Naturally occurring mutations to HCV protease inhibitors in treatment-naïve patients

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Abstract

Background: Protease inhibitors (PIs) to treat hepatitis C (HCV) virus infection have been approved and others are under development.

Results: The aims of this study were to illustrate natural polymorphisms in the HCV protease and measure the frequency of PI resistance mutations in different HCV genotypes from PI-naïve patients. Direct sequencing of HCV NS3/4A protease was performed in 156 HCV patients naïve to PIs who were infected with genotype 1a (n = 31), 1b (n = 39), 2 (n = 30), 3 (n = 33) and 4 (n = 23). Amino acid (aa) substitutions associated with HCV PI resistance were found in 17/156 (10.8%) sequences. Mutations V36L, T54S, V55A/I, and Q80K/L were observed in 29% of patients with genotype 1a, and V55F, Q80L/N and M175L in 10% of patients with genotype 1b. The mutation V158M was found in 3% of patients with genotype 2, D168Q was present in 100% of patients with genotype 3 and D168E was observed in 13% of patients with genotype 4. In addition, multiple aa polymorphisms not associated with PI resistance were detected in patients with genotypes 1a, 1b and 4.

Conclusions: Although major PI resistance mutations were not detected, other resistance mutations conferring low level resistance to PIs together with a number of natural polymorphisms were observed in proteases of PI naïve HCV patients. A more extensive analysis is needed to better evaluate the impact of baseline resistance and compensatory mutations in the efficacy of HCV PI treatment.

Keywords: Hepatitis C virus, HCV baseline resistance, Protease inhibitors, HIV/HCV co-infection, Genetic diversity

Background

Hepatitis C virus (HCV) infects more than 170 million people worldwide [1]. Treatment with pegylated interferon-α and ribavirin is burdened by adverse reactions in a significant proportion of patients [2] and a sustained virological response is achieved in only 50% of patients infected with genotype 1 [3] and 80% of patients infected with genotype 2 [4]. Recently, inhibitors of HCV non-structured serine–protease 3 (NS3/4A) have been approved (Telaprevir, Boceprevir), and others are under development (TMC435350, ITMN191, SCH900518, MK7009, BI-201335, MK5172, GS-9256, ABT 45, BMS-791325 and ACH-1625) [5-9]. However, selection of drug-resistant HCV variants has already been reported in protease inhibitor (PI)-treated individuals [7-14]. The degree of resistance appears to be related both to mutations at specific NS3 positions and to changes in amino acid (aa) residues [5,7,15].

The high HCV replication rate and lack of a proof-reading mechanism determine a natural variability, which promotes the rapid emergence of drug-resistant variants [15]. Natural aa changes in NS3 associated with reduced drug susceptibility have been observed in treatment naïve patients [10,16,17]. However, the clinical impact of baseline resistance and its influence on the ability of the virus to replicate in vivo remain unclear [15,16]. Recently, the sporadic transmission of naturally occurring NS3 resistance mutations was reported [16]. In addition, the impact of the frequency of baseline HCV PI resistance mutations in HIV/HCV co-infected patients with respect to HCV mono-infected patients is still debated [17-19]. The frequency of naturally
occurring NS3 aa substitutions associated with PI resistance in treatment naïve HCV patients infected with genotypes 1, 2, 3 and 4 was investigated.

**Materials and methods**

HCV PI-naïve patients referred to our hospital between 2010 and 2011 were included in the study. Patients were stratified according to HCV genotype and a comparable number of patients infected with HCV genotypes 1a, 1b, 2, 3 and 4 were sequentially enrolled in the study. Most (75%) were treated with pegylated Interferon-α and Ribavirin, while none had ever been treated with a PI for hepatitis C. For NS3 sequencing, surplus serum samples were prospectively collected from each patient. HCV genotypes were defined using the Versant HCV Genotype 2.0 Assay LiPa (Siemens Healthcare Diagnostic Inc., Tarrytown, NY USA). The NS3 region was sequenced to further subtype HCV strains and identify genotypes 1a/1b. Data were analyzed with the Blast program (http://blast.ncbi.nlm.nih.gov). The study was approved by the Ethics Committee of the Fondazione IRCCS Policlinico San Matteo (protocol no. 20080009620). Informed consent was obtained from all subjects prior to enrollment.

