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Association of γ-Glutamyl Transferase (GGT) Activity With Treatment and Clinical Outcomes in Chronic Hepatitis C (HCV)

James E. Everhart and Elizabeth C. Wright

Increased γ-glutamyl transferase (GGT) activity is associated with liver injury and with mortality in the general population. Less is known about its association with chronic hepatitis C (HCV) outcomes. We examined GGT as a predictor of both virological response to treatment and long-term clinical outcomes in the Hepatitis C Anti-viral Treatment Against Cirrhosis Trial (HALT-C). HALT-C enrolled patients with advanced liver disease (Ishak fibrosis score ≥3) in two phases: a lead-in to establish lack of sustained viral response with full dose pegylated interferon (IFN) and ribavirin followed by a 3.5-year randomized trial with low-dose IFN. Low-dose IFN did not prevent liver disease progression, and patients were then followed for up to an additional 5 years off therapy. Analyses were performed for 1,319 patients who had GGT measured prior to initiation of treatment. Increases in risk with each increase in quintile of GGT (10-57, 58-89, 90-139, 140-230, 231-2,000 IU/L) were determined by logistic regression for treatment response or Cox regression for clinical outcomes. Baseline GGT was associated with male sex, nonwhite ethnicity, diabetes and insulin resistance, interleukin (IL)28B rs12979860 CT and TT genotypes, and numerous markers of liver disease injury and severity. In the lead-in phase, increasing GGT was strongly associated with diminished week 20 response, end of treatment response, and sustained virological response in both univariate and multivariate analyses controlling for factors known to be associated with treatment response ($P < 0.0001$). GGT was also associated with all clinical outcomes in univariate and multivariate analysis ($P < 0.05$) except for hepatocellular carcinoma ($P = 0.46$ in multivariate analysis). *Conclusion*: GGT is an independent predictor of both virological response and clinical outcomes among patients with advanced liver disease due to HCV. (HEPATOLOGY 2013;57:1725-1733)

The enzyme γ-glutamyl transferase (GGT) catalyzes the transfer of a γ-glutamyl group from glutathione (GSH) and other γ-glutamyl compounds to amino acids or dipeptides. It also catalyzes hydrolysis of the γ-glutamyl bond. The enzyme is present in several organs, most notably the liver.[1] GGT activity is elevated in cholestatic liver disease, alcoholic and other fatty liver disease, and can be induced by a number of drugs, including barbiturates and phenytoin. GGT activity is not necessarily considered a routine test in the evaluation of liver disease because it is believed to contribute little diagnostic information. As a result, GGT is often not part of standard panels that include other liver enzymes (personal communication from seven hepatologists at academic sites). Perhaps because of its limited utility in diagnosis of liver disease, the prognostic significance of GGT may have been undervalued. For example, increased GGT activity been associated with increased mortality in the general population.[2-4] We examined the value of GGT activity as a predictor of treatment response and of liver disease outcomes among a large cohort of patients

Abbreviations: APRI, AST/Platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyl transferase; GSH, glutathione; HALT-C, Hepatitis C Anti-viral Treatment Against Cirrhosis Trial; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.

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with chronic hepatitis C who participated in the Hepatitis C Anti-viral Treatment Against Liver Disease Trial (HALT-C).

Patients and Methods

Study Design. HALT-C had two major treatment phases (clinical trials.gov identifier NCT00006164) and an observational phase.[5-7] A lead-in phase used full-dose pegylated interferon alpha 2a (Pegasys, Roche) and ribavirin to attempt to achieve sustained virological response (SVR) among patients with advanced liver disease (Ishak fibrosis score of 3 or greater on liver biopsy) who had previously been treated with standard interferon (IFN) with or without ribavirin. Patients who did not achieve SVR were eligible for the randomized phase, a controlled clinical trial of pegylated interferon alfa-2a at a dosage of 90 μg per week for 3.5 years, as compared with no treatment. The primary endpoint was progression of liver disease, as indicated by death, hepatocellular carcinoma (HCC), hepatic decompensation, or, for those with bridging fibrosis at baseline, an increase in the Ishak fibrosis score of 2 or more points. Most patients entered the randomized trial through the lead-in phase as nonresponders after 20 weeks of therapy (based on detectable hepatitis C virus (HCV) RNA by Roche Cobas Amplicor assay) or after subsequent breakthrough or relapse. Other patients entered the randomized phase as "express" patients by having failed to clear virus outside of the HALT-C lead-in. All patients also had liver biopsies scheduled at 18 months after randomization and at the end of treatment 42 months after randomization. Patients continued to be followed in the observational phase for clinical outcomes off therapy for as long as 5 additional years. The median duration of participation in the trial (time from randomization to first outcome or last time known to be outcome-free) was 6.0 years (range, 0-8.7 years). Informed consent in writing was obtained from each patient, and the study protocol was approved by the Institutional Review Committee of each of the participating centers.

