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CLINICAL STUDIES

YKL-40 genetic polymorphisms and the risk of liver disease progression in patients with advanced fibrosis due to chronic hepatitis C*

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Keywords
cirrhosis – decompensation – genetic polymorphisms – hepatitis C – interferon – virological response

Abbreviations
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHC, chronic hepatitis C; CTP, Child-Turcotte-Pugh; HALT-C, Hepatitis C Antiviral Long-Term Treatment against Cirrhosis Trial; HCC, hepatocellular carcinoma; *IL28B*, interleukin 28 B; INR, international normalized ratio; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; SVR, sustained virological response.

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Abstract

Background/Aims: The aim of this study was to explore the association of a functional YKL-40 promoter polymorphism (rs4950928) with baseline disease stage, response to antiviral therapy and risk of liver disease progression in a group of patients with chronic hepatitis C (CHC). *Methods:* YKL-40 promoter polymorphisms were determined in 456 Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial patients with bridging fibrosis or cirrhosis entering a prerandomization lead-in peginterferon/ribavirin 24-week treatment phase and in 462 patients followed for a mean of 3.8 years after randomization to maintenance peginterferon or observation. *Results:* Mean patient age was 49.5 years, 70.4% were men and 71.2% were Caucasian. The 17% frequency of the YKL-40 minor allele (T) was similar to that reported in the general population. YKL-40 genotype was associated significantly with baseline serum YKL-40 levels but was not associated with the likelihood of a virological response following 24–48 weeks of peginterferon/ribavirin therapy. Serum YKL-40 levels remained significantly lower during follow-up in the randomized TT homozygotes compared with CT heterozygotes and CC homozygotes ($P < 0.001$). Despite this association, YKL-40 genotype was not associated with the risk of clinical or histological liver disease progression. *Conclusions:* A reduced frequency of the protective YKL-40 promoter polymorphism was not observed in the HALT-C Trial patient population. The absence of an association between YKL-40 promoter polymorphisms and baseline liver disease severity as well as with the risk of liver disease progression over time suggests that this polymorphism is not associated with disease progression in CHC patients with established fibrosis.

YKL-40 is a fibroblast growth factor that contributes to the remodelling of inflamed tissues such as human liver and synovia via the degradation of low-density extracellular matrix (1–4). Secreted by activated macrophages, YKL-40 is believed to act as a chemoattractant for endothelial cells, can modulate angiogenesis during tissue repair and is expressed in multiple tissues including human liver (5, 6). The serum level of YKL-40 has been evaluated as a noninvasive marker of various chronic inflammatory and fibrotic liver diseases, including alcoholic liver disease and chronic hepatitis C (CHC) (1, 7, 8). The strong correlation between serum YKL-40 levels and hepatic mRNA levels in patients with CHC suggests that serum YKL-40 levels, in part, reflect ongoing hepatic fibrogenesis (1). In addition, among patients with CHC, serial measurement of serum YKL-40 levels can differentiate patients with slow from those with rapid progression of liver fibrosis (1). Genetic polymorphisms in the CHI3L1 gene located on chromosome

*The HALT-C Trial was registered with clinicaltrials.gov (#NCT00006164). This is publication #74 of the HALT-C Trial.

1q32.1 that encodes for YKL-40 were described recently (9, 10). In particular, a functional upstream promoter polymorphism of *CHI3L1* (rs4950928) has been associated with reduced serum levels of YKL-40 in multiple independent patient cohorts and with a reduced risk of asthma and bronchial hyperresponsiveness (10). In addition, the presence of this functional polymorphism in a large cohort of German patients with CHC was associated with a lower stage of liver fibrosis as well as lower serum YKL-40 levels (11).

The Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial was a prospective multicentre study of maintenance peginterferon in patients with CHC and advanced fibrosis who failed to respond to prior interferon-based treatment (12). During a 24-week lead-in phase, all patients were retreated with full-dose peginterferon alfa-2a and ribavirin (13). Patients with HCV RNA detectable at week 20 were categorized as nonresponders and were eligible for randomization, whereas week-20 virologic responders continued combination treatment for a full 48 weeks in the 'responder' arm of the trial (Fig. 1). Recently, in 513 HALT-C Trial patients participating in an ancillary study of serum fibrosis markers, we demonstrated that serum YKL-40 levels were associated strongly with the stage of hepatic fibrosis (14). In addition, lower baseline serum YKL-40 levels were associated independently with a higher likelihood of achieving a week-20 virological response during the lead-in phase (15). Furthermore, serum YKL-40 levels during treatment helped distinguish patients who went on to achieve a sustained virological response (SVR) from those who experienced virologic breakthrough or relapse. Finally, a multivariate model that included baseline serum YKL-40 levels was the best predictor of clinical outcomes in 462 patients followed for a median of 3.8 years in the randomized phase of the HALT-C Trial (16).

