Published in final edited form as:
J Hepatol. 2008 August ; 49(2): 184–191. doi:10.1016/j.jhep.2008.04.011.

Association of single nucleotide polymorphisms in interferon signaling pathway genes and interferon stimulated genes with the response to interferon therapy for chronic hepatitis C

Xiaowen Su[1], **Leland J. Yee**[2], **KyungAh Im**[2,3], **Shannon L. Rhodes**[1], **YongMing Tang**[1], **Xiaomei Tong**[1], **Charles Howell**[4], **Darmendra Ramcharran**[2], **Hugo R. Rosen**[5], **Milton W. Taylor**[6], **T. Jake Liang**[7], **Huiying Yang**[1,8], and **the Virahep-C Study Group**

1*Medical Genetic Institute, Cedars-Sinai Medical Center, Los Angeles, CA*

2*Department of Epidemiology, Graduate School of Public Health and Division of Infectious Diseases, Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA*

3*Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh; University of Pittsburgh, Pittsburgh, PA*

4*Department of Medicine, Division of Gastroenterology and Hepatology , University of Maryland School of Medicine, Baltimore, MD*

5*Integrated Program in Immunology and Hepatitis C Research Center, Division of Gastroenterology & Hepatology, University of Colorado, Denver, CO*

6*Department of Biology, Indiana University, Bloomington, IN*

7*Liver Diseases Branch, NIDDK, National Institutes of Health, Bethesda, MD*

Abstract

Background/aim—Interferon signaling pathway genes (IPGs) and interferon stimulated genes (ISGs) are associated with the host response to hepatitis C virus (HCV) infection. We studied single nucleotide polymorphisms (SNPs) in IPGs and ISGs for their associations with response to pegylated interferon α-2a (Peg-IFN-α) plus ribavirin therapy in HCV genotype-1 infected patients.

Methods—A two-stage study design was used. First, 91 SNPs from 12 IPGs and 9 ISGs were genotyped in a cohort of 374 treatment-naïve HCV patients and assessed for association with sustained viralogic response (SVR). Next, 14 potentially functional SNPs from the *OASL* gene were studied in this cohort.

Results—Three *OASL* SNPs (rs3213545 and rs1169279 from stage I, and rs2859398 from stage II), were significantly associated with SVR [rs3213545: p=0.03, RR=1.27 (1.03–1.58); rs1169279: p=0.02, RR=1.32 (1.05–1.65); rs2859398: p=0.02, RR=1.29 (1.04–1.61)] after adjusting for other covariates. Further analysis showed these 3 SNPs independently associated with SVR. Additionally, a similar trend towards the associations of these 3 SNPs with SVR was observed in a smaller, independent HCV cohort consisting of subjects from a number of clinical practice settings.

Corresponding Author: Dr. Leland J. Yee, A511 Crabtree Hall, 130 DeSoto Street , Pittsburgh, PA 15232, USA, E-mail: YeeL@edc.pitt.edu, FAX: +1 412-624-7397, Phone: 412-624-5326.
8Current address: Department of Discovery, Medicine & Epidemiology, AstraZeneca Pharmaceuticals LP, Wilmington, DE.

Conclusions—Our study suggests that *OASL* variants are involved in the host response to IFN-based therapy in HCV patients.

Keywords

pharmacogenetics; hepatitis C; interferon therapy; interferon signaling pathway genes; interferon-stimulated genes

Introduction

With approximately 170 million people infected worldwide, hepatitis C virus (HCV) is a major cause of chronic liver disease and the most common indication for liver transplantation in the United States [1]. Pegylated interferon-alfa (Peg-IFN-α) in combination with ribavirin is currently the standard-of-care treatment. Unfortunately, successful viral eradication occurs in only 50% of those treated [2]. Variation in response to interferon (IFN)-based therapy is consistently observed; specifically a lower response rate in African Americans (AA) compared to Caucasian Americans (CA) [3]. The biological mechanisms associated with response to Peg-IFN-α are not well understood, although host genetic factors are believed to play a role [2,4].

The administration of exogenous interferon provides antiviral action against HCV by signaling interferon stimulated gene (ISG) expression through IFN receptors and the Jak–STAT pathway [2], creating an anti-viral effect [5]. In addition, ribavirin is believed to regulate several ISGs leading to enhanced STAT1 binding to DNA [6].

