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View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.28366
Potential conflict of interest: Nothing to report.

Hepatitis C Genotype 4R Resistance-Associated Polymorphisms: The Achilles Heel of the Nonstructural 5A Inhibitors?

TO THE EDITOR:

We read with interest the review article reported by Lontok et al. on hepatitis C virus (HCV) resistance to direct antiviral agents (DAAs).[1] Nonstructural 5A (NS5A) class resistance-associated variants (RAVs; variants at any position associated with resistance to any NS5A inhibitor) may be associated with virological treatment failure (Fig. 1). Studies performed on NS5A RAVs in genotype (G) 4 patients who received a regimen based on sofosbuvir and NS5A inhibitors are very limited. In a recent multicenter study, it was shown that 2 of 3 patients who did not respond to sofosbuvir and

HCV Genotypes	NS5A Amino Acid Position								
	24	28	30	31	32	38	58	92	93
1a	K →G/N/R	I/M →A/G/T M→V	Q Q→E/H/R/G/K Q→L/T	L L→I/F L→M/V	P P→L/S	S S→F	H H→D	A A→T	Y Y→C/H/N/S Y→F
1b	Q	I/L/M L→T	H/Q/R R→H	I/L L→M L→F/V	P P→L/S	S	P P→D	A A→K	Y/C/F/S Y→H
2a	T T→A	F/L F→S L→F	K	L L→M/V	P	S	P	C C→R	Y Y→H
3a	S	M M→T	A A→K	L L→F/M/V	P	S	P	A	Y Y→H
4a/d	K	L L→V L→M*/V	L L→H/R*/S*/T*/V* →A	M M→V M→L*	P	S	P/T T→S*	A	Y →S →H/R/S*/T*
5a	Q	L L→I	Q	L L→F/V	P	S	P	A	T
6a	Q Q→H	F	R	L L→M L→V	P P→L/S	S	T T→N T→A/S	A	T

Five main major class NS5A RAVs
NS5A resistance < 10-fold
NS5A resistance 10 - <100-fold
NS5A resistance > 100-fold
* Resistance observed in combination with other RAVs

FIG. 1. NS5A class RAVs: variants at any position associated with resistance to any NS5A inhibitor.

ledipasvir combination had a G4-r subtype.[2] This result suggests that some subtypes have a different susceptibility to antiviral treatment. This loss of susceptibility could be attributed to the number of basal NS5A RAVs.

We aimed to demonstrate that NS5A RAVs number is more prevalent in certain HCV-specific subtypes. We collected from the NCBI, European, and Japanese HCV databases, 47 HCV-NS5A-G4 sequences, and proceeded to multiple sequence alignment using *Clustal W2*. We analyzed 16 subtypes 4a, seven 4b, three 4d, two 4m, three 4o, and 16 4r. We assessed prevalence by subtype of the main RAVs: L28M, L30R, L31M, P58T, and Y93H. Among the G4 subtypes, the subtype 4r is the only one with 75% of the strains having two or three RAVs.

The predominance of certain NS5A polymorphisms depending on G4 subtype was also reported by Zhou et al.[3] Indeed, 52 of 54 genotype 4d NS5A sequences harbored L30R, whereas L30Q was observed only in genotype 4f NS5A sequences. Only a few patients with treatment failure to sofosbuvir plus daclatasvir were available for resistance analysis.[3]

Because of the broad use of NS5A inhibitors as first-line DAA regimens, together with the high likeliness of selection of NS5A RAVs and persistence of these RAVs, a large prospective study on the respective role of the subtypes and of NS5A and NS5B RAVs on SVR12 should be conducted in patients treated with any Nucs or protease inhibitors and NS5A inhibitors combination.[4]

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View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.28611
Potential conflict of interest: Dr. Abergel consults for, is on the speakers' bureau of, and received grants from Gilead and MSD. He consults for and received grants from Janssen and Roche. He consults for AbbVie and Bristol-Myers Squibb.

REPLY:

We agree with Halfon et al. that more data on rare hepatitis C virus genotype (GT) subtypes are needed. Our analysis[1] was restricted to phase 2 and phase 3 trials with patients infected primarily with GT1a, GT1b, or GT2; the majority of existing cell culture phenotype data are for GT1a-based or GT1b-based replicons.

The strategy of assessing natural resistance-associated polymorphism prevalence in specific subtypes by searching public databases is good for hypothesis generation, but such sequence collections may not be sufficiently representative to support conclusions regarding the prevalence of polymorphisms in wild-type GT4 subtypes, especially given the small sample size (47 sequences in the context of more than 15 GT4 subtypes). To properly define a "wild-type" population requires a systematic and comprehensive approach. We were surprised to see 16 sequences assigned to GT4r. This result contradicts previous work in which only very few available GT4 sequences from public databases segregated to GT4r.[2]

The reference to "patients with treatment failure to sofosbuvir and daclatasvir" is inaccurate: the GT4 patients with treatment failure referred to in Zhou et al.[2] were treated with daclatasvir, pegylated interferon, and ribavirin. To our knowledge, no GT4 treatment failures on daclatasvir and sofosbuvir have been reported to date.

Information on how the data, some arguably not accurate, in figure 1 were derived is lacking. In GT3a replicons, the Y93H substitution confers >100-fold reduced susceptibility to several NS5A inhibitors, while a lower value is reported in the figure.[3-5] The fold-resistance range for GT4 variants with the L30S and L30R substitutions is not consistent with published