

Hepatitis C Virus Dynamics during Natural Infection Are Associated with Long-Term Histological Outcome of Chronic Hepatitis C Disease

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Background. The long-term dynamics of hepatitis C virus (HCV) infection and their association with hepatitis C disease are unknown.

Methods. Fifty-two treatment-naive subjects with chronic HCV genotype 1 infection were selected from the Alaska Natives and American Indians cohort. Viral RNA levels were measured in 223 specimens (mean, 4.3 specimens/subject) over 457 patient-years. Viral quasispecies diversity was analyzed in 187 specimens (mean, 3.6 specimens/subject) over 365 patient-years.

Results. Thirty-three subjects had minimal hepatic fibrosis, and 19 developed bridging fibrosis or cirrhosis. There was no significant difference in host variables, including alcohol consumption, between disease groups. Subjects with mild disease had higher serum RNA levels after 2 decades of infection ($P = .013$), greater fluctuations in RNA levels over time ($P = .04$), higher intraspecimen quasispecies diversity ($P = .001$), and higher rates of quasispecies diversification ($P = .004$) than did subjects with severe disease. On multivariate analysis, the odds of having severe disease were 15.3 (95% confidence interval, 2.3–99.6) times higher among persons with low quasispecies diversification rates compared with the odds among persons with high diversification rates.

Conclusions. Histological progression of hepatitis C is tightly associated with homogenization of HCV quasispecies, perhaps reflecting immune failure and/or selective outgrowth of aggressive viral variants.

The disease course of chronic hepatitis C is highly variable [1], and meta-analyses implicate host factors, such as alcohol use, age, and sex, as the most important risk factors for progression of hepatitis C [2]. Immunosuppression in the setting of HIV coinfection [3] and after liver transplantation [4] is also associated with hepatitis

C disease progression, perhaps indicating a protective role of host immunity. However, there is a paucity of longitudinal studies of hepatitis C virus (HCV) virology during natural infection. The consensus from cross-sectional studies is that serum HCV RNA levels are not associated with hepatitis C pathology at any specific time point of infection (reviewed in [5]). That immunosuppression is associated with higher HCV RNA levels [4, 6] is consistent with immune pressure playing a major role in determining the HCV viremia set point.

Immune pressure is also thought to be the primary force driving HCV quasispecies evolution in vivo [7, 8]. At least 4 different cross-sectional studies have concluded that high quasispecies diversity correlates with more severe liver disease [9–12], whereas at least 5 other studies have failed to find any relationship between HCV diversity and liver disease severity [13–17]. In contrast, a single small longitudinal study of HCV diversity over time found low rates of quasispecies diversification in subjects who developed severe liver dis-

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ease [18]. Furthermore, studies in liver transplant recipients and HIV-coinfected patients have been highly consistent in reporting that HCV quasispecies diversity decreases over time in subjects with progressive liver disease [19–25].

Given the conflicting results regarding the relationship between HCV quasispecies evolution and disease progression during natural infection, we studied the viral dynamics over time in 52 immunocompetent and treatment-naive subjects with chronic HCV genotype 1 infection and known disease status based on current liver biopsy results. The study was conducted in an extremely well-characterized cohort of Alaska Natives and American Indians (AN/AI cohort) with serum specimens that have been archived for >3 decades.

SUBJECTS, MATERIALS, AND METHODS

Study subjects. Details of the AN/AI cohort have been described elsewhere [26]. The Alaska Area Native Institutional Review Board (IRB), the Indian Health Service National IRB, 3 regional Alaska Native health boards, and the University of Washington IRB all approved the present study. Liver biopsies were performed only when clinically indicated and were scored for necroinflammatory activity and fibrosis by use of the Knodell scoring system [27]. Subjects classified as having severe disease had bridging fibrosis (stage 3) or cirrhosis (stage 4) documented by liver biopsy either before ($n = 4$) or within the period of virological testing ($n = 15$). Subjects classified as having mild disease had either no (stage 0) or mild (stage 1) fibrosis in liver biopsy samples obtained at an average interval of 0.1 years before study completion ($n = 33$). The estimated date of HCV infection was assigned on the basis of the date of first injection drug use or blood transfusion exposure (before July 1992) and was correlated with seroconversion data generated from stored serum specimens, as described elsewhere [28]. Of 355 HCV genotype 1–infected patients in the cohort, 52 were selected for longitudinal study of viral dynamics on the basis of the number of historical serum specimens available for testing (minimum of 3 specimens spanning at least 5 years), completion of a current liver biopsy, and absence of laboratory or clinical evidence of hepatitis B virus or HIV coinfection. Serum specimens were archived on a random basis.

