Hepatitis C Virus Envelope Glycoprotein Signatures Are Associated With Treatment Failure and Modulation of Viral Entry and Neutralization

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Background. A major challenge for antiviral treatment of hepatitis C virus (HCV) infection is viral resistance, potentially resulting from the high variability of HCV envelope glycoproteins and subsequent selection of strains with enhanced infectivity and/or immune escape.

Methods. We used a bioinformatics and functional approach to investigate whether E1/E2 envelope glycoprotein structure and function were associated with treatment failure in 92 patients infected with HCV genotype 1.

Results. Bioinformatics analysis identified 1 sustain virological response (R)–related residue in E1 (219T) and 2 non-SVR (NR)–related molecular signatures in E2 (431A and 642V) in HCV genotype 1a. Two of these positions also appeared in minimal networks separating NR patients from R patients. HCV pseudoparticles (HCVpp) expressing 431A and 642V resulted in a decrease in antibody-mediated neutralization by pretreatment sera. 431A/HCVpp entry into Huh7.5 cells increased with overexpression of CD81 and SR-BI. Moreover, an association of envelope glycoprotein signatures with treatment failure was confirmed in an independent cohort (Virahep-C).

Conclusions. Combined in silico and functional analyses demonstrate that envelope glycoprotein signatures associated with treatment failure result in an alteration of host cell entry factor use and escape from neutralizing antibodies, suggesting that virus-host interactions during viral entry contribute to treatment failure.

Keywords. interferon-alfa; resistance; ribavirin; therapy; outcome.

Hepatitis C virus (HCV) infection is a major public health burden, with 170 million people infected worldwide. The majority of HCV-infected individuals develop persistent infection that can progress to cirrhosis and hepatocellular carcinoma. Although treatment for chronic hepatitis C has markedly improved over the past decade, there continues to be a large unmet medical need in the area of hepatitis C prevention and therapy. Treatment with pegylated interferon alfa (PEG–IFN-alfa) and ribavirin is effective in clearing chronic HCV in <50% of patients infected with HCV genotype 1, the most common genotype in Europe and the United States [1]. Addition of the HCV protease inhibitors telaprevir or boceprevir improves the sustained virological response (SVR) in genotype 1–infected patients. However, treatment failures due to viral resistance and adverse events remain important challenges [2, 3]. Thus, a better understanding of the mechanisms mediating resistance is needed to further improve antiviral therapy.
Several host and viral factors have been identified as relevant to antiviral resistance [1]. Viral factors include genotype, load, and viral kinetics, such as rapid and early virological response [4]. Host-related factors linked to treatment failure include cirrhosis, steatosis, body weight, ethnicity, sex, age, diabetes [5], and individual genetic background, such as the presence of an IL28B polymorphism [6].

The highly variable HCV envelope glycoproteins E1 and E2 play a crucial role as targets of adaptive host immune responses [7]. Indeed, mutations in neutralization epitopes [8–10] or in epitopes targeted by CD4+ or CD8+ T lymphocytes [8] can lead to viral evasion from host immune responses. Furthermore, the ability of the HCV envelope to bind to blood cells may have an immunomodulating impact on treatment response [11–13]. As previously suggested by other authors [14–16], the difficulty in mounting an effective immune response against HCV envelope antigens with concomitant viral evasion may also contribute to treatment failure.

Furthermore, the glycoproteins E1 and E2 initiate HCV infection by mediating HCV attachment and entry in concert with host factors. Host cell entry factors include highly sulfated heparan sulfate (HS), CD81, scavenger receptor BI (SR-BI), claudin-1 (CLDN-1), occludin (OCLN), and receptor tyrosine kinases regulating viral coreceptor associations [17–24]. Within the envelope coding region, the hypervariable region 1 (HVR1) of E2 interacts with SR-BI and HS, is involved in the HCV/lipoprotein complex combining HVR1, SR-BI and high density lipoproteins during viral entry into host cells [25], and is a target of host immunity, with evolutionary rates in HVR1 higher among individuals who spontaneously clear HCV infection during acute hepatitis C [26].

