Cross-sectional Assessment of HCV NS5A Resistance-Associated Variants in Over 30,000 Samples Submitted for Drug Resistance Testing in the US

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I. Introduction

The first direct-acting antivirals (DAAIs) targeting NS5A were approved in 2014. NS5A inhibitors (NS5As) have become a major component of curative HCV treatment regimens. However, resistance-associated variants (RAVs) can impact NS5A efficacy and their identification in certain clinical situations is recommended in the current AASLD/IDSA Hepatitis C Guidance. Here we evaluated the presence of NS5A RAVs in over 30,000 clinical samples submitted for HCV NS5A resistance testing in the US from 2015 to 2017.

II. Methods

The entire NS5A region was amplified from plasma or serum samples by RT-PCR using GT1a, GT1b or GT3 primers. NS5A sequencing was performed using the illumina MiSeq platform with analysis at a threshold that was validated as equivalent to Sanger sequencing. Amino acid (aa) variants relative to either the H77 (GT1a), Con1 (GT1b) or S52 (GT3) reference sequence were determined and NS5A RAVs were enumerated (see list below). HCV genotype was assigned based on the NS5A sequence. Samples were also classified according to birth year cohort, gender and geographic region.

III. Results

Across greater than 30,000 samples evaluated:
• 86% were GT1a, 10.2% were GT1b, and 3.8% were GT3 (Figure 1).
• The most prevalent RAVs among GT1a viruses were aa28 (8%), aa30 (9%), aa31 (4%) and aa93 (6%), for GT1b were aa31 (13%) and aa93 (24%) and for GT3 were aa30 (5%) and aa93 (14%) (Figure 2).
• Where gender was identified, 68% were men (M) and 32% were women (F) (Figure 3). 0.6% were of unknown gender (U).

IV. Conclusions

The evaluation of a large database of HCV NS5A sequences derived from clinical samples submitted for routine resistance testing can provide important information on the distribution of individual RAVs and RAV combinations among men, women, geographic region and different birth cohorts. NS5A RAVs were more frequently observed in samples from men and among birth years 1930 - 1950. Of interest, there is a high co-incidence of Y93H and Q28H was observed in GT1a viruses (Figure 12). Sixty two percent of samples with Y93H also had a Q28H substitution which would predict high-level resistance to the currently approved NS5As.

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