Data from microarray or other gene expression studies have highlighted the importance of ISGs as well as genes of the interferon signaling pathway (IPGs) in the host response to HCV infection [7–11]. Furthermore, association studies have suggested that polymorphisms in these genes may play a role in differential drug response [12,13].

In the present study, we investigated associations between IPG and ISG candidate genes and the response to peginterferon-2a+ribavirin among treatment-naïve, chronic HCV genotype-1 infected patients.

Patients and Methods

Study Cohorts

1. HCV Cohort 1: Virahep-C Cohort—This study utilized patients from the Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C), a prospective, multi-center clinical study sponsored by the National Institutes of Health, conducted to gain a greater understanding of the mechanisms of resistance to antiviral therapy for chronic HCV infection. The details of this study have been described elsewhere [14]. Briefly, 401 HCV genotype-1 infected (196 AA and 205 CA) (Table 1), interferon treatment-naïve individuals were recruited from 8 clinical centers. All were treated with 180 mcg weekly pegylated interferon alpha-2α (Roche) plus 1000–1200 mg daily ribavirin, depending upon patient weight per standard protocol [14]. Patients were defined as having a sustained virologic response (SVR) if they had undetectable serum HCV-RNA (Roche Amplicor™ Assay; Alameda, CA) levels 6 months after the discontinuation of treatment. All other patients were defined as non-responders (NRs). Out of the 401 HCV patients from the Virahep-C cohort, 374 patients (180 AA and 194 CA) consented to the host genetic study. The genetic study protocol was approved by Institutional Review Board at each participating clinical center as well as Cedars-Sinai Medical Center.

2. HCV Cohort 2: Independent HCV Cohort—To evaluate associated gene(s) identified in Virahep-C cohort, we utilized data from a second cohort of chronic HCV patients receiving

IFN-based treatment from several clinical centers across the U.S. DNA samples were collected from 228 patients (68 AA and 160 CA) (Table 2) who were treated at (1) Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health; (2) University of Maryland; or (3) University of Colorado Health Sciences Center. These patients were chronically infected with HCV genotype-1, received IFN-based therapy (IFN-α only, the combination of standard IFN-α + ribavirin, or the combination of pegylated-IFN-α + ribavirin), and provided written consent to participate in studies of host genetics.

Two-stage Genotyping Strategy

In stage I, a total of 118 SNPs from 17 IPGs and ISGs were selected from the NCBI dbSNP database. These genes included: *STAT1, STAT2, IFNaR1, IFNaR2, IRF9, MX1, MX2, OAS1, OAS2, OAS3, OASL, IRF7, G1P2, G1P3, IFI35, PKR and IP10*. SNPs were selected with an average density of one per 3–5 kb interval, spanning a broad region including 5 kb flanking both 5'- and 3'- ends of the gene. In addition, selected SNPs had an overall minor allele frequency of ≥5% or nonsynonymous SNPs with an overall minor allele frequency of ≥0.5%. Illumina BeadArray technology (Illumina, San Diego) was used to genotype the selected SNPs. All SNPs were evaluated for their association with SVR. Any genes containing SNPs with a significant association (nominal $p<0.05$) with SVR after adjusting for other covariates were further investigated in stage II.

Based on the significant associations observed in stage I between two *OASL* SNPs and SVR, all known potentially functional *OASL* SNPs were evaluated in stage II. Specifically, we identified potential functional SNPs through a comprehensive database search that included: 1) a web-based database (TFSEARCH v1.3 http://www.cbrc.jp/research/db/TFSEARCH.html) scan for all SNPs in the region of a potential transcription factor binding site in the *OASL* gene promoter region, which included 5kb away from the transcription site of the *OASL* gene; 2) Ensembl Genomic Seqence Alignment database search for SNPs in any *OASL* intron regions conserved between human and mouse (http://www.ensembl.org/Homo_sapiens/geneseqalignview); 3) identification of all dbSNP catalogued SNPs in *OASL* exons; and 4) SNPs located at the intron-exon borders or potential splice sites. The 16 identified SNPs were genotyped using ABI TaqMan technology as previous described [15]. The probes and primers used are provided in Supplemental table 1.