Viral RNA was extracted from serum samples using the automatic Easy Mag extractor (Biomerieux, Lyon, France), and full-length HCV NS3/4A sequences were prospectively collected from each patient. HCV genotypes were defined using the Versant HCV GenoChip (http://www.virologyj.com/content/9/1/245). In detail, the primers were as follows: 1a-Forward 5'-GTCTCTGCRCGATTAGGCCGTGA-3' and 3-Reverse outer 5'-GTCAGTTGAGTGGCACTCATCAC-3' for genotype 1a; 1b-Forward outer 5'-GGAATGGTCTCCAAGGGGTG-3' and 3-Reverse inner 5'-CGAGACCTTGCGGTGGCAGT-3' for genotype 1b; 2-Forward outer 5'-GAGTACGTCATGATGGGGTGTCGAGC-3' and 3-Reverse inner 5'-GGAAATGTGTCCTCAAGGGGTTGA-3' and 1a-Reverse inner 5'-CATGGGCCCTTGGA-3' and 1b-Reverse inner 5'-CAGCCGTTGAGTGGCACTCATAC-3' for genotype 2; 3-Reverse outer 5'-GAGGCTCGGTGGTGCCATG-3' and 1a-Reverse inner 5'-GTGGAACCATGGCCTGCAA-3' and 3-Reverse inner 5'-CGAGGCGACTGCACCC-3' and 2-Reverse outer 5'-GAGGCTCGGTGGTGCCATG-3' for genotype 3; finally, 4-Forward outer 5'-GCCAGACCTTGGGTGGCAGT-3' and 3-Reverse outer 5'-CGACCTGGGTGTAGGCACCC-3' and 1b-Reverse inner 5'-GTGGAACCATGGCCTGCAA-3' and 3-Reverse outer 5'-CGAGGCGACTGCACCC-3' and 2-Reverse outer 5'-GCCAGACCTTGGGTGGCAGT-3' for genotype 4.

The phylogeny of the sequences was constructed using the Neighbour Joining method. The nucleotide substitution model was selected according to Akaike Information Criterion scores. A Neighbour Joining tree was constructed with MEGA 5 software [20] setting the Tamura 3-parameter as an evolutionary model with a heterogeneous rate among sites using gamma distribution for the relative rate. Branch support was assessed by bootstrap analysis with 1000 replicates. Bootstrap values of 70% were used as the cut off point for cluster analysis. The sequences reported in this study have been submitted to the GenBank database under accession numbers J × 170910 to J × 171065.

The GenBank accession numbers for reference sequences used to determine the HCV genotypes were as follows: M62321 (subtype 1a), NC004102 (subtype 1a; H77-US1977), D90208 (subtype 1b), D14853 (subtype 1c), D00944 (subtype 2a), D10988 (subtype 2b), D50409 (subtype 2c), AB031663 (subtype 2 k), D71763 (subtype 3a), D49374 (subtype 3b), D63821 (subtype 3 k), Y11604 (subtype 4a), GU085486 (subtype 4a), FJ025855 (subtype 4b), FJ025854 (subtype 4b), FJ462436 (subtype 4c) FJ462437 (subtype 4d), EU392172 (subtype 4d), EU392170 (subtype 4f), FJ462432 (subtype 4 g), FJ462438 (subtype 4 k), EU392171 (subtype 4 k) EU392173 (subtype 4 k), FJ839870 (subtype 4 l), FJ462433 (subtype 4 m), FJ462441 (subtype 4n), FJ462440 (subtype 4 o), FJ462431 (subtype 4p), FJ462434 (subtype 4q) FJ462439 (subtype 4r) FJ839869 (subtype 4 t), Y13184 (subtype 5a), Y12083 (subtype 5a), D84262 (subtype 6 b), D84263 (subtype 6 d), D84262 (subtype 6 g), D84265 (subtype 6 h), D84264 (subtype 6 k).
Results
The clinical and virologic characteristics of patients considered in the study are provided in Table 1. Fifteen patients (9.5%) were co-infected with HIV and treated with highly active antiretroviral therapy (HAART) (Table 1).