GGT activity was measured under code on stored frozen samples (−80°C) by Wako Pharmaceuticals (Richmond, VA) under a clinical trial agreement with the National Institute of Diabetes and Digestive and Kidney Diseases. The normal range was reported as 12-64 IU/L for men and 9-36 IU/L for women.

Patient Population. Of the 1,319 patients with GGT measurements, 770 participated in both the lead-in and randomized phases of the trial (blood sample drawn shortly before lead-in), 320 only in the lead-in phase (blood sample drawn shortly before lead-in), and 229 only in the randomized phase (blood sample drawn shortly before randomization). GGT results were available on 95.2% of lead-in patients and 95.1% of randomized patients. Values for the 1,319 patients were divided into quintiles and used throughout the analyses. The minimum was 10 IU/L, 20th percentile 58 IU/L, 40th percentile 90 IU/L, 60th percentile 140 IU/L, 80th percentile 231 IU/L, and maximum was 2,000 IU/L.

Statistical Analysis. Baseline associations with GGT quintiles were evaluated by assigning scores of 1 to 5 to the 5 quintiles and then using the Mantel-Haenszel chi-square test or an analysis of variance to test for trends with increasing GGT. Multivariate linear regression with backward selection was used to determine predictors of GGT quintile. Logistic regression with backwards selection was used to assess the association of GGT quintile and other variables with treatment response. Survival curves for clinical outcomes were estimated using the Kaplan-Meier method and the log-rank test for trends was used to test significance. Cox regression with backward selection was used to evaluate predictors of clinical outcomes. The analysis of change in GGT was based on the change from baseline to the time of the last biopsy, either 18 or 42 months after randomization. Analysis of variance was used to evaluate predictors of this change. For all analyses, the measurement closest to the baseline biopsy was considered the baseline GGT. All analyses were performed using SAS v. 9.3 (Cary, NC).

Results

Baseline Associations. Of the 1,090 patients who entered the lead-in phase and had GGT measured, enzyme activity was positively associated in univariate analysis with numerous other variables, including male sex, nonwhite ethnicity, diabetes, insulin resistance,

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history of smoking or drinking, current coffee consumption, IL28B rs12979860 T allele, numerous laboratory tests, low HCV RNA level, and several histological features (Table 1). Although PNPLA3 GG rs738409 genotype was strongly associated with hepatic steatosis ($P < 0.0001$, not shown), and steatosis was strongly associated with GGT ($P < 0.0001$), there was no association of the PNPLA3 genotype and GGT ($P = 0.31$). In multivariate linear regression with quintile of GGT activity as the dependent variable, the strongest associations with GGT activity were with male sex, IL28B rs12979860 CC allele, histologic hepatic steatosis, alanine aminotransferase (ALT), and alkaline phosphatase activities and serum ferritin concentration (Table 2).

An independent association of GGT activity with cirrhosis as the dependent variable was found in an analysis that added GGT quintile to a model with three variables (platelet count, aspartate aminotransferase [AST]/ALT ratio, and international normalized ratio [INR]) that had previously been shown to be associated with cirrhosis.[8] For each quintile increase in GGT activity, there was a corresponding 1.13 increase in the odds of cirrhosis (95% confidence interval [CI] 1.03-1.25, $P = 0.012$).

Treatment Response in the Lead-in Phase. In univariate analysis, the GGT activity quintile was strongly associated with lower week 12 early virological response, week 20 response, and with diminished SVR ($P < 0.0001$ for all) (Fig. 1). For example, 25.4% of patients in the lowest quintile of GGT had SVR, compared with only 6.9% in the highest quintile. In multivariate analysis increased GGT activity remained strongly associated with poorer treatment response when controlling for other independent predictors of response. For example, at week 20 of therapy the odds ratio for virological response per quintile increase in GGT activity was 0.63 (95% CI = 0.55-0.72, $P < 0.0001$) when controlling for cirrhosis, previous ribavirin use, AST/ALT, AST, albumin, platelet count, IL28B genotype rs12979860, HCV genotype 1, and log HCV RNA level of ≥ 6.

