

Association of *CTLA4* Polymorphisms with Sustained Response to Interferon and Ribavirin Therapy for Chronic Hepatitis C Virus Infection

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Cytotoxic T lymphocyte antigen-4 (CTLA4) suppresses cytotoxic T lymphocyte activity. We examined the associations of *CTLA4* single-nucleotide polymorphisms (SNPs) at promoter site -318 and exon-1 site 49 with clearance of hepatitis C virus (HCV) after treatment with combination interferon- α plus ribavirin (IFN- α + R) therapy in 79 white sustained responders (SRs) and 79 nonresponders (NRs). SRs had higher frequencies of 49G, alone (odds ratio [OR], 2.3; $P = .042$) and tightly linked with -318C in a haplotype (OR, 2.4; $P = .030$). Homozygosity for the -318C-49G haplotype was even more frequent among SRs (OR, 5.2; $P = .049$). Comparably strong associations persisted after multivariable analysis. Relationships were not seen with non-1 genotype viruses (OR, 0.93–1.25; $P > .25$). Virus load also declined more rapidly in carriers of both 49G ($P = .0095$) and the -318C-49G haplotype. *CTLA4* 49G in exon 1 alone and in a haplotype with -318C promoter is associated with sustained IFN α + R response in white patients with HCV genotype 1 infection.

Interferon- α (IFN- α)—the standard short-acting forms and the increasingly used newer long-acting pegylated

interferons [1]—in combination with ribavirin (IFN- α + R) is currently the standard regimen for the treatment of chronic hepatitis C virus (HCV) infection, but its cost and multiple, often debilitating side effects are significant drawbacks [2]. Moreover, only 40%–80% of suitable candidates treated with this regimen are sustained responders (SRs), which is defined as having achieved an undetectable HCV-RNA level at 6 months after discontinuation of therapy [3, 4]. The shortcomings of this regimen have prompted a search for factors that could predict those who would most likely benefit from it. Candidate predictors now include viral genotype, age, race, and early disappearance of HCV-RNA after initiation of therapy (viral dynamics) [5].

Clearance of HCV viremia naturally and in the context of IFN- α + R therapy have been correlated with a strong immune response. Both CD4⁺ T helper and CD8⁺ cytotoxic T lymphocyte (CTL) responses are important in the response to HCV infection [6–16]. Host genetic factors that govern these responses also may

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modify the course of HCV infection. Polymorphisms in HLA molecules, as well as cytokine genes, appear to be associated with natural clearance and histologic progression of HCV infection [17]. We and others have recently described the association of a variant in the interleukin-10 (*IL10*) gene promoter with both initial and sustained response to IFN- α + R therapy among patients chronically infected with HCV [18, 19].

The CTL antigen-4 (CTLA4), encoded by a gene on chromosome 2q33, is expressed on activated CD4⁺ and CD8⁺ T cells [20]. It binds to the ligands B7-1 (CD80) and B7-2 (CD86) [21–23] and down-regulates T cell function [22, 24–27]. Mice deficient in CTLA4 exhibit polyclonal T cell activation and proliferation [28–30]. A single nucleotide polymorphism (SNP) resulting in a C→T transition at position –318 of the *CTLA4* promoter has been described elsewhere [31]. In addition, a G→A transition at position 49 in exon 1 of the *CTLA4* gene encodes an alanine (Ala)→threonine (Thr) substitution in codon 17 of the leader peptide [32, 33].

Recent functional studies have produced consistent evidence that polymorphisms at position –318 of the promoter, position 49 in exon 1, and the resulting haplotypes formed by the variants of these 2 loci exert a differential functional effect on CTLA4-driven down-regulation of T cell activation [34–37].

Considering the possible importance of CTL responses in HCV clearance, the involvement of CTLA4 regulating those responses, and the evidence that variations in the *CTLA4* gene alter its expression, we examined whether the 2 potentially functional SNPs were associated with sustained response to IFN- α + R therapy in a group of individuals chronically infected with HCV.

SUBJECTS, MATERIALS, AND METHODS

Study subjects and viral genotyping. Among individuals with chronic HCV referred to the University of Alabama at Birmingham (UAB) Liver Center, 312 received IFN- α + R therapy under a clinical trial protocol. Suitability for therapy was determined by inclusion/exclusion criteria and guidelines established nationally and internationally [3, 4]. In brief, all subjects had compensated liver disease due to HCV, and none had other forms of chronic liver disease or other major comorbid conditions. All were seronegative for human immunodeficiency virus (HIV) type 1 and hepatitis B virus (HBV) [3, 4]. Demographics of the entire patient group have been described elsewhere [38].