Aa substitutions associated with HCV PI resistance were found in 50/156 (32%) sequences of PI naïve HCV patients (Table 2). Mutations V36L, T54S, V55A/I, and Q80K/L were observed in 29% of patients with genotype 1a, and V55F, Q80L/N and M175L in 10% of patients with genotype 1b. Mutation V158M was found in 3% patients with genotype 2, D168Q was present in 100% pts with genotype 3 and D168E was observed in 13% of patients with genotype 4 (Table 2). In addition, multiple aa polymorphisms not associated with PI resistance were detected in all genotypes (Table 2).

Amino acids at positions S138 and V170 reported to be correlated with PI resistance when there is a change from T to T/A [7], respectively, changed to S138C and V170I in patients with genotype 1b were not associated with PI resistance. In addition, all sequences of genotypes 2, 3 and 4 showed the mutation V36L, which has been associated with PI resistance in HCV patients with genotype 1. Of note, HCV PI resistance mutations in HCV/HIV co-infected patients were found in only one patient (Q80L).

A mutation at position 176 (S176N) different from that previously correlated with resistance (S176G) [11] was found in four patients with genotype 3. Previously reported [10] compensatory aa changes (I71V, I72T/F, and Q86P) were observed in both genotypes 1a and 1b. In individual genotype 1b strains, the resistance mutation V55F was associated with compensatory mutation T72I, mutation Q80L/N was associated with Q86P and mutation M175L was associated with T72I and Q86P. In contrast, the two compensatory mutations in genotype 1a were detected in the absence of resistance mutations.

Finally, a number of polymorphisms not associated with PI resistance, were detected between codon 4 and codon 179 in all genotypes (Table 3). In genotypes 1a, 1b and 4 multiple polymorphisms (4, 15 and 29, respectively) were detected, while 11 and 17 polymorphisms were found in genotypes 2 and 3, respectively. In detail, the number of aa changes for each natural polymorphic site in the different genotypes was 104 in genotype 1a, 186 in genotype 1b, 84 in genotype 2, 97 in genotype 3 and 255 in genotype 4.

The mean genetic diversity of NS3 was higher in genotype 4 (16.6%) than in genotype 1b (12.0%), 1a (10.4%), 2 (11.2%), and 3 (9.2%). Among patients infected with HCV genotype 1, sequences were equally distributed in HCV subtypes 1a and 1b. The number of sequences carrying mutations associated with PI resistance was 2-fold higher in subtype 1a with respect to subtype 1b (p = 0.07) (Figure 1). All sequences clustering within genotype 2, belonged to subtype 2c and only one sequence carried a mutation correlated with resistance. All sequences from patients infected with genotype 3 clustered in the subtype 3a, and all sequences showed the D168Q change (Figure 1). Among the HCV genotype 4 sequences, 14/23 (60.9%) belonged to subtype 4d, while, 6/23 (26.1%) were subtype 4a, 1/23 (4.3%) was subtype 4c and 2/23 (8.7%) clustered together with an uncommon subtype (Figure 1). Among these, one sequence exhibited 91.6% identity with HCV subtype 4 m, and the second exhibited 89.1% identity with HCV subtype 4 t (bootstrap value >99%) (Figure 1).

All HCV/HIV co-infected patients with genotype 4 clustered in the subtype 4c, while the mutations correlated with PI resistance were observed both in subtype 4c and subtype 4a (Figure 1). In particular, a mutation associated with PI resistance (D168E) was observed in two identical sequences (4-6706 m11 and 4-5208 m11) from different patients.