Among the covariates associated with treatment response, only IL28B genotype rs12979860 demonstrated a statistically significant interaction with GGT activity at all timepoints ($P < 0.05$). Therefore, the combined effect of GGT and IL28B genotype rs12979860 with treatment outcome was further examined (Fig. 2). As expected, CC homozygote patients had high rates of virological response. However, in this group there was not a statistically significant association of GGT activity with virological response. In contrast, CT heterozygote and TT homozygote patients had lower rates of virological response with increasing quintile of GGT. At the extremes, SVR occurred among 30% (74 of 250) of CC homozygote patients and in just 1 of 56 TT homozygote patients in the highest quintile of GGT activity.

Disease Outcomes. Of the 999 patients who entered the randomized phase and had GGT measured, enzyme activity was associated with the same variables as the patients who entered the lead-in (data not shown). In univariate Cox regression analyses, GGT quintile was associated with any clinical endpoint (hepatic decompensation, HCC, or death; $P < 0.0001$) as well as with death or liver transplantation ($P = 0.0003$) and with HCC ($P = 0.027$). The cumulative incidence for each clinical outcome after 7 years of observation is shown in Fig. 3. There were 518 patients in the randomized phase with GGT measured who were eligible to have a 2-point increase Ishak fibrosis score (baseline score of <5 and at least one follow-up biopsy). Among these patients, GGT activity was associated with a 2-point increase in Ishak fibrosis score on paired biopsies ($P < 0.0001$) (Fig. 3).

In multivariate Cox regression analyses, increasing GGT quintile was associated with increased risk of any clinical endpoint, death or transplantation, 2-point increase in Ishak fibrosis score (Table 3), and death alone (not shown) when controlling for features previously found to be associated with any endpoint (platelet count, AST/ALT, albumin, total bilirubin, and fibrosis stage) or with fibrosis progression (body mass index [BMI], platelet count, and hepatic steatosis). Association with HCC was not statistically significant ($P = 0.46$). There were no statistically significant interactions of GGT and other covariates with any clinical endpoint, but there was a small positive interaction of GGT and platelet count for increase in fibrosis ($P = 0.036$).

Among the four liver enzymes measured at baseline (GGT, ALT, AST, and alkaline phosphatase), GGT had the most robust association with response to therapy and with disease outcomes. Among the other enzymes, lower AST was an independent predictor of week 20 virological response and alkaline phosphatase was an independent predictor of predictor of HCC. ALT was neither an independent predictor of treatment response or of disease outcome.

Change in GGT Activity. We examined change in mean GGT activity with other variables for 809 patients who had GGT measured at baseline and at last biopsy (mean of 3.9 years). Mean GGT changed minimally during this period (−2.1 IU/L, $P = 0.72$),

Table 1. Association of Quintiles of GGT Activity (IU/L) With Patient Features Expressed as Means (SD) or Percent at Time of Entry to Lead-in Phase or Randomized Phase (for Express Patients)