By December 2000, ~170 individuals had completed therapy. In a nested case-control study, we included the first 79 consecutive white SRs and the first 79 consecutive white NRs who had completed therapy and consented to DNA genotyping. The number of black patients recruited into the protocol was too low to reach any meaningful conclusions. Individuals with non-1 genotype infections who responded (reached undetectable

viral RNA levels) by week 12 received treatment for a total of 6 months, whereas those with genotype-1 infections who responded by week 12 continued to receive therapy for 1 year.

We assessed HCV genotypes and HCV-RNA levels before, at weeks 4 and 12 during, and at 6 months after discontinuing treatment (National Genetics Institute, Los Angeles). Because this study was conducted before quantification of virus load in international units (IU) became routine, a conversion formula was used to approximate IU values from copies per milliliter: $\log_{10} \text{copies} = 1.066 (\log_{10} \text{IU}) - 0.0197$ [39]. An SR was defined as having had an undetectable plasma HCV RNA level at 6 months after discontinuation of therapy. This included individuals who were weakly positive for HCV-RNA at week 12 (<1000 copies/mL) but had no detectable HCV-RNA at 6 months after discontinuation of therapy (delayed responder). An NR was defined as having either detectable virus at week 12 of therapy, at which time treatment was discontinued, or detectable virus at 6 months after discontinuation of therapy, despite a virologic response during the initial course (flare-up). From trial patients who met these criteria, 79 SRs and 79 NRs were selected.

Alanine aminotransferase (ALT) levels were assessed by standard clinical laboratory techniques (Kirklin Clinic Laboratory). For categorical analyses, we used a threshold value of 1.5 times above the upper limit of normal (≥ 72 or < 72 IU/L), a commonly accepted definition of a “high” ALT level. HCV isolates were dichotomized as either 1 or non-1 genotype, because infections with genotype 1 viruses are less responsive and require a longer course of treatment [3, 4]. Baseline virus load was divided into “high” for individuals with a baseline virus load of 2×10^6 copies/mL (~850,000 IU) and “low” for individuals with a baseline virus load $< 2 \times 10^6$ copies/mL, the value adopted by most treatment algorithms for assessing probable response [1, 3, 4]. The threshold value for “older” and “younger” age was 40 years, as used by previous studies [3, 40].

DNA extraction and genotyping of allelic variants. Genomic DNA was extracted from whole blood using standard inorganic salt-out procedures. Polymerase chain reaction with sequence specific primers (PCR-SSP) was used to define the SNP in position 49 of exon 1 in the *CTLA4* gene. Four PCR-SSP reactions were used to type 2 *CTLA4* SNPs at nucleotide positions 49 in exon 1 and –318 in the promoter with each reaction, including a pair of SNP-specific primers (table 1) and a pair of control primers (C5, 5'-TgC CAA gTg gAg CAC CCA A-3') and (C3, 5'-gCA TCT TgC TCT gTg CAg AT-3') specific for human DRB1 intron 3 [41]. The first 2 SSP reactions defined the 49A/G alleles when each of the 49A and 49G-specific primers was paired with the general primer (SSPg). The second 2 SSP reactions combined one of the –318C- and –318T-specific primers with another general primer (SSPf). The PCR mix (10 μ L each) consisted of 1 \times buffer C (60 mM Tris-HCl [pH 8.5],

Table 1. Oligonucleotides used to define *CTLA4* polymorphisms by polymerase chain reaction (PCR) with sequence specific primers.

Oligo specificities	Oligo name	Oligo sequence (5'→3') ^a	Annealing positions ^b
CTLA4 49A (17 Thr)	SSP1	gCT CAg CTg AAC CTg gCT A	1223→1241
CTLA4 49G (17 Ala)	SSP2	CTC AgC TgA ACC Tgg CT g	1224→1241
CTLA4 general	SSPg	ACA gAg CCA gCC Aag CCA	1424→1441
CTLA4 -318C ^c	SSP3	CCA CTT AgT TAT CCA gAT CCT C	854→875
CTLA4 -318T ^c	SSP4	CCA CTT AgT TAT CCA gAT CCT T	854→875
CTLA4 general ^c	SSPf	gCT TTg ATC CAg ATA TgT ATT ACA C	1056→1080

^a Sequences corresponding to the single-nucleotide polymorphism sites are shown in boldface type.