Determination of HCV infection parameters. Chronic HCV infection was confirmed by detection of anti-HCV antibodies (EIA2.0 [Abbott Laboratories] and RIBA [Orthodiagnosics]), with confirmation of viremia by reverse-transcription polymerase chain reaction as described elsewhere [29]. Levels of HCV RNA in serum were determined by use of bDNA2.0 (Bayer) combined with an end-point dilution assay (Roche Amplicor; Roche Diagnostic Systems) [30], with a final detection limit of 40 IU/mL. Results were converted to international units per milliliter by use of the manufacturer's rec-

ommended conversion factor of 5.8 genome equivalents/mL to 1 IU/mL. Interspecimen variability was calculated as the average absolute value of differences between HCV RNA levels in consecutive serum specimens. HCV genotype was determined by 5' restriction fragment–length polymorphism analysis, as described elsewhere [31].

HCV quasispecies were characterized by heteroduplex tracking analysis (HTA) and nucleotide sequencing of the envelope 2 gene hypervariable region, as described elsewhere [19, 20, 32, 33]. A heteroduplex mobility ratio (HMR) was calculated as an estimate of HCV genetic diversity on the basis of the migration of probe–target hybrids in nondenaturing polyacrylamide gels (see Results). Comparison of HMR over time for individual patients allowed calculation of $\Delta\text{HMR}/\text{year}$, the normalized rate of change in quasispecies diversity per year. Nucleotide sequence analysis was performed using an Applied Biosystems model 377 automated sequencer (ABI). Nucleotide sequences were optimally aligned using the CLUSTAL W program [34, 35].

Statistics. Comparisons of proportions were made using the χ^2 or Fisher's exact test as appropriate. Comparisons of continuous variables were made using the Wilcoxon rank sum test or t test as appropriate. P values for comparisons within the severe or mild disease group between different time points of HCV infection were derived using a paired t test. All statistical analyses were performed using the software SAS (version 8.0; SAS Institute) and StatXact (version 4.0; Cytel Corporation). A history of heavy alcohol consumption was defined as self-reported consumption of >5 drinks/day during any period in their lifetime. For comparisons of the characteristics of the virological-study participants to those of the cohort of persons with HCV genotype 1 infection, alanine aminotransferase (ALT) levels reported within 1 year of the consent date for entry into the long-term HCV follow-up study were used in analyses. Multivariate analyses to identify independent factors associated with more severe disease at biopsy were conducted by use of logistic regression. Variables with a univariate $P \leq .25$ were considered in the multivariate models. The variables HCV infection length (3–10, 11–20, and ≥ 20 years), change in serum viral load over time (0.0–0.2, >0.2–0.4, and >0.4 log equivalents/mL/year), and $\Delta\text{HMR}/\text{year}$ (0.0–0.015, >0.015–0.035, and >0.035) were split into terciles for entry into the multivariate model. Forward selection was used to evaluate main effects. Variables were considered to be confounders and remained in the model if their exclusion changed the value of the coefficient(s) of interest by >15%. Because of sample size, multivariate models were limited to the consideration of 3 variables. P values were considered to be statistically significant at the .05 level.

RESULTS

AN/AI cohort. The 52 HCV genotype 1–infected study subjects were similar to the overall AN/AI cohort subset of genotype 1–infected persons with regard to age, sex, residence, alcohol consumption, and duration of HCV infection but were more likely to have an ALT level above the upper limit of normal ($P < .01$). The latter difference most likely occurred because liver biopsy was performed only as clinically indicated, such that persons who had elevated ALT levels preferentially underwent biopsy. Of the 52 study participants, 33 (63%) had mild disease at biopsy, and 19 (37%) had severe disease at biopsy. Subjects with mild disease did not differ from those with severe disease with regard to age at biopsy, sex, location, risk factor, genotype, alcohol consumption (either lifetime or at the time of biopsy), ALT level, estimated duration of HCV infection, or age at HCV infection (table 1). Viremia levels were determined in 223 historical serum specimens (mean, 4.3 specimens/person), for a total follow-up period of 457 patient-years (mean, 8.8 patient-years/person). Quasispecies analysis was performed on 187 historical serum specimens (mean, 3.6 specimens/person), for a total follow-up period of 365 patient-years (mean, 7.0 patient-years/person). There was no difference in the number of specimens and length of follow-up between those with mild and those with severe disease (table 1).