Table 1 Patient characteristics by HCV genotype

| Characteristic                          | 1a (n = 31) | 1b (n = 39) | 2 (n = 30) | 3 (n = 33) | 4 (n = 23) |
|-----------------------------------------|-------------|-------------|------------|------------|------------|
| Gender Male                             | 26 (83%)    | 20 (51%)    | 11 (36%)   | 26 (78%)   | 17 (85%)   |
| Female                                  | 5 (27%)     | 19 (49%)    | 19 (64%)   | 7 (22%)    | 3 (15%)    |
| Race Italian                            | 29 (93%)    | 38 (97%)    | 30 (100%)  | 31 (93%)   | 13 (65%)   |
| Others                                  | 2 (7%)      | 1 (3%)      | 0          | 2 (7%)     | 7 (35%)    |
| No. of patients HIV-1 co-infected       | 5           | 1           | 0          | 4          | 4          |
| receiving HAART (%)                     |             |             |            |            |            |
| Median HCV viral load (IU/mL log_{10})  | 5.66 (range 3.03-6.44) | 6.31 (range 4.2-6.75) | 5.96 (range 3.6-6.84) | 5.38 (range 2.97-6.58) | 5.66 (range 2.40-6.78) |
| in HCV mono-infected pts                |             |             |            |            |            |
| Median HCV viral load (IU/mL log_{10})  | 6.41 (range 4.73-6.77) | 5.95        | 0          | 6.54 (range 6.17-6.86) | 6.45 (range 6.42-6.52) |
| in HCV/HIV co-infected pts              |             |             |            |            |            |
**Discussion**

The identification of baseline resistance mutations to anti-HCV PIs is crucial for defining new therapeutic approaches. Natural polymorphisms in the HCV NS3/4A protease-coding region were analyzed in 156 patients including genotypes 1a, 1b, 2, 3, and 4. Relevant natural aa polymorphisms were found among the different genotypes and subtypes. The data presented are important not only to determine whether PI-resistant mutants are likely to be present in PI treatment-naive patients, but also for the examination of HCV protease among different genotypes and the possibility of eventually extending the PI treatment to non-genotype 1-infected patients. On the other hand, the study of preexisting viral variants to predict response to PIs for genotypes other than 1 might be misleading, since the molecular target structure could be considerably different between genotype 1 HCV and other genotypes. In fact, all recent clinical trials have been designed for treatment of HCV infections with genotype 1 [5,6,21]. Although culture systems for determining HCV susceptibility to PI compounds have been recently developed [4], the comparative genetic analysis of HCV strains in PI naïve patients infected with different virus genotypes may provide information useful for predicting treatment efficacy since, naturally occurring genotype-specific variations appear to have an effect in different HCV genotypes [22,23]. In addition, even though resistant viral variants exist at low frequency in untreated patients, specific NS3 protease mutations may have an important role in modulating resistance development and modifying viral fitness [22].

| NS3 Protease position | HCV variation in different genotypes (number of sequenced patients) |
|-----------------------|---------------------------------------------------------------|
|                       | 1a (n = 31) | 1b (n = 39) | 2 (n = 30) | 3 (n = 33) | 4 (n = 23) |
| 36 (R)                | V36L (2)    | V36         | L36       | L36       | L36       |
| 41 (R)                | Q41         | Q41         | Q41       | Q41       | Q41       |
| 43 (R)                | F43         | F43         | F43       | F43       | F43       |
| 54 (R)                | T54S (2)    | T54         | T54       | T54       | T54       |
| 55 (R)                | V55A/I (2)  | V55F (1)    | V55       | V55       | V55       |
| 79 (R)                | D79         | D79         | E79       | D79       | D79       |
| 80 (R)                | Q80K/L (3)  | Q80L/N (2)  | G80       | Q80       | Q80       |
| 109 (R)               | R109        | R109        | R109      | R109      | R109      |
| 138 (R)               | S138        | S138C (1)   | S138      | S138      | S138      |
| 155 (R)               | R155        | R155        | R155      | R155      | R155      |
| 156 (R)               | A156        | A156        | A156      | A156      | A156      |
| 158 (R)               | V158        | V158        | V158M (1) | V158      | V158      |
| 168 (R)               | D168        | D168        | D168      | D168Q (33) | D168E (3) |
| 170 (R)               | I170        | V170 (12)   | I170      | I170V (1) | I170      |
| 175 (R)               | L175        | M175L (1)   | L175      | L175      | L175      |
| 176 (R)               | E176        | E176        | D176      | S176N (4) | E176      |
| 71 (C)                | V71         | I71V/L (5)  | V71       | A71S (1)  | V71       |
| 72 (C)                | I72T/F (2)  | T72I/A/L (11)| T72       | L72F (1)  | N72C (2)  |
| 86 (C)                | P86         | Q86P (6)    | P86S (1)  | P86S (2)  | P86       |
| 88 (C)                | P88         | P88         | P88       | P88       | P88       |