^b Based on GenBank sequence M74363.

^c The -318C/T allele-specific PCR product can be further confirmed through digestion with *MseI*.

15 mM NH₄SO₄, and 2.5 mM MgCl₂), 50–70 ng of genomic DNA, 0.3 U of AmpliTaq polymerase, 120 nM each control primer, 250 nM each *TAPI*-specific primer (positive control), 0.4 mM each dGTP, dCTP, dTTP and dATP, 10% (vol/vol) glycerol, and 0.02% cresol red. PCR cycling began with 10 higher-stringency cycles of denaturing at 95°C for 25 s, annealing at 62°C for 45 s, and extension at 72°C for 45 s, followed by 22 additional lower-stringency cycles of denaturing at 95°C for 25 s, annealing at 58°C for 40 s, and extension at 72°C for 40 s. Half of each PCR product was loaded directly onto 1.5% agarose gels for electrophoresis, and the SSP-banding patterns were recorded on photographs of ethidium bromide-stained gels. PCR-SSP-based typing of the -318C/T variants were validated by 100% match with results from restriction fragment-length polymorphisms (RFLP), since -318T contains an *MseI* site (TTAA). Our strategy for typing -318C/T and 49A/G was similar to procedures developed independently by another group of investigators, as described elsewhere [35], except that the orientation of the 49A/G-specific primers differed and that our PCR-SSP was done at a higher stringency, as verified by close resemblance between primer melting temperatures and PCR annealing temperatures. PCR-SSP-based genotyping of *IL10* variants was performed according to procedures reported elsewhere [19].

Statistical analyses. To analyze proportions of individuals carrying a variant, the number of patients with that variant was divided by the total number of patients (N); SRs and NRs were compared with χ^2 statistics for contingency tables. Odds ratios (ORs), 95% confidence intervals, and *P* values (both maximum likelihood and Fisher's exact) were calculated as appropriate. For analysis of overall variant frequencies, because each individual carries 2 allelic variants at a given locus (1 per chromosome), each variant observed was counted separately, with the total number of observations divided by the total number of chromosomes (2N). At a given site 2 allelic variants are paired in any individual; whether they are the same 2 (homozygosity) or different, each pairing in individuals represents a correlation.

For this correlation, statistical adjustment was made, and *P* values were calculated using a "spatial correlation adjustment" in the PROC MIXED procedure in SAS [42].

Alleles at adjacent loci in linkage disequilibrium form haplotypes. To measure the strength of linkage disequilibrium between variants at the promoter and exon 1 loci, delta and χ^2 values were calculated according to standard methods described elsewhere [43]. Haplotypes were inferred from the relative values of these statistics for each allele combination. The putative promoter and exon-1 haplotypes were verified by comparison with previous studies [24, 44]. On pairs of chromosomes, 2 alleles at a single locus or 2 multilocus haplotypes form a genotype. For genotype frequencies, the number of patients with each was counted once and divided by the total number of patients (N).

Comparisons of the effects of carriage of variants on baseline virus load levels were tested using the Wilcoxon rank-sum test (2-tailed *P* value), including all 158 white patients with all viral genotypes. We used a multiple logistic regression model to adjust for covariates thought to modify therapeutic response using the entire cohort of 158 white patients and those with genotype-1 infections only. SRs to IFN- α + R were compared with NRs as the referent group.

To assess the effects of genetic variants on viral dynamics during the course of treatment, longitudinal analysis using mixed models was employed [42]. This time series analysis method is analogous to the repeated measures procedure based on analysis of variance (ANOVA). This form of longitudinal analysis is more robust than the conventional repeated measures procedure and allows for unequal measurement intervals. The SAS PROC MIXED procedure in SAS software (version 8.1) was used for longitudinal analysis, ensuring a parametric distribution appropriate for this statistical procedure by taking natural log-transformed virus load measurements (baseline, week 4, and week 12). For corroboration, we also compared the differences in virus load measurements at 3 time points: baseline, week 4, and week 12 of therapy.

Table 2. Baseline characteristics of white patients with hepatitis C virus infection enrolled in trials of interferon- α and ribavirin at the University of Alabama at Birmingham.