The aim of this study was to determine the relationship between YKL-40 promoter polymorphisms at rs4950928 and baseline serum YKL-40 levels in the HALT-C Trial patients who were enrolled in the serum fibrosis marker ancillary study. Anticipating that the YKL-40 promoter polymorphism T minor allele would be associated with lower serum YKL-40 levels, we also sought to determine whether this genetic polymorphism was associated with the likelihood of a virological response during the lead-in phase of this study. Finally, we evaluated the relationship between YKL-40 polymorphisms and the risk of clinical and histological disease progression in 462 subjects followed in the randomized phase of the trial.

Materials and methods

Eligible Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial subjects had detectable serum HCV RNA and bridging hepatic fibrosis (i.e. Ishak fibrosis score $\geq$ 3) or cirrhosis on a pretreatment liver biopsy and documented failure to achieve a sustained virological response to a prior course of interferon with or without ribavirin; all subjects had clinically compensated liver disease at entry (12, 13). Subjects were retreated with full-dose peginterferon-α2a, 180 μg/week, and ribavirin, 1.0–1.2 g/day, for 24 weeks in the 'lead-in phase' of this study. Subjects who remained viraemic at treatment week 20 met the definition of nonresponse and were eligible for randomization to maintenance peginterferon-α2a, 90 μg/week, vs no treatment for 3.5 years; subjects with undetectable HCV RNA at week 20, as determined by polymerase chain reaction (PCR) assay (Roche Molecular Systems, Pleasanton, CA, USA. COBAS Amplicor v 2.0, sensitivity of 100 IU/ml), were categorized as responders, were not eligible for randomization, but continued in the 'responder arm' of the trial and completed 48 weeks of full-dose (as tolerated) combination antiviral treatment. All HALT-C Trial participants entering the lead-in and randomized phase at 4 of the 10 HALT-C Trial clinical sites – the University of Michigan, University of Massachusetts/University of Connecticut, Massachusetts General Hospital and the Virginia Commonwealth University – were eligible for the serum fibrosis marker ancillary study and had additional serum and DNA collected; samples were frozen immediately at −80°C and stored at a central repository (SeraCare, Gaithersberg, MD, USA). This study was approved by local Institutional Review Boards, and all patients provided written informed consent for both the main trial and this ancillary study, including specific consent for genetic testing.

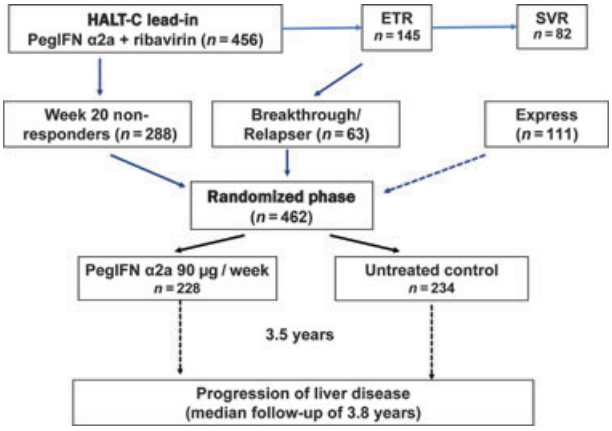

Fig. 1. Overview of study population. There were 456 patients with chronic hepatitis C and advanced fibrosis who were retreated with peginterferon-α2a/ribavirin for 24 weeks during the 'Lead-in' phase of the HALT-C Trial included in the current study. Two-hundred and eighty-eight patients who failed to clear HCV RNA at week 20 entered the randomized phase, whereas 63 patients who had a virological relapse/breakthrough after 24–48 weeks of peginterferon/ribavirin therapy also entered the randomized phase. Finally, there were 111 'Express' patients who had received peginterferon/ribavirin therapy and entered the randomized phase. All the subjects were followed for clinical and histological liver disease progression through a median follow-up of 3.8 years.

Laboratory and clinical assessment during the randomized phase

Routine baseline laboratory values (e.g. serum AST, ALT, albumin, bilirubin, platelet count) were obtained at local hospital laboratories. A baseline liver biopsy obtained within 12 months of enrolment was scored by consensus among a group of hepatopathologists for the degree of hepatic fibrosis and inflammation defined by the Ishak scoring system, and the degree of hepatic steatosis was estimated as grade 0–4 (17, 18). All patients were seen every 3 months during the randomized phase for laboratory and clinical assessment. In addition, annual liver ultrasounds were obtained to screen for hepatocellular carcinoma (HCC), and serum alpha-foetoprotein levels were obtained every 3 months. Clinical endpoints for this study included an increase in the Child-Turcotte-Pugh (CPT) score to $\geq$ 7 on two separate occasions 3 months apart, variceal bleeding, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, HCC or death. For the subgroup of patients with noncirrhotic fibrosis at baseline, histological progression was defined as a $\geq$ 2-point increase in the Ishak fibrosis score (12).

YKL-40 serum assay

Stored serum samples were tested for YKL-40 levels with a commercially available ELISA kit (Metra YKL-40, Quidel, San Diego, CA, USA) as described previously (Normal range: 24–125 ug/L) (14).