*R, position associated with primary resistance; C, position associated with compensatory mutations; (Lopez, 2008; Flint, 2009; Susser, 2009; Lentz, 2010; Verbimen, 2010; Romano, 2010, Halfon, 2011).

Reference strains for each genotype: M62321 (1a), D90208 (1b), D50409 (2c), D17763 (3a), and Y11604 (4a).

Letter on the left represents the wild type amino acid, on the right, the amino acid substitution. The number of patients with mutant HCV strains is indicated in brackets.

Polyorphism with no associated resistance.

Amino acid changes conferring resistance are reported in bold.

Table 2 Amino acid variations in the HCV NS3 protein associated with resistance mutations to HCV NS3 protease inhibitors, compensatory and enhanced replication.
| NS3 Protease position \(^a\) | HCV variation in different genotypes (number of sequenced patients) \(^b\) | NS3 Protease position \(^a\) | HCV variation in different genotypes (number of sequenced patients) \(^b\) |
|-------------------------|-----------------------------|-------------------------|-----------------------------|
|                         | 1\(a\) (n = 31) | 1\(b\) (n = 39) | 2 (n = 30) | 3 (n = 33) | 4 (n = 23) | 1\(a\) (n = 31) | 1\(b\) (n = 39) | 2 (n = 30) | 3 (n = 33) | 4 (n = 23) |
| 4 | T4P (14) | S67P/A (14) | A67VT (7) |
| 5 | AST/P (3) | A5G/C (6) | H69R (2) |
| 7 | S7A (12) | A7T/V (11) | T72A/I/L (13) |
| 10 | T10H/N (2) | L82G (3) | V83N/T/I |
| 11 | R11P (2) | V83N/T/I |
| 12 | G12A/R (3) | S91A/T (28) | A91S (5) | A91T (4) |
| 13 | L13M/W/T (13) | T95E/S (2) | R02K/T (15) |
| 14 | L14F (5) | E95D (3) | A95T/S (4) |
| 15 | D15G (28) | A/V98 T (15) |
| 16 | A16T (8) | S101A (8) |
| 18 | I18V (2) | S102A (3) | A102S (20) |
| 20 | S20G (4) | Y105F (14) |
| 24 | R24K (2) | V107I (4) |
| 26 | K26R (3) | D110E (5) | H110N (3) |
| 28 | Q28E/L (3) | I114V (3) | I114V (18) |
| 33 | V33I (2) | V33I (10) | V33I (13) | R117H/C (4) |
| 35 | V35I (5) | V35I (3) | R119Q (2) |
| 39 | A39T (3) | S122G (10) | S122T/C (5) | T122S (2) |
| 40 | A40T/S (9) | R123K (3) |
| 42 | S42T/F (5) | L127I (11) |
| 44 | L44M (2) | I132V/M (18) | L132I (2) |
| 46 | T46S/A (2) | S134T (13) |
| 47 | A47G/S (2) | P146S (2) |
| 48 | V48I (4) | V48I (11) | S147L (2) | S147L/A/R/T (5) | M147L/Q (15) |
| 49 | N49S (2) | V150A (29) | R150V/A (20) |
| 51 | V51A (2) | V51T/A (3) | A151V (3) |
| 56 | Y56F/C (4) | I153V/L (3) |
| 60 | S60A/T/P (7) | S166A/T (3) |
| 61 | T61S (2) | K61R (4) | N174S/G (23) | S174A/L (3) | S174T (2) |
| 64 | I64L (2) | V177I (3) |
| 65 | S65C (2) | A179T/V (2) |

\(^a\)Amino acid position associated with polymorphisms compared to reference sequences;

\(^b\)Reference strain accession numbers for each genotype: M62321 (1a), D90208 (1b), D50409 (2c), D17763 (3a), and Y11604 (4a).