Characteristic	Comparison of the entire cohort with white patients selected for the present study		Comparison of selected sustained responders (SRs) and nonresponders (NRs)	
	Entire cohort (n = 312)	Selected study group (n = 158)	SRs (n = 79)	NRs (n = 79)
Baseline virus load	$2.82 \times 10^6 \pm 1.96 \times 10^6$	$2.97 \times 10^6 \pm 1.98 \times 10^6$	$2.61 \times 10^6 \pm 2.05 \times 10^6$	$3.32 \times 10^6 \pm 1.84 \times 10^6$
IU ^a	$1.17 \times 10^6 \pm 8.34 \times 10^5$	$1.23 \times 10^6 \pm 8.42 \times 10^5$	$1.09 \times 10^6 \pm 8.70 \times 10^5$	$1.37 \times 10^6 \pm 7.86 \times 10^5$
Baseline ALT level, IU/L	105 \pm 81.9	105.2 \pm 82.8	110.6 \pm 93.6	99.4 \pm 71.6
Age in years at treatment	45 \pm 8.8	44.5 \pm 8.1	43.5 \pm 7.9	45.2 \pm 8.1
HCV genotype type-1/non-1, %	73.4/26.6	70.9/29.1	49.4/50.6	92.4/7.6
Male/female, %	55.3/44.7	57.3/42.7	51.3/48.7	63/37

NOTE. Data are mean \pm SD, unless otherwise indicated.

^a International units approximated using a conversion factor described elsewhere [39].

RESULTS

Patient selection and characteristics. Patients selected for the present study were quite representative of the entire UAB cohort in their characteristics at enrollment (table 2). SRs and NRs differed as expected in age, sex, virus load, viral genotype, and ALT—all characteristics previously reported to influence [3, 4] response to treatment. Among those with genotype 1 and non-1 infections, 39 and 40, respectively, were SRs, whereas 73 and 6, respectively, were NRs. Only 1 individual who was positive at week 12 achieved sustained response.

Distribution of CTLA4 promoter and exon 1 allelic variants and haplotypes. The frequencies of the CTLA4 promoter and exon 1 genotypes in white patients approximated Hardy-Weinberg equilibrium and were comparable with those reported elsewhere (for the promoter: C/C, 83.6%; C/T, 14.6%; and T/T, 1.8%; for exon 1: G/G, 10.4%; G/A, 44.8%; and A/A, 44.8%) [35, 44]. Likewise, this and earlier studies documented strong linkage disequilibrium between exon 1 position 49 and promoter -318 alleles [35, 44]; details on possible haplotype combinations are presented in table 3 [43].

In univariate analysis of white SRs with genotype-1 infections, 49G allele carriers (OR, 2.3; $P = .042$) and 49G/G genotype homozygotes (OR, 5.2; $P = .049$) were significantly more frequent among SRs (table 4). Carriers of the promoter -318C variant were frequent among both SRs and NRs, but more so among SRs. As measured by allele frequency based on 2N chromosomes, a 2-fold higher likelihood of response was seen with both the 49G (OR, 2.1; $P = .017$) and the -318C variant, either unadjusted or adjusted for correlation of alleles (data not shown). Carriage of the tightly linked -318C-49G haplotype also conferred a 2-fold higher likelihood of response (OR, 2.4; $P = .030$). The magnitude of the association of SR with homozygosity for the haplotype was even higher (OR, 5.2; $P = .04$). The nearly exclusive haplotype pairing of 49G with

-318C prevented separate analysis of 49G alone; every effect seen with 49G also occurred with the -318C-49G haplotype at the same magnitude. The dimorphic nature of the -318 and 49 SNPs produced reciprocal effects (inhibition of response) for the -318T and 49A variants and carrier frequencies. Neither the 49G nor the haplotype effect was observed in patients infected with non-1 genotype viruses ($P > .25$ for all).

Multivariable analyses. The associations of the 49G variant and the corresponding -318C+49G haplotype persisted after multivariable adjustment for baseline characteristics (sex, age at treatment, interaction between age at treatment and sex, baseline virus load, baseline ALT level, and possession of the IL10 [108] TCATA haplotype; table 5) in both the group of 158 patients and in the subgroup with genotype 1 infections. Control for effect of homozygosity for the IL10 (108) TCATA haplotype previously reported to be associated with SR to IFN- α + R [19] was precluded by the small number of individuals with this haplotype ($n = 5$). However, when considered jointly with the CTLA4 variant, carriage of a single IL10 (108) TCATA haplotype, also previously associated with SR, actually demonstrated a stronger association (OR, 3.0, when adjusted for

Table 3. Tests of linkage disequilibrium between CTLA4 promoter and exon 1 polymorphisms.

