# Beneficial *IL28B* genotype associated with lower frequency of hepatic steatosis in patients with chronic hepatitis C

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## Abstract

**BACKGROUND**—IL28B polymorphisms have been associated with both treatment induced and spontaneous clearance of hepatitis C virus (HCV). We previously found that LDL cholesterol levels were higher in chronic hepatitis C (CHC) patients with the CC genotype at the rs12979860 polymorphism, located proximal to the *IL28* gene. Here we analyzed the association of steatosis with *IL28B* genotype in treatment naïve patients with CHC.

**METHODS**—Two independent cohorts of 145 genotype 1 infected patients from an antifibrotic study and 180 genotype 1 patients from Duke were analyzed for presence and severity of steatosis in relation to the rs12979860 polymorphism at the *IL28B* locus. TaqMan assay based genotyping classified three groups CC, CT and TT.

**RESULTS**—CC genotype was associated with a lower prevalence of steatosis. In the antifibrotic study steatosis was found in 47.6% (50/105) of *IL28B* non-CC versus 22.5% (9/40; p=0.008) in CC patients. Similarly, steatosis was found in 67.4% (89/132) of non-CC patients compared to only 39.6% (19/48; p=0.001) of CC patients in the Duke cohort.

**CONCLUSIONS**—*IL28B* CC genotype is associated with less pronounced disturbances of lipid metabolism, as reflected both in serum lipoprotein levels and hepatic steatosis, in HCV infection.

## Keywords

chronic hepatitis C; genetic; lipids; IL28B; steatosis

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In a genome wide association study, SNPs in the *IL28B* region were found to be associated with treatment response to pegylated interferon and ribavirin in patients with hepatitis C virus (HCV infection [1], a finding which has subsequently been confirmed in multiple independent studies [2]. The SNP rs12979860 showed the strongest correlation with

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treatment response, and codes for either a T or a C leading to 3 different genotypes TT, CT, or CC. The CC genotype was the beneficial genotype associated with sustained response to peg-interferon plus ribavirin in hepatitis C.

The association between HCV infection and a reduction of lipid levels is well recognized. We previously studied the association of *IL28B* with different serum lipids, and found serum triglycerides to be lower and serum LDL cholesterol to be higher in IL28B CC patients [3], The effect on lipid levels in relation to *IL28B* genotype appeared to be stronger for HCV genotype 1 infection than for genotype 2 or 3 [3].

Here, we studied *IL28B*'s genotype in association with hepatic steatosis, another hallmark of CHC infection. Given the less pronounced effect on lowering cholesterol in *IL28B* CC compared to non CC genotypes, one might expect steatosis due to HCV infection to be lower in *IL28B* CC genotype patients compared to *IL28B* non-CC genotype patients with CHC. More importantly for steatosis, the finding of lower triglycerides in *IL28B* CC genotype patients with HCV, likewise argues for lower steatosis rates in these patients.

## METHODS

### Patient population

Patients for the current study were taken from two sources. One cohort of patients was from a recently completed multicenter Phase II clinical trial to assess the effectiveness of farglitazar, a PPAR γ agonist, as an antifibrotic agent among HCV-genotype 1 CHC patients who were non-responsive to prior interferon-based therapy [4]. Only 145 of the original 265 patients could be used for this study based on consent for IL28B genotyping (see table 1a), these patients did not show obvious differences to the original cohort [4].

The second cohort was from the Duke Hepatology Clinical Research Database and Repository [5]. In the Duke cohort, liver biopsies were from a time-point prior to initiation of any Peg-Interferon-therapy and for this analysis only lipid levels determined in a sample prior to initiation of any interferon therapy were considered. Characteristics of lipid determination and characterization of viral status and genotype has been described earlier [3]. Only genotype 1 patients with a biopsy prior to treatment initiation and with available steatosis scoring were included (see table 1b).

Samples were collected under fasting within the antifibrotic study, while those from the Duke cohort were collected under non-fasting conditions.

