2016

Polymorphisms and resistance mutations of hepatitis C virus on sequences in the European hepatitis C virus database

Dimas Alexandre Kliemann
Universidade Federal de Ciências da Saúde de Porto Alegre

Cristiane Valle Tovo
Universidade Federal de Ciências da Saúde de Porto Alegre

Ana Beatriz Gorini da Veiga
Universidade Federal de Ciências da Saúde de Porto Alegre

Angelo Alves de Mattos
Universidade Federal de Ciências da Saúde de Porto Alegre

Charles Wood
University of Nebraska - Lincoln, cwood1@unl.edu

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v22.i40.8910

World J Gastroenterol 2016 October 28; 22(40): 8910-8917
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Polymorphisms and resistance mutations of hepatitis C virus on sequences in the European hepatitis C virus database

Dimas Alexandre Kliemann, Cristiane Valle Tovo, Ana Beatriz Gorini da Veiga, Angelo Alves de Mattos, Charles Wood

Dimas Alexandre Kliemann, Cristiane Valle Tovo, Ana Beatriz Gorini da Veiga, Angelo Alves de Mattos, Graduate Program in Medicine: Hepatology - Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS 90050-170, Brazil

Dimas Alexandre Kliemann, Cristiane Valle Tovo, Hospital Nossa Senhora da Conceição, Porto Alegre, RS 90050-170, Brazil

Ana Beatriz Gorini da Veiga, Department of Basic Health Sciences, Laboratory of Molecular Biology, Graduate Program in Pathology - Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS 90050-170, Brazil

Charles Wood, School of Biological Sciences, Nebraska Center for Virology, University of Nebraska, Lincoln, NE 68511, United States

Author contributions: Kliemann DA designed this study, acquired and analyzed data; Kliemann DA and Wood C interpreted the data; Tovo CV, da Veiga ABG, de Mattos AA and Wood C contributed to writing of article, editing, reviewing and final approval of the article.

Institutional review board statement: The research protocol was approved by the Research Ethics Committee at Hospital Nossa Senhora da Conceição 12089/2012, approval report 12/2012.

Conflict-of-interest statement: The authors declare that no conflict of interest exists.

Data sharing statement: All available data can be obtained by contacting the corresponding author.

Manuscript source: Invited manuscript

Correspondence to: Dimas Alexandre Kliemann, MD, PhD, Hospital Nossa Senhora da Conceição - Serviço de Infectologia, Av. Francisco Trein, 596, bairro Cristo Redentor, Porto Alegre, Rio Grande do Sul 91350-170, Brazil. dimaskliemann@gmail.com
Telephone: +55-51-3357216
Fax: +55-51-3357216

Received: June 19, 2016
Peer-review started: June 21, 2016
First decision: August 8, 2016
Revised: September 12, 2016
Accepted: September 28, 2016
Article in press: September 28, 2016
Published online: October 28, 2016

Abstract

AIM

To evaluate the occurrence of resistant mutations in treatment-naïve hepatitis C virus (HCV) sequences deposited in the European hepatitis C virus database (euHCVdb).

METHODS

The sequences were downloaded from the euHCVdb (https://euhcvdb.ibcp.fr/euHCVdb/). The search was performed for full-length NS3 protease, NS5A and NS5B polymerase sequences of HCV, separated by genotypes 1a, 1b, 2a, 2b and 3a, and resulted in 798 NS3, 708 NS5A and 535 NS5B sequences from HCV genotypes

1a, 1b, 2a, 2b and 3a, after the exclusion of sequences containing errors and/or gaps or incomplete sequences, and sequences from patients previously treated with direct antiviral agents (DAA). The sequence alignment was performed with MEGA 6.06 MAC and the resulting protein sequences were then analyzed using the BioEdit 7.2.5. for mutations associated with resistance. Only positions that have been described as being associated with failure in treatment in *in vivo* studies, and/or as conferring a more than 2-fold change in replication in comparison to the wildtype reference strain in *in vitro* phenotypic assays were included in the analysis.

RESULTS

The Q80K variant in the *NS3* gene was the most prevalent mutation, being found in 44.66% of subtype 1a and 0.25% of subtype 1b. Other frequent mutations observed in more than 2% of the NS3 sequences were: I170V (3.21%) in genotype 1a, and Y56F (15.93%), V132I (23.28%) and I170V (65.20%) in genotype 1b. For the NS5A, 2.21% of the genotype 1a sequences have the P58S mutation, 5.95% of genotype 1b sequences have the R30Q mutation, 15.79% of subtypes 2a sequences have the Q30R mutation, 23.08% of subtype 2b sequences have a L31M mutation, and in subtype 3a sequences, 23.08% have the M31L resistant variants. For the NS5B, the V321L RAV was identified in 0.60% of genotype 1a and in 0.32% of genotype 1b sequences, and the N142T variant was observed in 0.32% of subtype 1b sequences. The C316Y, S556G, D559N RAV were identified in 0.33%, 7.82% and 0.32% of genotype 1b sequences, respectively, and were not observed in other genotypes.