-318 SNP	49 SNP	Haplotype frequency, %	Delta ^a	RO (95% CI)	P
T	A	8.5	0.02746	22.4 (3.0–165.9)	<.0001
T	G	0.3	0.00618	1.5 (5.1–87.0)	.30
C	G	33.0	0.05013	21.0 (5.1–87.0)	.0001
C	A	58.2	0.03967	2.9 (1.8–4.9)	.0001

NOTE. CI, confidence interval; RO, relative odds; SNP, single nucleotide polymorphism.

^a Delta (difference between observed and expected haplotype frequencies) is a measurement of the strength of linkage disequilibrium [43].

Table 4. Differential distribution of allele frequency, allele carriage, and genotype carriage for the -318 single nucleotide polymorphism (SNP), the 49 SNP, and the -318-49 haplotype among 39 white sustained responders (SRs) and 73 nonresponders (NRs) infected with genotype-1 hepatitis C virus.

Marker	SRs (n = 39)	NRs ^a (n = 73)	OR (95% CI)	P
Allele/haplotype (carriage) ^b				
-318C	38 (97.4)	70 (95.9)	3.8 (0.19-75.9)	.350
-318T	4 (10.3)	15 (20.5)	0.45 (0.14-1.48)	.186
49G	25 (64.1)	32 (43.8)	2.3 (1.0-5.1)	.042
49A	34 (87.2)	71 (97.2)	0.2 (0.04-0.9)	.049
-318C-49G	25 (64.1)	31 (20.5)	2.4 (1.1-5.4)	.030
Genotype (carriage)				
49G/G	5 (12.8)	2 (2.7)	5.2 (1.0-28.3)	.049
49A/A	14 (35.9)	41 (56.2)	0.4 (0.2-1.0)	.042
-318C-49G/-318C-49G	5 (12.8)	2 (2.7)	5.2 (1.0-29.2)	.049
-318C-49A/-318C-49A	12 (30.8)	33 (45.2)	0.5 (0.3-1.3)	.139

NOTE. Data are no. (%) of patients, unless otherwise indicated. CI, confidence interval; OR, odds ratio.

^a NRs served as the referent group for all calculations.

^b Analyses performed using allele frequencies based on 2N chromosomes (SRs [n = 78] and NRs [n = 146]) showed comparable ORs and P values.

49G carriage or -318C+49G carriage; P = .02) than was originally reported (OR, 1.6; P = .30) [19]. High level (multiplicative) interaction due to carriage of 49G or its haplotype plus carriage of *IL10* (108) TCATA was not detected (data not shown).

Longitudinal analyses. In an analysis of the effect of these markers on viral dynamics during the initial 12 weeks of therapy among the entire group of 158, 49G carriers and noncarriers showed significant differences in virus load (figure 1). Viral RNA concentration declined more rapidly in 49G carriers (P = .0095); lower levels were seen in carriers at each successive point, with the greatest difference occurring by week 4 (figure 1). Both the apparent acceleration in response among 49G carriers and the interaction between 49G carriage and time on viral dynamics were significant. Again, patients with the -318C+49G haplotype and with HCV genotype 1 only showed comparable longitudinal effects (data not shown).

DISCUSSION

Polymorphisms in *CTLA4* may augment the current array of predictors of therapeutic response to IFN- α + R. For genotype-1 infection in white SRs, we observed associations of the allelic variants and the homozygous genotypes consisting of the exon 1 49G and the linked promoter SNP -318C. Tight linkage disequilibrium of 49G with -318C accounted for the apparent advantage for SRs due to each marker individually and to the

corresponding -318C+49G haplotype; the 49G and the haplotype effects could not be distinguished from each other. The relationships of carriage of these markers were independent of previously described cofactors for response (e.g. age, sex, baseline virus load, and the *IL10* [108] TCATA polymorphism). Our finding that treated carriers with specific *CTLA4* variants reached lower levels of viremia more rapidly than treated persons not carrying them further attests to the likely biologic/clinical significance of the association.

In our study population, the apparent advantage of the one or both alleles, or the corresponding haplotype, was confined to infection with genotype-1 virus in white patients. Genotype-1 viruses may differ intrinsically from the others in the magnitude or quality of the T cell response they elicit. In a study of the differential effect of IFN on antiviral T cell responses, those infected with non-1 virus had stronger IFN-induced T cell responses to HCV core antigen [45].