\(^c\)Letter on the left represents the wild type amino acid, on the right, the amino acid substitution. The number of patients with mutant HCV strains is indicated in brackets.
decreased susceptibility to telaprevir \([7,24]\) and all sequences from genotype 3 showed the D168Q mutation which is known to decrease the activity of non-covalent HCV NS3 protease inhibitors against genotype 3 \([4, 14]\). Moreover, a higher number of polymorphic sites in HCV protease NS3/4A were observed in genotypes 1b and 4 compared with genotypes 1a, 2 and 3. Further studies are needed to better understand the potential implications on treatment of PI naïve patients with resistance at baseline which could influence the treatment failure rate. On the other hand the clinical role of compensatory mutations impacting the viral fitness of PI resistant strains \([25-27]\) requires additional investigation.

In contrast with reported observations \([28]\), HCV PI resistance mutations were not observed more frequently in HCV/HIV co-infected patients than HCV mono-infected patients. Phylogenetic analysis confirmed the greater heterogeneity of HCV genotypes 1b and 4, which may be explained by the presence of several divergent sequences with respect to other genotypes. In keeping with data from the Italian HCV genotype distribution \([29]\), subtype 4d strains were observed also in our series. In this data set, a wide distribution of mutations correlating with PI resistance was observed in all genotypes. A larger data set of HCV sequences including baseline data would clarify this finding.
In conclusion, i) the natural variability in all HCV viral populations (HIV co-infected or mono-infected) observed in our study confirms [30-32] and underlines their potential implication in the management of HCV treatment; ii) no major mutations associated with resistance to PIs were observed in HCV PI naïve patients, on the contrary, a consistent number of minor mutations which may reduce the efficacy of PIs were detected in genotypes 1a, 1b and also in genotypes 2, 3 and 4. Thus, the inclusion of patients with different genotypes in future larger clinical trials, would help define the efficacy of anti HCV PIs in patients infected with different genotypes and these data could be extended to design treatment protocols. In addition, further investigations are necessary to understand the utility of resistance analysis at baseline to evaluate response to HCV protease inhibitors.

Consent
Written informed consent was obtained from patients for publication of this manuscript and any accompanying images. A copy of the letter of consent is available for review by the Editor-in-Chief of this journal.

Competing interests
The authors declare that they have no financial or competing interests.

Authors’ contributions
SP study design, data analysis and paper writing. LF, AP, MG, LD sequencing.

