and other limitations in their discussion. They also note that the findings may only be generalizable to individuals of European ancestry, since the genetic instrument was designed by using data from predominantly European studies. Only a single variant was used as a genetic instrument, accounting for a modest 1.1% variance in liver fat content. The large population sample (279,013 individuals) and its relatively high minor allele frequency allowed for an acceptable genetic instrument (with an F-statistic above the arbitrary cut-off of 10), however the possibility of weak-instrument bias (leading to false negative results) still remains. The exposure being instrumented genetically (liver fat content) is a fairly distal consequence of the unidirectional perturbation from genetic variation through to mRNA to protein and metabolome, making MR studies of such a trait more susceptible to horizontal pleiotropy (whereby the association of a genetic instrument with a disease end point is explained by a parallel association with a different exposure to the one of interest). The heritability of computer tomography (CT) measured hepatic steatosis has previously been estimated at 26%-27%[14]. In future, larger genetic studies with imaging, outcome and genotype data (e.g. UK Biobank) should permit much larger genome-wide association studies on liver imaging, allowing for the generation of more powerful and accurate genetic tools for MR analysis of both liver fat content and liver fibrosis[15].

In summary, Laurisden and colleagues[8] confirm the observational association between liver fat content and CHD. When applying MR in a large population sample to investigate causality, this resulted in a negative study, suggesting that any association between liver fat content and CHD might not be causal. The availability of much larger studies with imaging, clinical outcome and genotype data will allow for more accurate and powerful genetic instruments for liver fat content (and potentially liver fibrosis), to further delineate the relationship between NAFLD and CHD.

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**Figure 1.**

**A)** The Mendelian randomization (MR) model: the causal role of an exposure (e.g. CT liver fat content as a proxy for NAFLD) on a disease (e.g. CHD) is being examined. A genetic variant (e.g. IL4M) is robustly associated with the exposure (continuous arrow) but not with measured or unmeasured confounders (dotted arrow). The genetic variant is also associated with the disease only through its effects on the exposure and not directly (dotted arrow). The model rests on three assumptions: (i) the genetic instrument is associated with the exposure or biomarker of interest (ii) the genetic instrument must not associate with confounders that are either known or unknown; (iii) the outcome is associated with the genetic instrument only through the effect of the exposure, and is in all other respects independent.

**B)** Summary figure of how MR can be considered analogous of the classical randomized controlled trial (RCT) ("treatment arms" shown in brackets) in the study by Lauridsen and colleagues[8]. Random allocation of alleles at conception and the unidirectional flow of information from the germline genome to exposure allow causal inference similar to the RCT framework. Genotype is generally unrelated to environmental exposure, thus reducing confounding.