Evaluation of Population Structure

We have previously evaluated population structure of the Virahep-C cohort [16]. Briefly, we used the structured association method developed by Pritchard and colleagues [17], employing genotyping data from 161 unlinked ancestry-informative SNPs and observed a strong correlation between self-reported race and individual admixture [16]. Consequently, we conducted our analyses using both self-reported race and estimates of individual admixture. Since we observed no significant differences in association results using either self-reported race or individual admixture estimates, we have chosen to report the results from self-reported race in the present study.

Statistical Methods

Proportions of SVR by baseline demographic and clinical characteristics were compared using the two-sided race-adjusted Mantel-Haenszel Chi-square test. The Cochran-Armitage test was used to examine a statistical trend between ordinal data and SVR. Modified Poisson regression with sandwich estimators of the variance were used for multivariable analyses and to adjust for potential confounders [18]. Results are reported in terms of relative risks (RR) with 95% confidence intervals (CI) along with the corresponding p-values. Statistical analyses were performed using SAS 8.02 (SAS Institute, Cary NC). Statistical significance was set at the

conventional level of α=0.05. Pairwise linkage disequilibrium (LD; D' and r^2) between SNPs were calculated with Haploview software [19].

Results

Demographic and clinical characteristics

Among Virahep-C participants, 374 (180 AA and 194 CA) consented to participate in host genetics studies. The characteristics of this group are presented in Table 1. AA patients had a significantly lower SVR (27.2%) as compared with CA patients (53.6%) (p=0.0001). Stratified by race, we observed significant associations between SVR and gender, baseline HCV viral level, Ishak fibrosis score, and proportion maximum Peg-IFN-α dosage.

Stage I: Associations between IFN signaling pathway and IFN stimulated genes with SVR

Out of the 118 selected SNPs, a total of 91 SNPs were genotyped successfully in 5 interferon signaling pathway genes and 12 interferon stimulated genes (Supplementary table 2). Three of the 91 SNPs (rs1141746 and rs1316896 from *GIP3* and rs2066816 from *STAT2*) were non-polymorphic in both racial groups and 3 SNPs (*PKR* rs2307478, *STAT2* rs2228259, and *OAS1* rs12298890) were non-polymorphic among CAs.

Out of the 91 SNPs genotyped successfully, 11 SNPs were observed to have a p-value less than 0.1 in univariable analysis with SVR in either the AA or CA group (Supplemental table 2), In race-adjusted analysis on these 11 SNPs, we observed two *OASL* SNPs (rs3213545 RR= 1.32; 95%CI: 1.04–1.67; p=0.03 and rs1169279 RR= 1.30; 95%CI: 1.01–1.67; p=0.04) and 1 *STAT2* SNP (rs2066811 RR=1.59; 95%CI: 1.02–1.47; p=0.04) to be significantly associated with SVR (Table 3). To determine if these three SNPs were independently associated with SVR, we included the SNPs along with other covariates previously reported to be significantly associated with SVR [14] in a multivariable regression model (Table 4). A statistically significant association remained after adjustment for other covariates for carriers of the *OASL* rs3213545 T allele (RR=1.27; 95%CI: 1.03–1.58; p=0.03) and for carriers of the *OASL* rs1169279 A allele (RR=1.32; 95%CI: 1.05–1.65; p=0.02). However, the carriers of *STAT2* rs2066811-G failed to retain its statistical significance (RR=1.40; 95%CI: 0.94–2.10; p=0.10).

Since the *STAT2* rs2066811-G allele is common in AAs (16.9%) and very rare in CAs (0.3%), we performed multivariable regression analysis for G allele carriage in the AA group alone, but did not observe a statistically significant association between this SNP and SVR after adjusting for other covariates (RR=1.41; 95%CI: 0.91–2.17; p=0.12).

Stage II: High density genotyping of the *OASL* gene

In order to further explore the potential functional significance of *OASL* gene polymorphisms in relation to SVR, we genotyped all known SNPs of potential functional significance in the *OASL* gene (Supplemental table 3). Among the 16 identified SNPs, 14 SNPs were successfully genotyped; rs12811390 of intron 2 and rs10083129 in the promoter region failed genotyping. Among the 14 successfully genotyped SNPs, 3 SNPs were not polymorphic in our cohort (rs12315068, rs28360476 and rs3861793). Of the remaining 11 SNPs, 2 resulted in synonymous amino acid changes; 4 were located in the promoter region and are believed to be within potential transcriptional factor biding sites; 1 SNP was located in the 3' untranslated region (3'-UTR); 1 SNP was in the downstream 3' end, and 3 SNPs were located in introns are considered potentially functionally significant because they are also located in a conserved region in the mouse.