Table 5. Multivariable analyses of the effects of the 49G allele and -318C-49G haplotype carriage on sustained response to interferon- α plus ribavirin in 158 white patients.

Multivariable model	OR (95% CI)	P
49G		
49G allele carriers	2.0 (1.02-3.97)	.04
Baseline virus load	1.7 (0.85-3.40)	.14
Baseline ALT level	0.98 (0.487-1.96)	.95
Sex	0.04 (0.002-0.91)	.04
Age at treatment	0.27 (0.03-2.7)	.26
<i>IL10</i> (108) TCATA allele carriage	3.0 (1.2-7.4)	.02
Interaction between sex and age	4.1 (0.77-22.2)	.1
-318C-49G		
-318C+49G haplotype carriers	2.0 (1.0-3.9)	.04
Baseline virus load	1.7 (0.87-3.5)	.12
Baseline ALT level	1.0 (0.49-2.0)	.95
Sex	0.04 (0.002-0.9)	.04
Age at treatment	0.26 (0.025-2.65)	.25
<i>IL10</i> (108) TCATA allele carriage	3.0 (1.2-7.3)	.02
Interaction between sex and age	4.2 (0.77-22.3)	.1
49G among those with genotype-1 infections only		
49G allele carriers	2.5 (1.1-6.0)	.04
Baseline virus load	2.2 (0.946-5.14)	.07
Baseline ALT level	1.4 (0.58-3.6)	.43
Sex	0.3 (0.009-9.51)	.49
Age at treatment	0.41 (0.02-7.0)	.54
<i>IL10</i> (108) TCATA allele carriage	2.6 (0.81-8.5)	.11
Interaction between sex and age	1.1 (0.18-6.4)	.94

NOTE. ALT, alanine aminotransferase; CI, confidence interval; IL, interleukin; OR, odds ratio.