Although the variability in HVR1 and the covariance networks in structural and nonstructural proteins have been linked to antiviral treatment response [27, 28], the functional role of envelope variability in the response to antiviral treatment is unknown. We therefore investigated whether the high variability of HCV envelope glycoproteins contributes to antiviral resistance during IFN-based therapy by selection of strains with enhanced entry and/or escape from host immune responses. To address this question, we used a bioinformatics approach to map envelope glycoprotein signatures in a cohort of 92 patients infected with HCV genotype 1 who were receiving PEG–IFN-alfa/ribavirin therapy and then investigated the functional relevance of the key identified signatures, using state-of-the-art cell culture models for HCV entry.

**METHODS**

**Patients**

Pretreatment serum samples were collected from 92 patients infected with HCV genotype 1 (49 had genotype 1b infection, and 43 had genotype 1a infection), of whom 44 were from Centre Hospitalier Universitaire (CHU) Strasbourg, 7 were from CHU Tours, 3 were from CHU Clermont-Ferrand, 16 were from CHU Bobigny, 6 were from CHU Villejuif, 5 were from CHU Toulouse, 5 were from Hôpital Pitié–Salpêtrière (Paris), 4 were from CHU Bordeaux, and 2 were from CHU Brest (Table 1). Approval of the study was obtained by the Comité de Protection des Personnes–CPP d’Alsace (19/11/2008, DC-2008-829), in accordance with the guidelines of the Declaration of Helsinki. Virological response was evaluated 4

<table>
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<th>Characteristic</th>
<th>R (n = 42)</th>
<th>NR (n = 50)</th>
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<tr>
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<tr>
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<td>2.7±2.2</td>
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Data are no. (%) of patients or median value (interquartile range), unless otherwise indicated. Sustained virological response was defined as an undetectable HCV RNA level 6 months after the end of treatment. Abbreviations: ALT, alanine transaminase; HCV, hepatitis C virus; ULN, upper limit of normal.

* No or minimal fibrosis corresponds to an activity grade of ≤1 plus a fibrosis stage of ≤1, moderate to severe fibrosis corresponds to an activity grade of ≥2 or a fibrosis stage of 2 or 3, regardless of the activity grade; and cirrhosis corresponds to a fibrosis stage of 4.
and 12 weeks after the start of treatment and 6 months after the end of treatment and was classified into 2 groups, SVR (R) and non-SVR (NR), and into 2 subgroups, null virological response (NR0) and rapid virological response (RVR; Supplementary Table 1).

Moreover, 94 HCV genotype 1 sequences from the well-characterized Virahep-C cohort [29] were analyzed in a validation approach. A total of 48 patients treated with PEG-IFN-alfa/ribavirin were classified in the R group, and 46 patients were classified in the NR group [29].

**Reverse Transcription Polymerase Chain Reaction (PCR) and Direct Sequencing**

HCV strains were investigated in plasma collected at baseline for all patients. HCV RNA (330 ng per reaction) was subjected to reverse transcription, and complementary DNA was then amplified using the proofreading LongRange PCR enzyme mix and published primers [30], generating amplicons including the genes encoding E1/E2 for direct sequencing.

**Bioinformatics Analyses of HCV E1/E2 Sequences**

First, investigation of molecular signatures (ie, amino acids) linked to virological response to treatment was performed by comparing patient groups and subgroups according to treatment efficacy, using the VESPA program (available at: http://hcv.lanl.gov/content/sequence/VESPA/vespa.html). The frequency and relative proportions of 1 residue at each position for the different patient (sub)groups were taken into account. An amino acid was retained in a first screening if it was over-represented in 1 group or subgroup (ie, if it represented ≥60% of the amino acids at this position) and appeared less frequently in the comparative patient group or subgroup (ie, if it represented <50% of the amino acids at this position; Table 2).