HCV RNA levels and progression of liver disease. Quantification of HCV RNA in all samples from the study population indicated that mean viral RNA levels in serum increased with each successive decade of infection (figure 1A), in concordance with the findings of a previous study by Eyster et al. in patients with hemophilia [6]. HCV RNA levels were significantly higher during the fourth decade of infection than during the first decade ($P = .04$). When analyzed according to disease group, mean HCV RNA levels in all specimens from subjects with mild disease were not different than mean levels in all specimens from subjects with severe disease ($P = .39$) (figure 1B). However, HCV RNA levels were significantly higher in the mild disease group than in the severe disease group in all specimens obtained after 2 decades of infection ($P = .013$) (figure 1C). A significant increase in viral RNA levels was observed over time in the mild disease group ($P = .001$) but not in the severe disease group. Overall, specimens from subjects with mild disease had significantly greater interspecimen variability in HCV RNA levels than did specimens from subjects with severe disease ($P = .04$) (figure 1D).

HCV quasispecies and disease outcome. HCV quasispecies that were infecting subjects with advanced fibrosis were characterized over time and were compared with quasispecies that were infecting subjects with mild liver disease. Figure 2A demonstrates a highly significant correlation between the 2 methods used to characterize HCV quasispecies, HTA and nucleotide

sequencing ($R^2 = 0.8326$; $P < .001$). Figure 2B illustrates HCV quasispecies tracking in serial specimens from 2 subjects, one with severe disease and the other with mild disease, over identical 16-year intervals using the HTA technique. Quasispecies genetic diversification is reflected by the increasing upward shift in the probe-target hybrids on electrophoresis. The lack of shift in the radioactive band over a 16-year period in the subject who developed severe disease is remarkable considering the high rate of evolution associated with the HCV hypervariable region (reviewed in [36]).

Figure 2C shows a box plot comparing intraspecimen quasispecies diversity (expressed as HMR) for the 2 patient groups according to decade of infection. Subjects in the mild disease group had increasing quasispecies diversity within individual specimens with each successive decade of infection ($P < .001$), whereas the opposite trend was observed in subjects in the severe disease group. Differences between groups were highly significant after 2 decades of infection ($P = .001$). Subjects with mild disease had significantly greater rates of quasispecies diversification than did subjects with severe disease over the entire study interval ($P = .004$) (figure 2D). Finally, higher rates of quasispecies diversification correlated with higher mean viral

Table 1. Comparison of demographic characteristics of persons with mild and severe liver disease.

Characteristic	Disease		P
	Mild ^a (n = 33)	Severe ^b (n = 19)	
Age at biopsy, mean, years	42.7	43.5	.56
Sex, % female	70 (n = 23)	53 (n = 10)	.22
Urban/rural split, % urban	76 (n = 25)	68 (n = 13)	.57
Risk factor, %			1.00
IDU	55 (n = 18)	58 (n = 11)	
BT	21 (n = 7)	21 (n = 4)	
Other or none	24 (n = 8)	21 (n = 4)	
Genotype, %			.25
1a	64 (n = 21)	79 (n = 15)	
1b	36 (n = 12)	21 (n = 4)	
Alcohol at biopsy, %			
>50 g/day	12 (n = 4)	11 (n = 2)	1.00
Any	55 (n = 18)	32 (n = 6)	.11
Length of HCV infection at consent, mean, years	12.6	16.4	.18
Age at HCV infection, mean, years	30.1	26.1	.35
ALT level at biopsy, median, U/L	83	105	.11

NOTE. ALT, alanine aminotransferase; BT, blood transfusion; HCV, hepatitis C virus; IDU, injection drug use.

^a Histological stage 0 (no fibrosis) or 1 (minimal fibrosis) liver disease.

^b Histological stage 3 (bridging fibrosis) or 4 (cirrhosis) liver disease. Repeat liver biopsies performed in 17 subjects over a mean follow-up of 7 years showed no change in histological disease status in 15 of 17 cases.