We excluded patients co-infected with hepatitis B virus or HIV-1; and patients with poor quality DNA, defined as >10% failed genotypes in prior test-genotyping across a minimum of 10 single nucleotide polymorphisms (SNPs).

Hepatic steatosis was classified according to the Brunt classification: Absent or Grade 0 (<5% of hepatocytes affected), grade 1 (5-33% of hepatocytes affected), grade 2 (33-66% of hepatocytes affected); and grade 3 (>66% of hepatocytes affected) [6]. This study, the database and repository were approved by the Duke University Institutional Review Board. All patients provided written informed consent and the study was conducted in accordance with provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

### *IL28B* Genotyping

The genomic region associated with HCV response in Ge *et al.*[1] contains several highly correlated SNPs around the *IL28B* gene. We selected the most strongly associated SNP, rs12979860, located upstream of this gene for genotyping in our cohort using the 5' nuclease

assay with allele specific TaqMan probes [7]. Based on this TaqMan assay, there are three possible results referred to as genotypes at this locus, and thus each sample was defined as CC, CT or TT ("*IL-28B*-type"). Genotyping was performed in the Duke Institute for Genome Sciences and Policy Genotyping Core. Genotyping calls were manually inspected and verified prior to release. Hardy-Weinberg Equilibrium was assessed in Caucasians and African Americans separately.

164 patients of the treatment naïve Duke cohort with genotype 1 HCV infection were also genotyped for IL18B rs8099917, where genotype TT was described as beneficial compared to GT or GG [2].

## Statistical analysis

SPSS statistical software, Version 12.1 (SPSS Inc. Chicago, Illinois, USA) was used. Chi-square testing or Fisher's exact test when appropriate was applied for testing the association of *IL28B* genotype with steatosis. T-test was used for testing difference in lipid levels in relation to steatosis and *IL28B*. Logistic regression analysis was performed to evaluate *IL28B* genotypes role for presence of steatosis when controlled for covariates. *IL28B* genotypes were coded to test a recessive model, comparing patients homozygous (CC) to those with one or no copies of the C allele (CT/TT).

We perfomed an exploratory analysis in the antifibrotic cohort and an independent confirmatory analyzes in the Duke Cohort. We also describe the data after controlling for gender, lipid levels, BMI, HCV viral load, race, and HCV genotype.

Finally, an exploratory assessment of the effect of steatosis and pre-treatment lipids compared to rs12979860 genotype as a predictor of response to PEG-IFN/RBV treatment was done using a multivariable logistic regression model controlling for age, gender, race, and fibrosis.

# RESULTS

## Association of *IL28B* genotype with Steatosis in the Antifibrotic Study

One hundred-forty-five patients (of 265 all HCV genotype-1 patients in the total antifibrotic study) were available for genotyping (121 were Caucasians, 12 African American and 12 various ethnic groups). Steatosis was significantly more frequent in *IL28B* non-CC vs. CC patients (47.6% [50/105] vs. 22.5% [9/40]; p=0.008), with no difference between *IL28B* CT and *IL28B* TT patients (48.1% [37/77] vs. 46.4% [13/28]). As there was no difference between *IL28B* CT and *IL28B* TT patients, we limited the analysis to IL28B CC versus non-CC patients. Severity of steatosis showed similar trend (p=0.02; see figure 1). Similar results were obtained if analysis was limited to Caucasian patients only (22.2% [8/36] vs. 47.1% [40/85], p=0.014).

In comparing patients with hepatic steatosis to those without, univariate analysis showed that patients with steatosis were less likely to carry the *IL28B* CC genotype (15.5% versus 36%; see table 1), had higher BMI (29.5 to 27.5), were younger (50.6 years versus 52.6 years) and had higher triglyceride levels (110.9 mg/dl versus 95.0mg/dl; p=0.012; see table 1).