The most relevant signatures which were explored in functional tests were those combining at least the 2 previous frequency features, plus different distributions between subtypes (1a/1b) in order to highlight subtype-specific residues according to treatment efficacy, and differential predicted antigenicity (based on humoral immunogenicity predicted by Antheprot 2000 v6.0 software; available at: http://antheprot-phil.ibcp.fr) [31].

Second, a complementary approach was used because amino acids can interact with other residues within HCV envelope proteins, with a potential indirect impact of these interactions on treatment. To distinguish between NR patients and R patients, pairs of covarying amino acid positions were calculated within each group [28], and pairs with an S value of at least 0.5 were retained. Because there are many covarying amino acid combinations [28], we used the following optimality criteria to determine the combinations that could separate NR patients from R patients: (1) combinations containing the fewest number of pairs or (2) combinations that maximized a measure of the total correlation value (calculated as the weight of each pair, given by correlation value S, subtracted from the maximum correlation value over all pairs), using binary integer programming (bintprog, Matlab R2011a). The networks were extracted from covarying pairs determined separately for genotypes 1a and 1b, and covariances were calculated over all individuals within each genotype, for the NR patients versus the R patients. Moreover, response groups were separated by determining a single amino acid combination exhibited by a pair in one group that was not contained in the other group, with separate analyses performed for the NR and R groups.

**Multivariate Analysis**

A multivariate analysis including all data available from our cohort as possible predictors was performed to determine whether the presence of mutations is independent from other predictive factors.

**Cell Lines**

HEK 293T and Huh7.5 cells were cultured as described elsewhere [32, 33]. Huh7.5 cells individually overexpressing HCV entry factors have been described previously [10].

**HCV Pseudoparticle Production, Entry, and Neutralization**

Plasmids derived from the HCV-1a (H77-AB67037) reference strain expressing HCV E1/E2 were used as templates to introduce molecular signatures by site-directed mutagenesis.
HCV pseudoparticles (HCVpp) were produced by transfecting HEK 293T cells [32–34], using the lentiviral CMV-Gag-Pol packaging construct, a luciferase reporter plasmid, and plasmids encoding HCV E1/E2 of the reference strain 1a, with or without a single molecular signature. Expression of E1/E2 was assessed in producer cell lysates by immunoblotting [9, 10]. The relative amounts of HCVpp were normalized using p24 antigen quantification. HCV entry assays were performed as described elsewhere [9]. For neutralization assays, HCVpp were preincubated with either HCV-positive sera from patients infected with a HCV strain containing the respective molecular signature or with HCV-negative control serum [9, 32, 33]. Neutralization titers were defined as the highest dilution giving a specific neutralization of >50% [9, 32].

**Statistical Analysis**
Statistical analyses for entry and neutralization experiments were performed using the Friedman test, a nonparametric version of repeated measures analysis of variance.

**RESULTS**

**Bioinformatics Analysis Identifies the Association of HCV Envelope Signatures 219T, 431A, and 642V With Treatment Outcome**
The cohort comprised 92 patients infected with genotype 1 (43 with genotype 1a and 49 with genotype 1b) treated with PEG–IFN-alfa/ribavirin. To assess associations between envelope signatures and treatment outcome, the patients were grouped according to SVR: 42 were in the R group (25 with genotype 1b and 17 with genotype 1a), and 50 were in the NR group (24 with genotype 1b and 26 with genotype 1a). Figure 1 shows a representation of the amino acid frequency on HCV E2 from residues 384 to 480 for HCV 1a and 1b strains in the 92 patients. A high variability in HVR1 was observed for HCV 1a and 1b, while HVR2 had the highest variability among HCV 1b strains. Bioinformatics analysis identified 3 signatures associated with treatment outcome of HCV genotype 1a: 219T in E1, which was associated with R, and 431A and 642V in E2, which were associated with NR (Table 2).

