Anstee QM, Liu YL, Day CP, Reeves HL. Reply to: HCC and liver disease risk in homozygous *PNPLA3* p.I148M carriers approach monogenic inheritance. *Journal of Hepatology* 2015, 62(4), 982-983.

Reply to: HCC and liver disease risk in homozygous *PNPLA3* p.I148M carriers approach monogenic inheritance

To the Editor:

We read with interest the thought-provoking letter from Krawczyk *et al.* [1] in response to our recent publication in the *Journal of Hepatology* describing the strong genetic association between the *PNPLA3* rs738409 (C>G, p.I148M) variant and the development of NAFLD-associated hepatocellular carcinoma (HCC) [2]. We agree that the observed magnitude of effect is striking: comparison of genotype frequencies between a NAFLD-HCC patient cohort and an unselected UK general population sample (the MRC/Wellcome Trust UK 1958 Birth Cohort) demonstrated a 12-fold increased risk of HCC [OR 12.19; 95% CI 6.89–21.58; p <0.0001] for rs738409 minor (G) allele homozygotes relative to C-allele homozygotes [2]. Krawczyk *et al.* compare the effect of *PNPLA3* to the monogenic condition, hereditary haemochromatosis due to *HFE* rs1800562 (A>G, p.C282Y). As their detailed comparison demonstrates, carriage of either variant confers a similar risk of HCC [1].

Although statistical techniques to assess proportion of variability explained by genetic variations are imperfect [3], our data indicates a Population Attributable Risk (PAR) of *PNPLA3* rs738409 for HCC of 55% and an AUROC of 0.68 attributable to this variant [2]. Further supporting the independent role of *PNPLA3* rs738409 as a risk factor for HCC, it is telling that other genetic variants that are strong modifiers of NAFLD severity and fibrosis progression such as *TM6SF2* [4,5], do not appear to confer an additional, independent increased risk of HCC [4]. We agree with Krawczyk *et al.* that *PNPLA3* is an important genetic risk factor for liver disease. For the moment, we would however caution against a move towards considering "*PNPLA3*-associated NAFLD", or by extension "*PNPLA3*-associated HCC", as distinct, monogenic conditions analogous to *HFE* in haemochromatosis but rather suggest that *PNPLA3* should be considered a strong modifier within a complex, polygenic disease trait that is subject both to genetic and substantial environmental influence (e.g. due to dietary factors and the intestinal microbiota [6]).

Given the mounting evidence of an association between *PNPLA3* and HCC [7,8], it is timely to consider the clinical utility of *PNPLA3* genotyping to assist in patient risk stratification. It has been suggested that, assuming a sensitivity of 80%, an odds ratio of >50 is required to control false-positive rates to an acceptable level of <10% [9]. A re-analysis of the data from Liu *et al.* [2] to assess sensitivity/specificity is shown in Table 1. Based on these data, the use of *PNPLA3* genotyping alone to *positively* predict risk of HCC is unlikely to be tenable. However, even in this HCC-enriched dataset, the *negative predictive value* was substantially greater, both within the NAFLD *vs.* NAFLD-HCC comparison and the NAFLD-HCC *vs.* unselected background population comparison, and so it may be that knowledge of *PNPLA3* rs738409 genotype has utility to select out those individuals least likely to develop HCC and therefore least likely to benefit from surveillance. As Krawczyk *et al.* suggest, studies addressing the interaction between *PNPLA3* and *HFE* in determining HCC risk would be of great interest. Given the increasing prevalence of NAFLD-HCC and consequent clinical need for improved risk-stratification care pathways [10], we would also suggest that large, prospective studies are needed urgently to validate our findings and to determine the utility and health-economic merits of a multi-factorial risk stratification that incorporates *PNPLA3* rs738409 genotype along with other recognised risk factors for HCC.

Conflict of interest

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

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Table 1. Reanalysis of the data from Liu *et al.* [2] focusing on clinical utility of *PNPLA3* genotype testing in HCC risk prediction.

Cohorts (n)	Genotype comparison	Sensitivity	Specificity	PPV*	NPV*
NAFLD-HCC (100) *vs.* NAFLD cohort (275)	GG *vs.* CC	51%	79%	47%	82%
	CG/GG *vs.* CC	72%	45%	32%	82%
NAFLD-HCC (100) *vs.* background UK Pop^{n} (1476)	GG *vs.* CC	51%	92%	28%	97%
	CG/GG *vs.* CC	72%	59%	10%	97%

CI, confidence interval; HCC, hepatocellular carcinoma; NASH, non-alcoholic steatohepatitis; N, number; OR, odds ratio; p, protein. Risk allele frequencies according to http://www.ncbi.nlm.nih.gov/snp/.