When all four statistically significant parameters from the univariate analysis (age, BMI, *IL28B* CC vs. non-CC and triglycerides) were analyzed in a logistic regression model, all except age remained independently associated with steatosis, where BMI and triglycerides were positively associated with steatosis while *IL28B* CC genotype was associated with less steatosis (see table 2a).

## Association of *IL28B* genotype with Steatosis in the Duke Database study

**Genotype 1—**As all patients from the antifibrotic study were HCV genotype 1, we similarly only analyzed the HCV genotype 1 infected patients within the Duke cohort. One-hundred and eighty treatment naïve patients with *IL28B* genotype from the Duke Database with genotype 1 HCV infection had information on steatosis in their liver biopsy prior to initiation of any antiviral therapy.

Steatosis was more frequent in the patients from the Duke cohort (60.0% [108/180; CI: 52.7 – 66.9%] versus 40.7% [59/145; CI: 33.0 – 48.8%] in the antifibrotic study cohort; p=0.001). Similar to the antifibrotic study cohort, the Duke cohort showed a significant difference between CC and non-CC patients (39.6% [19/48] vs. 67.4% [89/132]; p=0.001) in relation to presence of steatosis. In contrast but consistent with the antifibrotic cohort, no difference between CT patients and TT patients was seen (68.4%.[67/98] vs 64.7% [22/34]; n.s.,). Limiting the analysis to only the 130 Caucasian patients, gave similar results (36.6% [15/41] vs. 62.5% [58/89], p=0.004 or p=0.002 one sided), and no significant difference between CT and TT patients (66.28% [47/71] vs. 61.1% [11/18] n.s.).

In the Duke cohort, cholesterol levels were higher in the CC genotype patients with biopsy results on steatosis available than in the non-CC genotype patients (though not statistically, supplemental table 2b) similar to the combined cohort reported earlier [3], and as in the antifibrotic study cohort alone (see supplemental table 2a).

Duke patients with steatosis differed from those without steatosis in several parameters (see table 2b), of which only *IL28B* and BMI were concordant with the findings from the antifibrotic study (see table 2a). Age showed an opposite direction compared to the antifibrotic cohort, and triglyceride levels were not significantly different in the Duke cohort, most likely due to the non-fasting state of patients within this cohort. Apo A1 was significantly different between patients with steatosis in the Duke cohort only.

One-hundred and thirty seven patients had data on all 5 parameters. When these five parameters were entered into a binary logistic regression model, only BMI, fibrosis and *IL28B* were significantly associated with steatosis. *IL28B* was associated with less steatosis while BMI and fibrosis both were associated with steatosis. When Apolipoprotein B and age were excluded as not significant parameters, only *IL28B* (p=0.026) and fibrosis (p=0.001) remained significant (see table 3b)

## IL28B rs8099917 in relation to steatosis and its role on steatosis in comparison with 12979860

164 patients for the treatment naïve Duke cohort with genotype 1 were analyzed for rs8099917, where 97 patients had genotype TT, 59 had GT and 8 and GG. Steatosis was present in 45.4%, 32% and 40%, respectively (p=0.2). Likewise in Binary logistic regression, rs12979860 was relevant while rs8099917's p=value was 0.137 in univariate analysis but 0.851 after inclusion of rs12979860, which was significant in univariate and multivariate analysis.

## IL28B, steatosis and treatment response

54 genotype 1 patients underwent therapy with pegylated interferon and ribavirin. Rs12979860 CC genotype was associated with significantly increased odds ratio of response. Presence of steatosis showed a trend but was not significantly associated with failure to achieve SVR, The role of steatosis showed a stronger trend towards significance when IL28B genotype was included. Therefore the association of steatosis with reduced

chance of SVR to pegylated interferon and ribavirin is not explained by *IL28B* genotype's association with lower frequency of steatosis (see table 4).