References
1. Lauer GM, Walker BD: Hepatitis C virus infection. N Engl J Med 2001, 345:51–52.
2. Manns MP, McHutchinson JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albright JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001, 358:958–965.
3. Hadziyannis SJ, Sette HJ, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackill AM, REGAHSYS International Study Group. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004, 140:346–355.
4. Gottweis JM, Scheel TK, Jensen TB, Ghanem L, Bulh J: Differential efficacy of protease inhibitors against HCV genotypes 2a, 3a, 5a and 6a NS3/4A protease recombinant viruses. Gastroenterology 2011, 141:1067–1079.
5. Sarrazin C, Kieffer TL, Bartels D, Hanzelka B, Müh U, Welker M, Wincheringer D, Zhou Y, Chu HW, Lin C, Weeink C, Reesink H, Zeuzem S, Kwong AD: Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology 2007, 132:1767–1777.
6. Kieffer TL, Sarrazin C, Miller JS, Welker MW, Forestier N, Reesink HW, Kwong AD, Zeuzem S, Telaprevir and pegylated interferon-alpha2a inhibit wild-type and resistant genotypes 1 hepatitis C virus replication in patients. Hepatology 2007, 46:631–639.
7. Lenz O, Verberink T, Lin TI, Vigen L, Cummings MD, Lindberg J, Berke JM, Dehertogt P, Fransen E, Scholliers A, Vermeiren K, Ivens T, Raboison P, Edlund M, Storrn S, Viang L, de Kock H, Fanning GC, Simmen KA: In vitro resistance profile of the hepatitis C virus NS3/4A protease inhibitor TMC435. Antimicrob Agents Chemother 2010, 54:1878–1887.
8. Romano KP, Ali A, Roys WE, Schiffer CA: Drug resistance against HCV NS3/4A inhibitors is defined by the balance of substrate recognition versus inhibitor binding. PNAS 2010, 107:20986–20991.
9. Haffton P, Locarnini S: Hepatitis C virus resistance to protease inhibitors. J Hepatol 2012, 55:192–206.
10. López-Labrador FX, Moya A, González-Candelas F: Mapping natural polymorphisms of hepatitis C virus NS3/4A protease and antiviral resistance to inhibitors in worldwide isolates. Antivir Therapy 2008, 13:481–494.
11. Flint M, Mullens S, Deatly AM, Chen W, Miller LZ, Raikoon R, Broom C, Emini EA, Hove AH: Selection and characterization of hepatitis C virus replicons dually resistant to the polymerase and protease inhibitors HCV-796 and boceprevir (SCH503043). Antimicrob Agents Chemother 2009, 53:401–411.
12. Susser S, Welsch C, Wang Y, Zettler M, Domingues FS, Kasey U, Hughes E, Raikoon R, Tong X, Herrmann E, Zeuzem S, Sarrazin C: Characterization of resistance to the protease inhibitor boceprevir in hepatitis C virus-infected patients. Hepatology 2009, 50:1709–1718.
13. Verberink T, van Marck H, Vandenbroucke L, Vigen L, Claes M, Lin TI, Simmen K, Natsys J, Fanning G, Lenz O: Tracking the evolution of multiple in vitro hepatitis C virus replicon variants under protease inhibitor selection pressure by 454 deep sequencing. J Virol 2010, 84:11124–11133.
14. Guo Z, Prongay A, Tong X, Fischmann T, Bogen S, Velazquez F, Venkataraman S, Njoroge FG, Madison V: Computational study of the effects of mutations A156T, D168V, and D168Q on the binding of HCV protease inhibitors. PLoS Comput Biol 2008, 4:1655–1663.
15. Thompson AJ, McHutchison JG: Antiviral resistance and specifically targeted therapy for HCV (STAT-C). J Viral Hepat 2009, 16:377–387.
16. Kuntzen T, Timm J, Berical A, Lennon N, Berlin AM, Young SK, Lee B, Heckerman D, Carlson J, Reyor LL, Kleyman M, McMahon CM, Birch C, Schulze Zur Wiesch J, Ledlie T, Koehnsen M, Kodira C, Roberts AD, Lauer GM, Rosen HR, Bihl F, Cerny A, Spengler U, Liu Z, Xing Y, Schneidewind A, Maday ME, Flackenstein JF, Park WM, Galagan E, Nusbaum C, Walker BD, Heckerman D, Carlson J, Neyts J, Fanning T, Venkataraman S, Njoroge FG, Madison V: Computational study of the effects of mutations A156T, D168V, and D168Q on the binding of HCV protease inhibitors. PLoS Comput Biol 2008, 4:1655–1663.
17. Trimoulet P, Belzunce C, Faure M, Wiltkop L, Reigadas S, Dupon M, Ragnaud JM, Fleurey H, Neau D: Hepatitis C virus (HCV) protease variability and anti-HCV protease inhibitor resistance in HIV/HCV-coinfected patients. HIV Med 2011, 12:506–509.
18. Haffton P, Bourlière M, Krithi H, Penaranda G, Martineau A, Oulès V, Courcambeck J, Philibert P: Mutation rate in hepatitis C virus NS5 protease is not influenced by HIV-1 protease inhibitor therapy. AIDS 2008, 22:1694–1696.
19. Morisca G, Bagaglio S, Uberti-Foppa C, Galli L, Lazarin A: Detection of hepatitis C patients with natural resistance to NS5A protease inhibitors in HIV/HCV-coinfected individuals treated with antiretroviral therapy. J Acquir Immune Defic Syndr 2009, 51:106–108.
20. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S: MEGAS: molecular evolutionary genetics analysis using maximumlikelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011, 28:2731–2739.
21. Pawlotsky JM: Treatment failure and resistance with direct-acting antiviral drugs against hepatitis C virus. Hepatology 2011, 53:1742–1751.
22. Welsh C, Schweizer S, Shimakami T, Domingues FS, Kim J, Lemon SM, Antes I: Ketoamide resistance and hepatitis C virus fitness in val55

