I. Introduction

- HCV genotype (GT) and subtype are associated with differences in clinical outcomes, response to treatment and epidemiology.
- HCV recombinant strains have been described previously. The most well studied probably being the RF1_2k/1b with a homologous recombination breakpoint near the NS2/NS3 border.
- A better understanding of recombination in HCV is of practical importance and evolutionary interest.
- We carried out an analysis of a number of inter-subtype and inter-genotype recombinant HCV.

II. Methods

- Recombinant HCV strains were identified during routine drug resistance testing of clinical specimens using amplification approaches that were not intentionally designed to detect recombinants. We report only those events that were successfully amplified.
- NS3/4A, NS5A, NS5B or upstream sequences amplified with GT1a, GT1b, GT2 or pan-genotypic primers were subjected to Nextera Tagmentation and sequenced on the Illumina MiSeq platform.
- A subtyping routine was used to align a random subset of reads to a large library of reference HCV strains.
- Consensus sequences were generated and compared to reference sequences (Bootscan, Simplot) to define recombination breakpoints.
- Replication capacity and NS5A inhibitor susceptibility was assessed for the GT1b Con1 replicon containing various GT1a/GT1b NS5A recombinant sequences, with and without NS5A inhibitor resistance-associated variants (RAVs), relative to the GT1a H77 NS5A reference sequence.

III. Results

Figure 1. Recombination breakpoints in the NS5A region of GT1a/1b recombinant viruses

- Twenty six GT1a/1b recombinant viruses were identified from diverse geographic collection centers (Figures 1, 3).
- Recombination breakpoints mapped at NS5A amino acid (aa) 386 for 21 viruses and aa 366 for 4 viruses, relative to H77. Each of these 25 recombinants shared the same GT1b recombination site at aa 348 relative to Con1 (Figure 1).
- Two GT1a/1b recombinants exhibited breakpoints at GT1a aa 277 (H77) and GT1b aa 214 or 222 (Con1) (Figure 1).
- One inter-genotype recombinant GT1a/4 had a breakpoint in NS5A (Figure 2).
- Four inter-genotype recombinants had breakpoints upstream of NS3. Two of these were GT2b/1a, one was GT2a/1b, one was GT2k/1b (Figure 2).
- Recombinant GT1a/1b NS5A sequences inserted into a Con1 replicon confer lower replication capacity relative to the reference GT1a H77 NS5A sequence (Figure 4).
- The NS5A inhibitor susceptibility of replicons containing recombinant NS5A regions correlated with the absence or presence of NS5A RAVs (Figure 4).

Figure 2. Recombination breakpoints in inter-genotype recombinant viruses

- Independent identification of similar recombinant strains from multiple samples derived from diverse geographic collection sites suggests the existence of circulating recombinant forms.
- Broad geographical distribution and non-negligible prevalence suggest that recombinant strains should be taken into account in epidemiology studies, diagnosis and antiviral therapy decisions.
- The prevalence of recombinant forms may be underestimated based on current genotyping methods that are unlikely to detect recombination and resistance assays that are not intentionally designed to detect recombinants.

Figure 3. Recombinant strains were identified from diverse geographic collection centers

Figure 4. Replication capacity and NS5A inhibitor susceptibility of GT1a/1b NS5A recombinants

V. Acknowledgements

Monogram Biosciences Clinical Reference Laboratory.