We observed only one SNP (rs2859398), located in the promoter region at position −2875bp, to be significantly associated with SVR but only in CAs and not in AAs. The frequency of the C allele differed significantly between responders (22.2%) and non-responders (14.4%) (*P*=0.04; Supplemental table 3) before adjustment for covariates. In multivariable regression analysis adjusting for potential confounding variables, carriage of the rs2859398-C allele was significantly associated with SVR (RR=1.29; 95%CI: 1.04–1.61; p=0.02) (Table 4).

In order to understand whether the three associated *OASL* SNPs are in strong linkage disequilibrium (LD) with each other or whether each contributes to the association with SVR independently, we first performed LD analysis. As shown in Supplemental table 4, among CAs, all 3 SNPs are moderately associated with each other (r^2 ranges from 0.46–0.75). In AAs, only SNPs rs3213545 and rs2859398 showed moderate linkage (r^2 =0.77), while these two SNPs are not associated with the third SNP rs1169279 (r^2 =0.12–0.13). Thus it is likely that some of these SNPs may contribute to the association with SVR independently.

To evaluate the association between SVR and the *OASL* gene as a whole, we performed analysis by grouping patients into two groups: those carrying no minor allele of associated SNPs (value=0) and those carrying at least one minor allele of the three associated SNPs (value=1). As show in Table 5, there was a significant association between patients with at least one minor allele of the three SNPs and SVR after adjusting for other covariates (RR=1.31; 95%CI: 1.03–1.66; p=0.03).

Three *OAS L* SNPS association with SVR in the HCV cohort 2

Table 2 presents the basic demographic profile of the HCV cohort 2. Among the 228 individuals receiving IFN-based therapy for chronic HCV genotype 1 infection, 30% were AA. We observed a similar trend of association for carriage of the rs1169279-A, rs3213545-T and rs2859398-C alleles and SVR in this validation cohort, although the p-values were of borderline significance, p=0.09 after adjusting for other covariates, respectively for the same categorical groupings described above (Table 5).

Discussion

With a two-stage genotyping strategy and two sets of samples, we observed positive associations between three alleles in the *OASL* gene (rs1169279-A allele, rs3213545-T allele and rs2859398-C allele) and SVR. This association was consistent in both AA and CA patients and the significance was improved after adjustment for potential confounding variables. There was independent association with SVR among these 3 associated alleles. A similar trend of association for these SNPs with SVR was also observed in a smaller independent HCV patient cohort.

Generally speaking, the present study supports previous reports that polymorphisms in ISGs may be important in the clearance of hepatitis C virus, both naturally and in the context of therapy [12,20,21]. In addition to the *OASL* gene, we also observed statistically significant associations with SVR in either AA or CA alone for several SNPs in the ISPs as well as the ISGs. For example, a nonsynonymous SNP rs2066811 in *STAT2* was significantly associated with SVR in AA patients, but not in CA patients even though there is a similar trend in both groups. It should be noted that the G allele of this SNP was very rare among CA patients and more common in AA patients (G allele frequency: 0.3% in CA and 16.9% in AA). Therefore, for some genes tested in the current study, although a robust significant result was not obtained, they deserve further evaluation in additional HCV cohorts. Such genes include *STAT1, STAT2, OAS2, PKR, MX2, IFNαR1* and *IFNαR2*.

The interferon-induced 2'-5'- oligoadenylate synthetases (OAS) are important in the antiviral response [22]. The human OAS family contains OAS1, OAS2, OAS3 and OASL [23]. With the 2'-5' oligoadenylate catalytic activity in innate viral clearance and its induction by interferon, OASL is recognized as a significant molecule in antiviral response. IFN-α/IFN-β and IFN-γ bind to their cognate receptors, and lead to the formation of IFN-stimulated gene factor 3, a protein that activates transcription of the *STAT1* gene and, by doing so, induces the formation of homodimers between STAT1, STAT2, and p48, (or of STAT1 homodimers,) respectively. These homodimer complexes translocate to the nucleus and produce transcriptional up-regulation of OAS family members. OAS is only activated by double-stranded RNA (dsRNA) while RNase L is activated by 2'-5'-oligoadenylate (2–5 A), which degrades dsRNA. OASL is postulated to interfere with the 2–5A system through blocking OAS activation [23–25]. Therefore, OASL may have a negative effect on anti-viral function of the OAS isozymes.

