Introduction:
Chronic liver disease is a major cause of morbidity and mortality in HIV-infected individuals. A direct correlation between HIV RNA levels and liver fibrosis has been reported, suggesting that HIV plays an integral role in liver disease progression. The existence of distinct HIV subpopulations has been documented in the genital tract, blood, and brain. However, liver-specific HIV quasispecies have not been extensively explored. Therefore, this study utilized phylogenetic and statistical analyses to examine intrapatient HIV variability in the liver.

Methods:
Sequence data from four HIV-infected patients were examined for evidence of compartmentalization. Alignments were performed using the neighbor-joining (NJ) approach implemented in ClustalX2. Statistical robustness was confirmed by bootstrap analysis using 1000 replicates. Viral Epidemiology Signature Pattern Analysis (VESPA) was used to determine the frequency of each amino acid in liver-derived viral variants versus viral variants from another tissue. Compartmentalization was assessed using Mantel’s Test, p-values ≤0.05 were considered significant.

Results:
Analyses were performed on HIV gp120, nef, and the V3 loop sequences. Distinct phylogenetic clustering of liver variants was observed in all three regions, suggesting unique selective pressures that act on specific portions of the viral genome (Fig 1-3). As expected, sequences from the brain and CSF exhibited clustering; however, this pattern was not observed in V3 variants from any other tissue (Fig. 1). Distinct clustering of liver variants was also observed for nef and gp120 sequences (Fig 2-3). Evidence for significant viral compartmentalization was supported further by Mantel’s Tests results, with a p-value <0.05 for 22 of 28 comparisons involving the liver (78.6%). Signature sequence analysis identified specific amino acid differences between viral variants from the liver and all other tissues. The frequency of amino acid differences between V3 variant paired comparisons ranged from 5 in liver-kidney and liver-spleen comparisons to 10 in liver-brain and liver-CSF comparisons (Table 1). Signature liver-associated amino acids from nef sequences were identified for patients AM (mean=2.14, range 1-8), AZ (mean=1.5, range 1-2), and DY (mean=3.5, range 2-4). Liver-specific amino acids were also identified for gp120 sequences from the same three patients (data not shown).

Conclusions:
Recent research suggests a role for HIV in liver disease. This study provides evidence of HIV compartmentalization in the liver, and identifies signature amino acids that characterize liver-specific quasispecies. These data strongly suggest that HIV variants differ in their ability to infect various cell types. Furthermore, HIV mutation is required for attachment, entry, and replication within a given cell type. Thus, these investigations may provide additional insight into antiviral treatment response, immune response, and selective pressures unique to the hepatocellular microenvironment. The phenotypic implications of liver-specific HIV mutations should be further examined, as they may aid in the development of HIV-associated liver disease treatments.