the single nucleotide exchange −16934T>A is associated with differences in TLR2 functionality. Of note, a search for possible transcription factor binding sites with TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) did not provide evidence that the −16934T>A mutation itself leads to differences in *TLR2* gene expression. In contrast, the number of GT repeats in intron 2 of the *TLR2* gene has been shown to be linked with differential cytokine responses [1]. Thus, the difference in −16934A>T allele frequencies between patients with and without SBP is more likely to reflect association with the numbers of *TLR2* GT repeats rather than an effect of the SNP itself. Since Bruns *et al.* did not report on the *TLR2* GT repeat polymorphism, we do not know if a similar association also exists in their patients' sample. In particular, alleles with long GT repeats (>20) – the risk factor for SBP in our study – are found more often in association with both the homozygous −16934T/T and the heterozygous −16934A/T genotypes. Interestingly, the distribution of alleles shown by Bruns and co-workers indicates SBP to have occurred most often among heterozygous carriers of the −16934A>T polymorphism. This particular feature and the overall small number of observed SBP episodes (25/119 = 21%) may have resulted in the fact that 16934T/T homozygosity was not confirmed by the work of Bruns and co-workers.

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.

Reference

[1] Veltkamp M, Wijnen PA, van Moorsel CH, Rijkers GT, Ruven HJ, Heron M, et al. Linkage between Toll-like receptor (TLR) 2 promotor and intron polymorphisms: functional effects and relevance to sarcoidosis. Clin Exp Immunol 2007;149:453–462.

Hans Dieter Nischalke*
Department of Medicine I,
University Hospital Bonn,
University of Bonn,
Sigmund Freud Str. 25, 53127 Bonn,
Germany
**Tel.: +49 228 287 51416; fax: +49 228 287 51419.*
E-mail address: nischalke@ukb.uni-bonn.de

Frank Grünhage
Department of Internal Medicine II,
Saarland University Hospital,
Homburg,
Germany

Ulrich Spengler
Department of Internal Medicine I,
University Hospital Bonn,
Bonn,
Germany

Beate Appenrodt
Department of Internal Medicine I,
University Hospital Bonn,
Bonn,
Germany
Department of Internal Medicine II,
Saarland University Hospital,
Homburg,
Germany

Interaction between *IL28B* and *PNPLA3* genotypes in the pathogenesis of steatosis in chronic hepatitis C non genotype-3 patients

To the Editor:

Steatosis is frequently observed in patients with chronic hepatitis C (CHC), and is associated with fibrosis progression and treatment failure [1,2]. Tillmann *et al.* recently reported a negative association between the interleukin 28B (*IL28B*) rs12979860 CC genotype, predicting sustained virological response [3], and steatosis in genotype-1 CHC [4]. However, whether this association was independent of other genetic factors was not evaluated.

The patatin-like phospholipase domain-containing 3 (*PNPLA3*) rs738409 polymorphism is a strong determinant of hepatic fat accumulation and steatohepatitis [5,6], but also influences steatosis and fibrosis progression in CHC [7–9]. A previous study [7] also reported an association between *IL28B* rs12980275 genotype and steatosis in CHC non genotype-3 patients, but the rs12979860 *IL28B* polymorphism was not tested and the interaction with *PNPLA3* genotype was not analyzed in details.

We now confirm the negative association of rs12979860 CC with histologically-determined steatosis (111/184, 60% *vs.* 266/383, 69%; $p = 0.036$) and steatosis grade >1 (17/184, 9% *vs.* 70/383 18%; $p = 0.0059$) in 567 naïve, consecutive, non genotype-3 patients from referral centers in Milan and Vienna, without excessive alcohol intake (>i.e. 60/40 g/day for M/F), HBsAg positivity, and HIV infection. Sixty-five percent of patients were genotype-1, and 20% had cirrhosis. A similar trend was observed for all genotypes. Importantly, as shown in Table 1, the association between *IL28B* genotype and steatosis was independent of acquired risk factors, and of the *PNPLA3* GG genotype. Interestingly however, the rs12979860 CC genotype protected form steatosis in patients positive, but not in those negative for the *PNPLA3* G variant at risk (49/78, 63% *vs.* 144/187, 77%, $p = 0.022$ and 62/106, 58% *vs.* 74/196, 62%, $p = 0.52$, respectively). Therefore, data suggest the possibility that an interaction occurs between *IL28B* and *PNPLA3* genotypes in the pathogenesis of steatosis in CHC non genotype-3 patients.

Table 1. Independent predictors of steatosis in 567 non genotype-3 CHC patients.

