Interleukin-6 (IL-6) haplotypes and the response to therapy of chronic hepatitis C virus infection

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Abstract

Chronic hepatitis C virus (HCV) infection affects nearly 170 million individuals worldwide. Treatment of HCV with pegylated interferon-α-2a is successful in eradicating virus from only 30%–80% of those treated. Interleukin-6 (IL-6) is an important cytokine involved in the immune response to infectious agents and in vitro studies suggest that host genetic variation, particularly haplotypes, may affect IL-6 expression. We examined the contribution of haplotypes in the IL-6 gene on sustained viral response (SVR) to therapy for chronic HCV infection. We observed the IL-6 T-T-G-G-G-G-C-A-G-A haplotype to be associated with a lower risk of achieving SVR among Caucasian Americans (CAs) (RR=0.80; 95% C.I.: 0.66–0.98; p=0.0261). Using a sliding window approach, the rs1800797-(G)-rs1800796-(G)-rs1800795-(G) haplotype was associated with a reduced chance of SVR (RR=0.79; 95% C.I.: 0.66–0.94; p=0.0081), as was the rs1800796-(G)-rs1800795-(G)-rs2069830-(C) haplotype (RR=0.78; 95% C.I.: 0.66–0.94; p=0.0065) among CAs. Overall, the rs1800797-(G)-rs1800796-(G)-rs1800795-(G) haplotype was independently associated with a reduced chance of SVR (RR=0.78; 95% C.I.: 0.62–1.0; p=0.0489) after adjustment for potential confounding factors. Our findings further illustrate the complexity of IL-6 genetic regulation and the potential importance of haplotypes on IL-6 expression. Our findings provide additional support for the potential importance of genetic variation in the IL-6 gene and the response to HCV therapy.

Introduction

Hepatitis C virus (HCV) infection affects an estimated 170 million individuals worldwide, and 5 million in the United States, where it is currently recognized as the most prevalent blood-borne infection and the leading indication for a liver transplant.1, 2
HCV with pegylated interferon-α-2a is successful in eradicating virus from only 30%–80% of those treated, with individuals infected with the more resilient genotype-1 virus having markedly lower response rates than those with non-genotype-1 infections.3, 4 Additionally, differences in outcome have been described by race, with African Americans (AAs) having significantly lower response rates than Caucasian Americans (CAs).5–7

Expressed in a number of different cell types, including, hepatocytes, macrophages, B-cells and T-cells, Interleukin-6 (IL-6) is a pleiotropic cytokine important in the immunologic response to infections. IL-6 plays an important role in HCV infection as well as the response to IFN therapy. In addition to interacting with crucial components of the interferon response pathways, IL-6 is an activator of acute phase proteins in hepatocytes.8 A recent study has suggested the potential importance of IL-6 in the treatment response of HCV patients to interferon-based therapy.9

Functional studies of IL-6 genetics suggest that its expression is complex. A number of single nucleotide polymorphisms (SNPs) have been described within the IL-6 gene that form conserved haplotypes.10, 11 While a number of these SNPs may have an impact on IL-6 expression,10, 11 studies suggest that IL-6 regulation is complex, with haplotypes playing a critical role in IL-6 expression.11 In the present study we examined whether host genetic diversity in the IL-6 gene is associated with the response to therapy for chronic HCV, with an emphasis on the role of IL-6 haplotypes.

Results

Table 1 presents the baseline demographics of the participants in this study. Briefly, slight differences in age were observed in the NIDDK (p=0.034, Wilcoxon rank-sum test) and the overall combined cohort by race (p=0.027, Wilcoxon rank-sum test), indicating that AAs are slightly older than CAs in our cohorts. Additionally, slight differences in fibrosis score were observed in the NIDDK cohort by race (p=0.024, chi-square test), indicating higher proportion of AAs exhibited a worse baseline fibrosis category (>=3) than CAs. Whereas, the majority of AAs and CAs in Virahep-C cohort were in the milder fibrosis (<3) category. Nonetheless, we analyzed data by combining both cohort due to the small number of AA subjects in NIDDK cohort.

Among AA participants, 86 (47.8%) had HCV genotype 1a infections, 81 (45.0%) genotype 1b infections, and 13 (7.2%) a genotype-1 virus that could not be subgenotyped or mixed 1a/1b infection. Among CA participants, 111 (57.2%) had HCV genotype 1a, 55 (28.4%) genotype 1b, and 28 (14.4%) a genotype-1 virus that could not be subgenotyped or mixed 1a/1b infection. Participants in the NIDDK cohort were restricted to genotype 1-infected individuals.