Testing for YKL-40 promoter polymorphisms

DNA was extracted at SeraCare from frozen whole blood. Genotyping for the single tag nucleotide polymorphism (SNP) rs4950928 of the *CHI3L1* promoter polymorphism accounting for the upstream at −131G → C point mutation on genomic DNA was performed with allele-specific real-time PCR at a high-throughput facility (Celera Diagnostics, Alameda, CA, USA). The YKL-40 genotypes were classified as CC homozygous for the major allele, CT heterozygous or TT homozygous for the minor allele. From prior publications and HapMap data, we expected the distribution of YKL-40 genotypes in Caucasians and African Americans to be similar. Therefore, to maximize power, we performed the analyses in all of the patients grouped together but also performed all of the analyses in Caucasians alone.

Statistical analyses

Log-transformation of non-normally distributed variables including serum YKL-40 levels was undertaken when needed. Continuous variables are presented as means ± standard deviation; we derived additive linear models for the number of minor alleles (including a continuous variable for the number of minor alleles) to compare values by genotype (e.g. test of trend). To assess changes in serum YKL-40 values over time, we used random effects models that were fit in SAS based on proc mixed that included a random effect of time as well as the interaction of time with genotype (SAS Institute, Cary, NC, USA).

To predict the risk of clinical progression in liver disease by YKL-40 promoter polymorphism, we used Cox proportional hazards regression, and data were censored at the patient's last follow-up visit or at 1400 days (i.e. 3.8 years) after randomization, whichever occurred first. To assess the relationship between histological progression and YKL-40 genotype, we relied upon complementary log-log regression analysis. All analyses were performed at the Data Coordinating Center (New England Research Institutes, Watertown, MA, USA) with SAS statistical software version 9.2 (SAS Institute).

Results

Lead-in analysis population

The baseline features of the 456 patients enrolled in the lead-in phase of the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial who underwent genetic testing are provided in Table 1. Overall, 283 (68.0%) patients were CC homozygous at the major allele, 123 were CT heterozygotes (29.6%) and 10 (2.4%) were TT homozygotes. The CT and TT genotypes were in Hardy–Weinberg equilibrium ($P = 0.43$) in the overall study population as well as in the Caucasian subgroup ($P = 0.48$). In addition, the 17% overall frequency of the T allele in these HALT-C Trial patients is similar to that reported in other patient populations (10, 11).

The age, demographic features and baseline laboratory markers of liver disease severity were similar in the three YKL-40 genotype subgroups with the exception of race/ethnicity, serum ALT and BMI. Furthermore, the histological severity of liver disease was similar, as was the proportion with oesophageal varices, in all three subgroups. As expected, baseline serum YKL-40 levels were significantly lower in the TT subjects compared with the CT or CC subgroups (1.96 vs 2.25 vs 2.47, $P < 0.001$, trend test). Having shown previously that lower baseline serum YKL-40 levels were independently associated with week-20 virologic responses, we also examined the relationship between YKL-40 genotype and week-20 virologic response. The likelihood of a week-20 virologic response was similar for the CC and CT genotypes but somewhat higher for the TT group, although the difference did not reach statistical significance (Table 1). In addition, baseline serum YKL-40 levels differed according to subgroup with those in the TT subgroup being lowest (Table 1). Serum YKL-40 levels also decreased significantly by week 24; however, the magnitude of decline in serum YKL-40 levels did not differ according to subgroup (Fig. 2A). In addition,

Table 1. Baseline features of the lead-in HALT-C Trial patients with YKL-40 promoter polymorphism data