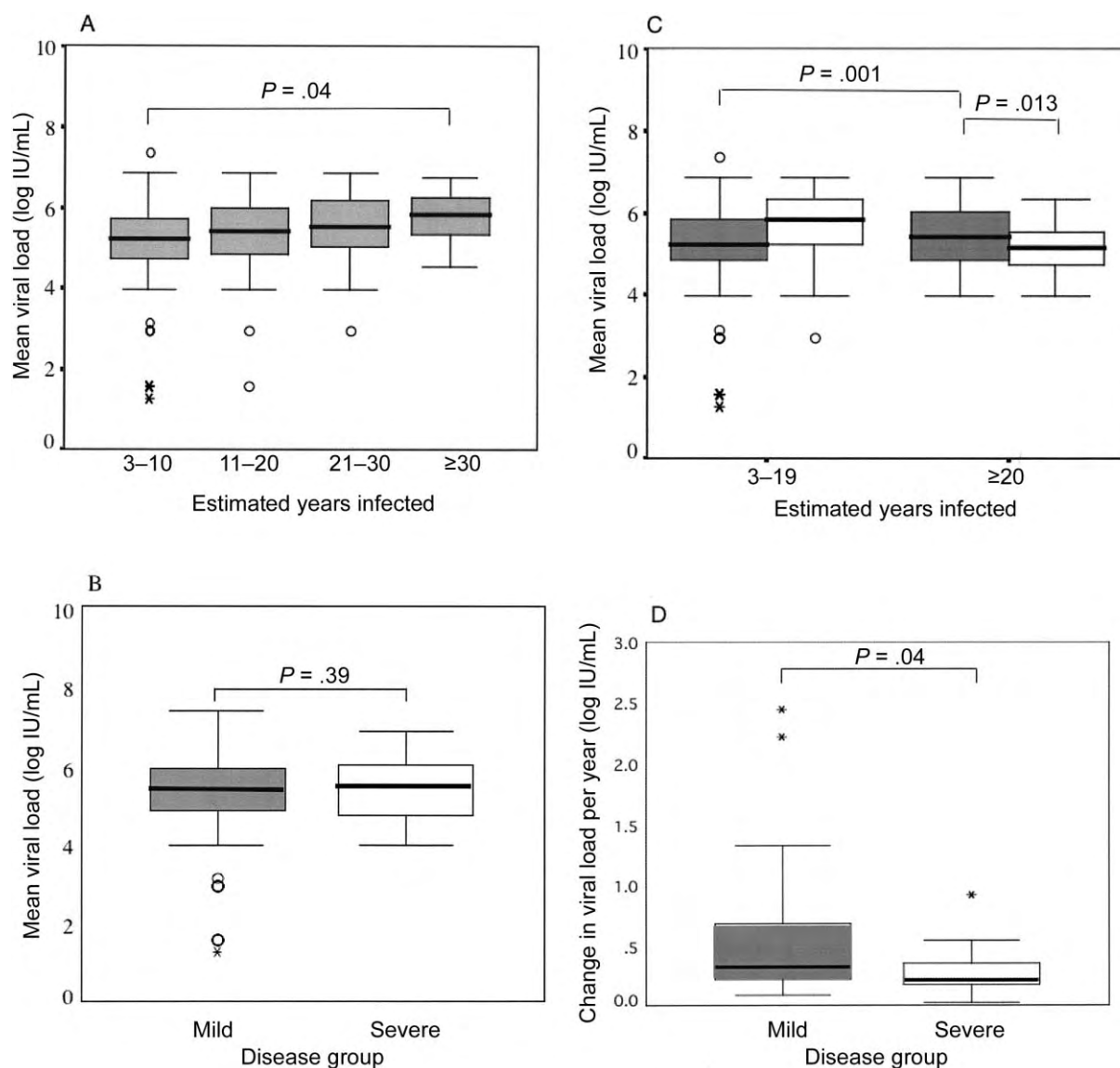


Figure 1. Mean levels of hepatitis C virus (HCV) RNA in serum, according to years after initial infection. Data were derived from all 223 study specimens. Panel A compares HCV RNA levels according to decade of infection for all 52 HCV genotype 1–infected study subjects, and panels B–D compare HCV RNA levels according to disease status for 33 study subjects with mild disease (*shaded boxes*) and 19 study subjects with severe disease (*white boxes*). When HCV RNA levels in all specimens were compared (*B*), no difference was found between the mild and severe disease groups. When the data were analyzed according to disease status and duration of infection at the time the sample was obtained (*C*), HCV RNA levels were not significantly different between the 2 disease groups until after the second decade of infection, at which time subjects with mild disease had higher HCV RNA levels than did subjects with severe disease. Subjects with mild disease had a highly significant increase in HCV RNA levels over time, whereas subjects with severe disease did not. Subjects with mild disease also had significantly greater changes in HCV RNA levels between specimens than did those with severe disease over the course of the study (*D*). Horizontal lines within the boxes indicate median values; lower and upper box limits indicate the 25th and 75th percentiles; vertical bars indicate the largest and smallest value that is not an outlier; and symbols indicate outliers.

load over time ($P = .02$) but not with change in viremia or R slope of viremia (data not shown). Table 2 summarizes comparisons of HCV dynamics according to disease group for the 52 subjects.