Next, we performed a multivariate analysis to determine whether the presence of the signatures is independent from other predictive factors. Multivariate analysis taking into account all parameters displayed in Table 1 demonstrated that the 219T mutation correlated with R ($P = .06$), lower baseline viral load ($P = .05$), and genotype 1a ($P = .016$); that the 431A mutation was associated with NR ($P < .001$) and female sex ($P = .03$); and that the 642V mutation was associated with NR ($P = .04$), genotype 1a ($P = .006$), and female sex ($P = .02$). Interestingly, these data point to an additional association of defined mutants with sex and excluded dependence from other factors.

When the study was initiated in 2008, the link between IL28B polymorphism and HCV clearance was not yet uncovered [6]. Because the inclusion of patients and the collection of sera had been completed at the time of this discovery, IL28B status could not be determined, owing to the absence of material and consent for genetic analyses.

To investigate whether envelope signature was associated with virological response in an independent second cohort, we analyzed HCV genotype 1 sequences from the Virahep-C cohort [29]. VESPA analyses identified 3 signatures in very similar regions of envelope glycoproteins as the ones identified in the French cohort. The signatures identified in the Virahep-C cohort included 216A (E1, R), 438V (E2, NR), and 482Q (E2, NR; Table 2). These data corroborate the concept of the presence of envelope signatures and outcome and confirm the impact of the identified envelope glycoprotein regions for this association.

**Covariance Networks on E1/E2 Separate Patients According to Virological Response**
To further confirm the relevance of the identified signatures, we used a network approach to investigate multiple amino acid positions that reflect response. Because amino acids can interact with other residues within HCV envelope proteins, with the potential for these interactions to influence viral phenotype and treatment response, we calculated covarying amino acid pairs in E1/E2 for each genotype and for the NR and R groups [28]. Despite roughly similar numbers of individuals infected with 1a ($n = 43$) and 1b ($n = 49$), there were twice as many covarying pairs for 1a sequences, compared with 1b sequences (1095 vs 516, respectively). The 1a sequences also exhibited amino acid pairs with higher covariances than for 1b (maximum $S$ value, 6.9 vs 2.8, respectively). We extracted the dominant aspects by determining optimal networks that either minimized the number of pairs used to separate response groups or that to a certain extent maximized the total covariance. Fewer pairs were required to separate NR patients from SVR patients for 1a, compared with 1b, regardless of optimality criteria (5–7 pairs for 1a vs 6–9 pairs for 1b). Combination of all pairs that appeared in these optimal networks produced the overall network for each genotype displayed in Figure 2. Interestingly, 2 of the 3 positions of HCV genotype 1a identified by VESPA (219 and 431) also appear as nodes in the corresponding minimal network separating NR from R (Figure 2A).

**Molecular Signatures Associated With Treatment Outcome Modulate Viral Entry**
Next, we investigated whether the identified signatures altered the phenotype of virus-host interactions during viral entry.
and antibody-mediated neutralization. We generated HCV pseudoparticles (HCVpp) of genotype 1a expressing envelope glycoproteins containing the identified signatures. Figure 3A shows entry efficiency into Huh7.5 cells of HCVpp bearing identified signature positions, compared with wild-type (wt, without signature) reference strains of genotype 1a. HCVpp entry markedly and significantly decreased when HCVpp contained the R-related signature 219T (P < .0001), whereas HCVpp entry increased for the NR-related signatures 431A and 642V (P = .0001 and P < .0001, respectively; Figure 3A).