Variable	YKL-40 CC genotype	YKL-40 CT genotype	YKL-40 TT genotype	Overall*	*P*-value
Number	283	123	10	456	
Age	49.1 (7.1)	49.3 (6.8)	46.9 (5.2)	49.2 (7.1)	0.81
Male (%)	203 (71.7)	86 (69.9)	8 (80.0)	324 (71.1)	0.98
Race					
Caucasian (%)	203 (71.7)	103 (83.7)	10 (100.0)	338 (74.1)	0.002†
African American (%)	64 (22.6)	13 (10.6)	0 (0)	93 (20.4)	
Other (%)	16 (5.7)	7 (5.7)	0 (0)	25 (5.5)	
Duration of infection (years)	26.5 (7.8)	28.2 (7.6)	24.9 (3.8)	26.9 (7.7)	0.21
Lifetime alcohol (drinks)	18 717 (31 674)	17 312 (21 453)	15 234 (15 609)	17 441 (27 778)	0.58
Lifetime smoking (pack-years)	15.8 (17.9)	14.8 (16.4)	16.8 (20.9)	15.2 (17.2)	0.70
Diabetes (%)	76 (26.9)	23 (18.7)	5 (50.0)	117 (25.7)	0.55
Mean BMI (kg/m^2)	30.0 (5.8)	28.5 (4.7)	30.0 (4.8)	29.6 (5.5)	0.04
% With oesophageal varices	32 (15.2%)	17 (19.5%)	3 (50.0%)	55 (16.5%)	0.08
% With prior IFN and ribavirin	198 (70.0%)	85 (69.1%)	4 (40.0%)	310 (68.0%)	0.25
Laboratory features					
Log_{10} HCV RNA (IU/ml)	6.44 (0.51)	6.41 (0.65)	6.50 (0.52)	6.44 (0.54)	0.74
% Genotype 1	247 (87.3%)	111 (90.2%)	9 (90.0%)	406 (89.0%)	0.41
AST (IU/ml)	89.7 (66.3)	96.0 (71.8)	116.0 (110.1)	93.2 (70.0)	0.20
ALT (IU/ml)	107.1 (66.4)	124.6 (98.9)	145.1 (132.4)	114.7 (81.1)	0.02
AST/ALT	0.87 (0.29)	0.82 (0.23)	0.82 (0.22)	0.85 (0.27)	0.07
Total bilirubin (mg/dl)	0.77 (0.45)	0.77 (0.41)	0.85 (0.55)	0.77 (0.43)	0.86
INR	1.03 (0.10)	1.03 (0.09)	1.05 (0.11)	1.03 (0.10)	0.87
Albumin (g/L)	3.84 (0.37)	3.90 (0.34)	3.80 (0.39)	3.87 (0.36)	0.36
Platelets $\times 10^3/mm^3$	177.5 (69.9)	169.7 (59.0)	151.4 (56.7)	174.2 (65.5)	0.14
Log_{10} YKL-40 (ug/L)	2.47 (0.43)	2.25 (0.41)	1.96 (0.49)	2.39 (0.44)	<0.001
Baseline histology					
Mean Ishak Fibrosis score	4.00 (1.26)	3.99 (1.24)	4.40 (0.97)	4.01 (1.25)	0.64
% Ishak 5/6	103 (36.4%)	46 (37.4%)	3 (30.0%)	168 (36.8%)	0.96
Mean HAI	7.47 (2.11)	7.27 (2.11)	7.80 (1.99)	7.43 (2.08)	0.64
Steatosis (% $\geq$ 2)	118 (41.7%)	45 (36.6%)	5 (50.0%)	185 (40.6%)	0.60
Virologic response					
N (%) with week-20 virologic response	105 (37.1)	45 (36.6)	7 (70.0)	169 (37.1)	0.33
N (%) with week-48 virologic response‡	88/98 (89.8)	39/42 (92.9)	7/7 (100.0)	145/158 (91.8)	0.85
N (%) with week-72 SVR§	47/105 (44.8)	26/45 (57.8)	3/7 (42.9)	82/169 (48.5)	0.34

*40 patients missing genotype data. Data presented as mean (SD) or *n* (%).

†*P*-value tests whether percent Caucasian differs by genotype.

‡There were 169 with virologic response; 158 had complete week-48 data and of those, 147 had genotype data.

§There were 169 with virologic response for which week-72 SVR was relevant.

amongst the 158 patients in the responder arm who received a full 48-week course of peginterferon and ribavirin, the likelihood of a week-48 and week-72 virologic response did not differ by YKL-40 genotype (data not shown). However, assessment of serial serum YKL-40 levels amongst the 158 lead-in responders demonstrated a significant decline over time and a difference in levels according to subgroup, but the magnitude of decline in serum YKL-40 levels did not differ according to subgroup (Fig. 2B).

Randomized phase analysis

The mean age of the 462 HALT-C Trial subjects entering the randomized phase in this study was 49.5 years; 70% were men, 71% were Caucasian and 49% were randomized to receive low-dose peginterferon (Table 2, Fig. 1). Seven (1.7%) were TT minor allele homozygotes, 119 (28.5%) were CT heterozygotes and 292 (70.0%) were CC major allele homozygotes. The distribution of YKL-40 genotypes was in Hardy–Weinberg equilibrium overall ($P = 0.19$) and in the Caucasian-only subset ($P = 0.24$). Baseline demographics of the three groups of patients were similar, with the exception that all of the TT homozygotes, but not the other subgroups, were Caucasian. In addition, laboratory markers of disease severity as well as baseline histological features were similar in the three groups except for higher serum AST and ALT levels in the TT subgroup. Baseline serum YKL-40 levels, however, were significantly lower in the TT subgroup compared with the CT and CC subgroups (2.20 vs 2.34 vs 2.50, $P < 0.001$, trend test).