Comparison of hypervariable region sequences by disease group. Figure 3A summarizes sequence analysis of 244 hy-

pervariable region clones isolated from 7 randomly selected subjects with severe hepatitis C compared with 196 clones isolated from 7 randomly selected subjects with mild hepatitis C (GenBank accession numbers EF204599–EF204766). Viral quasispecies from subjects with mild disease had higher interspecimen DNA diversity (figure 3A) and greater interspecimen

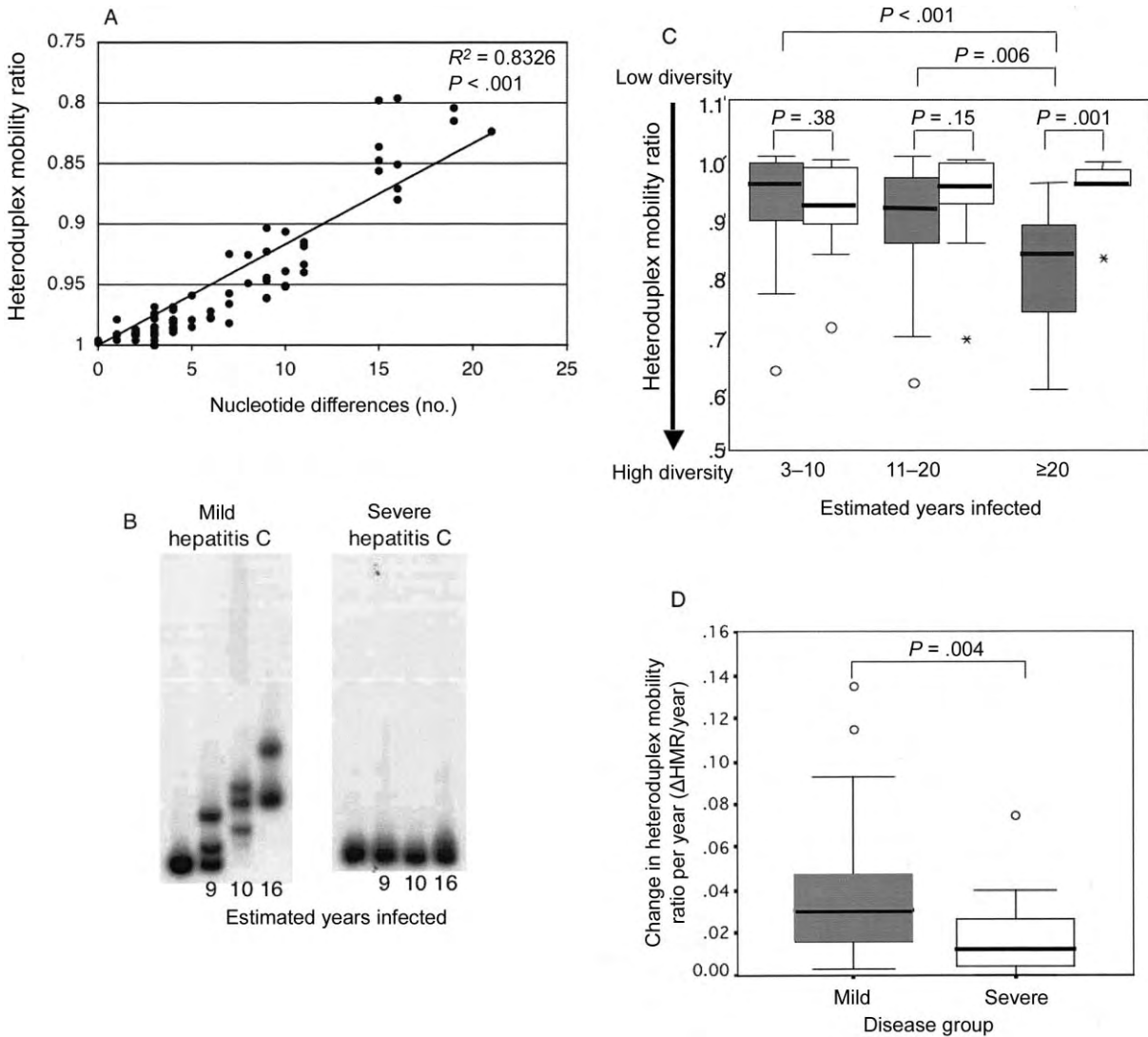


Figure 2. Correlation between hepatitis C virus (HCV) quasispecies genetic diversification and liver disease severity. Panel A compares data generated by heteroduplex mobility analysis (presented as heteroduplex mobility ratio; *vertical axis*) and nucleotide sequencing (*horizontal axis*) of 75 HCV hypervariable region clones. The R^2 value for the correlation was 0.8326 ($P < .001$). Panel B shows an autoradiogram with representative results from heteroduplex tracking analysis of HCV quasispecies in serial samples obtained over a 16-year interval from one subject who developed mild hepatitis C (*left series*) and another subject who developed severe hepatitis C (*right series*). The upward shifting band indicates the genetic divergence of HCV over time in the patient with mild disease. In contrast, no genetic change was detected in the hypervariable region over the 16-year period in the subject with severe disease. The first lane of each series contains the homoduplex control (probe hybridized to itself). Panel C compares intraspecimen quasispecies diversity in subjects with mild disease (*shaded boxes*) vs. severe disease (*white boxes*), according to decade of infection. Data were generated by heteroduplex mobility analysis of hypervariable region polymerase chain reaction amplicons; decreasing values on the vertical axis represent increasing quasispecies diversity. Significant increases in HCV quasispecies diversity occurred over time in specimens from subjects in the mild disease group, whereas diversity decreased over time in specimens from subjects in the severe disease group. A highly significant difference in cumulative quasispecies diversity was evident between the 2 patient groups after 2 decades of HCV infection. Panel D compares rates of change in quasispecies genetic diversity in sequential specimens over time ($\Delta\text{HMR}/\text{year}$) according to disease group. Subjects with mild hepatitis C had significantly greater rates of change in HCV quasispecies diversity over time than did those with severe hepatitis C. Horizontal lines within the boxes indicate median values; lower and upper box limits indicate the 25th and 75th percentiles; vertical bars indicate the largest and smallest value that is not an outlier; and symbols indicate outliers.