In the current study, three *OASL* SNPs were observed to be associated with SVR. Among them, one is a silent mutation located in the exon 2 at codon 136. In sequence comparison analysis using ESE Finder V3.0 (Cold Spring Harbor Laboratory) [26], the sequence surrounding this SNP was predicted to be a binding site for SF2/ASF splicing factor protein as reported by Yakub [25]. The sequence with the allele C matched with a 7-nt exonic splicing enhancer (ESE) consensus sequence, ctctCgt. When the sequence of rs3213545 changed to allele T, as ctctTgt, this specific region was no longer recognized as SF2/AFF consensus ESE site. Mutation within an ESEs has been reported to result in reduction of the level of full-length transcripts and the corresponding proteins [27]. Thus, it is likely that T allele carriers of this SNP may have lower expression of OASL as compared with C allele carriers. Such lower OASL activity may consequently increase the antiviral activity of OAS isozymes due to their inhibitory effect, leading to an increased clearance of virus.

Another SNP (rs2859398) associated with increased SVR is located in the *OASL* promoter region at position −2875bp. Through a bioinformatics sequence comparison analysis using Transcription Element Search System (TESS v6.0) (http://www.cbil.upenn.edu/tess/), several human transcription element binding sites were observed within the specific sequence around the T allele of the SNP. In contrast, after this SNP was changed from T to C, this specific sequence was no longer recognized as human transcription elements binding site. Some of these transcription element binding sites, such as C/EBP beta, are important in the regulation of genes involved in immune and inflammatory responses, such as the IL-1 response element in the IL-6 gene and the regulatory regions of several acute-phase and cytokine genes [28, 29]. This polymorphism may represent another mechanism for the *OASL* gene to influence the antiviral effect during IFN-based therapy.

The associations and trends observed in the Virahep-C sample were also identified in the second sample (HCV cohort 2). There were several important differences with respect to these two cohorts. First, cohort 2 was comprised of a smaller proportion of AAs (30%) and a smaller sample size (N=228), compared to the Virahep-C cohort (AAs=48.1% and N=374). Additionally, cohort 2 consisted of a more heterogeneous group of patients attending clinical centers throughout the U.S. and was not composed of a specially selected group of patients who fulfilled specific criteria for entry in to a clinical study. Furthermore, this cohort sample consisted of individuals who were treated with IFN-α only, the combination standard IFN-α + ribavirin, or pegylated-IFN-α + ribavirin, and consisted of individuals who were both treatment-naïve and treatment-experienced. Even with such a diverse patient sample, we still observed a borderline significant association between these three SNPs and SVR. Such robust association supports an important role for the *OASL* gene in a patient's response to IFN-based therapy.

It is also important to note a couple of limitations to our study. We have adjusted for the most important and commonly utilized clinical confounding variables such as baseline viral level in our analyses. However, additional factors such as interferon sensitivity determining region (ISDR) and NS5A sequences may play an important role on therapeutic response. However without systematic and complete data on these variables on the participants in our study for multivariable analyses, residual confounding from these factors may exist. Additionally, future studies will need to address the biological function of the SNPs identified in the study. In particular, such studies will need to take in to account the complexity of polymorphisms and gene expression, taking in to account post-translational modifications that may also affect expression.

In summary, we report three SNPs of the *OASL* gene to be associated with sustained viral response in patients with chronic HCV infection. Two of these SNPs are predicated to influence gene expression through bioinformatics analyses. We observed a similar trend of associations with SVR for these SNPs in another HCV study cohort. Further functional studies are warranted to demonstrate whether these SNPs influence the expression of the *OASL* and to understand the mechanism by which these polymorphisms might influence an individual's response to INF-based therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

HCV, hepatitis C virus; AA, African Americans; CA, Caucasian Americans; SVR, sustained virologic response.