Among the SNPs genotyped, rs13447445, rs13447446, and rs 2069829 were monomorphic and therefore not included in the analyses. Figure 1.A. summarizes the SNPs genotyped in the present study and those included in haplotype construction. Figure 1.B. presents the 10-SNP haplotypes that occurred with a frequency >5% in each race and their respective associations with SVR. The $\text{IL-6 T-T-G-G-G-C-A-G-A}$ haplotype was associated with a
lower risk of achieving SVR among CAs (RR=0.80; 95% C.I.: 0.66–0.98; p=0.0261). A similar trend was observed among AAs for this haplotype (RR=0.70; 95% C.I.: 0.44–1.13), but the association was not statistically significant (p=0.1275).

Figure 2.A presents the associations for the 3-SNP sliding window analysis among AAs. None of the haplotypes tested were significantly associated with SVR. Figure 2.B presents the associations for the 3-SNP sliding window analysis among CAs. Frames containing SNP 4 (rs1800797), SNP 5 (rs1800796), and SNP 6 (rs1800795) had significant associations with SVR (sets 3, 4, and 5 in Figure 2.B.). In particular, the rs1800797-(G)-rs1800796-(G)-rs1800795-(G) haplotype (set 4) was associated with a reduced chance of SVR (RR=0.79; 95% C.I.: 0.66–0.94; p=0.0081), as was the rs1800796-(G)-rs1800795-(G)-rs2069830-(C) haplotype (set 5) (RR=0.78; 95% C.I.: 0.66–0.94; p=0.0065).

The rs1800797-(G)-rs1800796-(G)-rs1800795-(G) haplotype (set 4) was independently associated with a reduced chance of SVR (RR=0.78; 95% C.I.: 0.62–1.0; p=0.0489) after adjustment for potential confounding factors including race, baseline viral level, fibrosis score, gender and the interaction between race and baseline viral level (Table 2). Table 3 presents the associations for individual SNPs.

**Discussion**

Using a systematic analysis of IL-6 haplotypes, we observed consistent associations of haplotypes in this gene with a reduced likelihood of SVR. In particular, we observed the 10-SNP haplotype **IL-6 T-T-G-G-G-G-C-A-G-A** haplotype associated with a lower risk of achieving SVR among CAs (RR=0.80; 95% C.I.: 0.66–0.98; p=0.0261). Using a sliding window approach, we observed frames containing rs1800797, rs1800796, and rs1800795 to be associated with SVR. In particular, the rs1800797-(G)-rs1800796-(G)-rs1800795-(G) haplotype was associated with a reduced chance of SVR (RR=0.79; 95% C.I.: 0.66–0.94; p=0.0081), as was the rs1800796-(G)-rs1800795-(G)-rs2069830-(C) haplotype (RR=0.78; 95% C.I.: 0.66–0.94; p=0.0065). All associations were only observed among CAs.

Our sliding window analyses further suggested that the region containing the SNPs rs1800797, rs1800796, rs1800795 and rs2069830 may be an important region in the IL-6 gene, with several haplotypes in this region associated with a lower likelihood of SVR. In *vitro* functional studies support the fact that this region may be of regulatory importance.11, 12

Previous studies of genetic variation in IL-6 and the response to HCV therapy have focused on single nucleotide polymorphisms. Natterman and colleagues described an association between carriage of high producing genotypes (ie., the GG or GC genotypes)10 of the C-174G (rs1800795) polymorphism and a greater likelihood of SVR.12 In contrast, our haplotypic observations were of lowered likelihood of SVR. A functional study of IL-6 genetics by Terry et al., suggests that IL-6 regulation is complex and that small differences on the haplotype level may have a significant impact on IL-6 expression. For example, they described a $(-597G)+(-572G)+(-174G)$ clone containing a 9/11 base pair A$n$T$n$ repeat to
have greater levels of expression than one containing a 10/10 repeat.11 These observations reinforce the importance of haplotypes in IL-6 expression.

The present study underscores the importance of examining genetic haplotypes in addition to individual SNPs. Our findings build upon those of Nattermann and colleagues and collectively, both studies suggest that host genetic variation in the IL-6 gene may be important in the response to therapy for HCV. Additional studies are needed to fully elucidate the complex functional aspects of this gene.