CONCLUSION

HCV mutants resistant to DAAs are found in low frequency, nevertheless they could be selected and therapy could fail due resistance substitutions in HCV genome.

Key words: Hepatitis C virus resistance; Quasispecies; Direct antiviral agents; Polymorphisms; Drug resistance

Core tip: Chronic hepatitis C virus (HCV) infection is a significant cause of morbidity and mortality. The main therapeutic targets are the NS3/4A protease, NS5B polymerase, and NS5A replication complex. Pre-existence of resistance associated variants to direct antiviral agents (DAA) reduces sustained virologic response rates. Despite the low frequency of mutations, this resistant population is likely to be selected in patients undergoing therapy with DAA. Even though HCV variants resistant to DAA targeting one viral protein remain susceptible to DAA targeting another viral protein, combination therapy could fail due to selection of HCV with resistance substitutions in multiple targets.

Kliemann DA, Tovo CV, da Veiga ABG, de Mattos AA, Wood C. Polymorphisms and resistance mutations of hepatitis C virus on sequences in the European hepatitis C virus database. *World J Gastroenterol* 2016; 22(40): 8910-8917 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i40/8910.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i40.8910

INTRODUCTION

Chronic hepatitis C virus (HCV) infection affects around 180 million people worldwide and is a significant cause of liver-related morbidity and mortality[1] until recently. Interferon-α in combination with Ribavirin was the mainstream treatment regimen but eligibility and safety of the interferon-based therapies were low, and consequently the overall effectiveness of the treatment was very limited. Fortunately, the development of new direct-acting antiviral (DAA) drugs against HCV has progressed significantly and resulted in oral interferon-free therapies[2].

The three main therapeutic targets for HCV infection are the NS3/4A protease, the NS5B polymerase, and the NS5A replication complex. The first series of interferon-free regimens, including combinations of simeprevir (SMV), sofosbuvir (SOF), paritaprevir, daclatasvir, ledipasvir (LDV), ombitasvir (OMV), dasabuvir (DSV), grazoprevir (GZR) and elbasvir have already been approved and recommended by the European Association for the Study of the Liver (EASL) and by the American Association for the Study of Liver Diseases (AASLD)[3,4].

HCV variants infecting the human population show extreme genetic diversity, which is partly explained by the long evolutionary association between the virus and its human host. HCV exists in the host as a swarm of related quasispecies. This diversity is a result of the error-prone viral polymerase combined with rapid viral replication, which, in turn, enables the virus to rapidly overcome the host immune responses and to become resistant to antiviral drugs[5]. The selection of resistance-associated amino-acid variants (RAV) from HCV quasispecies is dependent on drug-, host- and virus-related factors. The potency of the drug itself is primarily influenced by viral susceptibility, by previous exposure to the drug and by the genetic barrier to resistance. The ability of a RAV to persist and to induce treatment failure (relapse, non-response or viral breakthrough) is related to its fitness or its replication capacity as compared to the wild-type virus[6,7].

Resistance to DAAs is driven by the selection of mutations at different positions in the NS3 protease, NS5B polymerase and NS5A protein[8,9]. Each compound or drug family induces a specific mutation profile that may be characteristic of the viral genotype/subtype. Furthermore, each class of DAA is characterized by a difference in the genetic barrier to resistance. Even though the specific resistance mutation for each individual agent in the drug class differs, there is a

great concern about the possibility of cross-resistance between compounds in the same inhibitor class, especially for the NS3 protease and NS5A inhibitors[10].

The ability to detect RAV depends primarily on the different types of the sequencing technologies used, including population-based sequencing, clonal sequencing and deep sequencing. The sensitivities for detection by these three approaches were reported to be approximately 25%, 5% and 0.5%, respectively, and the presence of viral mutants below the detection levels might be missed[11]. For HCV the frequency of routine testing of drug resistance prior to the use of the new treatment regimens is not known. Some guidelines[4] suggest that routine monitoring for HCV drug resistance-associated variants during therapy should not be recommended and there is no consensus on the utility of pre-treatment resistance testing.

Currently, there is a large number of HCV sequences available on public databanks, however they have not been analyzed to correlate HCV genotypes and viral genomic characteristics with drug resistance phenotypes[11]. Three HCV databases are currently available to provide insight into the basic biology, immunology, and evolution of the virus: the Japanese database (http://s2as02.genes.nig.ac.jp), the European database (http://euhcvdb.ibcp.fr) and the American database (Los Alamos National Laboratory) (http://hcv.lanl.gov).

The objective of this study is to evaluate the occurrence of polymorphisms and resistant mutations in the NS3, NS5A and NS5B regions in treatment-naïve HCV sequences deposited in the European hepatitis C virus database (euHCVdb). This analysis will provide insights into the levels of circulating drug resistance, which may affect the success of the therapeutic regimens.