Variable	1st Quintile 10-57	2nd Quintile 58-89	3rd Quintile 90-139	4th Quintile 140-230	5th Quintile 231-2000	*P* Value for Trend
N patients	228	216	219	208	219	
Patient characteristics						
Age years	50.4 (7.3)	50.3 (7.9)	50.1 (7.3)	50.0 (7.4)	49.0 (6.7)	0.059
Male (%)	57.9%	70.4%	73.5%	75.5%	83.1%	<0.0001
Race (%)						<0.0001
White	82.5%	78.7%	74.4%	72.6%	62.1%	
Black	9.7%	10.7%	11.9%	19.7%	25.1%	
Hispanic	4.4%	7.9%	10.1%	5.8%	11.4%	
Other	3.5%	2.8%	3.7%	1.9%	1.4%	
BMI (kg/m^2)	29.3 (5.8)	29.6 (5.0)	29.9 (5.4)	30.2 (5.8)	29.8 (5.0)	0.14
Diabetes (%)	17.1%	21.8%	23.7%	25.5%	32.0%	0.0002
Previous ribavirin treatment (%)	70.6%	65.7%	68.5%	76.0%	77.6%	0.013
Esophageal varices (N=743)	22.6%	23.9%	23.2%	25.0%	28.7%	0.22
Prescription medication use						
Any, %	72.4%	72.2%	80.8%	78.9%	79.5%	0.021
Antiepileptic, %	3.1%	4.2%	4.1%	3.9%	2.7%	0.81
Ever smoked, % (N=1066)	78.9%	71.4%	71.0%	78.5%	81.6%	0.19
Ever drank, % (N=1085)	78.0%	80.9%	83.4%	88.0%	89.5%	0.0001
Drinking at baseline, %	14.9%	15.7%	20.1%	20.2%	22.8%	0.015
Lifetime drinks (N=1085)	18,451 (35,392)	16,056 (22,619)	18,728 (31,667)	20,013 (36,342)	19,817 (27,330)	0.32
Coffee cups per day (%) (N=848)						0.0011
Nondrinkers	15.7%	14.6%	13.5%	13.0%	18.1%	
<1,	20.2%	30.4%	31.0%	27.8%	33.7%	
1-<3	42.7%	38.0%	41.5%	46.3%	40.4%	
≥ 3 cups/day	21.4%	17.0%	14.0%	13.0%	7.8%	
Laboratory values						
IL28B rs12979860 (N=983)						<0.0001
CC, %	45.4%	34.3%	22.7%	13.1%	10.1%	
CT, %	41.0%	49.0%	54.0%	57.4%	61.8%	
TT, %	13.7%	16.7%	23.2%	29.5%	28.1%	
PNPLA3 rs738409 (N=989						0.31
CC, %	54.9%	53.5%	54.0%	51.6%	53.2%	
CG, %	39.8%	36.4%	37.0%	41.3%	35.3%	
GG, %	5.3%	10.1%	9.0%	7.1%	11.4%	
Hemoglobin (g/dL)	14.6 (1.4)	15.0 (1.4)	15.0 (1.4)	15.2 (1.4)	15.4 (1.2)	<0.0001
Neutrophils ($X10^3/mm^3$)	3.3 (1.4)	3.3 (1.4)	3.2 (1.3)	3.1 (1.3)	2.9 (1.2)	0.0013
Platelets ($X10^3/mm^3$)	181 (65)	167 (60)	169 (66)	170 (70)	155 (61)	0.0002
AST (IU/L)	63.1 (45)	74.7 (42)	90.6 (51)	97.6 (63)	126.7 (84)	<0.0001
ALT (IU/L)	85.2 (66)	101.9 (61)	113.3 (76)	126.0 (88)	154.6 (102)	<0.0001
Alkaline phosphatase (IU/L)	79.5 (28)	86.2 (33)	100.0 (41)	104.4 (42)	118.3 (52)	<0.0001
APRI	1.04 (1.12)	1.37 (1.26)	1.61 (1.49)	1.75 (1.70)	2.39 (2.23)	<0.0001
AST/ALT	0.80 (0.26)	0.80 (0.28)	0.87 (0.30)	0.85 (0.30)	0.88 (0.29)	0.0006
AFP (ng/mL)	6.0 (8.4)	12.9 (26.4)	16.5 (26.1)	16.3 (25.7)	24.9 (32.0)	<0.0001
Total bilirubin, mg/dL	0.68 (0.33)	0.81 (0.44)	0.79 (0.41)	0.80 (0.42)	0.87 (0.42)	<0.0001
Albumin (g/L)	3.95 (0.38)	3.96 (0.39)	3.84 (0.38)	3.87 (0.38)	3.82 (0.42)	<0.0001
Prothrombin time (INR)	1.02 (0.09)	1.04 (0.13)	1.04 (0.11)	1.03 (0.09)	1.04 (0.11)	0.23
Ferritin (ng/mL)	172 (139)	266 (254)	294 (261)	421 (394)	571 (554)	<0.0001
Serum bile acids (μmol/L)	11.1 15.3)	12.3 (13.4)	19.0 (23.3)	19.2 (23.3)	21.5 (22.5)	<0.0001
HOMA IR (N=1087)	12.5 (21)	14.1 (22)	14.7 (18)	17.0 (30)	20.0 (26)	0.0004
HCV genotype						0.18*
Genotype 1, %	84.7%	89.8%	89.5%	90.9%	88.6%	
Genotype 2, %	9.7%	3.7%	2.3%	4.3%	4.1%	
Genotype 3, %	4.8%	5.6%	6.9%	2.9%	4.6%	
Other genotype, %	0.9%	0.9%	1.4%	1.9%	2.7%	
HCV RNA (Log_{10} IU/mL)	6.49 (0.62)	6.45 (0.56)	6.37 (0.51)	6.41 (0.45)	6.39 (0.47)	0.023
Histologic findings (N=1088)						
Hepatic steatosis (%)						<0.0001
<1	39.9%	20.5%	13.7%	9.6%	10.1%	
1-5	40.8%	48.4%	48.0%	36.1%	34.9%	