**Methods**

**Study Population and clinical data**

This study utilized participants from the Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C), a National Institutes of Health (NIH)-funded multi-center study aimed at understanding the mechanisms of resistance to antiviral therapy for chronic HCV infection among genotype-1-infected interferon treatment-naive individuals. Additional emphasis was placed on identifying factors that may contribute to differences in outcome by race among AAs and CAs.5 All subjects were born in the United States (US) and race was determined by a self-administered questionnaire.

We also utilized participants who were attending the NIH Clinical Center (N-113). Individuals who were treatment-naive, infected with HCV genotype-1, and underwent IFN-based therapy (pegylated interferon-α + ribavirin or IFN-α only) were included in the present analysis. Available demographic data including self-reported race, sex, patient age and response to interferon were included in multivariable analyses.

Sustained virologic response (SVR) was defined as having undetectable HCV-RNA 6 months after the discontinuation of therapy. The Roche Amplicor Assay version 2 with a lower limit of detection of 600 IU/mL was used in this study. Participants received a liver biopsy within 6 months prior to the start of therapy. Biopsies were scored by a single pathologist who was blinded to patient outcome using the Ishak modified histological activity index (HAI) score.13 To facilitate categorical analysis, biopsy scores were dichotomized as ≥3 and <3. All participants from both the Virahep-C study and the NIH clinical center provided written consent to participate in host genetics studies.

**Statistical analysis**

Proportions of SVR by baseline demographic and clinical characteristics were compared using the two-sided race-adjusted Mantel-Haenszel Chi-square test. The non-parametric Wilcoxon rank-sum test was used to compare racial differences in distributions of continuous data (such as age, baseline viral level, proportion of peginterferon). Unadjusted associations between individual haplotype (2N) and haplotype carrier (N) frequencies, as well as allele and allele carrier frequencies for examination of individual loci, with SVR were summarized using relative risk (RR) estimates, 95% confidence intervals (95% C.I.) and p-values.
Multiple Poisson regression models with sandwich estimators of the variance were utilized to adjust for potential confounding factors. Potential confounding factors were selected based on previously published findings and data that were collected across both cohorts: included race, baseline viral levels, Ishak score, gender. The SAS program, version 9 was used for all analyses. Statistical significance was set at $\alpha=0.05$.

**Genotyping**

Among the 401 individuals treated in the Virahep-C Study, a subset of 373 consented to participate in host genetics studies and had DNA available for genotyping. Details of the Virahep-C host genetics ancillary study have been published previously. Single nucleotide polymorphisms in the IL-6 gene were selected using the HapMap database. Initially, rs1880242, rs2056576, rs2069827, and rs2069845 were selected using HapMap version 1 and genotyped using the Illumina Beadarray system (Illumina Corporation, San Diego, CA) for patients in both cohorts. Additionally, rs2069860, rs13306435 and rs2069850 were genotyped using allelic discrimination in the NIDDK cohort, but not the Virahep-C study.

To increase the density of SNP coverage for the IL-6 gene, we further genotyped rs1554606, rs1800795, rs1800797, rs2069830, and rs2069837 by allelic discrimination using the ABI 7900 Sequence Detection System using TaqMan technology (Applied Biosystems, Foster City, CA) for participants from both cohorts. Additional genotyping for rs13447445, rs13447446, rs2069829 and rs1800796 was conducted using the fluorogenic 5’-nuclease TaqMan allelic discrimination assay on a 7900HT Real time PCR instrument (Applied Biosystems, Foster City, CA) in both cohorts. All probes and reagents were purchased from Applied Biosystems (Foster City, CA). All genotype calls were determined by two independent investigators, and only concordant calls were used.

**Haplotype construction**

We constructed haplotypes using the SAS Genetics module (SAS® v9.2) using a stepwise version of the EM algorithm (SAS® version 9.1; Cary, NC) separately by race. Due to the nominal sample size of the NIDDK cohort (N=22 AAs and N=91 CAs), participants from both cohorts were combined for haplotype construction and to improve statistical power for analyses. Since rs2069860, rs13306435 and rs2069850 were genotyped only in the NIDDK cohort and not the Virahep-C cohort, these SNPs were not included in haplotype construction to ensure that haplotypes were comparable across both cohorts. In addition, we conducted stratified analyses by cohort to determine whether the directions of associations are similar by cohort.