MATERIALS AND METHODS

HCV database

The sequences were downloaded from the euHCVdb (https://euhcvdb.ibcp.fr/euHCVdb/). This bank provides important data about the HCV sequences (*e.g.* genotype, genomic region, viral proteins and their functions, known 3-dimensional structures) and ensures consistency of the annotations, which enables reliable keyword queries. Users can extract subsets of sequences obtained by Sanger sequencing matching particular criteria or enter their own sequences and analyze them with various bioinformatics programs available on the same server. The euHCVdb is mainly oriented towards protein sequence, structure and function analyses and structural biology of HCV, and is re-built every month from an up-to-date database by an automated process[12].

The search was performed for full-length NS3 protease, NS5A and NS5B polymerase sequences of HCV separated by genotypes 1a, 1b, 2a, 2b and 3a. These subtypes were chosen due to their worldwide prevalence and presence in drug trials, specifically genotype 1 with protease inhibitors (PI) and genotype 3 with polymerase inhibitor. Reference strains for the three genotypes were obtained (1a: AF009606, 1b: D90208, 2a: D00944, 2b: D10988 and 3a: D17763). Sequences containing missing data, such as gaps and sequencing errors, and sequences from patients previously treated with DAA were excluded from the analysis. To ensure the quality of the analysis, sequences with stop codons in the NS5B gene or with ambiguities consisting of more than 2 bases per nucleotide position or more than 2 ambiguities per codon at individual drug resistance-associated position were also excluded.

Alignment and edition of the sequences

The sequence alignment was performed with MEGA 6.06 MAC[13] followed by sequence editing, exclusion of sequences with missing data, and translation of the nucleic acids sequences into amino acids. The resulting protein sequences were then analyzed using BioEdit 7.2.5. to identify mutations associated with resistance[14].

Analysis of natural polymorphisms

Known mutations associated with resistance to protease-, NS5A complex- and polymerase-inhibitors were used to search for polymorphism patterns among HCV genotypes[15]. Only positions that have been described as being associated with failure in treatment in *in vivo* studies, and/or as conferring a more than 2-fold change in replication in comparison to the wildtype reference strain in *in vitro* phenotypic assays were included in the analysis.

RESULTS

Database search

The search resulted in 831 NS3, 869 NS5A and 6,065 NS5B sequences from HCV genotypes 1a, 1b, 2a, 2b and 3a. After the exclusion of incomplete sequences and those containing errors and/or gaps, and from patients previously treated with DAA, 798 sequences were included in the NS3 dataset. There were 313 from genotype 1a, 412 from genotype 1b, 19 from genotype 2a, 26 from genotype 2b and 28 from genotype 3a. There were 699 sequences identified in the NS5A dataset, with 272 from genotype 1a, 353 from genotype 1b, 19 from genotype 2a, 26 from genotype 2b and 29 from genotype 3a. For the NS5B polymerase there were 535 HCV sequences: 165 from genotypes 1a, 307 from genotype 1b, 19 from genotype 2a, 24 from genotype 2b and 20 from genotype 3a. Notably, the NS5B region has more than 5300 incomplete sequences deposited into this databank.

Mutation analyses

Mutation analyses were performed for positions

Table 1 Main amino acid substitutions found in the hepatitis C virus NS3 protease

	Amino acid (frequency %)											
	HCV genotype											
	1a			1b			2a		2b		3a	
Position	wt	Variants		wt	Variants		wt	Variants	wt	Variants	wt	Variants
36	V (98.08)	L (1.6)	M (0.32)	L (0.74)	I (0.25)	V (99.01)	L (100)	-	L (100)	-	L (100)	-
80	Q (54.37)	K (44.66)	R (0.97)	Q (93.37)	K (0.25)	L (6.39)	G (100)	-	G (100)	-	Q (100)	-
155	R (99.36)	K (0.64)	-	R (99.50)	P (0.50)	-	R (100)	-	R (100)	-	R (100)	-
156	A (100)	-	-	A (100)	-	-	A (100)	-	A (100)	-	A (100)	-
168	D (99.36)	E (0.32)	G (0.32)	D (98.77)	A (0.25)	E (0.98)	D (100)	-	D (100)	-	Q (100)	-

Amino acids in bold are associated with resistance. wt: Wild-type; HCV: Hepatitis C virus.

where resistance-associated amino acid substitutions have been described in the literature for conferring resistance to DAA. Amino acid substitutions related to HCV resistance to DAA are described below.

Frequency of resistance-associated variants

NS3/4A PI (Table 1): The available PI are more effective against HCV genotype 1 than to other genotypes due to natural polymorphisms in the NS3 region of the latter, therefore they are only used in the treatment of patients carrying HCV genotype 1. Thus, our analysis discusses mainly the findings for the genotype 1 dataset; nevertheless, the results for the other genotypes are shown in Table 1. The Q80K variant was the most prevalent mutation, found in 44.66% of the subtype 1a, and in 0.25% of subtype 1b sequences; the variant V80L was also observed in 6.39% of the latter. Other positions with frequencies higher than 2% were I170V (3.21%) in genotype 1a, and Y56F (15.93%), V132I (23.28%) and I170V (65.2%) in genotype 1b.