When limiting the analysis to Caucasian patients only, rs8099917 was significantly associated with steatosis as 39 of 60 (65%) non-TT patients had steatosis compared to 30 of 64 (46.9%) TT patients (p=0.048). However, when rs8099917 TT vs. non-TT and rs12979860 CC vs. non-CC were evaluated in a multivirate model, rs8099917 became irrelevant. However, all 46 rs12979860 CC patients tested for rs8099917 were also rs8099917 TT, while the remaining non-CC patients were either rs8099917 TT or non-TT.

## DISCUSSION

When comparing *IL28B* rs12979860 and rs8099917 within the initial GWAS by Ge et al [1] rs8099917 was among the top three SNPs and even the top SNP within the Caucasians in relation to treatment response. In this study we found no significant association of rs8099917 with steatosis while we found a significant association with rs12979860. This would fit with two paper by the group of Sarrazin et al. who found a stronger role for rs2979860 vs. rs8099917 in a genotype 2/3 cohort [8], and in a genotype 1 cohort [9]. However, In Japan, no difference between rs8099917 and rs12979860 was reported [10]. Interestingly, in the current study of dominantly Caucasian patients, no significant association of rs8099917 with steatosis was observed, but an association with rs12979860.

We earlier demonstrated significant higher LDL-cholesterol and apolipoprotein B levels but lower triglyceride levels in CC patients with HCV compared to non-CC patients [3]. Hepatic steatosis is another frequent disturbance of lipid metabolism in patients with hepatitis C [11,12]. In the present study, we demonstrate that the presence of steatosis was lower in CC compared to non-CC patients in two independent HCV genotype 1 patient cohorts and a trend in a genotype 2/3 cohort. Though the relative small cohorts are a limitation, but in both cohorts the difference is significant, making a finding by chance unlikely. Furthermore, in 89 patients, who had received some form of therapy or where information regarding therapy was not available within the database showed similar results, with p-value of 0.03 for lower steatosis in CC vs. non-CC patients (data not shown). Importantly, this finding was independent from the lipid changes and provides further evidence that *IL28B* CC genotype is associated with less pronounced disturbances of lipid metabolism, as reflected both in serum lipoprotein levels and hepatic steatosis, in hepatitis C infection. Furthermore, these changes in lipid metabolism would be consistent with lower intrinsic interferon activity in *IL28B* CC patients, and explain the better response to treatment, as the difference of interferon activity from before to on treatment would be greater in the *IL28B* CC patients.

The higher serum lipid levels but lower steatosis in "*IL28B*" CC patients could be explained by more efficient export of lipids from hepatocytes in the setting of the beneficial CC genotype. But it furthermore, would fit with the hypothesis of higher intrahepatic interferon levels in presence on non-CC genotype. As discussed earlier [3], higher interferon expression is likely to lead to suppression of lipoprotein lipase, which would result in lower conversion of VLDL to LDL, and subsequent higher steatosis. Exogenous interferons have been shown to lower LDL cholesterol, and raise triglyceride in VLDL, concomitant with suppression of lipoprotein lipase in patients with known HCV infection [13,14,15], but also in individuals without known history of HCV [16].

Cholesterol synthesis and HCV-viral production, both, have been associated with microRNA-122 (miR-122) levels in the liver [17,18]. However, one would expect increased not decreased steatosis with elevated lipid synthesis especially in the context of miR-122 [19]. However, it is known that HCV-infection itself leads to decreases in serum cholesterol,

still HCV infection is associated with steatosis. Thus, we hypothesize that HCV-infection might actually engage miR-122 for its life cycle and thereby decrease miR-122 availability for lipid production. We have, however, no samples available to prove this hypothesis that miR-122 is differently regulated, and thus cannot address this subject in our population. Though conflicting data have been presented, some studies have found steatosis to be associated with failure to achieve sustained viral response [20,21,22]. Our study was too small to reliably evaluate the independent role of steatosis. However, it appears that earlier reports on failure to achieve sustained virological response in the presence of steatosis might be partially attributable to non-CC genotype of *IL28B*.