(Continued)

TABLE 1. (Continued)

Variable	1st Quintile 10-57	2nd Quintile 58-89	3rd Quintile 90-139	4th Quintile 140-230	5th Quintile 231-2000	P Value for Trend
6-33	17.1%	23.7%	31.5%	40.4%	42.2	
>=34	2.2%	7.4%	6.9%	13.9%	12.8%	
Cirrhosis (%)	25.4%	35.2%	43.8%	42.8%	42.9%	<0.0001
Ishak fibrosis score	3.6 (1.2)	4.0 (1.2)	4.2 (1.3)	4.1 (1.3)	4.3 (1.2)	<0.0001
Ishak inflammation score	7.0 (2.2)	7.4 (2.0)	7.7 (2.0)	7.6 (2.0)	7.9 (2.1)	<0.0001
Periportal injury	2.5 (1.0)	2.7 (0.9)	2.8 (1.0)	2.7 (0.9)	2.9 (0.9)	<0.0001
Parenchymal injury	2.7 (0.9)	2.5 (0.8)	2.6 (0.9)	2.7 (0.8)	2.8 (0.9)	<0.0001
Portal inflammation	2.2 (0.7)	2.2 (0.8)	2.2 (0.8)	2.2 (0.7)	2.2 (0.7)	0.96
Mallory bodies (%)	6.1%	7.0%	13.2%	16.8%	25.7%	<0.0001
Hepatocellular iron grade >1 (%)	7.0%	8.4%	8.2%	10.1%	11.0%	0.11
Adherence during lead-in						
Pegylated IFN > 80% of prescribed dose	71.1%	74.5%	67.1%	63.5%	65.3%	0.025
Ribavirin > 80% of prescribed dose	65.4%	69.9%	63.5%	68.8%	68.0%	0.67
Pegylated IFN and ribavirin > 80% of prescribed dose	50.4%	58.8%	50.7%	51.9%	49.3%	0.41

N=1,090 unless otherwise noted. APRI, AST/platelet ratio index. *Genotype 1 versus others.

and was unaffected by treatment assignment ($P = 0.47$). Change in GGT activity was positively correlated with changes in histological steatosis ($r = 0.21$, $P < 0.0001$), alkaline phosphatase activity ($r = 0.24$, $P < 0.0001$), ALT activity ($r = 0.31$, $P < 0.0001$), serum ferritin concentration ($r = 0.25$, $P < 0.0001$), and modestly with Ishak inflammation score ($r = 0.078$; $P = 0.026$), but not with change in fibrosis score ($r = -0.041$, $P = 0.25$), change in BMI ($r = 0.03$, $P = 0.39$), AST/ALT ratio ($r = 0.05$; $P = 0.15$), or platelet count ($r = -0.04$; $P = 0.26$). Results were similar irrespective of treatment assignment. Particularly striking was the association with change in steatosis and with alcohol drinking, which were independently associated with change in GGT ($P < 0.01$). The mean change in GGT activity was −42 IU/L for the 274 patients who had a decrease in steatosis, −3 IU/L for the 430 patients with no change, and 44 IU/L for the 189 patients with an increase in steatosis ($P < 0.0001$). The mean change in GGT was −54 IU/L for the 44 patients who reported stopping drinking, −3 IU/L for the 737 patients whose drinking status did not change, and 37 IU/L for the 89 patients who reported that they had started drinking ($P = 0.019$). The association with change in GGT was accentuated when both variables were considered together. The mean change in GGT was −112 IU/L for the 14 patients who stopped drinking and steatosis decreased, 4 IU/L for the 332 patients with no change in drinking or steatosis, and 191 IU/L for the 16 patients who started to drink and steatosis increased.