Because molecular signatures may modulate HCV E1/E2 interactions with host entry factors and because different use of host entry factors has been identified as a mechanism of viral evasion in patients with acute and chronic infection [10], we studied whether the molecular signatures may modulate viral entry by different use of CD81, SR-BI, CLDN-1, and OCLN. We thus studied viral entry of HCVpp displaying the individual molecular signatures in Huh7.5 cells stably overexpressing the 4 main cell entry factors individually Figure 3B. Overexpression of either CD81 (P = .0007) or SR-BI (P = .0007), but
not of CLDN-1 or OCLN, significantly and specifically increased entry of HCVpp bearing the NR-related signature 431A by a median 2.6-fold (vs 1.6-fold for wt) or 2.4-fold (vs 1.6-fold for wt), respectively, compared with parental Huh7.5 cells (Figure 3C). No specific increase was observed with any of the cell lines for HCVpp displaying the other signatures (data not shown). Thus, the increased entry of HCVpp containing the NR-related signature 431A may be the result of a modulation of CD81 and SR-BI use.

Envelope Signatures Alter Envelope Antigenicity and Confer Escape From Neutralizing Antibodies

To study the functional impact on viral evasion from patients’ immune responses, we first analyzed the predicted antigenicity of molecular signatures by Antheprot software. A lower antigenicity was predicted for NR-associated residues 431A and 642V than other amino acid potentially present at these positions (Figures 4A and 4B), while the R-related molecular signature 219T demonstrated higher antigenicity (Figure 4C).

To address the functional relevance of these findings in a state-of-the-art model system for antibody-mediated neutralization, we incubated patient sera containing envelope-specific antibodies with HCVpp expressing envelope proteins containing the identified envelope signatures. Introduction of the NR-related residue 431A into the H77-1a reference strain resulted in a marked and significant decrease in HCVpp neutralization by serum from patients infected with HCV strains containing the respective molecular signature (median neutralization titers, 1:100 for HCVpp bearing 431A vs 1:800 for wt HCVpp; P = .002; Figure 5). A decrease in sensitivity to neutralization was also observed for NR-related signature 642V (median titer, 1:20 for HCVpp bearing 642V vs 1:100 for wt HCVpp; P = .01; Figure 5). No changes in neutralization were observed for the 1a R-related signature 219T (Figure 5). These data demonstrate that molecular signatures associated with treatment failure can change the antigenicity of viral envelopes and impair the sensitivity to antibody-related neutralization.

DISCUSSION

HCV entry is required for initiation and maintenance of infection and is a major target for host immune responses [33]. An important role for HCV entry and antibody-mediated
neutralization has been demonstrated for viral evasion in chronic infection and reinfection of liver grafts [8, 9, 32]. In this study, we show for the first time that HCV entry and evasion from neutralizing antibodies are associated with resistance to antiviral therapy. By use of a bioinformatics and functional approach, we identified 3 molecular signatures in the envelope glycoproteins of HCV genotype 1a that were associated with HCV entry and resistance to antiviral therapy. The molecular signatures are associated with increased viral entry and increased stability of viral entry factors.

Figure 3. Molecular envelope signatures associated with treatment outcome influence viral entry in the hepatitis C virus pseudoparticles (HCVpp–Huh7.5 cell culture model). A, Entry of mutated HCVpp (bearing signature), compared with entry of wild-type (wt) HCVpp (without signature). HCVpp entry was analyzed by luciferase reporter gene expression. Entry levels of mutated HCVpp are expressed as percentage of viral entry, with compared with wt (which is equal to 100%). Data are mean values ± SD from at least 4 independent experiments performed in triplicate (H77-1a wt vs A219T, H77-1a wt vs D431A, and H77-1a wt vs A642V). B, Entry factor expression in SR-BI-, CD81-, CLDN1-, or OCLN-transduced Huh7.5 cells. The relative overexpression of each entry factors was determined by flow cytometry and is indicated as fold-expression change, compared with parental Huh7.5 cells. Data are mean values ± SD from 1 experiment performed in duplicate. Overexpression of either SR-BI, CD81, CLDN1, or OCLN did not affect the stability or proportion of other HCV entry factors at the cell surface (data not shown). C, Receptor dependency of HCVpp bearing signature 431A (D431A) and HCVpp without signature (wt). Parental (ie, control) and transduced Huh7.5 cells were incubated with wt or mutated HCVpp. Viral entry is expressed as the fold-change in viral entry, compared with the parental cells. Overexpression of either CD81 or SR-BI increased entry of HCVpp bearing the NR-related signature 431A by a median 2.6-fold (vs 1.6-fold for wt) or the non–sustain virological response (NR)–related molecular signature (MS) 431A (H77-1a D431A; A); a 632-652 amino acid pattern in E2 with either amino acid 642A or the NR-related MS 642V (B); and a 209-229 amino acid pattern in the E1 sequence, with either amino acid 219A or a sustain virological response–related MS 219T (C). Predicted antigenicity profiles were generated using Antheprot 2000 v6.0 software [31]. The height of the peaks corresponds to the relative antigenicity.