We further evaluated linkage disequilibrium (LD) among genotyped loci by examining the pair-wise LD between each locus within each race using the SAS® genetics module and the Grasp program. Utilizing these data, we generated haplotypes consisting of 3, 4, and 5 consecutive, overlapping SNPs. Using a “sliding window” approach, we determined the association between carriage of smaller haplotype blocks and SVR by race in order to further refine specific regions of the IL-6 gene that may be contributing to the association with SVR. Only haplotypes with a frequency >5% within a race were analyzed for that race.
Evaluation of population structure

Using data from 161 ancestry-informative single nucleotide polymorphisms (SNPs), we derived estimates of individual admixture for participants in the genetics study, and utilized the structured association method to evaluate the population structure. We observed two distinct ancestral groups that had a strong correlation with self-reported race. Consequently, we used self-reported race in these analyses.

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Figure 1. Targeted SNPs and IL-6 haplotypes

Part A. summarizes the single nucleotide polymorphisms (SNPs) targeted in the present study. Due to the fact that several additional SNPs were genotyped in the NIDDK cohort and not in Virahep-C, only SNPs common to both cohorts were used for haplotype estimation. SNPs not included in haplotype construction are in parenthesis. Note: the figure is not to scale. Part B. presents the haplotypes within each race with a frequency ≥5%, the observed frequencies, association with sustained viral response (SVR), the corresponding 95% confidence interval (95% C.I.), and p-value for the association.

| Haplotypes among AA | Frequency\^\^\| \| Non- | SRV | SVR | RR\| \| 95% C.I. | p-value |
|---------------------|-----------------|-----|-----|-----|-----------------|--------|
| T-T-G-G-G-C-A-G-A    | Overall 23.0%   | 16.4%| 25.1%| 0.70 | (0.44, 1.13)   | 0.1275 |
|                      | SVR            |     |     |     |                 |        |
|                      | Non-SVR        |     |     |     |                 |        |
| T-C-G-G-G-G-C-A-G-A  | 14.3% | 18.9%| 13.1%| 1.32 | (0.86, 2.03)   | 0.2267 |
| T-C-G-G-G-G-C-A-T-G  | 12.3% | 13.4%| 11.9%| 1.20 | (0.72, 1.98)   | 0.4978 |
| T-C-G-G-G-G-C-G-G-A  | 11.0% | 13.7%| 10.0%| 1.21 | (0.76, 1.94)   | 0.4385 |

| Haplotypes among CA | Frequency\^\^\| \| Non- | SRV | SVR | RR\| \| 95% C.I. | p-value |
|---------------------|-----------------|-----|-----|-----|-----------------|--------|
| T-T-G-G-G-G-C-A-G-A | Overall 36.3%   | 30.9%| 41.1%| 0.80 | (0.66, 0.98)   | 0.0261 |
|                      | SVR            |     |     |     |                 |        |
|                      | Non-SVR        |     |     |     |                 |        |
| G-C-G-A-G-C-C-A-T-G  | 25.1% | 24.2%| 25.8%| 0.93 | (0.75, 1.15)   | 0.4775 |

\^ SNPs in parenthesis are excluded from haplotype frequency estimation.
\^ With SVR and observed haplotype frequency
\^ Expected haplotype frequencies
**Figure 2.A. African Americans.**