Acknowledgements

Members of Virahep-C contributing to the study include: ***from the Beth Israel Deaconess Medical Center, Boston, MA*:** Nezam Afdhal, MD (Principal Investigator), Tiffany Geahigan, PA-C, MS (Research Coordinator); ***from the New York-Presbyterian Medical Center, New York, NY:*** Robert S. Brown, Jr., MD, MPH (Principal Investigator), Lorna Dove, MD, MPH (Co-Investigator), Shana Stovel, MPH (Study Coordinator), Maria Martin (Study Coordinator); ***from the University of California, San Francisco, San Francisco, CA:*** Norah Terrault, MD, MPH (Principal Investigator), Stephanie Straley, PA-C, Eliana Agudelo, PA-C, Melissa Hinds, BA (Clinical Research Coordinator), Jake Heberlein (Clinical Research Coordinator); ***from Rush University, Chicago, IL:*** Thelma E. Wiley, MD (Principal Investigator), Monique Williams, RN (Study Coordinator); ***from the University of Maryland, Baltimore, MD:*** Charles D. Howell, MD (Principal Investigator), Kelly Gibson (Project Coordinator), Karen Callison, RN (Study Coordinator), Jane Lewis, RN (Study Coordinator); ***from the University of Miami, Miami, FL:*** Lennox J. Jeffers, MD (Principal Investigator), Shvawn McPherson Baker, PharmD (Co-Investigator), Maria DeMedina, MSPH (Project Manager), Carol Hermitt, MD (Project Coordinator); ***from the University of Michigan, Ann Arbor, MI:*** Hari S. Conjeevaram, MD, MS (Principal Investigator), Robert J. Fontana, MD (Co-Investigator), Donna Harsh, MS (Study Coordinator); ***from the University of North Carolina, Chapel Hill, NC:*** Michael W. Fried, MD (Principal Investigator [K24 DK066144]), Scott R. Smith, PhD (Co-Investigator), Dickens Theodore, MD, MPH (Co-Investigator), Steven Zacks, MD, MPH, FRCPC (Co-Investigator), Roshan Shrestha, MD (Co-Investigator), Karen Dougherty, NP (Co-Investigator), Paris Davis (Study Coordinator), Shirley Brown (Study Coordinator); ***from St. Louis University, St. Louis, MO:*** John E. Tavis, PhD (Principal Investigator), Adrian Di Bisceglie, MD (Co-Investigator), Ermei Yao, PhD (Co-Investigator), Maureen Donlin, PhD (Co-Investigator), Nathan Cannon, BS (Graduate Student), Ping Wang, BS (Lab Technician); ***from Cedars-Sinai Medical Center, Los Angeles, CA:*** Huiying Yang, MD, PhD (Principal Investigator), George Tang, PhD (Project Scientist), Dai Wang, PhD (Project Scientist); ***from the University of Colorado Health Sciences Center, Denver, CO:*** Hugo R. Rosen, MD (Principal Investigator), James R. Burton, MD (Co-Investigator), Jared Klarquist (Lab Technician); ***from Veteran's Administration, Portland, OR:*** Scott Weston (Lab Technician); ***from Indiana University, Bloomington, IN:*** Milton W. Taylor, PhD (Principal Investigator), Corneliu Sanda, MD (post-doctoral associate), Takuma Tsukahara, MS (statistician), Mary Ferris (lab assistant); ***from the Data Coordinating Center, Graduate School of Public Health at the University of Pittsburgh, Pittsburgh, PA:*** Steven H. Belle, PhD (Principal Investigator), Richard A. Bilonick, PhD (Statistician), Geoffrey Block, MD (Co-Investigator), Jennifer Cline, BS (Data Manager), Marika Haritos, MS (Statistician), KyungAh Im, MS (Statistician), Stephanie Kelley, MS (Data Manager), Sherry Kelsey, PhD (Co-Investigator), Laurie Koozer, BA

(Project Coordinator), Sharon Lawlor, MBA (Data Coordinator), Stephen B. Thomas, PhD (Co-Investigator), Abdus Wahed, PhD (Statistician), Yuling Wei, MS (Project Coordinator), Leland J. Yee, PhD (Consultant), Song Zhang, MS, MD (Statistician); ***from the National Institute of Diabetes and Digestive and Kidney Diseases:*** Patricia Robuck, PhD, MPH (Project Scientist), James Everhart, MD, MPH (Scientific Advisor), Jay H. Hoofnagle, MD (Scientific Advisor), Edward Doo, MD (Scientific Advisor), T. Jake Liang, MD (Scientific Advisor), Leonard B. Seeff, MD (Scientific Advisor); ***from the National Cancer Institute:*** David E. Kleiner, MD, PhD (Central Pathologist).