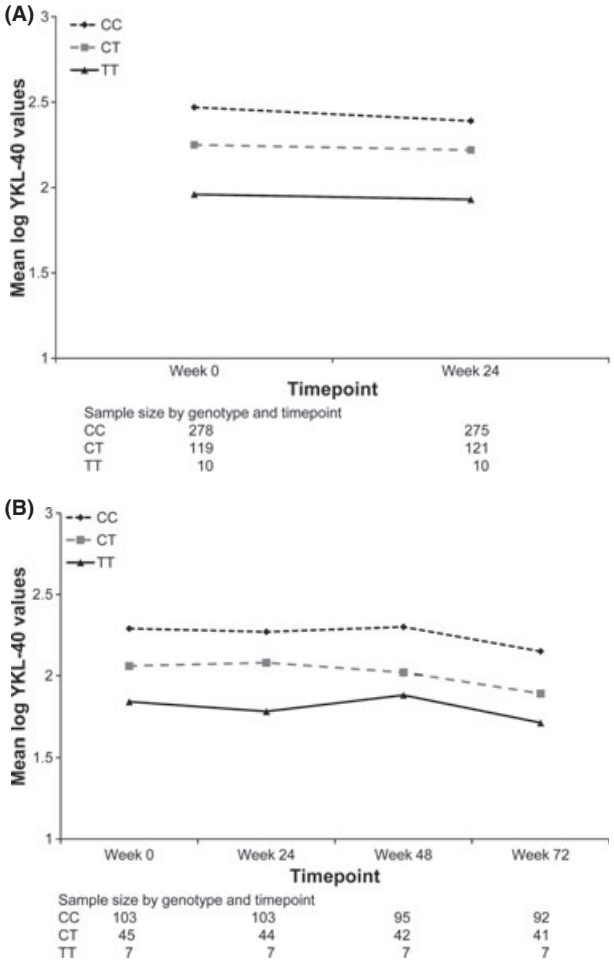

Fig. 2. Serum YKL-40 levels and YKL-40 genotype in the lead-in/responder arm of HALT-C. (A) The baseline serum YKL-40 levels were significantly lower in patients with the TT genotype compared with those with the CT and CC genotype ($P < 0.001$). In addition, the serum YKL-40 levels significantly decreased by week 24 ($P < 0.001$) and remained significantly lower in the subjects with the TT genotype compared with those with the CT and CC genotype ($P < 0.001$). (B) In the 158 subjects with a virological response during the lead-in phase who received a full 48-week course of peginterferon and ribavirin, the serum YKL-40 levels significantly decreased over time ($P < 0.001$) and remained significantly lower in the subjects with the TT genotype compared with those with the CT and CC genotype ($P < 0.001$).

Clinical and histological outcomes

During a mean follow-up of 51 months, a primary clinical outcome developed in 69 (15%) of the 462 randomized patients, including Child-Turcotte-Pugh increase ($n = 40$), hepatocellular carcinoma ($n = 7$), ascites ($n = 32$), encephalopathy ($n = 15$), variceal bleeding ($n = 8$) and death ($n = 24$). No significant relationship emerged, however, between YKL-40 genotype and the risk of a clinical outcome (Table 3). In addition, analyses conducted in patients stratified by treatment group during the randomized phase failed to demonstrate an association of YKL-40 genotype with clinical liver disease progression. Finally, analysis of the 1050 randomized patients also failed to demonstrate a significant association between YKL-40 genotype and risk of developing a clinical outcome (see Supporting Information Table S1). A worsening of hepatic fibrosis, defined as an increase in the Ishak fibrosis score of $\geq$ 2 points at month 24 or 48 compared to baseline, was a primary endpoint in the HALT-C Trial for patients with noncirrhotic fibrosis. Among the 280 patients with a pretreatment Ishak fibrosis score of < 5, 209 had a follow-up biopsy that was adequate for analysis, and, among these, 191 also had YKL-40 genetic data available. Among these 191 patients, 90 were randomized to maintenance peginterferon while 101 were not treated. During follow-up, 70 (33.5%) patients had worsening hepatic fibrosis, whereas 139 had stable or unchanged Ishak fibrosis scores, but the distribution of YKL-40 genotypes was not significantly different in subjects with and without histological progression (Table 4). In addition, an analysis of the 935 randomized HALT-C Trial patients with YKL-40 genetic data available showed, similarly, no association with clinical outcomes nor with histological outcomes in 432 subjects with paired biopsies (see Table S1). In addition, analyses stratified by treatment group failed to demonstrate associations with YKL-40 genotype.

During the randomized phase of the trial, serum YKL-40 levels remained consistently lower in subjects with the TT genotype than in subjects with the CT and CC genotypes (Fig. 3). In fitting a model of serial YKL-40 levels over time, we found that the YKL-40 genotype, the baseline serum YKL-40 level and the effect of time were all significant (all $P < 0.001$). No interaction was apparent, however, between YKL-40 genotype and serum YKL-40 levels over time.