| SNP: | SNP1 | SNP2 | SNP3 | SNP4 | SNP5 | SNP6 | SNP7 | SNP8 | SNP9 | SNP10 |
|------|------|------|------|------|------|------|------|------|------|-------|
| Set 1 | T    | T    | G    | Freq.: 0.43 | RR=0.86 (95% C.I.: 0.61 - 1.22); p=0.4032 |
| 1    | T    | C    | G    | Freq.: 0.40 | RR=1.27 (95% C.I.: 0.91 - 1.78); p=0.1611 |
| 1    | G    | C    | G    | Freq.: 0.14 | RR=0.80 (95% C.I.: 0.47 - 1.37); p=0.4055 |
| Set 2 | C    | G    | G    | Freq.: 0.49 | RR=1.16 (95% C.I.: 0.83 - 1.63); p=0.3847 |
| 2    | T    | G    | G    | Freq.: 0.44 | RR=0.90 (95% C.I.: 0.64 - 1.28); p=0.5651 |
| Set 3 | G    | G    | G    | Freq.: 0.85 | RR=1.39 (95% C.I.: 0.79 - 2.33); p=0.2457 |
| 3    | G    | G    | C    | Freq.: 0.08 | RR=0.62 (95% C.I.: 0.27 - 1.40); p=0.2182 |
| Set 4 | Freq.: 0.84 | G    | G    | G    | RR=1.36 (95% C.I.: 0.79 - 2.33); p=0.2457 |
| 4    | Freq.: 0.08 | G    | C    | G    | RR=0.60 (95% C.I.: 0.26 - 1.36); p=0.1916 |
| 4    | Freq.: 0.07 | A    | G    | C    | RR=0.99 (95% C.I.: 0.51 - 1.92); p=0.9751 |
| Set 5 | Freq.: 0.74 | G    | G    | C    | RR=1.12 (95% C.I.: 0.74 - 1.67); p=0.5925 |
| 5    | Freq.: 0.10 | G    | G    | T    | RR=1.27 (95% C.I.: 0.77 - 2.10); p=0.5665 |
| 5    | Freq.: 0.08 | C    | G    | C    | RR=0.59 (95% C.I.: 0.26 - 1.35); p=0.1807 |
| 5    | Freq.: 0.07 | G    | C    | C    | RR=0.86 (95% C.I.: 0.42 - 1.78); p=0.6846 |
| Set 6 | RR=0.90 (95% C.I.: 0.60 - 1.34); p=0.5987 | G    | C    | A    | Freq.: 0.72 |
| 6    | RR=1.21 (95% C.I.: 0.69 - 2.10); p=0.5198 | G    | C    | A    | Freq.: 0.10 |
| 6    | RR=1.23 (95% C.I.: 0.69 - 2.19); p=0.4972 | G    | T    | A    | Freq.: 0.09 |
| 6    | RR=0.96 (95% C.I.: 0.44 - 2.10); p=0.9083 | C    | C    | A    | Freq.: 0.06 |
| Set 7 | RR=0.89 (95% C.I.: 0.63 - 1.25); p=0.4955 | C    | A    | G    | Freq.: 0.48 |
| 7    | RR=1.01 (95% C.I.: 0.70 - 1.46); p=0.9582 | C    | A    | T    | Freq.: 0.29 |
| 7    | RR=1.24 (95% C.I.: 0.77 - 1.99); p=0.3896 | C    | G    | G    | Freq.: 0.11 |
| 7    | RR=1.33 (95% C.I.: 0.82 - 2.15); p=0.2660 | T    | A    | G    | Freq.: 0.10 |
| Set 8 | Freq.: 0.57 | RR=0.97 (95% C.I.: 0.69 - 1.36); p=0.8524 | A    | G    | A    |
| 8    | Freq.: 0.30 | RR=0.97 (95% C.I.: 0.67 - 1.41); p=0.8907 | A    | T    | G    |
| 8    | Freq.: 0.12 | RR=1.24 (95% C.I.: 0.77 - 1.99); p=0.3896 | G    | G    | A    |
Three-SNP sliding windows and the corresponding associations with sustained virologic response (SVR) are presented. **Part A.** presents the results among African Americans (AAs), while **Part B.** presents the results among Caucasian Americans (CAs). For each 3-SNP block, the haplotype frequency, association with SVR (Relative Risk: (RR)), 95% C.I. interval and p-values are given.