Financial support: This study was a cooperative agreement funded by the NIDDK and co-funded by the National Center on Minority Health and Health Disparities (NCMHD), with a Cooperative Research and Development Agreement (CRADA) with Roche Laboratories, Inc. Grant numbers: U01 DK60329, U01 DK60340, U01 DK60324, U01 DK60344, U01 DK60327, U01 DK60335, U01 DK60352, U01 DK60342, U01 DK60345, U01 DK60309, U01 DK60346, U01 DK60349, U01 DK60341. Other support: National Center for Research Resources (NCRR), Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, General Clinical Research Centers Program grants: M01 RR00645 (New York Presbyterian), M02 RR000079 (University of California, San Francisco), M01 RR16500 (University of Maryland), M01 RR000042 (University of Michigan), M01 RR00046 (University of North Carolina). Additional support was provided to LJY by 1KL2 RR024154-01.

References

1. Brown RS, Hepatitis C. liver transplantation. Nature 2005;436:973–978. [PubMed: 16107838]
2. Feld JJ, Hoofnagle JH. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. Nature 2005;436:967–972. [PubMed: 16107837]
3. Jeffers LJ. Treating hepatitis C in African Americans. Liver Int 2007;27:313–322. [PubMed: 17355451]
4. Gao B, Hong F, Radaeva S. Host factors and failure of interferon-alpha treatment in hepatitis C virus. Hepatology 2004;39:880–890. [PubMed: 15057887]
5. Sen GC. Viruses and interferons. Annu Rev Microbiol 2001;55:255–281. [PubMed: 11544356]
6. Zhang Y, Jamaluddin M, Wang S, Tian B, Garofalo RP, Casola A, et al. Ribavirin treatment up-regulates antiviral gene expression via the interferon-stimulated response element in respiratory syncytial virus-infected epithelial cells. J Virol 2003;77:5933–5947. [PubMed: 12719586]
7. Giannelli G, Guadagnino G, Dentico P, Antonelli G, Antonaci S. MxA and PKR expression in chronic hepatitis C. J Interferon Cytokine Res 2004;24:659–663. [PubMed: 15684819]
8. Bigger CB, Guerra B, Brasky KM, Hubbard G, Beard MR, Luxon BA, et al. Intrahepatic gene expression during chronic hepatitis C virus infection in chimpanzees. J Virol 2004;78:13779–13792. [PubMed: 15564486]
9. Chen L, Borozan I, Feld J, Sun J, Tannis LL, Coltescu C, et al. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. Gastroenterology 2005;128:1437–1444. [PubMed: 15887125]
10. Di Bona D, Cippitelli M, Fionda C, Cammà C, Licata A, Santoni A, et al. Oxidative stress inhibits IFN-alpha-induced antiviral gene expression by blocking the JAK-STAT pathway. J Hepatol 2006;45:271–279. [PubMed: 16595158]
11. Luo S, Cassidy W, Jeffers L, Reddy KR, Bruno C, Howell CD, et al. Interferon-stimulated gene expression in black and white hepatitis C patients during peginterferon alfa-2a combination therapy. Clin Gastroenterol Hepatol 2005;3:499–506. [PubMed: 15880320]
12. Knapp S, Yee LJ, Frodsham AJ, Hennig BJ, Hellier S, Zhang L, et al. Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of MxA, OAS-1 and PKR. Genes Immun 2003;4:411–419. [PubMed: 12944978]
13. Suzuki F, Arase Y, Suzuki Y, Tsubota A, Akuta N, Hosaka T, et al. Single nucleotide polymorphism of the MxA gene promoter influences the response to interferon monotherapy in patients with hepatitis C viral infection. J Viral Hepat 2004;11:271–276. [PubMed: 15117331]
14. Conjeevaram HS, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucas TE, Afdhal N, et al. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. Gastroenterology 2006;131:470–477. [PubMed: 16890601]
15. Goodarzi MO, Guo X, Taylor KD, Quiñones MJ, Samayoa C, Yang H, et al. Determination and use of haplotypes: ethnic comparison and association of the lipoprotein lipase gene and coronary artery disease in Mexican-Americans. Genet Med 2003;5:322–327. [PubMed: 12865761]