Table 2. Cross-Sectional Associations With of GGT Activity at Initial Examination

Variable	Parameter Estimate	SE	F-statistic	Pr>F
Intercept	-0.673	0.281	5.77	0.017
Male	0.612	0.087	49.00	<0.0001
Non-Hispanic black	0.214	0.104	4.21	0.040
IL28B rs12979860 CC	-0.804	0.084	91.26	<0.0001
Genotype 1	0.261	0.110	5.60	0.018
ALT per 100 IU	0.477	0.069	47.16	<0.0001
Alkaline phosphatase per 100 IU	0.715	0.092	60.95	<0.0001
AST/ALT	0.876	0.171	26.3	<0.0001
AFP per 10 ng/mL	0.048	0.016	9.53	0.002
APRI	-0.082	0.035	5.63	0.018
Log_{10} ferritin	0.601	0.086	48.46	<0.0001
Hepatic steatosis (4 levels)	0.343	0.042	67.33	<0.0001

Multivariate linear regression with backwards selection based on $P < 0.05$ (n=981). APRI, AST/platelet ratio index.

Discussion

The current report includes several new and confirmatory findings regarding the prognostic significance

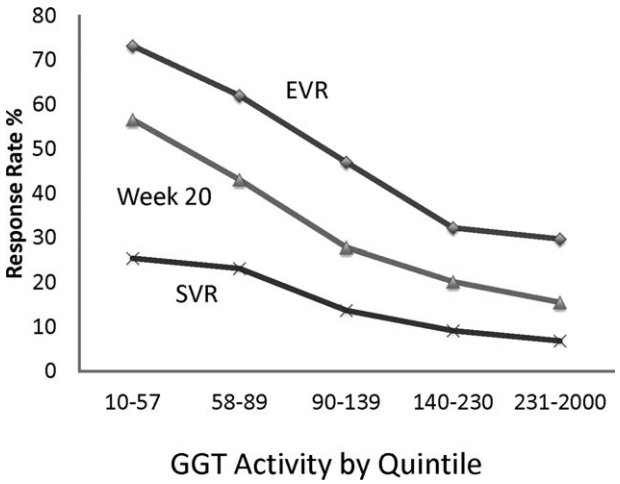

Fig. 1. Association of quintile of GGT activity (IU/L) with probability of virological response during the lead-in phase of HALT-C. EVR, early virological response; SVR, sustained virological response.

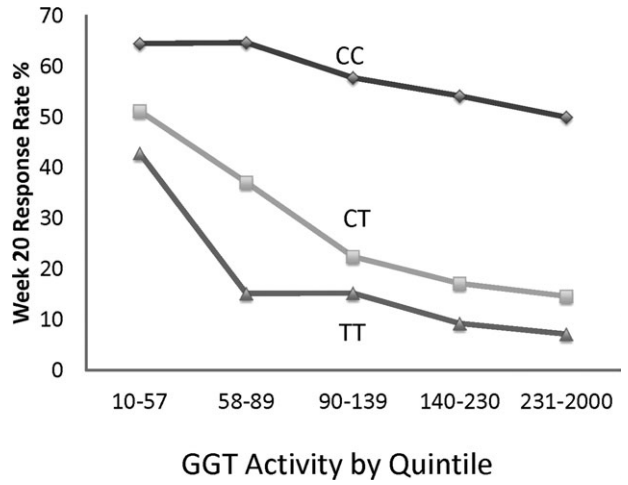

Fig. 2. Joint association of quintile of GGT activity (IU/L) and IL28B rs12979860 allele with week 20 virological response during the lead-in phase of HALT-C.

of GGT activity in chronic HCV. These findings pertain both to treatment response and to disease outcome.

Treatment Response. We confirmed that higher GGT activity is associated with lower probability of virological response to IFN-based treatment for HCV.[9-14] Compared with previous studies, HALT-C was unique in its size, prospective patient characterization, and in the homogeneity of the patient population, all of whom had advanced fibrotic liver disease and previous treatment with IFN. All patients underwent a uniform treatment protocol and careful measurement of adherence, neither of which had a meaningful effect on the association of virological response with GGT. A new finding concerned the associations with IL28B rs12979860 genotype and GGT activity with treatment outcome. IL28B rs12979860 is a noncoding single nucleotide polymorphism (SNP) residing 3 kb upstream of the IL28B gene, which encodes IFN-λ3.[15] GGT was strongly associated with the T allele, whose presence reduces the likelihood of response to therapy. While this finding confirms that of another study,[16] it must be noted that previous nonresponse to prior treatment could have biased the distribution of patients towards an overrepresentation of patients with TT genotype and high GGT. Of greater significance, patients with at least one copy of the T allele had poorer virological response with increasing GGT. In fact, patients homozygous for the T allele and in the highest quintile of GGT had a very low on treatment response rate (Fig. 2) and almost no chance of sustained virological response. In contrast, CC homozygotes had a favorable response rate that was relatively unaffected by GGT activity. It is not clear why GGT activity appears to potentiate the effect of the rs12979860 genotype.