In conclusion, *IL28B* CC is associated with absence of steatosis, which was independent from serum lipids.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## List of Abbreviations

| | |
|---|---|
| **LDL** | low density lipoprotein |
| **HCV** | hepatitis C virus |
| **VLDL** | very low density lipoprotein |
| **HIV** | human immunodeficiency virus |
| **PEG-IFN** | pegylated interferon |
| **RBV** | ribavirin |

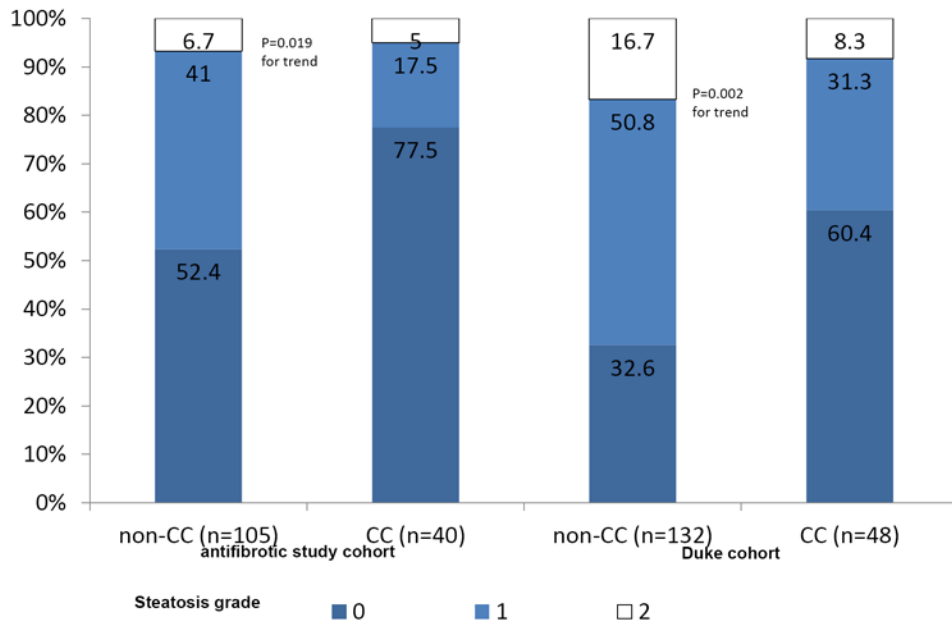| **SVR** | sustained virologic response |
| **SNP** | single nucleotide polymorphism |
| **Apo** | apolipoprotein |

**Figure 1. Severity of Steatosis in relation to IL28B genotype (rs12979860)**

**Table 1**

Baseline characteristics for patients from the antifibritic study (A) and the Duke cohort (B)

| A | This cohort (n=145) | Original Farglitzar Study (n=265) | | |
|---|---|---|---|---|
| | | Original Placebo (n=88) | Original Farglitzar 0.5mg (n=89) | Original Farglitzar 0.5mg (n=88) |
| Age | 51.8 (± 6.8) | 52.1 (± 5.6) | 51.8 (± 7.3) | 51.4 (± 5.8) |
| Gende,r male sex | 90 (62.1%) | 53 (60%) | 53 (60%) | 59 (67%) |
| BMI | 28.3 (± 4.6) | 29.7 (± 5.5) | 27.9 (± 4.2) | 29.2 (± 6.0) |
| Race W, B, A, o | | | | |
| White | 122 (84.1%) | 63 (72%) | 67 (76%) | 68 (77%) |
| Black | 12 (8.3%) | 12 (14%) | 14 (16%) | 9 (10%) |
| Asian | 4 (2.8) | 11 (13%) | 6 (7%) | 7 (8%) |
| other | 7 (4.8%) | 2 (2%) | 2 (2%) | 4 (2%) |
| Fibrosis score (Ishak) | | | | |
| 2 | 50 (34.5%) | 35 (41%) | 31 (35%) | 32 (36%) [*] |
| 3 | 69 (47.6%) | 37 (43%) | 42 (48%) | 41 (47%) |
| 4 | 26 (19.9%) | 14 (16%) | 14 (17%) | 14 (16%) [**] |
| IL28B | | | | |
| CC | 40 (27.6%) | | | |
| CT | 77 (53.1%) | | | |
| TT | 28 (19.3%) | | | |