### Figure 2.B. Caucasian Americans.

| SNP Set | SNP1 | SNP2 | SNP3 | SNP4 | SNP5 | SNP6 | SNP7 | SNP8 | SNP9 | SNP10 |
|---------|------|------|------|------|------|------|------|------|------|-------|
| Set 1   | T    | T    | G    | Freq.: 0.39 | RR=0.87 (95% C.I.: 0.72-1.05); p=0.1306 |
|         | G    | C    | G    | Freq.: 0.36 | RR=1.05 (95% C.I.: 0.88-1.27); p=0.5755 |
|         | T    | C    | G    | Freq.: 0.17 | RR=0.92 (95% C.I.: 0.72-1.18); p=0.5226 |
|         | G    | C    | T    | Freq.: 0.07 | RR=1.79 (95% C.I.: 1.07-1.85); p=0.0343 |
| Set 2   | T    | G    | G    | Freq.: 0.38 | RR=0.84 (95% C.I.: 0.69-1.02); p=0.0685 |
|         | C    | G    | G    | Freq.: 0.27 | RR=1.10 (95% C.I.: 0.90-1.33); p=0.3552 |
|         | C    | G    | A    | Freq.: 0.27 | RR=0.94 (95% C.I.: 0.77-1.16); p=0.5796 |
|         | C    | T    | A    | Freq.: 0.06 | RR=1.16 (95% C.I.: 1.13-1.88); p=0.0181 |
| Set 3   | G    | G    | G    | Freq.: 0.59 | RR=0.85 (95% C.I.: 0.71-1.01); p=0.0730 |
|         | G    | A    | G    | Freq.: 0.28 | RR=0.98 (95% C.I.: 0.80-1.19); p=0.8168 |
|         | T    | A    | G    | Freq.: 0.08 | RR=1.44 (95% C.I.: 1.12-1.86); p=0.0206 |
|         | G    | G    | C    | Freq.: 0.06 | RR=1.35 (95% C.I.: 1.02-1.78); p=0.0714 |
| Set 4   | G    | G    | G    | Freq.: 0.57 | RR=0.79 (95% C.I.: 0.66-0.94); p=0.0081 |
|         | A    | G    | C    | Freq.: 0.34 | RR=1.12 (95% C.I.: 0.94-1.34); p=0.2154 |
|         | G    | C    | G    | Freq.: 0.06 | RR=1.35 (95% C.I.: 1.02-1.79); p=0.0688 |
| Set 5   | G    | G    | C    | Freq.: 0.58 | RR=0.78 (95% C.I.: 0.66-0.94); p=0.0065 |
|         | G    | C    | C    | Freq.: 0.36 | RR=1.20 (95% C.I.: 1.00-1.43); p=0.0474 |
|         | C    | G    | C    | Freq.: 0.06 | RR=1.35 (95% C.I.: 1.02-1.79); p=0.0688 |
| Set 6   | G    | C    | A    | Freq.: 0.57 | RR=0.84 (95% C.I.: 0.71-0.99); p=0.0479 |
|         | C    | C    | A    | Freq.: 0.36 | RR=1.20 (95% C.I.: 1.00-1.43); p=0.0474 |
|         | G    | C    | G    | Freq.: 0.07 | RR=1.05 (95% C.I.: 0.75-1.46); p=0.7902 |
| Set 7   | G    | A    | C    | Freq.: 0.55 | RR=0.87 (95% C.I.: 0.73-1.04); p=0.1211 |
|         | C    | A    | T    | Freq.: 0.37 | RR=1.14 (95% C.I.: 0.96-1.37); p=0.1402 |
|         | C    | G    | G    | Freq.: 0.07 | RR=1.02 (95% C.I.: 0.72-1.44); p=0.9285 |
| Set 8   | G    | A    | A    | Freq.: 0.55 | RR=0.88 (95% C.I.: 0.74-1.31); p=0.1638 |
|         | A    | G    | A    | Freq.: 0.37 | RR=1.12 (95% C.I.: 0.94-1.33); p=0.2266 |
|         | A    | T    | G    | Freq.: 0.07 | RR=1.02 (95% C.I.: 0.72-1.44); p=0.9285 |

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**Figure 2. “Sliding window” analysis**

Three-SNP sliding windows and the corresponding associations with sustained virologic response (SVR) are presented. **Part A.** presents the results among African Americans (AAs), while **Part B.** presents the results among Caucasian Americans (CAs). For each 3-SNP block, the haplotype frequency, association with SVR (Relative Risk: (RR)), 95% C.I. interval and p-values are given. SNP1= rs1880242; SNP2= rs2056576; SNP3=rs2069827; SNP4=rs1800797; SNP5=rs1800796; SNP6=rs1800795; SNP7= rs2069830;
SNP8=rs2069837; SNP9=rs1554606; SNP10=rs2069845. Only haplotypes with a frequency ≥5% are presented. Analyses was conducted using the Grasp program and SAS.
Baseline characteristics of the Virahep-C, NIDDK and the cohorts combined.