Disease Outcomes. Among patients with chronic HCV, higher GGT activity has been associated with more severe liver disease in a number of cross-sectional studies.[17-23] GGT is also a component of two scores that were constructed for noninvasive evaluation of fibrosis stage.[24,25] However, cross-sectional studies provide inconclusive evidence that GGT is associated with liver disease progression. There are far fewer studies that have evaluated GGT in disease progression, and they were conducted among smaller or more heterogeneous patient populations than HALT-C.[26,27] A common problem with studies of disease progression is that few included GGT in the evaluation of risk factors, perhaps because it was not measured routinely. Therefore, it is significant that the current study demonstrated a statistically robust, increased rate of fibrosis progression and of clinical outcomes among patients with higher GGT. In particular, GGT activity was independent of previously established predictors, including histological features, for fibrosis progression and liver disease outcomes in the HALT-C cohort.[28] GGT activity was also associated with increased risk of death and of liver transplantation. The association was not seen for HCC, perhaps because of different pathways to development of HCC than for other outcomes. However, other studies found an independent association of GGT activity and HCC in the general population and among patients with HCV.[27,29,30]

It is uncertain why GGT is associated with poorer prognosis with chronic hepatitis C, as well as greater

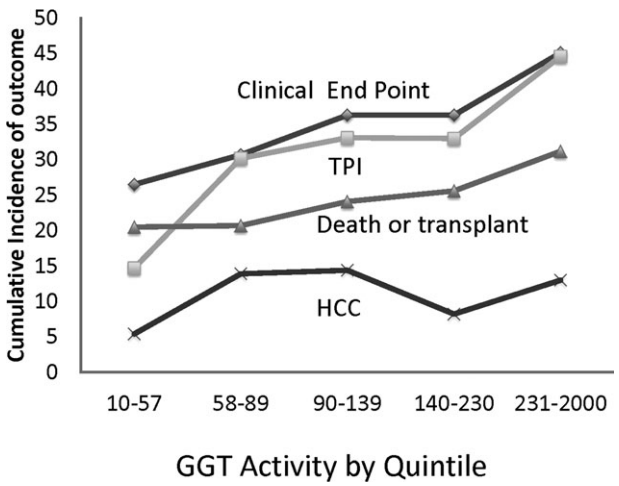

Fig. 3. Cumulative incidence of outcomes according to quintile of GGT activity (IU/L): increase of at least two points (TPI) in Ishak fibrosis score on paired biopsies and of any clinical endpoint (hepatic decompensation, hepatocellular carcinoma, or death), death or transplantation, and HCC within 7 years.

Table 3. Association of GGT Activity (per quintile) With Outcomes

		Univariate Analysis*			Multivariate Analysis		
	Number of Outcomes/ Number at Risk	Hazard Ratio	95% CI	*P*-value	Hazard Ratio	95% CI	*P*-value
Any endpoint†	299/999	1.21	1.11-1.31	0.0001	1.14‡	1.05-1.24	0.0024
Death	133/999	1.21	1.07-1.38	0.0023	1.15‡	1.01-1.30	0.035
Death or liver transplant	202/999	1.19	1.08-1.32	0.0006	1.13‡	1.02-1.25	0.019
Hepatocellular carcinoma (HCC)	83/997	1.17	1.00-1.37	0.053	1.07§	0.89-1.28	0.46
2 point fibrosis score increase	146/518	1.24	1.11-1.39	0.0002	1.15|	1.02-1.30	0.023

*Adjusted for treatment group.

†Death, decompensated liver disease, or HCC.

‡Adjusted for treatment group, baseline platelet count, AST/ALT ratio, albumin, total bilirubin, and Ishak fibrosis score.

§Adjusted for treatment group, cirrhosis, BMI, baseline platelet count, AST, and smoking history.