| B | This "Duke cohort"(n=145) | Larger "Duke lipids cohort" (n=634)[4] |
|---|---|---|
| Age | 50.3 (±6.8) | |
| Gende,r male sex | 125 (69.4%) | 62% |
| BMI | 29.3 (±5.5) | Unknown |
| Race W, B, A, o | | |
| White | 130 (72.2%) | 71% |
| Black | 45 (25%) | 29% |
| other | 5 (2.8%) | |
| Fibrosis score (MetavirIshak) | | |
| 0-1 | 54 (30%) | 2% |
| 2 | 61 (33.9%) | 39% |
| 3-4 | 65 (26.1%) | 33% |
| IL28B | | |
| CC | 48 (26.7%) | |
| CT | 98 (54.4%) | |
| TT | 34 (18.9%) | |

[*] Includes 2 patients withF1 fibrosis,

[**] included 3 patients with F5 fibrosis

**Table 2**

Comparison of patient with and without hepatic steatosis in antifibrotic cohort (A) and Duke Cohort (B)

| A | *n*/N | Steatosis n (%) | No steatosis n (%) | P value |
|---|---|---|---|---|
| Females | 59/86 | 22 (37.3%) | 33 (38.4%) | n.s. |
| Caucasians | 58/86 | 48 (82.8%) | 73 (84.9%) | n.s. |
| *IL28B* CC | 59/86 | 9 (15.3%) | 31 (36.0) | 0.008 |
| Age in years | 59/86 | 50.64 ± 6.8 | 52.62 ± 6.7 | 0.031 |
| Body Mass Index (BMI) | 59/86 | 29.5 ± 4.7 | 27.5 ± 4.3 | 0.01 |
| Metavir: Fibrosis | 59/86 | 1.90 ± 0.76 | 1.81 ± 0.70 | n.s. |
| LDL-C (mg/dL) | 53/73 | 89.9 ± 38.3 | 93.8 ± 25.8 | n.s. |
| Apo C3 (mg/dL) | 53/73 | 11.5 ± 3.4 | 10.5 ± 2.3 | n.s. |
| Apo B (mg/dL) | 53/73 | 73.3 ± 22.4 | 70.5 ± 15.8 | n.s. |
| Apo_A1_Ln | 53/73 | 139.8 ± 30.3 | 138.6 ± 27.1 | n.s. |
| Triglycerides (mg/dL) | 53/69 | 110.9 ± 45.5 | 95.0 ± 49.6 | 0.012 |
| B | *n*/N | Steatosis n (%) | No steatosis n (%N) | P value |
| Females | 66/107 | 32 (29.9%) | 20 (30.3%) | n.s. |
| Caucasians | 72/108 | 73 (67.6%) | 57 (79.2%) | n.s. |
| *IL28B* CC | 72/108 | 19 (17.6%) | 29 (40.3) | 0.001 |
| Age in years | 59/86 | 48.9 ± 6.8 | 46.1 ± 8.5 | 0.021 |
| Body Mass Index (BMI) | 55/98 | 30.3 ± 5.4 | 27.4 ± 5.3 | 0.002 |
| Metavir: Fibrosis | 71/108 | 2.50 ± 1.1 | 1.70 ± 1.1 | <0.001 |
| LDL-C (mg/dL) | 46/98 | 96.04 ± 30.1 | 105.7 ± 32.2 | n.s. 0.157 |
| HDL-C (mg/dL) | 51/94 | 45.2 ± 1.29 | 50.3 ± 15.8 | n.s. 0.061 |
| Apo C3 (mg/dL) | 47/98 | 9.0 ± 4.4 | 9.8 ± 4.0 | n.s. 0.066 |
| Apo B (mg/dL) | 47/98 | 76.1 ± 21.9 | 81.3 ± 25.8 | n.s. 0.453 |
| Apo_A1_Ln | 47/98 | 134.3 ± 26.1 | 143.2 ± 29.5 | 0.031 |
| Cholesterol (mg/dL) | 41/94 | 167.3 ± 37.4 | 179.1 ± 33.9 | n.s. 0.056 |
| Triglycerides (mg/dL) | 47/98 | 138.4 ± 70.6 | 135.1 ± 90.0 | n.s. |