	OR	95% CI	*p*
Age (yr)	1.04	1.02-1.05	<0.0001
BMI (kg/m^2)	1.20	1.13-1.28	<0.0001
PNPLA3 rs738409 GG	3.36	1.50-8.70	0.0025
IL28B rs12979860 CC	0.57	0.38-0.85	0.0060

Evaluation of the combined effect of *IL28B* and *PNPLA3* genotypes on treatment outcomes may permit to discriminate whether steatosis has indeed a causal role in determining resistance to antivirals, or the association is an epiphenomenon due to the confounding association of steatosis with *IL28B* rs12979860 CC, as suggested by Tillmann *et al.* [4].

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.

References

[1] Leandro G, Mangia A, Hui J, Fabris P, Rubbia-Brandt L, Colloredo G, et al. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. Gastroenterology 2006;130:1636–1642.

[2] Marabita F, Aghemo A, De Nicola S, Rumi MG, Cheroni C, Scavelli R, et al. Genetic variation in the interleukin-28B gene is not associated with fibrosis progression in patients with chronic hepatitis C and known date of infection. Hepatology. 2011;54:1127–1134.

[3] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.

[4] Tillmann HL, Patel K, Muir AJ, Guy CD, Li JH, Lao XQ, et al. Beneficial IL28B genotype associated with lower frequency of hepatic steatosis in patients with chronic hepatitis C. J Hepatol 2011;55:1195–1200.

[5] Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461–1465.

[6] Valenti L, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, et al. Homozygosity for the PNPLA3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1209–1217.

[7] Cai T, Dufour JF, Muellhaupt B, Gerlach T, Heim M, Moradpour D, et al. Viral genotype-specific role of PNPLA3, PPARG, MTTP and IL28B in hepatitis C virus-associated steatosis. J Hepatol 2011;55:529–535.

[8] Valenti L, Rumi M, Galmozzi E, Aghemo A, Del Menico B, De Nicola S, et al. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. Hepatology 2011;53:791–799.

[9] Trepo E, Pradat P, Potthoff A, Momozawa Y, Quertinmont E, Gustot T, et al. Impact of PNPLA3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. Hepatology 2011;54:60–69.

Luca Valenti*
Centro Malattie Metaboliche del Fegato,
Department of Internal Medicine,
Università degli Studi Fondazione Ca' Granda IRCCS,
Ospedale Maggiore Policlinico,
Padiglione Granelli, Via F Sforza 35, 20122 Milano,
Italy
**Tel.: +39 0255033301; fax: +39 0250320296*
E-mail address: luca.valenti@unimi.it

Alessio Aghemo
A.M. Migliavacca Center for Liver Disease,
First Division of Gastroenterology,
Università degli Studi,
Fondazione IRCCS Ospedale Maggiore Policlinico "Ca' Granda" IRCCS,
Milan,
Italy

Albert Friedrich Stättermayer
Internal Medicine,
Department of Gastroenterology and Hepatology,
University of Vienna,
Vienna,
Austria

Reply to: "Interaction between *IL28B* and *PNPLA3* genotypes in the pathogenesis of steatosis in chronic hepatitis C non genotype-3 patients"

To the Editor:

We thank Dr. Valenti *et al.* for raising an additional point that *PNPLA3* might be associated with steatosis in the setting of HCV infection after controlling for *IL28B* [1]. They demonstrate in their cohort of 567 treatment naïve patients that both *IL28B* (rs12979860) and *PNPLA3* (rs738409) may contribute to steatosis. Furthermore, in their study the beneficial effect of *IL28B* was limited to the patients without the *PNPLA3* risk allele "G" as rs738409. A role of *PNPLA3* GG genotype for steatosis in HCV infected patients has been observed in several studies, especially when excluding genotype 3 patients [2–5]. In the Duke cohort, we did not find a significant difference for steatosis in relation to *PNPLA3*, (25/52 *vs.* 67/127, $p = 0.623$). However, in the European cohort, the rs738409 SNP was used instead of SNP rs2281135. The SNP rs2281135 is considered a tagging SNP (rs2281135) for the likely causal SNP (rs738409) within the *PNPLA3* region. Thus, as we had a tagging SNP (rs2281135) for the likely causal SNP (rs738409) within the *PNPLA3* region only for the Duke cohort, we cannot rule out that the lack of a significant difference is due to the lack of accuracy of a surrogate SNP. Interestingly though, when grouped according to *PNPLA3* rs2281135 GG *vs.* non-GG, the role of IL28B remained significant in the *PNPLA3* rs2281135 GG patients only ($p = 0.002$), while it was only a trend in the non-GG patients ($p = 0.46$).