AMINO ACID SUBSTITUTIONS ASSOCIATED WITH TREATMENT FAILURE OF HEPATITIS C VIRUS INFECTION

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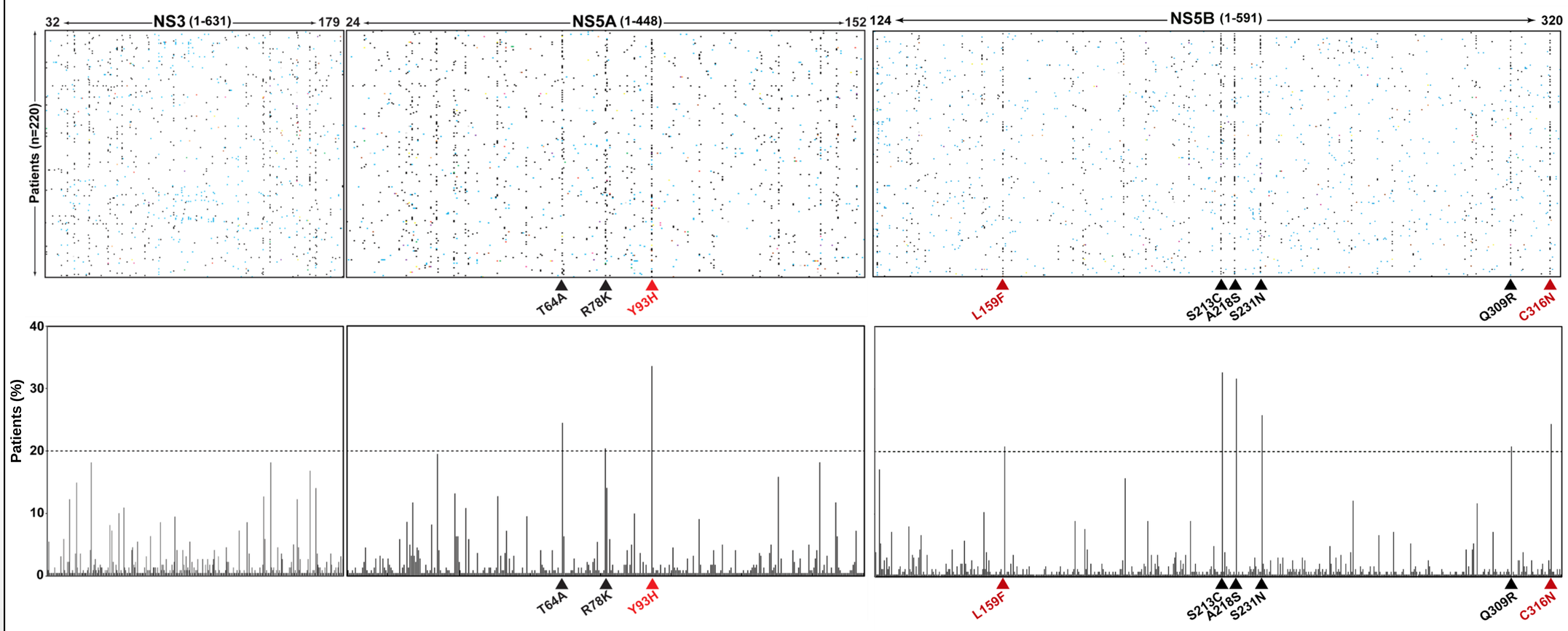
Despite the high sustained virological response rates achieved with current directly-acting antiviral agents (DAAs) against hepatitis C virus (HCV), around 2% to 5% of patients do not achieve such a response. Identification of amino acid substitutions associated with treatment failure requires analytical designs, such as subtype-specific ultra-deep sequencing (UDS) methods for HCV characterization and patient management. By deep sequencing analysis of 220 subtyped HCV samples from infected patients who failed therapy, collected from 39 Spanish hospitals, we determined amino acid sequences of the DAA-target proteins NS3, NS5A and NS5B, by UDS of HCV patient samples, in search of resistance-associated substitutions (RAS). Using this procedure, we have identified six highly represented amino acid substitutions (HRSs) in NS5A and NS5B of HCV, which are not *bona fide* RAS. They were present frequently in basal and post-treatment virus of patients who failed therapy to different DAA-based therapies. Contrary to several RAS, HRSs belong to the acceptable subset of substitutions according to the PAM250 replacement matrix. Coherently, their mutant frequency, measured by the number of deep sequencing reads within the HCV quasispecies that encode the relevant substitutions, ranged between 90% and 100% in most cases. Also, they have limited predicted disruptive effects on the three-dimensional structures of the proteins harboring them. The information on HRSs that will be gathered during sequencing should be relevant not only to help predict treatment outcomes and disease progression but also to further understand HCV population dynamics, which appears much more complex than thought prior to the introduction of deep sequencing.

Heat map of amino acid substitutions and their distribution among patients after treatment failure with DAAs

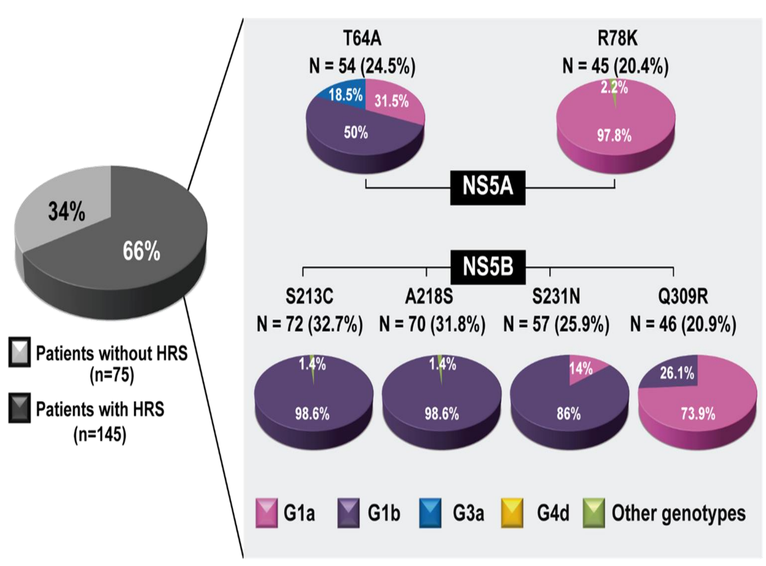


We defined as highly represented substitutions (HRS) whose that are not *bona fide* RAS, and that are present in more than 20% of the patients.