Figure 4. Molecular envelope signatures associated with treatment failure modulate predicted antigenicity. Predicted antigenicity levels are shown for a 421–441 amino acid pattern in the E2 sequence of H77-1a reference strain, containing either amino acids 431D (H77-1a; wild-type wt) or the non–sustain virological response (NR)–related molecular signature (MS) 431A (H77-1a D431A; A); a 632-652 amino acid pattern in E2 with either amino acid 642A or the NR-related MS 642V (B); and a 209-229 amino acid pattern in the E1 sequence, with either amino acid 219A or a sustain virological response–related MS 219T (C). The predicted antigenicity levels were generated using Antheprot 2000 v6.0 software [31].
Figure 5. Molecular signatures associated with treatment failure confer viral escape from antibody-mediated neutralization. Hepatitis C virus pseudoparticles (HCVpp) expressing envelopes containing molecular signatures (described in Figure 3) were preincubated with HCV-positive sera from patients who were infected by a HCV strain containing the respective molecular signature or with HCV-negative control serum. Neutralization titers were defined as the highest dilution giving a specific neutralization above 50% [9, 32]. For each case, median neutralization titers of the wild-type (wt) and the mutant HCVpp are shown (data are derived from 3 experiments performed in triplicate). The scale used is a logarithmic scale. Asterisks denote statistically significant differences in neutralization titers between wt and mutant HCVpp.

associated with treatment failure in a cohort of 92 patients infected with genotype 1. Two of these positions also appeared in minimal networks separating NR patients from R patients. Functional analyses using HCVpp expressing the NR patient–specific signatures 431A and 642V demonstrated that introduction of these residues into nonrelated HCV strains resulted in a decrease in antibody-mediated HCVpp neutralization by pretreatment sera and in a signature-dependent modulation of HCVpp entry into Huh7.5 cells overexpressing CD81 and SR-BI.

Interestingly, envelope regions containing the identified molecular signatures uncovered in this study have been reported to play an important functional role in virus-host interactions during entry and antibody-mediated neutralization. The identified positions 431 and 642 are next to 2 N-glycosylation sites at position 430 and 645 of E2 [35, 36]. Furthermore, these residues are part of an immunogenic, highly conserved, and conformational epitope within the domain B of E2 recognized by neutralizing human monoclonal anti-envelope antibodies [37]. A single amino acid substitution at position 431 resulted in escape from human neutralizing monoclonal anti-E2 CBH-2–mediated neutralization, while the mutation A642G has been reported to inhibit binding of CBH-2 [37]. The positions 431 and 642 are also close to 2 disulfide bonds (C429-C552) and (C644-C607) that are involved in E2 folding and play a role in its tertiary structure [36]. Amino acid 431 is located in the top β-sheet of the E2 DI domain, exposed at the virion surface and containing most of the CD81 binding determinants; position 642 is located in E2 DIII, another surface-exposed domain of E2 also containing CD81 binding determinants [36]. It is of interest to note that our study did not identify residues associated with treatment outcome in genotype 1b patients. This finding is most likely due to the markedly higher genetic variability of envelope glycoproteins within HCV 1b strains (see Figure 1 and covariance results). Additional functional studies using recombinant viruses or pseudotypes expressing patient-derived viral envelope glycoproteins are required to identify functionally relevant mutations not identified with bioinformatics analysis.