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Chronic kidney disease (CKD) and NAFLD: Time for awareness and screening

To the Editor:

We read with interest the article by Allen *et al.* reporting on the high prevalence and associated mortality of chronic kidney disease (CKD), in patients who had undergone liver transplantation [1].

We believe a thorough interpretation of these findings, which are consistent with recent liver transplant literature, should take into account the evolving aetiological spectrum of liver disease in both patients candidate for liver transplantation as well as in those with milder stage disease [2]: in either case, non-alcoholic steatohepatitis (NASH) is as an emerging risk factor for renal dysfunction, as compared with other aetiologies of cirrhosis. An analysis of the United Network Organ Sharing (UNOS) database, during the years 2002–2011, shows NASH-related cirrhosis as an increasing indication for simultaneous liver-kidney transplantation (SLKT) [3]. A concerning observation was the reduced 5-year liver graft, kidney graft, and patient survival in NASH patients in comparison to transplantation for other causes of cirrhosis (HR of 2.50 (95% CI 1.10–5.80), 2.30 (1.10–5.10), and 2.20 (1.02–5.79) respectively), for loss of liver transplant, loss of kidney graft, and death. These findings are consistent with European data suggesting NASH as a risk factor for severe renal impairment after liver transplantation; Houlihan *et al.* [4] reported more rapid loss of renal function in patients receiving a liver transplant for NASH-related cirrhosis in comparison to other aetiologies of cirrhosis (OR for incident CKD in NASH *vs.* controls of 2.43 (95% CI 1.05–5.63)) 2 years after liver transplantation, independent of BMI, diabetes, hypertension, hepatocellular carcinoma, and tacrolimus levels. Remarkably, 35% of patients transplanted for NASH-related cirrhosis progressed to stage 3b-4 CKD within 2 years after liver transplantation, compared to 10% of patients transplanted for other aetiologies of cirrhosis.

The evidence connecting NAFLD to CKD is also emerging in the general population: we recently subjected studies, assessing the association of NAFLD with CKD, to meta-analysis [5], which included 33 studies (63,902 participants, 16 population-based and 17 hospital-based, 20 cross-sectional and 13 longitudinal). For 20 studies, individual participant data were obtained and NAFLD was defined by liver histology in 13 studies (2205 participants). NAFLD was associated with an increased prevalence (OR 2.12, 95% CI 1.69–2.66) and incidence (HR 1.79, 95% CI 1.65–1.95) of CKD. In non-cirrhotic biopsy-proven NAFLD patients, NASH was associated with a higher prevalence (OR 2.53, 95% CI 1.58–4.05) and incidence (HR 2.12, 95% CI 1.42–3.17) of CKD than simple steatosis. Similarly, NAFLD with advanced fibrosis was associated with a higher CKD prevalence (OR 5.20, 95% CI 3.14–8.61) and incidence (HR 3.29, 95% CI 2.30–4.71) than NAFLD with milder fibrosis stages. Remarkably, the severity of NAFLD was positively associated with CKD stages, with NASH and advanced fibrosis conferring a greater risk of developing stage 5 CKD (renal failure) than simple steatosis and milder fibrosis stages, respectively.

In all analyses, the magnitude and direction of effects remained unaffected by diabetes status (present *vs.* absent) and after adjustment for other traditional risk factors for CKD (including age, obesity, metabolic syndrome components, smoking, hypertension, race).

The prevalence of CKD, as well as NAFLD, is continuously rising in concert with the epidemic of its risk factors, including diabetes, obesity and metabolic syndrome. Although early recognition and treatment of CKD have been shown to reduce the staggering cost of CKD and related hospitalizations [6], CKD often goes unrecognized, and in the Third National Health and Nutrition Survey (NHANES III), among all individuals with moderately decreased GFR (stage 3 CKD), the awareness approached 8.2% [7].

Since current therapeutic options to reverse CKD in liver transplant recipients are limited, the findings discussed above emphasize the need for practicing hepatologists to recognize early CKD in chronic liver disease patients and in NAFLD in particular, to optimize the management of these patients by delaying renal disease progression. From a therapeutical stand-point, promising preliminary evidence from randomized trials suggest several available pharmacological classes provide incremental benefits on renal disease progression over others, with statins [8] and angiotensin receptor blockers slowing renal function decline and ameliorating proteinuria [9], in addition to the