amino acid distances (figure 3B) than did viral quasispecies from subjects with severe disease, although the results were of borderline statistical significance, possibly because of small sample size. The ratio of nonsynonymous to synonymous mu-

tations in the hypervariable region sequences from subjects in the AN/AI cohort was consistently <1 and did not differ significantly between study groups (0.62 vs. 0.85 for mild vs. severe disease). Comparison of amino acid use by site revealed 6 highly

Table 2. Comparison of hepatitis C virus (HCV) dynamics over time, according to disease outcome.

Characteristic	Disease		P
	Mild (n = 33)	Severe (n = 19)	
Viral load			
Time points, no.	4.6	4.7	.85
Length of follow-up, years	8.4	11.2	.58
At baseline, mean, log IU/mL	4.9	5.0	1.00
At biopsy, mean, log IU/mL	5.2	5.4	.88
Variability through time, log IU/mL	0.35	0.21	.04
Mean through time, log IU/mL	5.1	5.3	.40
Slope through time	0.13	0.08	.31
Quasispecies^a			
Time points, no.	3.7	3.5	.52
Length of follow-up, years	6.8	8.8	.88
HMR, mean	0.906	0.945	.02
Δ HMR/year, mean	0.039	0.018	<.01

^a The heteroduplex mobility ratio (HMR) is an estimate of HCV quasispecies diversity. A lower HMR value indicates higher diversity. Δ HMR/year is the rate of change in HCV quasispecies diversity per year (diversification rate).

conserved residues irrespective of disease group: position 385 (threonine); 389, 390, and 406 (all glycines); 403 (phenylalanine); and 409 (glutamine) (figure 4). In contrast, the consensus amino acid differed between disease groups at 14 of 27 hypervariable region sites (figure 4B), and the frequency of consensus amino acid use was significantly different at 5 additional sites (388, 392, 395, 403, and 410); thus, in all 70% of sites had significant differences in amino acid use between disease groups (figure 4). The mean number of different amino acids found per site was 5.5 versus 4.6 for subjects with mild versus severe disease. Consensus amino acid use in subjects with severe disease was stable over time at 22 (81%) of 27 positions, compared with 17 (63%) of 27 of positions in subjects with mild disease; the percentages were 93% and 74%, respectively, when taking into account conservative amino acid changes. Phylogenetic analyses of evolution within the hypervariable region indicated that, in the majority of cases (12/14), HCV quasispecies clustered according to time point (figure 4C), as opposed to viral sequences being interspersed over time (data not shown).

Multivariate analysis. The above virological investigations implicate the rate of change in HCV viremia and the rate of change in HCV quasispecies (expressed as Δ HMR/year) as 2 variables that may be important in defining disease progression in hepatitis C. However, >1 variable likely influences hepatitis C disease progression. Therefore, sex, genotype, history of heavy alcohol consumption and alcohol consumption at time of biopsy, estimated length of HCV infection, the measure of change in viremia over time, and mean Δ HMR/year were considered in a multivariate model described in Subjects, Materials, and

Methods. The single most significant factor in predicting stage of liver disease in this multivariate model was the rate of HCV quasispecies evolution, mean Δ HMR/year. After accounting for mean Δ HMR/year, the *P* values for change in viremia, for sex, and for genotype were 0.16, 0.23, and 0.22, respectively. The odds among persons with a low and medium mean Δ HMR/year of having severe liver disease were 10.7 (95% confidence interval [CI], 1.8–62.5) and 4.8 (95% CI, 0.8–27.6) times the odds among persons with a high mean Δ HMR/year, respectively. These odds ratios were 11.2 and 4.9 after adjustment for estimated duration of HCV infection (*P* = .28), 11.4 and 4.4 after adjustment for any alcohol consumption at the time of biopsy (*P* = .12), and 15.3 and 4.7 after adjustment for a history of heavy alcohol consumption (*P* = .62).