Discussion

Host, environmental and genetic factors are thought to explain, in part, the highly variable rate of hepatic fibrosis and clinical progression of liver disease in patients with chronic hepatitis C (CHC). Recent studies based on exploratory genome-wide association methodologies have led to the identification of a series of genes that may increase an individual patient's risk of progressing to advanced fibrosis and cirrhosis (19, 20). In addition, hypothesis-driven genetic association studies involving a limited number of polymorphisms in genes that are linked biologically to hepatic fibrogenesis have been reported, but their results have not been validated consistently in independent cohorts (21, 22). Hepatic YKL-40 mRNA levels correlated strongly with serum YKL-40 levels in a prospective study of 87 untreated patients with CHC that were followed prospectively over 10 years (1). In addition, recent reports demonstrated that a functional polymorphism in the promoter region

Table 2. Baseline features of the randomized HALT-C Trial patients with YKL-40 promoter polymorphism data

Variable	YKL-40 CC genotype	YKL-40 CT genotype	YKL-40 TT genotype	Overall	*P*-value
Number	292	119	7	462*	
Age (years)	49.7 (6.9)	49.5 (6.7)	48.6 (5.9)	49.5 (7.0)	0.71
Gender (% male)	201 (68.8)	90 (75.6)	5 (71.4)	325 (70.4)	0.21
Race					
Caucasian (%)	199 (68.2)	96 (80.7)	7 (100)	329 (71.2)	0.003†
African American (%)	77 (26.4)	17 (14.3)	0 (0)	110 (23.8)	
Other (%)	16 (5.5)	6 (5.0)	0 (0)	23 (5.0)	
Duration of infection (years)	27.0 (7.4)	27.8 (7.6)	26.3 (5.7)	27.1 (7.4)	0.51
Lifetime alcohol (drinks)	19 355 (31 039)	19 139 (24 303)	15 537 (10 236)	18 514 (28 054)	0.83
Lifetime smoking (pack-years) (%)	17.0 (18.1)	16.0 (16.6)	18.9 (22.9)	16.5 (17.5)	0.75
Diabetes (%)	81 (27.7)	31 (26.1)	4 (57.1)	129 (27.9)	0.65
Mean BMI	30.2 (5.7)	29.5 (5.2)	29.2 (4.5)	29.9 (5.5)	0.20
% With oesophageal varices	50 (17.7%)	24 (20.9%)	3 (42.9%)	83 (18.6%)	0.17
% Lead-in nonresponders	181 (62.0%)	78 (65.6%)	2 (28.6%)	288 (62.3%)	0.83‡
% Breakthrough/relapsers	40 (13.7%)	14 (11.8%)	4 (57.1%)	63 (13.6%)	
% Express	71 (24.3%)	27 (22.7%)	1 (14.3%)	111 (24.0%)	
Laboratory features					
Log_{10} HCV RNA (IU/ml)	6.48 (0.48)	6.53 (0.51)	6.52 (0.30)	6.50 (0.47)	0.45
% Genotype 1	270 (92.5%)	114 (96.6%)	6 (85.7%)	433 (93.9%)	0.31
AST (IU/ml)	85.1 (49.0)	94.3 (64.8)	138.3 (141.1)	90.0 (60.2)	0.03
ALT (IU/ml)	100.8 (63.2)	119.2 (95.6)	164.3 (172.2)	108.7 (79.7)	0.01
AST/ALT	0.89 (0.28)	0.84 (0.22)	0.86 (0.18)	0.88 (0.26)	0.16
Total Bilirubin (mg/dl)	0.75 (0.37)	0.75 (0.35)	0.57 (0.25)	0.74 (0.36)	0.59
INR	1.03 (0.10)	1.04 (0.09)	1.10 (0.11)	1.03 (0.10)	0.08
Albumin (g/L)	3.80 (0.38)	3.86 (0.34)	3.64 (0.36)	3.83 (0.37)	0.52
Platelets $\times 10^3/mm^3$	177.7 (72.4)	168.0 (56.3)	136.5 (62.8)	174.3 (67.1)	0.10
Log_{10} YKL-40 (ug/L)	2.50 (0.40)	2.34 (0.39)	2.20 (0.57)	2.45 (0.41)	<0.001
Baseline histology					
Mean Ishak Fibrosis score	4.05 (1.26)	4.11 (1.23)	4.43 (1.40)	4.08 (1.25)	0.48
% Ishak 5/6	111 (38.0%)	50 (42.0%)	3 (42.9%)	182 (39.4%)	0.45
Mean HAI	7.66 (2.08)	7.44 (1.91)	7.29 (2.29)	7.56 (2.03)	0.28
Steatosis (% $\geq$ 2)	130 (44.5%)	52 (43.7%)	3 (42.9%)	204 (44.2%)	0.87
Randomized phase outcomes					
N (%) Peginterferon	148 (50.7)	56 (47.1)	2 (28.6)	228 (49.4)	0.28
N (%) Clinical progression	44 (15.1)	20 (16.8)	2 (28.6)	69 (14.9)	0.42
Duration of follow-up (days)	1220 (431)	1249 (401)	1023 (621)	1228 (429)	>0.99

Presented as mean (SD) or *n* (%). *T*-test for continuous variables and chi-sq *P*-values for categorical variables.

*44 missing YKL-40 genotype data.

