Genetic diversity of Hepatitis C virus within a chronically infected HCV patient

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**Introduction**

The global epidemic of hepatitis C virus (HCV) is among the leading causes of preventable death worldwide. In the United States, an estimated 2.7 to 2.9 million people have chronic HCV infection [1].

As a positive stranded RNA virus, HCV has high mutation rates and can be classified into six major genotypes and approximately 80 subtypes. HCV shows a genetic diversity even in one individual.

Circulating Hepatitis C virus (HCV) genotypes in Alaska have not been characterized. Genotyping HCV isolates is of specific clinical interest because genotyping is a significant predictor of the response to antiviral therapy and serves as a guideline for therapy duration.

**Objective**

To examine hepatitis C virus (HCV) genome variation within a single patient by targeting two conserved regions for genotypic sequencing and phylogenetic analysis.

**Model**

HCV viral genome is approximately 9.6 kb in length. The two regions selected for genotyping are the 5' untranslated region (UTR), a highly conserved region, and non-structural 5B (NS5B); both are often used for genotyping analysis.

**Methods**

Serum from a chronically infected HCV-naive patient was collected from the Alaska State Virology laboratory, from which multiple hepatitis C virus sequences covering the 5' untranslated region (UTR) region were sequenced and aligned.

**Figure 2** Schematic of methodology: (i) RNA extraction, (ii) cDNA synthesis, (iii) cloning, and (iv) plasmid DNA preparation for sequencing [4,5].

**Preliminary Results**

**Figure 3** Quantitative real time PCR amplification plot comparing three cDNA libraries. With respect to threshold cycle (CT=26.59), we evaluated efficiency of our cDNA-library (3rd Library), which was 39x more effective than the reference library.

**Figure 4** M13 PCR was utilized to screen colonies indicating positive plasmid insertion following TA cloning. A 216-bp fragment covering the 5'UTR region was amplified and cloned, generating several hundred TA colonies. Expected fragment size was 372 bp, which is indicated above.

**Discussion & Future Direction**

We have begun aligning individual sequence data obtained from a fragment covering the 5'UTR region.

Results currently indicate that there are two primary sequence variations with about 50% conservation. This suggests high levels of HCV genetic diversity within this patient sample.

**Future Direction**

Our goal is to complete screening and alignment of the 5'UTR region and repeat the process at the NS5B region to obtain a more comprehensive representation of HCV genome variation. Once alignment is complete, a phylogenetic tree analysis can be conducted.

We aim to utilize next-generation sequencing technology to sequence the patient HCV genome as a comparison to our phylogenetic analysis.

**Reference**

5. PCR4-TOPO. 2014, www.snapgene.com/resources

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