DISCUSSION

The present study's major contribution is the demonstration that, during natural infection, the dynamic changes in levels and diversity of HCV genomes in serum differ over decades of infection between subjects developing severe versus mild hepatitis C disease. Most significant was the difference in HCV quasispecies between disease groups: both HCV hypervariable region diversity and the rate of hypervariable region diversification over time were significantly lower in specimens from subjects with severe disease.

In at least one respect, these findings are not new. Studies in liver transplant recipients have been highly consistent in reporting significant associations between low-diversity HCV infections and increased rates of liver disease progression [19–25]. In this model, evolution of low-diversity HCV quasispecies within months after liver transplantation is a strong predictor of rapidly progressive allograft fibrosis. In contrast, disease recurrence is markedly delayed in those recipients with genetically diverse quasispecies. In a recent study of HCV quasispecies over time in HIV-coinfected patients with hemophilia and chronic hepatitis C, Qin et al. [25] also reported lower quasispecies diversification rates in subjects progressing to end-stage liver disease relative to control subjects with compensated chronic hepatitis C. However, results in immunosuppressed populations do not necessarily predict similar findings during natural infection. In this regard, at least 10 previous studies have examined HCV quasispecies relative to liver disease in cohorts of subjects with natural infection, with only a single study reporting that low quasispecies diversification associates with worse disease outcome. Although only 6 cases were studied, patients underwent multiple serial liver biopsies and HCV quasispecies were analyzed in multiple serum specimens over time, the latter aspect being similar to our present design. All other studies have been cross-sectional, with one notable exception: a retrospective study by Duffy et al. [17], who compared HCV sequence changes in 18 subjects infected via a com-