†*P*-value tests whether percent Caucasian differs by genotype.

‡*P*-value tests whether proportion of lead-in nonresponders differs by genotype.

Table 3. Risk of clinical liver disease progression by YKL-40 promoter polymorphism at rs4950928

	All patients (n = 462)	With clinical progression (n = 69)	Without clinical progression (n = 393)	Hazard ratio (95% CI)*	*P*-value
Homozygous major (CC)	292 (69.9%)	44 (66.7%)	248 (70.5%)	1 Ref	0.47
Heterozygous (CT)	119 (28.5%)	20 (30.3%)	99 (28.1%)	1.19 (0.75, 1.89)	
Homozygous minor (TT)	7 (1.7%)	2 (3.0%)	5 (1.4%)		
Missing rs4950928	44	3	41		

*HR calculated assuming an additive model and a continuous variable for genotype status.

of the YKL-40 gene was associated with reduced serum YKL-40 levels in multiple independent patient populations and with reduced gene expression in peripheral blood cells (10, 11, 23). Furthermore, based on both qualitative and quantitative genetic analyses, Berres *et al.* (11) showed that in patients with CHC, histological fibrosis scores and serum YKL-40 levels were associated after adjustment for other co-factors with YKL-40

Table 4. Risk of histological fibrosis progression by YKL-40 promoter polymorphism at rs4950928

	All patients (n = 209)	With fibrosis progression (n = 70)	Without fibrosis progression (n = 139)	Hazard ratio (95% CI)*	P-value
Homozygous major (CC)	138 (72.3%)	49 (75.4%)	89 (70.6%)	1 Ref	0.64
Heterozygous (CT)	50 (26.2%)	14 (21.5%)	36 (28.6%)	0.88 (0.53, 1.48)*	
Homozygous minor (TT)	3 (1.6%)	2 (3.1%)	1 (<1%)		
Missing rs4950928	18	5	13		

*Hazard ratio calculated assuming an additive model to compare the 3 genotype groups.

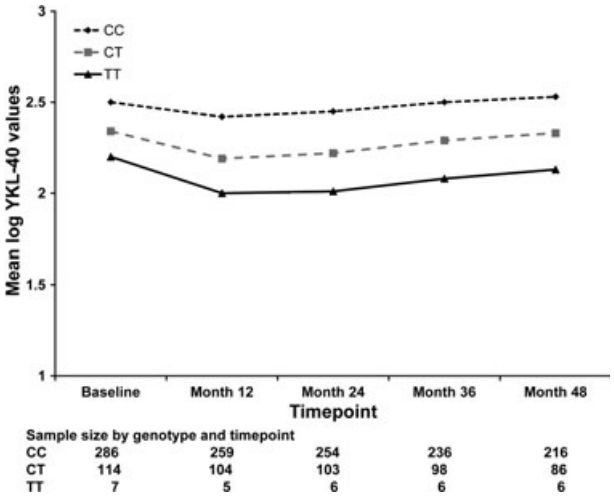

Fig. 3. Serum YKL-40 levels stratified by YKL-40 genotype during the randomized phase of the HALT-C Trial. At baseline, the serum YKL-40 levels were significantly lower in subjects with the TT minor allele compared with the CT heterozygotes and CC homozygotes ($P < 0.0001$). Although the serum YKL-40 levels significantly increased in each subgroup over time ($P < 0.0001$), they remained consistently lower in the CC subgroup compared with others ($P < 0.001$).

promoter polymorphisms. Having obtained extensive serum YKL-40 data in a large group of well characterized patients with CHC enrolled in the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial, we sought to determine whether significant associations existed between YKL-40 promoter polymorphisms and serum YKL-40 levels as well as with baseline liver disease severity, response to antiviral therapy, and clinical and histological progression of liver disease (14, 15).

The distribution of YKL-40 promoter polymorphisms in the HALT-C Trial patients was similar to that reported in HapMap general population controls as well as in another large study of patients with asthma (10). These data suggest that contrary to the findings of Berres *et al.* (11), YKL-40 promoter polymorphisms are not protective against the development of advanced liver fibrosis. However, other host and environmental co-factors for fibrosis progression that were present in many HALT-C Trial patients – e.g. history of heavy alcohol consumption, increased body mass index and diabetes mellitus – may have overshadowed the protective effect of a YKL-40 promoter polymorphism (TT) associated with lower serum YKL-40 levels. In addition, the limited number of patients with the favourable genotype (TT) as well as the selection of patients with advanced fibrosis for entry into the HALT-C Trial may have precluded the detection of an association between YKL-40 genotype and baseline disease severity. Furthermore, the presence of advanced liver fibrosis in and of itself may have reduced the clearance of YKL-40 from the circulation, leading to spuriously high serum levels even in patients with the TT genotype. In support of these concepts, the study by Berres *et al.* (11) included patients with CHC who had a broader distribution of fibrosis scores, ranging from normal (stage 0) to cirrhosis (stage 6), as compared to the more limited distribution of fibrosis states in the current study (confined to Ishak stages 3–6); in addition, none of the patients included in the study by Berres *et al.* (11) were reported to have consumed excessive amounts of alcohol, while many HALT-C Trial subjects, although abstinent at trial entry, had a history of high-level lifetime alcohol use. Furthermore, the previously reported association between serum YKL-40 levels and YKL-40 genotype was restricted to patients with mild hepatic fibrosis (11), presumably because advanced hepatic fibrosis can result in reduced clearance of YKL-40 and other serum fibrosis markers. A significant association between baseline serum YKL-40 levels and YKL-40 genotype was observed in the 456 patients entering the lead-in phase as well as the 462 *randomized* HALT-C Trial patients (Tables 1 and 2). The persistence of low serum YKL-40 levels over time in the lead-in patients with genotype TT compared with those with genotype TC and CC also suggests the presence of a strong and persistent association between YKL-40 genotype and circulating levels of YKL-40 (Fig. 2).