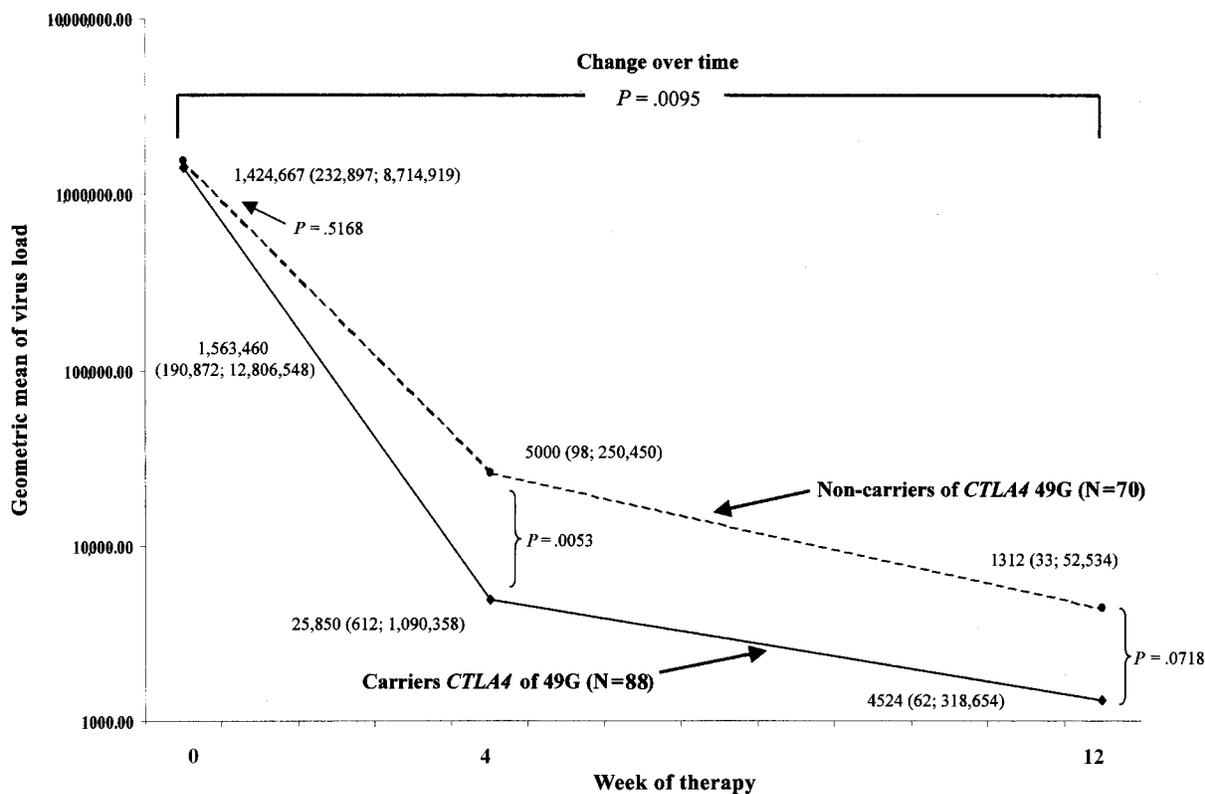


Figure 1. Effects of *CTLA4* 49G variant carriage on viral dynamics during the first 12 weeks of therapy among the 158 white patients included in the present study. The geometric mean of hepatitis C virus (HCV) RNA levels (copies/mL) among 88 G carriers and 70 non-G carriers are plotted (log scale) against the duration of therapy (weeks). Measurements (mean \pm SD) are shown for weeks 0, 4, and 12. Effects of the $-318C-49G$ haplotype were virtually identical (data not shown).

Any of several mechanisms may account for our observations. First, the polymorphisms, either individually or as a haplotype, may confer a difference with respect to gene expression. Carriage of the $-318T$ allele, compared with $-318C/-318C$ homozygotes, has been associated with increased levels of *CTLA4* mRNA [37]. Similarly, 49G has been shown to have a reduced effect on *CTLA4* up-regulation [36]. Another study of the functional effects of the haplotypes formed by these 2 loci demonstrated increased expression of *CTLA4* by the $-318T-49A$ haplotype [35]. Thus, investigative efforts to date consistently suggest that $-318C$, 49G, or the $-318C-49G$ haplotype plays a role in down-regulating *CTLA4*, presumably thereby amplifying the T cell response in some individuals. Alternatively, since *CTLA4* ligands B7-1 (CD80) and B7-2 (CD86) may play a role in the Th1/Th2 developmental pathways, polymorphism in *CTLA4* could shift the Th1/Th2 balance [46–48]. A third possibility is that these SNPs are in linkage disequilibrium with an adjacent marker that, by itself or as part of the haplotype, may alter *CTLA4* expression and/or function. Such a marker could instead account for the association by some other mechanism that enhances the T cell responses believed to gov-

ern both natural acute viral clearance and equilibrium concentration of virus during IFN- α + R therapy [6, 49].

Our study had certain limitations. First, the small number of patients infected with non-1 HCV genotypes precluded meaningful analysis of associations between *CTLA* polymorphisms and response to therapy in those individuals. Second, the study was conducted before assays for plasma HCV concentration were standardized or results were reported in IU [50]. An HCV-RNA copy has not been officially defined, measurement variability between assays still exists, and the correlation of RNA copies with IU is uncertain [39, 50–55]. The factor we used to convert measured levels of HCV-RNA into international units only approximates the true value. Future studies attempting to replicate our observed epidemiologic associations may be more informative if they measure virus loads directly in international units. Third, this study was conducted in a single white population; the associations of *CTLA4* polymorphism with response to IFN therapy should be examined in other ethnic groups and populations.

As more genetic variants that affect interferon response are identified, rapid genotyping of those markers, presumably in-

cluding *CTLA4* variants, may become useful along with other prognostic factors, such as viral genotype in guiding the management of HCV infection. Such genetic testing could well provide a fast and cost-efficient alternative to direct measurement of cytokine levels.

In summary, successful viral clearance during therapy for HCV infection appears to depend on robust T cell responses facilitated by the reduction of CTLA4 levels, and diminished in vitro expression of CTLA4 has been found in the presence of *CTLA4* polymorphisms associated with improved viral clearance. With further clinical validation of this phenomenon, it will remain to be determined precisely how the *CTLA4* alleles/haplotype, along with the *IL10* (108) TCATA haplotype, influence the pathogenesis of HCV infection and its prognosis after antiviral therapy. Meanwhile, beyond the implication that the costimulatory pathway involving CTLA4 affects the outcome of therapy for HCV infection, there is the prospect that the *CTLA4* gene or its product might represent a separate target for therapeutic intervention.

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