The percentage of patients carried any one of the HRS was statistically significant relative to those showing any other amino acid substitution (excluding the RAS) within the protein regions analyzed.

The six HRS that have been identified are indicated in black, while the RAS that were present in more than 20% of the patients are indicated in red. Dot color intensity in the top panels correspond to frequency, which is high for most HRS in mutant spectra.

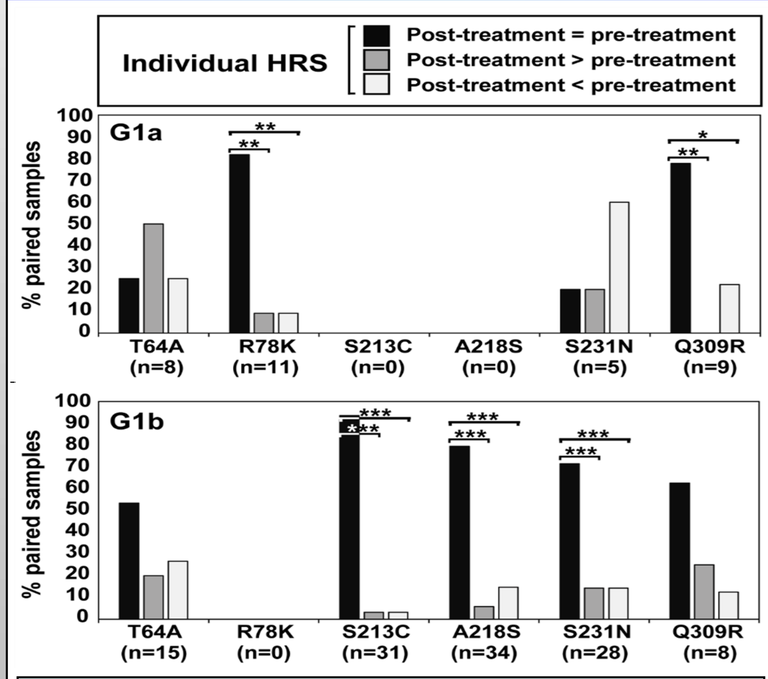
Distribution of HRS among subtypes



Most of the patients selected at least one HRS.

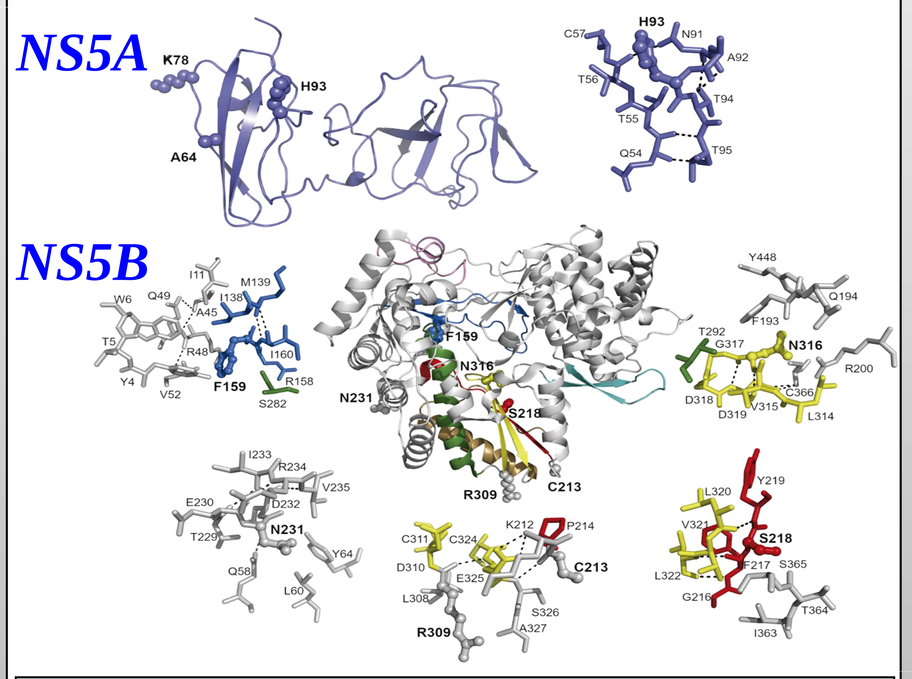
As previously described for RAS, HRS show a certain degree of specificity according to the subtype.

HRS in pre-treatment samples



HRS at the time of DAA treatment failure are largely determined by their presence before treatment.

Location of HRS in the NS5A and NS5B structure



RAS have a predicted distorting effect on the structure of the NS5A and NS5B, while HRS can be easily accommodated without causing major distortions.

- **HRS were selected by more than 20% of patients who failed treatment, independently of the therapeutic regimen they received.**
- **HRS were found both in basal samples and post-treatment samples.**
- **HRS are predicted to be tolerated by the virus.**
- **They can help to understand the population dynamics of HCV.**
- **They may have potential predictive value in relation to the response to treatment with DAAs.**

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