The V36L and V36M RAVs were identified in 1.6% and 0.32% of genotype 1a sequences, respectively, and in 0.74% and 0% of genotype 1b, respectively. The T54S variant was observed in 0.97% of genotype 1a and in 0.5% of genotype 1b sequences. The R155K variant was observed in 0.64% of genotype 1a sequences and was not observed in genotype 1b. There were two genotype 1b sequences (0.5%) with P substitution at position 155. Finally, no RAV A156T mutation was found in the 831 NS3 sequences analyzed.

The prevalence of resistant variants for the PI was found to be low in the dataset. Amino acid substitutions conferring resistance to these drugs were observed at NS3 position 168; the most frequent mutation was D168E, which was found in 0.32% of subtype 1a and in 0.98% of subtype 1b sequences. The prevalence of known NS3 variants enriched for by GZR was found to be low: F43S (0.31%) and Y56H (0%) in the whole dataset; the NS3 Q41R mutation was not observed.

NS5A replication complex inhibitors (Table 2): For subtype 1a there were a total of 272 NS5A sequences in our dataset. Mutations L23M (0.37%), M28T (0.75%), Q30H (1.47%), Q30R (0.37%), L31M (1.12%), P58S (2.21%) and Y93C (0.37%) were observed, whereas no variants were observed at NS5A position 32. Of the 353 subtype 1b sequences analyzed, 0.28% had the L23I mutation, 2.27% had L28M mutation, 5.95% had R30Q mutation, 3.40% had M31L mutation, 3.68% had P58S mutation, and 4.25% had the Y93H mutation. Of 19 subtype 2a sequences analyzed, one (5.26%) sequence had the Q30R mutation, 3 (15.79%) sequences had the M31L mutation, and one (5.26%) sequence had the H58P mutation. For subtype 2b a total of 26 sequences were analyzed, 6 (23.08%) with the L31M and one (3.85%) with the S58P mutation. In subtype 3a, for which 28 sequences were analyzed, the resistant variants M28I, A30L and P58R were found, each in a different sequence (3.57%) of the dataset. Only M31L was found in more than one sequence (23.08%) for this subtype. No mutation was found in the NS5A sequence at position 32 of any subtype.

NS5B polymerase inhibitors (Table 3): The NS5B S96T, C223H/Y, and S282T variants were not observed in any sequence in the present study, and the NS5B N142T variant was observed in 0.32% of the subtype 1b sequences. The V321L RAV was identified in 0.6% of genotype 1a sequences and in 0.32% of genotype 1b sequences.

The C316Y, S556G, and D559N RAVs were identified in 0.33%, 7.82% and 0.32% of genotype 1b sequences, respectively, and were not observed in other genotypes. The M414T and Y448H RAVs were not found in any of the 535 NS5B sequences analyzed.

Variants at NS5B positions 495 and 496 known to confer resistance polymerase inhibitors were not observed; on the other hand, the NS5B A421V and V499A substitutions were found in both subtypes 1a and 1b. The A421V mutation occurred in 9.64% of subtype 1a and in 4.55% of subtype 1b sequences. The V499A variant was the dominant amino acid substitution in subtype 1a sequences (95.15%), while for subtype 1b it was observed in only 9.74% of the sequences; there has been no reported evidence for negative clinical impact of the V499A.

Table 2 Main amino acid substitutions found in the hepatitis C virus NS5A protease

	Amino acid (frequency %)																							
	HCV genotype																							
	1a								1b								2a				2b				3a			
Position	wt								wt								wt				wt				wt			
28	M	94.38	I	0.37	T	0.75	V	4.49	L	97.17	M	2.27	V	0.52			F	100			L	100			M	96.46	I	3.57
30	Q	98.16	H	1.47	R	0.37			R	92.35	K	1.13	L/M	0.28	Q	5.95	K	94.74	R	5.26	K	100			A	96.46	L	3.57
31	L	98.88	M	1.12					L	96.03	M	3.40	I	0.57			M	84.21	L	15.79	M	76.92	L	23.08	L	100		
93	Y	98.90	C	0.37	H	0.74			Y	95.75	H	4.25					Y	100			Y	100			Y	100		

Amino acids in bold are associated with resistance. wt: Wild-type; HCV: Hepatitis C virus.

DISCUSSION

The resistance to direct antiviral therapy has been a major problem in a number of chronic viral infections. While much attention has been given to studies about HIV infection and resistance to antiviral therapy[16], the extent of mutations in the development of drug resistance in infection by HCV is less studied. The presence of HCV mutations is mainly due to factors such as selection pressure, error-prone replication (because of RNA polymerase's poor fidelity) and the high replication capacity of the virus. It is believed that any mutant can be generated continuously in a HCV-infected patient[17]. Hence, selected variants are considered to be pre-existent mutants generated during the natural HCV life cycle. The incidence of resistant variants is variable and depends on the binding domain, as well as on the different HCV populations, genotypes and subtypes.