\|Adjusted for BMI, platelet count, and hepatic steatosis.

severity of other liver diseases and with a number of diverse conditions, such as cardiovascular disease, type 2 diabetes, various malignancies, and overall mortality.[2-4,30-37] One likely reason is that GGT is a marker of oxidative stress, especially in its relationship to GSH metabolism. GSH is a major antioxidant, for which the liver is a net synthesizer.[38] Maintenance of a pool of reduced GSH is especially important during periods of oxidative stress. Extracellular GSH and its oxidized form, GSH disulfide, are broken down to their constituent amino acids by GGT and then transported back into cells for resynthesis of GSH. As the only enzyme of the γ-glutamyl cycle located on the outer surface of the plasma membrane, GGT plays a key role in GSH homeostasis by providing cysteine, the rate-limiting substrate, for intracellular synthesis of GSH.[39] It has been suggested that catabolism of GSH by GGT results in prooxidant metabolites.[40] As an adaptive response to exposure to oxidants, the expression of GGT increases, although the mechanisms for induction are uncertain.[41,42] At the population level, GGT activity has been positively associated with C-reactive protein, a general marker for increased oxidative stress.[43]

Other Associations. It is interesting that GGT activity was associated with fibrosis stage and cirrhosis at baseline and predicted fibrosis progression, but a change in fibrosis score was not associated with change in GGT. Nor was a change in GGT activity correlated with changes in platelet count or AST/ALT, which are markers of development of cirrhosis. These findings suggest that GGT is a marker of disease activity, and not merely a reflection of disease severity, such as platelet count, which declines as cirrhosis and portal hypertension develop. This finding provides additional, albeit indirect evidence that GGT reflects a state of oxidative stress in chronic HCV. It is also interesting that ALT was not independently associated with treatment response or with disease progression and that AST was associated with week 20 virological response but not disease progression. Thus, in the setting of HCV associated advanced liver disease, GGT has greater prognostic significance than ALT or AST.

Given the prognostic significance of GGT, we examined other patient characteristics with which GGT was associated, a few of which are stressed here. The mechanisms whereby hepatic steatosis and elevated GGT are associated are not entirely clear, but several have been proposed.[44] For example, fatty liver could cause hepatocellular damage that would simulate the synthesis of GGT. Alternatively, excess fat in the liver could enhance oxidative stress, leading to overconsumption of GSH with a compensatory increase in GGT synthesis. Finally, a higher GGT production could be secondary to a low-grade hepatic inflammation induced by hepatic steatosis. PNPLA3 genotype was strongly related to steatosis and steatosis strongly related to GGT, but there was not an association of PNLP3 with GGT activity, which was also the case in at least one other study.[22] Thus, it appears that the mechanism for the relationship of PNPLA3 with steatosis is likely different from that of steatosis with GGT activity. A change in GGT activity was also correlated with change in alcohol consumption, which was expected, given our previous report that demonstrated a change in hepatic steatosis with a change in drinking.[45] It is interesting that a change in alcohol was associated with GGT change independent of a change in steatosis. GGT change was inversely associated with coffee consumption, as found in other studies that were not restricted to patients with HCV.[46-48] Furthermore, coffee consumption has previously been found to be associated with improved treatment response and slower disease progression in HALT-C. This association deserves greater scrutiny. Ferritin concentration was strongly associated with both baseline GGT and with a change

in GGT activity. Ferritin is a marker of oxidative stress, which may explain the strong correlation with GGT activity.[49]

HALT-C provided a strong platform for evaluation of prognostic factors in chronic HCV. Advantages included a large sample size, relatively homogeneous population, careful attention to uniform data collection on a wide variety of variables, and a high patient retention rate. Although GGT activity was strongly associated with treatment response and with disease progression, these results are not necessarily generalizable to all patients with HCV. HALT-C was restricted to patients with advanced liver disease who had not cleared virus with IFN treatment and were motivated to participate in a clinical trial. Furthermore, as new treatments are introduced, GGT may not be as strong a predictor of treatment response. However, given the results of this and other studies, it is likely, that GGT will be associated with poorer response at least as long as IFN-based therapy is used.

In conclusion, GGT activity was found to predict treatment response and liver disease outcomes in a large cohort of patients infected with chronic HCV and advanced fibrotic disease. Although confirmation from other studies is needed regarding the prognostic significance of GGT, results of the current study suggest that greater attention should be given to GGT activity.

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