Our functional data indicate that 431A and 642V individually enhance HCV entry, potentially in part through a differential CD81 use, and contribute to escape from neutralizing antibodies. Different receptor use with concomitant evasion from host neutralizing responses has been previously identified to confer viral escape during liver graft and chronic HCV infection [10]. This work confirmed in the cell culture–derived HCV (HCVcc) system the functional relevance of 3 E2 residues discovered by using HCVpp [10]. Enhanced viral entry by mutations at positions 447, 458, and 478 of the emerging viral variant reinfecting the liver graft was the result of an increase CD81 use, a key determinant of viral evasion from antibodies at postbinding steps [10]. Finally, the position 219 in E1 has been shown to be crucial for entry of HCVpp and HCVcc [38]. Together, the identification of nonresponse-related 431A and 642V and response-related 219T molecular signatures that modulate HCV entry and neutralization in vitro uncover a possible mechanism for treatment failure in HCV genotype 1a–infected patients, where strains with better viral fitness and escape from humoral immunity could contribute to resistance and persistence of viral infection.

The association of defined envelope glycoprotein signatures with treatment outcome was confirmed in the Virahep-C cohort. Although the envelope positions were not identical (Table 2), they were located very close to each other in structurally or functionally related envelope glycoprotein regions or epitopes. The residues E1 216 (Virahep C) and E1 219 (French cohort) are almost at the identical position, in a functional E1 region crucial in HCV entry [38]. The E2 438 (Virahep-C) and E2 431 (French cohort) are within the same E2 structural region (domain I) [36] and within the same B cell epitope encompassing amino acids 430–444 [37]. Collectively, these comparative analyses highlight envelope glycoprotein regions that are relevant for viral evasion during treatment. Our data indicate that the envelope signatures showed the strongest association with rapid virological response or null response (Table 2). This is most likely because pretreatment sequences were analyzed and because these mutations were most relevant to mechanisms of evasion occurring early during antiviral treatment.
Since the virus rapidly mutates during the 12-month treatment period, it is likely that new mutations contributing to treatment failure emerge at later time points.

Of note, each individual molecular signature identified in our cohort affected HCV entry and neutralization, suggesting that minimal changes within the HCV envelope may have an impact on virus-host interactions. It has been reported that single mutations in E2 may drastically modify HCV infectivity and/or viral sensitivity to antibody-mediated neutralization (G451R, 2a [39]; S501N/V506A, 1a [40]; and N415Y/E655G, 1a [41]).

In addressing the association of genetic variability and treatment outcome, Aurora et al identified covarying amino acid positions linked together within networks, differing between patients who did and those who did not respond to treatment. Despite their different approach, which involved studying the full-length genome, thereby precluding direct comparison with our results, these positions included amino acid covarying pairs located in E1/E2 [28]. This study suggested a role for genetic variability and treatment outcome, but the absence of functional studies in cell culture models for HCV infection did not allow any conclusions regarding the functional and mechanistic relevance of their findings.

In conclusion, our results demonstrate that envelope glycoprotein signatures are associated with treatment failure. Combined in silico and functional analyses demonstrate that NR-related signatures result in increased HCV entry with concomitant escape from neutralizing antibodies. Our results suggest a role for the humoral response during or for treatment-induced virological response, which was not, to our knowledge, previously reported. Thus, these findings also may be argued to include B-cell epitopes of the viral envelope into the immunogens and vectors of therapeutic vaccine approaches described previously [42]. Taken together, our data suggest that virus-host interactions during viral entry contribute to treatment failure and highlight the relevance of HCV entry as an antiviral target to address and overcome antiviral resistance.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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