With the exception of NS5B nucleoside analogues, the current DAAs target the NS3, as well as the allosteric sites of NS5B and NS5A, which all have a low threshold of resistance[10,18]. Data from both replicon analysis and from clinical trials have consistently identified viral mutations that can be associated with antiviral treatment failure[19]. A recently published analysis found that 58.7% of the HCV sequences deposited in GenBank harbored at least one dominant resistance variant[20]. In the present study, the overall prevalence of patients with variants resistant to DAAs was found to be low.

The frequency of variants resistant to NS5A-inhibitors ranged from 0% to 4.25%. Variants resistant to polymerase-inhibitors were observed mainly in genotype 1b and occurred in up to 7.82% (palm site), 2.41% (thumb site), and 9.74% (finger-loop site) of the sequences. These data corroborate other studies reported in the literature[15,21], and a comparasion with an analysis performed in Los Alamos databank[11] showed similar results (Table 4).

Pre-existing dominant resistance mutations in the NS3 region are more common in treatment-naïve patients infected with genotype 1a (cumulative incidence 8.6% *vs* 1.4%)[22]. Within NS3, the resistant Q80K mutation, which is based on available data only relevant for SMV and ASV, was the most prevalent (44.66% genotype 1a, 0.25% genotype 1b) and this result corroborates the recent findings of Pol *et al*[1,23] with European patients where, Q80K was observed in 34.7% and 2.1% of subtype 1a and 1b patients, respectively. The mutation I170V, present in 3.21% of genotype 1a and 65.20% of the genotypes 1b sequences analyzed, has been reported as not showing any influence on protease inhibitor activity[24]. Therefore, considering the actual recommendations in EASL and AASLD guidelines, up to 45% of patients with genotypes 1 have resistance mutations that can lead to treatment failure using PI.

The prevalence of resistant variants in the context of the NS5A inhibitors is highly dependent on viral subtype due to several positions having different baseline amino acids in each subtype[15]. Resistance against DVC, OMV, LDV is more common in genotype 1b (up to 4.25% of the sequences), but it can also occur in genotype 1a in less than 1.5% of the sequences. Furthermore, a broad cross-resistance between NS5A inhibitors is expected by the selection of mutations at codons 31 and/or 93 causing a loss in susceptibility to the majority of these compounds[24]. Other researchers also determined Y93H as most frequent baseline NS5A RAV in genotype 1b (6%-23%), followed by L31M (3%-4%)[24,25], whereas NS5A RAVs occurred at low frequencies in genotype 1a. Across the HCV genotypes, variation is observed at several of the residues identified as important sites for resistance, and substitutions M28L, Q30R, H58P that were found in genotype 1b; M28F, Q30K, L31M, H58P in genotype 2a; M28L, Q30K, L31M, H58P in genotype 2b and Q30A in genotype 3a could be defined as natural polymorphisms that distinguish those genotypes from 1a.

Table 3 Main amino acid substitutions found in the hepatitis C virus NS5A protease

	Amino acid (frequency %)														
	HCV genotype														
	1a				1b								2a	2b	3a
Position	wt				wt								wt	wt	wt
282	S	99.40	R	0.60	S								S	S	S
316	C				N	37.02	C	62.34	**R/Y**	**0.32**			C	C	C
556	S				N	89.24	D	0.98	**G**	**7.82**	N	1.95	G	G	G

Amino acid in bold are associated with resitance. wt: Wild-type; HCV: Hepatitis C virus.

Table 4 Resistance associated variants conferring resistance to direct antiviral agents[1]

	euHCVdb		Los Alamos	
	1a	1b	1a	1b
DAA	RAV	RAV	RAV	RAV
NS3				
Simeprevir	V36M (0.32%)		V36M (0.44%)	
	Q80K (44.66%)	Q80L (6.39%)	Q80K (36.62%)	Q80L (6.02%)
	S122G (4.49%)	S122G (9.07%)	n.a	n.a
	R155K (0.64%)		R155K (0.88%)	
	D168E (0.32%)	D168E (0.98%)	D168E (0.29%)	D168E (0.80%)
				D170T (0.20%)
Paritaprevir	R155K (0.64%)		R155K (0.88%)	
	D168E (0.32%)	D168E (0.98%)	D168E (0.29%)	D168E (0.80%)
Grazoprevir	A156T (0.00%)	A156T (0.00%)	A156T (0.00%)	A156T (0.00%)
	D168E (0.32%)	D168E (0.98%)	D168E (0.29%)	D168E (0.80%)
NS5A				
Ledipasvir	M28T (0.75%)		n.a	n.a
	Q30H (1.47%)			
	Q30R (0.37%)			
	L31M (1.12%)			
	Y93H (0.74%)	Y93H (4.25%)		
	Y93C (0.37%)			
Daclatasvir	M28T (0.75%)		n.a	n.a
	Q30H (1.47%)			
	Q30R (0.37%)			
	Y93H (0.74%)	Y93H (4.25%)		
Ombitasvir	M28V (4.49%)	Y93H (4.25%)	n.a	n.a
Elbasvir	Q30H (1.47%)			
	L31M (1.12%)			
	Y93H (0.74%)	Y93H (4.25%)		
NS5B				
Sofosbuvir	S282T (0.00%)	S282T (0.00%)	S282T (0.00%)	S282T (0.00%)
Dasabuvir		C316N (37.02%)		C316N (36.17%)
		C316Y (0.32%)		C316Y (0.30%)
		N556G (7.82%)	N556G (0.42%)	N556G (8.21%)