n/N reflect with number of patients with steatosis (n) to without steatosis (N), not all data were available for all patients

**Table 3**

Binary logistic regression analysis concerning presence of steatosis

**A) Multivariate analysis concerning the presence of steatosis in antifibrotic therapy cohort**

| | unadjusted | | | adjusted | | | Adjusted excluding age | | |
|---|---|---|---|---|---|---|---|---|---|
| | p-value | Odds ratio | 95.0% C.I. | p-value | Odds ratio | 95.0% C.I. | p-value | Odds ratio | 95.0% C.I. |
| *IL28B* rs12979860 CC | 0.007 | 0.32 | 0.14 - 0.74 | 0.015 | 0.30 | 0.11 - 0.79 | 0.012 | 0.29 | 0.11 - 0.76 |
| BMI | 0.011 | 1.10 | 1.02 - 1.19 | 0.045 | 1.10 | 1.00 - 1.20 | 0.016 | 1.11 | 1.02 - 1.22 |
| Triglycerides | 0.043 | 2.48 | 1.03 - 5.94 | 0.022 | 3.22 | 1.18 - 8.76 | 0.036 | 2.78 | 1.07 - 7.22 |
| age | 0.092 | 0.96 | 0.91 – 1.01 | 0.17 | 0.97 | 0.90 – 1.02 | | | |

**B) Multivariate analysis concerning the presence of steatosis in Duke cohort**

| | unadjusted | | | adjusted | | | Adjusted [*] | | |
|---|---|---|---|---|---|---|---|---|---|
| | p-value | Odds ratio | 95% C.I | p-value | Odds ratio | 95% C.I | p-value | Odds ratio | 95% C.I |
| BMI | .004 | 1.12 | 1.04 - 1.22 | .047 | 1.096 | 1.00 - 1.20 | .069 | 1.09 | 0.99 - 1.19 |
| Apo_AI | .098 | 0.99 | 0.97 - 1.00 | .111 | 0.987 | 0.97 - 1.00 | | | |
| Fibrosis | <0.001 | 2.23 | 1.47 - 3.37 | .001 | 2.179 | 1.37 - 3.47 | .001 | 2.15 | 1.40 - 3.32 |
| Age | .397 | 1.023 | 0.97 - 1.08 | .790 | 0.992 | 0.93 - 1.05 | | | |
| *IL28B* rs12979860 CC | .08 | 0.33 | 0.15 - 0.75 | .041 | 0.383 | 0.15 - 0.96 | .026 | 0.38 | 0.15 - 0.95 |

When limited to the 99 Caucasian patients with data on triglycerides, only IL28B CC genotype was independently associated with steatosis (p=0.021).

[*] adjusted with the significant parameters (BMI, Fibrosis and CC) included only

**Table 4**

Treatment response in relation to steatosis and *IL28B* CC

| | unadjusted | | | Adjusted | | |
|---|---|---|---|---|---|---|
| | **p-value** | **Odds ratio** | **95% C.I** | **p-value** | **Odds ratio** | **95% C.I** |
| Steatosis | 0.126 | 0.40 | 0.07 - 1.40 | 0.056 | 0.10 | 0.01 - 1.06 |
| *IL28B* rs12979860 CC | <0.001 | 29.56 | 4.93 – 177.36 | 0.001 | 60.99 | 5.70 – 652.10 |