| Factor                        | Virahep-C Cohort (n=374) | NIDDK Cohort (n=413) | Combined cohort (n=487) |
|-------------------------------|--------------------------|----------------------|------------------------|
|                              | AA | CA | p*   | AA | CA | p*   | AA | CA | p*   |
| Age (mean years ± SD)         |    |    |      |    |    |      |    |    |      |
| n=180                         | 48.7 ± 7.1 | 47.0 ± 8.6 | 0.062 | 52.8 ± 7.6 | 48.3 ± 8.8 | 0.034 | 49.2 ± 7.2 | 47.4 ± 8.6 | 0.027 |
| Gender                        |    |    |      |    |    |      |    |    |      |
| Male                          | 118 (65.6%) | 126 (65.0%) | 0.902 | 6 (27.3%) | 41 (45.1%) | 0.129 | 68 (33.7%) | 109 (38.3%) | 0.3 |
| Female                        | 62 (34.4%) | 68 (35.1%) | 16 (72.7%) | 50 (55.0%) | 134 (66.6%) | 176 (61.8%) |
| Fibrosis Score (Ishak)        |    |    |      |    |    |      |    |    |      |
| < 3                           | 115 (64.3%) | 117 (60.3%) | 0.433 | 7 (33.3%) | 55 (60.4%) | 0.024 | 122 (61.0%) | 172 (60.4%) | 0.886 |
| ≥ 3                           | 64 (35.8%) | 77 (39.7%) | 14 (66.7%) | 35 (39.6%) | 78 (39.0%) | 113 (39.7%) |
| Baseline viral level (log10 IU/mL) mean ± S.D. | 6.2 ± 0.7 | 6.3 ± 0.8 | 0.13 | 6.0 ± 0.4 | 5.7 ± 0.6 | 0.056 | 6.2 ± 0.7 | 6.1 ± 0.8 | 0.68 |
| Proportion of peginterferon taken in first 24 weeks (mean ± S.D.) | 0.82 ± 0.28 | 0.87 ± 0.26 | 0.02 | N/A | N/A | N/A | N/A | N/A |
| Response to therapy           |    |    |      |    |    |      |    |    |      |
| NR                            | 131 (72.8%) | 90 (46.4%) | <.0001 | 20 (90.9%) | 62 (68.1%) | 0.032 | 151 (74.8%) | 152 (53.3%) | <0.001 |
| SVR                           | 49 (27.2%) | 104 (53.6%) | 2.9 (9.1%) | 29 (31.9%) | 51 (25.3%) | 133 (46.7%) |

Note: all participants in Virahep-C and the NIDDK were infected with HCV genotype-1.

Abbreviations: SD= standard deviation; SVR=sustained virologic responders; NR= non-responders; N/A=data not available for the NIDDK cohort, and therefore not calculated for the combined cohort.

*P-values represent comparisons for variables within each cohort. Mantel-Haenszel test was used for categorical data, otherwise Wilcoxon rank-sum test was used for continuous data (such as age, baseline viral level, and proportion of peg interferon).
Table 2

Multivariable model of *IL-6* haplotypes carriage adjusting for potential confounding factors.

| Factor                                      | RR **   | 95% C.I. ** | P †     |
|---------------------------------------------|---------|-------------|---------|
| *IL-6* G-G-G haplotype carriage*           | 0.78    | 0.62 – 1.0  | 0.0489  |
| CA race                                    | 1.74    | 1.33 – 2.29 | <0.0001 |
| Baseline viral level (log_{10})            | 0.58    | 0.43 – 0.78 | 0.0002  |
| Ishak fibrosis score (≥3 vs. <3)           | 0.89    | 0.83 – 0.96 | 0.0041  |
| Male gender                                | 0.84    | 0.68 – 1.04 | 0.1147  |
| Interaction of CA race and baseline viral level | 1.55    | 1.12 – 2.15 | 0.0085  |

* IL-6 G-G-G = rs1800797-(G)-rs1800796-(G)-rs1800795-(G) haplotype (set 4)

** RR = Relative Risk; 95% C.I = 95% confidence interval

† based on Modified poisson regression model
Table 3

Associations between individual alleles and sustained viral response (SVR) in the combined Virahep-C and NIDDK cohort by race. Part B.1 presents the associations between allele frequency and SVR. Part B.2 presents the associations of allele carriage and SVR.