[1]DAAs recommended by the EASL and AASLD guidelines 2015. RAV: Resistance associated variants; DAAs: Direct antiviral agents; AASLD: American Association for the Study of Liver Diseases.

In contrast to NS3 PI, NS5B-non-nucleoside-inhibitors and NS5A-inhibitors where resistance mutations are subtype-dependent, little is known about NS5B nucleos(t)ide analogs genotype- and subtype-dependent resistance mutations. Several nucleotide analogs have shown very promising results and SOF is the first DAA in this drug class to gain regulatory approval[9,26], followed by DSV. In the present analysis, NS5B RAV were not detected in genotype 1a, whereas in genotype 1b, NS5B RAV were found in more than one third of the individuals (C316N in 37.02% and S556G in 7.82%) conferring low to medium resistance to DSV. In mixed cohorts consisting of American and European patients, while the S556G mutation was observed in frequencies of 0.5%-16%, the C316N RAVs occurred in frequencies lower than that observed in this study (11%-18%), at baseline in genotype 1b samples[15].

The S282T is the *in vitro* signature resistance mutation that conveys decreased susceptibility to SOF in the replicon system. Although the S282T substitution requires only a single nucleotide change, this variant was not found in any of the NS5B sequences analyzed in this study, neither in a previous study based on sequences from the Los Alamos databank[11]; in a study that analyzed 1459 HCV sequences from GenBank, this mutation was found in only one sequence[20].

With the currently in-use DAAs recommended by EASL and AASLD guidelines, our analyses suggest that it is possible that virologic failure could occur in half of the patients with HCV genotype 1a receiving SMV in combinations with pegylated-intereferon and ribavirin. In addition, more than 7% of the patients with HCV genotype 1b receiving DSV could also fail to respond to treatment, and the presence of variants with resistant mutations in the NS5A region should affect almost 5% of the treated individuals.

If in one hand the analysis of a public databank may not reflect the real prevalence of RAV in the population, on the other hand the abundance of information deposited in databank's sequences allows the identification of potentially unknown polymorphisms in populations not submitted to new HCV treatments. Since it is impossible to correlate criteria of inclusion in databanks with population data, epidemiological studies are necessary to determine the real prevalence of RVA in the population.

Although the potential to confer resistance to DAAs of the majority of the amino acid substitutions identified in our analyses is not known, the ability of HCV to rapidly evolve under drug selection pressure and the presence of baseline natural polymorphisms associated with resistance to DAA should be considered as possible threats to the success of these new therapies. The real impact of these constitutive RAV on the possibility of SVR with DAA remains unclear and undefined and some of these RAV apparently disappear after therapy (NS3/NS4 RAV) while others remain in the viral population (NS5 RAV). Globally, clinical significance of these constitutive RAV remains obscure

In summary, there are many relevant clinical questions that still need to be answered regarding HCV resistance to DAAs, mainly due to the limited available data and the large number of DAA approved or soon to be approved for clinical use. Perhaps resistance mutations in the new interferon-free DAA era may not have significant clinical impact initially[27,28], nonetheless the presence of a minor drug-resistance population will likely affect the success of the therapy upon the expansion and prolonged use of DAA regimens, and the relevance of pre-existing resistance mutations for responses to Interferon-free DAA therapies needs to be further investigated. Therefore, testing for drug resistance variants prior to the initiation of treatment will be needed in the very near future in order to help guide the selection of the most optimized treatment option.

COMMENTS

Background

Chronic hepatitis C virus (HCV) infection is a significant cause of morbidity and mortality worldwide. The main therapeutic targets against HCV are the viral NS3/4A protease, NS5B polymerase, and NS5A replication complex. While much attention has been given to HIV infection and resistance to antiviral therapy, the extent of mutations in the development of drug resistance in HCV infection is less studied. The presence of HCV mutations is mainly due to factors such as selection pressure, error-prone replication (because of RNA polymerase's poor fidelity) and the high replication capacity of the virus. It is believed that any mutant can be generated continuously in HCV-infected patients. Hence, selected variants are considered to be pre-existent mutations generated during the natural HCV life cycle. The incidence of resistant variants is variable and depends on the binding domain, as well as on the different HCV populations, genotypes and subtypes and pre-existence of resistance associated variants to direct antiviral agents (DAAs) reduces sustained virologic response rates. A recently published analysis found that 58.7% of the HCV sequences deposited in the GenBank harbored at least one dominant resistance variant.