In addition to pursuing associations between YKL-40 promoter polymorphisms and liver disease progression, we explored the potential role of YKL-40 promoter polymorphisms in predicting the outcomes of antiviral therapy. Multiple recent reports have demonstrated the importance of interleukin-28B (*IL28B*) gene polymorphisms in predicting the likelihood of achieving viral suppression during and after peginterferon and ribavirin therapy in treatment-naïve patients with CHC (24, 25).

In addition, a prior analysis of HALT-C Trial patients demonstrated that the same *IL28B* polymorphisms are associated significantly with the likelihood of achieving a week-20 virological response as well as an sustained virological response (SVR) following 48 weeks of therapy (26). In the current study, we did not find a significant association of the YKL-40 genetic polymorphisms with week-20 (on treatment response), week-48 (end-of-treatment response) or week-72 (SVR) virological responses. In addition, when we analysed YKL-40 polymorphisms in the entire cohort of 935 HALT-C Trial lead-in patients with available genetic data (n = 675 Caucasian), we did not see an association between this genetic marker and virological response (data not shown). Therefore, our prior observations that baseline serum YKL-40 levels are an independent predictor of virological response during the lead-in phase of the HALT-C Trial may reflect the association of lower serum YKL-40 levels with less severe liver disease, which is known to influence virological responsiveness (15, 16). Nonetheless, studies of YKL-40 genotype in treatment-naïve HCV patients with CHC may be worthwhile in HCV-infected patients with a broader distribution of disease severity.

We also explored the relationship between YKL-40 promoter polymorphisms and the risk of clinical and histological liver disease progression over time in a large cohort of well characterized and prospectively monitored patients with CHC. In the current HALT-C Trial subset, however, we observed no significant association between YKL-40 genotype and the risk of clinical or histological liver disease progression (Tables 3 and 4). When we also analysed YKL-40 genotype in a larger cohort of 935 randomized HALT-C Trial patients who had available genetic data, we also failed to find an association of YKL-40 polymorphisms with clinical and histological disease progression (Table S1). Nonetheless, serum YKL-40 levels remained significantly lower throughout follow-up monitoring in subjects with the TT genotype compared to subjects with the CT and CC genotypes (Fig. 3). Because we showed previously that baseline serum YKL-40 levels are associated with the risk of liver disease progression, our current data suggest that serial measurements of serum YKL-40 levels, not YKL-40 gene polymorphisms, may prove to be a useful prognostic marker in patients with CHC and advanced fibrosis. Our study findings are consistent with other analyses of the HALT-C Trial cohort that also failed to demonstrate a genetic predisposition to clinical or histological progression of liver disease over time (27, 28). Absence of an association may have resulted, in part, from the selection of a cohort of patients with advanced fibrosis for inclusion in the HALT-C Trial. In support of this notion, recent reports demonstrate that a panel of seven genetic polymorphisms, 'CRS-7', is associated with the risk of fibrosis progression in patients with mild CHC but is less useful in patients with more advanced fibrosis (29, 30).

In conclusion, our study results failed to support a reduced frequency of the YKL-40 promoter polymorphism associated with reduced serum YKL-40 levels in our population of patients with CHC and advanced fibrosis. This observation suggests that the previously reported association of the YKL-40 promoter polymorphism with liver disease severity (11) is most useful in patients with CHC who lack other risk factors or environmental co-factors for advanced fibrosis. Our prior observation that lower baseline serum YKL-40 levels were associated with improved responsiveness to peginterferon and ribavirin was probably secondary to an association of baseline liver disease severity with serum YKL-40 levels, rather than to an association with YKL-40 gene expression *per se*. Absence of an association between YKL-40 promoter polymorphisms and the risk of clinical and histological liver disease progression also suggests that this locus may be more important in the initiation and/or development of early fibrosis than in the progression of more advanced, established liver fibrosis.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Risk of clinical and histological fibrosis progression by YKL-40 promoter polymorphism at rs4950928 in the overall HALT-C Trial.

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