### Combined Virahep-C Study + NIDDK

| SNP          | AA       | CA       | 95% C.I. | p*       | 95% C.I. | p*       |
|--------------|----------|----------|----------|----------|----------|----------|
| **B.1. Allele Frequency** |          |          |          |          |          |          |
| rs1880242-G  | 0.92     | 0.58–1.46| 0.7197   | 1.17     | 0.97–1.40| 0.0972   |
| rs1880242-T  | 0.97     | 0.84–1.13| 0.7197   | 0.86     | 0.72–1.03| 0.0972   |
| rs2056576-C  | 1.14     | 0.81–1.61| 0.4654   | 1.15     | 0.95–1.39| 0.149    |
| rs2056576-T  | 1.04     | 0.93–1.17| 0.4654   | 0.87     | 0.72–1.05| 0.149    |
| rs2069827-G  | 0.60     | 0.25–1.44| 0.3069   | 0.74     | 0.57–0.96| 0.0532   |
| rs2069827-T  | 0.77     | 0.40–1.47| 0.3069   | 0.69     | 0.45–1.06| 0.0532   |
| rs1800797-A  | 1.01     | 0.52–1.95| 0.9835   | 1.09     | 0.91–1.31| 0.3713   |
| rs1800797-G  | 1.00     | 0.80–1.26| 0.9835   | 0.92     | 0.77–1.10| 0.3713   |
| rs1800796-C  | 0.58     | 0.26–1.33| 0.1662   | 1.38     | 1.04–1.82| 0.0558   |
| rs1800796-G  | 0.87     | 0.74–1.02| 0.1662   | 1.44     | 0.93–2.23| 0.0558   |
| rs1800795-C  | 0.91     | 0.47–1.79| 0.7898   | 1.19     | 0.99–1.42| 0.0578   |
| rs1800795-G  | 0.97     | 0.79–1.19| 0.7898   | 0.84     | 0.71–1.00| 0.0578   |
| rs2069830-C  | 0.80     | 0.49–1.29| 0.3694   | N.C      | N.C      | N.C      |
| rs2069830-T  | 0.91     | 0.74–1.14| 0.3694   | N.C      | N.C      | N.C      |
| rs2069837-A  | 0.85     | 0.53–1.36| 0.5015   | 0.95     | 0.68–1.33| 0.7831   |
| rs2069837-G  | 0.94     | 0.77–1.14| 0.5015   | 0.96     | 0.70–1.31| 0.7831   |
| rs1554606-G  | 1.04     | 0.72–1.51| 0.8204   | 0.88     | 0.73–1.05| 0.1537   |
| rs1554606-T  | 1.01     | 0.90–1.15| 0.8204   | 0.89     | 0.75–1.05| 0.1537   |
| rs2069845-A  | 1.01     | 0.71–1.45| 0.9427   | 0.89     | 0.74–1.07| 0.2137   |
| rs2069845-G  | 1.00     | 0.89–1.14| 0.9427   | 0.90     | 0.77–1.06| 0.2137   |
| **B.2. Allele carriage** |          |          |          |          |          |          |
| rs1880242-G  | 0.90     | 0.53–1.52| 0.6899   | 1.28     | 0.94–1.74| 0.1036   |
| rs1880242-T  | 1.03     | 0.19–5.70| 0.9769   | 0.84     | 0.62–1.13| 0.2760   |
| rs2056576-C  | 1.17     | 0.62–2.20| 0.6253   | 1.31     | 0.86–2.00| 0.1813   |
| SNP          | AA RR   | 95% C.I. | p*      | CA RR   | 95% C.I. | p*      |
|--------------|---------|----------|---------|---------|----------|---------|
| rs2056576-T  | 0.84    | 0.51–1.38| 0.4997  | 0.87    | 0.67–1.12| 0.2793  |
| rs2069827-G  | N.C     | N.C      | N.C     | N.C     | N.C      | N.C     |
| rs2069827-T  | 1.69    | 0.69–4.11| 0.303   | 1.43    | 1.09–1.89| 0.0274  |
| rs1800797-A  | 1.01    | 0.51–2.00| 0.9829  | 1.04    | 0.81–1.35| 0.7387  |
| rs1800797-G  | N.C     | N.C      | N.C     | 0.78    | 0.57–1.08| 0.1789  |
| rs1800796-C  | 0.58    | 0.25–1.35| 0.1761  | 1.48    | 1.10–1.20| 0.0276  |
| rs1800796-G  | N.C     | N.C      | N.C     | 1.38    | 0.28–6.89| 0.6604  |
| rs1800795-C  | 0.94    | 0.47–1.89| 0.8691  | 1.14    | 0.88–1.47| 0.3278  |
| rs1800795-G  | N.C     | N.C      | N.C     | N.C     | N.C      | N.C     |
| rs2069830-T  | 1.39    | 0.82–2.34| 0.2371  | N.C     | N.C      | N.C     |
| rs22069837-A | N.C     | N.C      | N.C     | 0.46    | 0.41–0.53| 0.2844  |
| rs22069837-G | 1.25    | 0.74–2.10| 0.4089  | 1.02    | 0.71–1.46| 0.9182  |
| rs1554606-G  | 0.75    | 0.37–1.51| 0.4419  | 0.72    | 0.54–0.96| 0.0441  |
| rs1554606-T  | 0.84    | 0.52–1.34| 0.4610  | 1.08    | 0.83–1.40| 0.5678  |
| rs2069845-A  | 0.66    | 0.35–1.26| 0.2403  | 0.75    | 0.56–1.01| 0.0893  |
| rs2069845-G  | 0.83    | 0.52–1.33| 0.4314  | 1.07    | 0.82–1.40| 0.5927  |

N.C. = not calculated due to low frequency of individuals with the allele

* chi-square test