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COMMENTARY

Inherited susceptibility to non-alcoholic fatty liver disease

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Abbreviations

DHS	Dallas Heart Study
HOMA-IR	HOMA of insulin resistance
NAFLD	Non-alcoholic fatty liver disease
SNP	Single nucleotide polymorphism

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function tests and affects >25% of the population in Western countries [1]. It is defined histologically as fat accumulation in the liver exceeding 5–10% by weight in people consuming less than 14 units of alcohol per week without evidence of viral, toxin-induced or autoimmune liver disease [2]. In addition to being very common, it has attracted considerable scientific and medical attention for two main reasons. First, a significant number of people with simple fatty liver (steatosis) go on to manifest hepatitis (non-alcoholic steatohepatitis), fibrosis and, ultimately, cirrhosis [1]. Second, it is strongly associated with all components of the metabolic syndrome [2], and many would argue that it is causally implicated in hepatic insulin resistance and metabolic dyslipidaemia (high triacylglycerol and low HDL-cholesterol levels).

As with the accumulation of any substrate in a given tissue, fat accretion in the liver is a consequence of an imbalance between fatty acid influx/synthesis and disposal. Fatty acids in hepatic triacylglycerols are derived from three major sources [3]; dietary chylomicron remnants account for ~15%, NEFAs released from adipose tissue during lipolysis or as spillover from lipoproteins hydrolysed at a rate exceeding fatty acid uptake into adipocytes account for as much as ~60%, and newly synthesised fatty acids account for up to ~25%, at least in insulin-resistant states. The relative contribution of splanchnic lipolysis vs peripheral fat lipolysis remains unclear, although elegant studies by Jensen and colleagues suggest that, in lean people, splanchnic lipolysis accounts for as little as ~5% of portal vein NEFAs, increasing to ~20% in viscerally obese people [4]. In the fasting state, de novo lipogenesis accounts for <5% of liver fatty acids, but this small pool appears to be significantly increased in insulin-resistant states [3], where the combination of hyperinsulinaemia and excess glucose flux drive lipogenesis via two key transcription factors, sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate-responsive element-binding protein (CHREBP) [5]. It is also important to bear in mind that the relative contributions of these pathways change considerably between the fed and fasting state, de novo lipogenesis typically increasing in the fed state. Triacylglycerols may also accumulate secondary to decreased fat oxidation or lipoprotein-mediated triacylglycerol export. Mutations in *APOB* and *MTP* are inherited examples of defects in lipoprotein formation and triacylglycerol export [6].

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Obesity is strongly correlated with liver steatosis, and studies in monozygotic twins discordant for obesity suggest that acquired obesity increases liver fat independently of genetic background [7]. Although this doesn't exclude a genetic contribution, it does support the idea that obesity is a major factor in the development of NAFLD.

The standard suggestion is that NEFA levels are increased in obesity, leading to increased NEFA flux to the liver. This is probably overly simplistic as fasting NEFA levels are not necessarily increased in obesity [8]. Nevertheless, insulin-stimulated NEFA suppression may be reduced, leading to 24 h increases in NEFA flux to the liver. Obesity is also associated with hyperinsulinaemia and increased de novo lipogenesis [3].

Some of the best evidence for a genetic contribution to NAFLD comes from data indicating that ethnicity has a substantial effect on liver fat accumulation. Petersen et al. showed that lean, apparently healthy Asian-Indian men have more liver fat and are more insulin resistant than BMI- and age-matched white individuals [9]. In the Dallas Heart Study (DHS), which reported on 2,287 participants, the incidence of liver steatosis was significantly higher in Hispanic-Americans (45%) than in black Americans (24%) or white Americans (33%) [10]. In order to identify the genetic factors that contribute to interindividual differences in hepatic fat content, Romeo et al. performed a genome-wide survey of non-synonymous variants in the DHS participants [11]. Data for the three ancestry groups were combined into one analysis to increase statistical power, and the use of a chip based on non-synonymous variants alone was expected to increase the likelihood of finding causative variants as compared with genome-wide single nucleotide polymorphism (SNP) chips that include intronic and intergenic SNPs, as functional implications are harder to demonstrate for these. A single variant in *PNPLA3* (rs738409) was strongly associated with hepatic fat content ($p=5.9\times10^{-10}$) and with hepatic enzyme (alanine aminotransferase) levels ($p=3.7\times10^{-4}$), suggesting that the variant also increases the risk of steatohepatitis [11]. This relationship remained highly significant after adjusting for BMI, and, interestingly, the SNP was not associated with plasma triacylglycerol or HDL-cholesterol levels. There was no association between the SNP and fasting indices of insulin sensitivity (HOMA of insulin resistance [HOMA-IR]) in either the DHS samples or a larger ($n=14{,}821$) study (Atherosclerosis Risk in Communities [ARIC] study). The investigators went on to sequence the coding regions of *PNPLA3* in 160 individuals with the highest, and 160 with the lowest, liver fat levels [11]. Although the total number of variants was similar in the two groups, all three individuals with probable null variants were in the high-fat group, a finding that suggests that loss of function mutations increase liver fat content. This work also led to the identification of a common variant (rs6006460) associated with lower hepatic fat in black Americans (rs6006460 is rare [<1%] in Hispanic-Americans and white Americans) [11]. These two variants (rs738409 and rs6006460) together explain 72% of the differences among the three ancestry groups with respect to hepatic fat content.