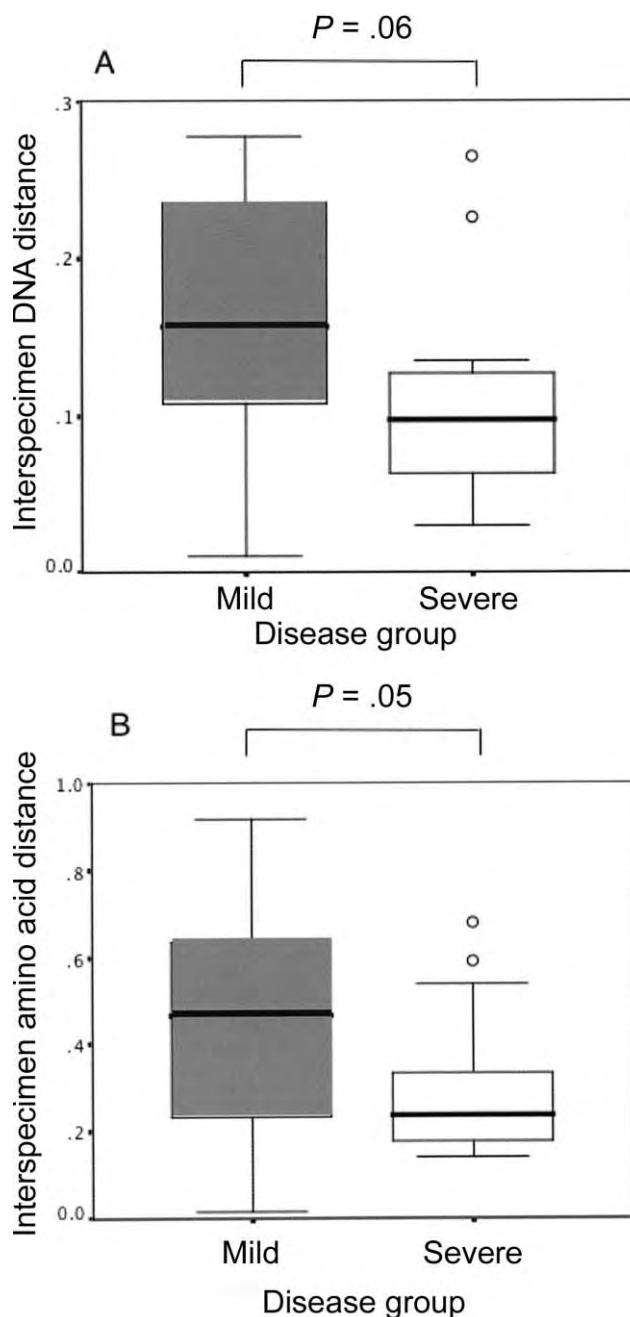


Figure 3. Interspecimen DNA (A) and amino acid (B) distance, according to disease group. DNA sequence analysis was performed on 440 hypervariable region clones present in serial serum specimens from 7 randomly selected subjects with mild disease (*shaded boxes*) and from 7 randomly selected subjects with severe disease (*white boxes*). Intra- and intersample DNA distances were estimated by generating a distance matrix based on all pairwise comparisons of sequences, using the DNADIST program Kimura 2 parameter option [35]. Both interspecimen DNA distances ($P = .06$) and interspecimen amino acid distances ($P = .05$) appeared to be greater in specimens from subjects in the mild disease group than in specimens from subjects in the severe disease group. The borderline statistical significance of the results may have been due to the small sample size. Amino acid sequences were deduced from the DNA sequences. Horizontal lines within the boxes indicate median values; lower and upper box limits indicate the 25th and 75th percentiles; vertical bars indicate the largest and smallest value that is not an outlier; and symbols indicate outliers.

mon source—HCV-contaminated anti-D immunoglobulin—in 1977 and 1978. The authors concluded that HCV genetic heterogeneity was unrelated to disease activity. Despite its attractive design, the study included only 6 subjects with severe disease,

and only 1 specimen was analyzed per case. Perhaps more importantly, only a single HCV isolate was studied, and quasispecies analyses were not performed.

We were unable to distinguish the mechanisms driving qua-

served between hypervariable regions amplified from patients with mild versus severe disease. Although the meaning of these differences is not known, the findings are of potential interest from the therapeutic vaccine perspective. Considerable evidence argues that HCV hypervariable region diversification is driven by positive humoral selection [7, 40–44], which most likely explains why quasispecies diversification is decreased in immunosuppressed subjects. Unfortunately, anti-hypervariable region immune responses were not evaluated in the present study. Nonetheless, our findings raise the intriguing possibility that anti-hypervariable region humoral responses may be protective in the chronically infected host. Several lines of evidence suggest that host immunity plays a protective role in hepatitis C. Immunosuppression (both during HIV coinfection and after liver transplantation) is associated with liver disease acceleration. CD4 T cell counts are inversely associated with hepatitis C disease severity in coinfecting subjects [45, 46]. HCV-specific CD4 T cell responses are stronger in subjects with mild versus severe posttransplantation hepatitis C disease [47]. Host immune responses play a critical role in the spontaneous resolution of acute hepatitis C in both humans and the chimpanzee experimental model (reviewed in [48]). HCV diversification during the acute phase of infection is associated with the establishment of chronicity [49]. For these reasons, we speculate that quasispecies diversification during the chronic phase may be essential for continued persistence in the face of effective host pressure.

In the present study, overall levels of HCV RNA in serum did not differ between study groups. However, subjects with mild disease had a significant increase in HCV RNA levels over time, whereas those with severe disease did not, and, by the third decade of infection, subjects with mild disease had significantly higher HCV RNA levels than did subjects with severe disease. Our group data on HCV viremia over time agree with those from Eyster et al.'s study in patients with hemophilia [6] and from Fanning et al.'s study in the Irish women's cohort [50], both of which showed that HCV RNA levels in serum increase over time. However, previous longitudinal studies have not reported differences in HCV viremia over time according to disease group. In immunosuppressed populations, HCV RNA levels are increased by 8-fold or more compared with natural infection [4, 45], yet hypervariable region diversity goes down, suggesting that viral diversification is not necessarily the result of increasing viral loads.

Our study has potential limitations. Variability and sampling error in studies of hepatic disease with a histological end point have been noted in the literature. We minimized this variability in the present study by using 2 categories of disease status, rather than the 5 histological score end points. Because of the retrospective nature of this study, viral RNA in specimens frozen in our serum bank may have been subject to degradation

over time. However, the number of years of specimen storage did not differ between the 2 disease groups ($P = .81$). Consequently, effects of degradation would likely have been similar between our 2 study groups. The present analyses were limited to the HCV hypervariable region and lacked both immunological and immunogenetic data. For example, when studying larger genetic regions of the HCV anti-D cohort clade, Ray et al. [43] demonstrated divergent HCV evolution away from consensus sequences in persons having HLA alleles associated with specific epitopes and toward consensus epitope sequences in persons lacking the restricting allele. Future studies in the AN/AI cohort will analyze multiple HCV genes in the context of immune responses.

In summary, we have demonstrated that HCV dynamics differ in treatment-naïve, immunocompetent subjects with severe versus mild hepatitis C disease. Although the data suggest that adaptation of HCV to the infected host may be linked to hepatitis C pathogenesis, the present study could not distinguish between the involvement of homogeneous quasispecies in disease and simple outgrowth of highly fit variants in response to changes in the permissiveness of the infected host. Nonetheless, because low-diversity quasispecies are associated with greater disease severity, monitoring viral diversity over time may prove to be a reasonable alternative to more-invasive histological monitoring in the clinical setting.

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