Research frontiers

It is expected that in the near future a method able to detect all the mutations in the HCV genome will be available, making it possible to decide which DAA can be used to treat hepatitis C in a specific patient.

Innovations and breakthrough

This study showed a low frequency of mutations but a high number of polymorphisms of HVC genome which can impact in patients receiving treatment with DAAs.

Applications

Although the potential to confer resistance of the majority of the amino acid substitutions identified in our analyses is not known, the ability of HCV to rapidly evolve under drug selection pressure and the presence of baseline natural polymorphisms associated with resistance to DAAs should be considered as possible threats to the success of these new therapies.

Peer-review

This manuscript analyzed the occurrence of polymorphisms and resistant mutations in NS3, NS5A and NS5B regions in treatment-naive HCV sequences deposited in the European hepatitis C virus database.

REFERENCES

1 **Pol S**, Vallet-Pichard A, Corouge M, Mallet VO. Hepatitis C: epidemiology, diagnosis, natural history and therapy. *Contrib Nephrol* 2012; **176**: 1-9 [PMID: 22310776 DOI: 10.1159/000332374]

2 **Cornberg M**, Manns MP. New kids on the block--step by step to an ideal HCV therapy. *Lancet* 2015; **385**: 1050-1052 [PMID: 25468162 DOI: 10.1016/S0140-6736(14)62008-0]

3 **European Association for Study of Liver**. EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol* 2015; **63**: 199-236 [PMID: 25911336 DOI: 10.1016/j.jhep.2015.03.025]

4 **AASLD/IDSA HCV Guidance Panel**. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology* 2015; **62**: 932-954 [PMID: 26111063 DOI: 10.1002/hep.27950]

5 **Gray RR**, Salemi M, Klenerman P, Pybus OG. A new evolutionary model for hepatitis C virus chronic infection. *PLoS Pathog* 2012; **8**: e1002656 [PMID: 22570609 DOI: 10.1371/journal.ppat.1002656]

6 **Welsch C**, Jesudian A, Zeuzem S, Jacobson I. New direct-acting antiviral agents for the treatment of hepatitis C virus infection and perspectives. *Gut* 2012; **61** Suppl 1: i36-i46 [PMID: 22504918 DOI: 10.1136/gutjnl-2012-302144]

7 **Schneider MD**, Sarrazin C. Antiviral therapy of hepatitis C in 2014: do we need resistance testing? *Antiviral Res* 2014; **105**: 64-71 [PMID: 24583028 DOI: 10.1016/j.antiviral.2014.02.011]

8 **Kieffer TL**, George S. Resistance to hepatitis C virus protease inhibitors. *Curr Opin Virol* 2014; **8**: 16-21 [PMID: 24852142 DOI: 10.1016/j.coviro.2014.04.008]

9 **Soriano V**, Vispo E, de Mendoza C, Labarga P, Fernandez-Montero JV, Poveda E, Treviño A, Barreiro P. Hepatitis C therapy with HCV NS5B polymerase inhibitors. *Expert Opin Pharmacother* 2013; **14**:

1161-1170 [PMID: 23621117 DOI: 10.1517/14656566.2013.795543]
10 **Poveda E**, Wyles DL, Mena A, Pedreira JD, Castro-Iglesias A, Cachay E. Update on hepatitis C virus resistance to direct-acting antiviral agents. *Antiviral Res* 2014; **108**: 181-191 [PMID: 24911972 DOI: 10.1016/j.antiviral.2014.05.015]
11 **Alves R**, Queiroz AT, Pessoa MG, da Silva EF, Mazo DF, Carrilho FJ, Carvalho-Filho RJ, de Carvalho IM. The presence of resistance mutations to protease and polymerase inhibitors in Hepatitis C virus sequences from the Los Alamos databank. *J Viral Hepat* 2013; **20**: 414-421 [PMID: 23647958 DOI: 10.1111/jvh.12051]
12 **Combet C**, Penin F, Geourjon C, Deléage G. HCVDB: hepatitis C virus sequences database. *Appl Bioinformatics* 2004; **3**: 237-240 [PMID: 15702954]
13 **Tamura K**, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013; **30**: 2725-2729 [PMID: 24132122 DOI: 10.1093/molbev/mst197]
14 **Aloia AL**, Locarnini S, Beard MR. Antiviral resistance and direct-acting antiviral agents for HCV. *Antivir Ther* 2012; **17**: 1147-1162 [PMID: 23188771 DOI: 10.3851/IMP2426]
15 **Bartels DJ**, Sullivan JC, Zhang EZ, Tigges AM, Dorrian JL, De Meyer S, Takemoto D, Dondero E, Kwong AD, Picchio G, Kieffer TL. Hepatitis C virus variants with decreased sensitivity to direct-acting antivirals (DAAs) were rarely observed in DAA-naive patients prior to treatment. *J Virol* 2013; **87**: 1544-1553 [PMID: 23152524 DOI: 10.1128/JVI.02294-12]
16 **Jazwinski AB**, Muir AJ. Direct-acting antiviral medications for chronic hepatitis C virus infection. *Gastroenterol Hepatol* (N Y) 2011; **7**: 154-162 [PMID: 21528041]
17 **Echeverría N**, Moratorio G, Cristina J, Moreno P. Hepatitis C virus genetic variability and evolution. *World J Hepatol* 2015; **7**: 831-845 [PMID: 25937861 DOI: 10.4254/wjh.v7.i6.831]
18 **Lindström I**, Kjellin M, Palanisamy N, Bondeson K, Wesslén L, Lannergard A, Lennerstrand J. Prevalence of polymorphisms with significant resistance to NS5A inhibitors in treatment-naive patients with hepatitis C virus genotypes 1a and 3a in Sweden. *Infect Dis* (Lond) 2015; **47**: 555-562 [PMID: 25851241 DOI: 10.3109/23744235.2015.1028097]
19 **Fridell RA**, Qiu D, Wang C, Valera L, Gao M. Resistance analysis of the hepatitis C virus NS5A inhibitor BMS-790052 in an in vitro replicon system. *Antimicrob Agents Chemother* 2010; **54**: 3641-3650 [PMID: 20585111 DOI: 10.1128/AAC.00556-10]
20 **Chen ZW**, Li H, Ren H, Hu P. Global prevalence of pre-existing HCV variants resistant to direct-acting antiviral agents (DAAs): mining the GenBank HCV genome data. *Sci Rep* 2016; **6**: 20310 [PMID: 26842909 DOI: 10.1038/srep20310]
21 **Ogishi M**, Yotsuyanagi H, Tsutsumi T, Gatanaga H, Ode H, Sugiura W, Moriya K, Oka S, Kimura S, Koike K. Deconvoluting the composition of low-frequency hepatitis C viral quasispecies: comparison of genotypes and NS3 resistance-associated variants between HCV/HIV coinfected hemophiliacs and HCV monoinfected patients in Japan. *PLoS One* 2015; **10**: e0119145 [PMID: 25748426 DOI: 10.1371/journal.pone.0119145]
22 **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, Koehrsen M, Kodira C, Roberts AD, Lauer GM, Rosen HR, Bihl F, Cerny A, Spengler U, Liu Z, Kim AY, Xing Y, Schneidewind A, Madey MA, Fleckenstein JF, Park VM, Galagan JE, Nusbaum C, Walker BD, Lake-Bakaar GV, Daar ES, Jacobson IM, Gomperts ED, Edlin BR, Donfield SM, Chung RT, Talal AH, Marion T, Birren BW, Henn MR, Allen TM. Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naïve patients. *Hepatology* 2008; **48**: 1769-1778 [PMID: 19026009 DOI: 10.1002/hep.22549]
23 **Dietz J**, Susser S, Berkowski C, Perner D, Zeuzem S, Sarrazin C. Consideration of Viral Resistance for Optimization of Direct Antiviral Therapy of Hepatitis C Virus Genotype 1-Infected Patients. *PLoS One* 2015; **10**: e0134395 [PMID: 26317755 DOI: 10.1371/journal.pone.0134395]
24 **Suzuki Y**, Ikeda K, Suzuki F, Toyota J, Karino Y, Chayama K, Kawakami Y, Ishikawa H, Watanabe H, Hu W, Eley T, McPhee F, Hughes E, Kumada H. Dual oral therapy with daclatasvir and asunaprevir for patients with HCV genotype 1b infection and limited treatment options. *J Hepatol* 2013; **58**: 655-662 [PMID: 23183526 DOI: 10.1016/j.jhep.2012.09.037]
25 **Suzuki F**, Sezaki H, Akuta N, Suzuki Y, Seko Y, Kawamura Y, Hosaka T, Kobayashi M, Saito S, Arase Y, Ikeda K, Kobayashi M, Mineta R, Watahiki S, Miyakawa Y, Kumada H. Prevalence of hepatitis C virus variants resistant to NS3 protease inhibitors or the NS5A inhibitor (BMS-790052) in hepatitis patients with genotype 1b. *J Clin Virol* 2012; **54**: 352-354 [PMID: 22658798 DOI: 10.1016/j.jcv.2012.04.024]
26 **Gerber L**, Welzel TM, Zeuzem S. New therapeutic strategies in HCV: polymerase inhibitors. *Liver Int* 2013; **33** Suppl 1: 85-92 [PMID: 23286851 DOI: 10.1111/liv.12068]
27 **Lawitz E**, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383**: 515-523 [PMID: 24209977 DOI: 10.1016/S0140-6736(13)62121-2]
28 **Sulkowski MS**, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, McPhee F, Sherman D, Hindes R, Symonds W, Pasquinelli C, Grasela DM. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med* 2014; **370**: 211-221 [PMID: 24428467]

P- Reviewer: Bolhassani A, Cao GW, Grassi A, Lee HC, Rezaee-Zavareh MS
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Zhang FF

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327