In this edition of *Diabetologia*, Kotronen et al. [12] report the results of a validation study in which the *PNPLA3* SNP rs738409 (I148M) was shown to be associated with increased liver fat content in 291 Finnish individuals [12]. Like Romeo et al. [11], they used magnetic resonance spectroscopy to non-invasively measure liver fat content. They confirmed the association of the *PNPLA3* SNP with increased liver fat content and higher liver enzyme levels. They also confirmed that this relationship was independent of BMI and fasting indices of insulin sensitivity. Although this was a smaller study, the authors obtained additional phenotypic information in the form of gold standard measures of hepatic and peripheral insulin sensitivity with hyperinsulinaemic–euglycaemic clamps in 109 participants. Here, too, they found no association of the *PNPLA3* variant with hepatic insulin sensitivity.

PNPLA3 encodes a non-secreted protein called adiponutrin, the precise biological function of which remains unclear. The highest levels of adiponutrin are found in the adipocyte fraction of white adipose tissue, but it is also present in the liver; mRNA levels are low in the fasting state and are significantly increased by carbohydrate refeeding in both tissues [13, 14]. It was originally identified as a member of the calcium-independent phospholipase A_2 family of proteins, but in addition to triacylglycerol hydrolase activity, it appears to have significant acylglycerol transacylase activity (including transfer of fatty acids to mono- and diacylglycerol) [15]. The fact that *Pnpla3* expression is induced by insulin and carbohydrate refeeding suggests that it may have a predominantly lipogenic function in the liver. This hypothesis would also be consistent with the increase in *Pnpla3* expression noted in the fatty liver of *ob*/*ob* mice [14]. In humans, white adipose tissue levels of *PNPLA3* mRNA are increased in obesity [16], and Kotronen et al. [12] also found increased levels in the liver of obese individuals.

In summary, these observations suggest that, although obesity remains a major determinant of liver fat accumulation, genetic factors almost certainly influence susceptibility to accumulate fat in the liver. Like many recent genome-wide association studies, they also highlight the power of this 'hypothesis-free' approach for the generation of novel hypotheses about complex disease states. The work of Romeo et al. [11] and Kotronen et al. [12] raises a number of intriguing questions related to the biological activity of wild-type and mutant adiponutrin in white

adipose tissue and liver. A particularly interesting and surprising finding was the apparent lack of an association of the *PNPLA3* SNP rs738409 with hepatic insulin sensitivity, despite its convincing associations with liver fat content and liver enzyme levels (inflammation). The correlation coefficient generally reported for liver fat and hepatic insulin sensitivity is similar to that reported in the paper by Kotronen et al. (r=−0.62), and yet both reports related to rs738409 suggest that it is not associated with insulin sensitivity. While the study by Kotronen et al. may not have had sufficient power to identify small effects, the results are consistent with those observed by Romeo et al. [11], who found no association between rs738409 and HOMA-IR despite more than 90% power. Is it possible that the *PNPLA3* variant increases liver triacylglycerol content whilst reducing diacylglycerol levels and/or the levels of other lipid species in the liver by accelerating transacylation of diacylglycerol to triacylglycerol or reducing triacylglycerol hydrolase activity? Answers to these and other related questions will no doubt shortly follow and should inform our understanding of the molecular pathogenesis of NAFLD and its metabolic sequelae.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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