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variants of the NS3 serine protease. Antimicrob Agents Chemother 2012, 56:1907–1915.

23. Thibeault D, Bousquet C, Gingras R, Lagacé L, Maurice R, White PW, Lamare D: Sensitivity of NS3 serine proteases from hepatitis C virus genotypes 2 and 3 to the inhibitor BILN 2061. J Virol 2004, 78:7352–7359.

24. Zhou Y, Bartels DJ, Hanzerka BI, Müh U, Wei Y, Chu HM, Tiggas AM, Brennan DL, Rao BG, Swenson L, Kwong AD, Lin C: Phenotypic characterization of resistant Val36 variants of hepatitis C virus NS3-4A serine protease. Antimicrob Agents Chemother 2008, 52:110–120.

25. Lin C, Luong YP, Rao BG, Wei YY, Brennan DL, Fulghum JR, Hisao HM, Ma S, Maxwell JP, Cottrell KM, Femi RB, Gates CA, Kwong AD: In vitro resistance studies of hepatitis C virus serine protease inhibitors, VX-950 and BILN 2061: structural analysis indicates different resistance mechanisms. J Biol Chem 2004, 279:17508–17514.

26. Yi M, Tong X, Skelton A, Chase R, Chen T, Prongay A, Bogen S, Saksena AK, Njoroget FG, Veselenak RL, Pyles RB, Bourne N, Malcolm BA, Lemon SM: Mutations conferring resistance to SCH6, a novel hepatitis C virus NS3/4A protease inhibitor. Reduced RNA replication fitness and partial rescue by second-site mutations. J Biol Chem 2006, 281:8205–8215.

27. Liu R, Abid K, Pichardo J, Pazienza V, Ingravallo P, Kong R, Agraval S, Bogen S, Saksena A, Cheng KC, Prongay A, Njoroget FG, Barouby M, Negro F: In vitro antiviral activity of SCH446211 (SCH6), a novel inhibitor of the hepatitis C virus NS3 serine protease. J Antimicrob Chemother 2007, 59:51–58.

28. Eshun-Wilson I, Plas HV, Prozesky HW, Zeier MD, Nachega J, Taljaard JJ: Combined antiretroviral treatment initiation during hospitalization: outcomes in South African adults. AIDS 2009, 51:106–108.

29. Argentini C, Dettori S, Villano U, Guadagnino V, Infantolino D, Dentico P, Coppola RC, Rapcetta M: Molecular characterization of HCV genotype 4 isolates circulating in Italy. J Med Virol 2000, 62:89–90.

30. Vallet S, Gounou F, Nsouba B, Legrand-Quillen MC, Goudeau A, Picard B: Genetic heterogeneity of the NS3 protease gene in hepatitis C virus genotype 1 from untreated infected patients. J Med Virol 2005, 75:528–537.

31. Winters MA, Welles SL, Holodny M: Hepatitis C virus protease gene diversity in patients coinfected with human immunodeficiency virus. J Virol 2006, 80:4196–4199.

32. Franco S, Pareta M, Aparicio E, Cloet B, Martinez MA: Genetic and catalytic efficiency structure of an HCV protease quasispecies. Hepatology 2007, 45:899–910.

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