16. Yee LJ, Tang YM, Kleiner DE, Wang D, Im K, Wahed A, et al. Myxovirus-1 and protein kinase haplotypes and fibrosis in chronic hepatitis C virus. Hepatology 2007;46:74–83. [PubMed: 17526009]
17. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics 2000;155:945–959. [PubMed: 10835412]
18. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol 2004;159:702–706. [PubMed: 15033648]
19. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265. [PubMed: 15297300]
20. Hijikata M, Mishiro S, Miyamoto C, Furuichi Y, Hashimoto M, Ohta Y. Genetic polymorphism of the MxA gene promoter and interferon responsiveness of hepatitis C patients: revisited by analyzing two SNP sites (−123 and −88) in vivo and in vitro. Intervirology 2001;44:379–382. [PubMed: 11805446]
21. Hijikata M, Ohta Y, Mishiro S. Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt −88) correlated with the response of hepatitis C patients to interferon. Intervirology 2000;43:124–127. [PubMed: 10971132]
22. Marie I, Rebouillat D, Hovanessian AG. The expression of both domains of the 69/71 kDa 2',5' oligoadenylate synthetase generates a catalytically active enzyme and mediates an anti-viral response. Eur J Biochem 1999;262:155–165. [PubMed: 10231377]
23. Rebouillat D, Hovanessian AG. The human 2',5'-oligoadenylate synthetase family: interferon-induced proteins with unique enzymatic properties. J Interferon Cytokine Res 1999;19:295–308. [PubMed: 10334380]
24. Hartmann R, Norby PL, Martensen PM, Jorgensen P, James MC, Jacobsen C, et al. Activation of 2'-5' oligoadenylate synthetase by single-stranded and double-stranded RNA aptamers. J Biol Chem 1998;273:3236–3246. [PubMed: 9452437]
25. Yakub I, Lillibridge KM, Moran A, Gonzalez OY, Belmont J, Gibbs RA, et al. Single nucleotide polymorphisms in genes for 2'-5'-oligoadenylate synthetase and RNase L inpatients hospitalized with West Nile virus infection. J Infect Dis 2005;192:1741–1748. [PubMed: 16235172]
26. Cartegni L, Wang J, Zhu Z, Zhang MQ, Krainer AR. ESEfinder: A web resource to identify exonic splicing enhancers. Nucleic Acids Res 2003;31:3568–3571. [PubMed: 12824367]
27. Ryther RC, Flynt AS, Harris BD, Phillips JA 3rd, Patton JG. GH1 splicing is regulated by multiple enhancers whose mutation produces a dominant-negative GH isoform that can be degraded by allele-specific small interfering RNA (siRNA). Endocrinology 2004;145:2988–2996. [PubMed: 14988388]
28. Rosati M, Valentin A, Patenaude DJ, Pavlakis GN. CCAAT-enhancer-binding protein beta (C/EBP beta) activates CCR5 promoter: increased C/EBP beta and CCR5 in T lymphocytes from HIV-1-infected individuals. J Immunol 2001;167:1654–1662. [PubMed: 11466389]
29. Yang Y, Pares-Matos EI, Tesmer VM, Dai C, Ashworth S, Huai J, et al. Organization of the promoter region of the human NF-IL6 gene. Biochim Biophys Acta 2002;1577:102–108. [PubMed: 12151100]

Table 1

Characteristics of the 374 Virahep-C study participants who consented to participate in genetics studies.

	All (n=374) n	%SVR	AA (n=180) n	%SVR	CA (n=194) n	%SVR	p-value††
Age (years) †							
> 48	181	38.7%	93	24.7%	88	53.4%	0.586
≤ 48	193	43.0%	87	29.9%	106	53.8%	
Gender (n, %)							
Male	244	36.5%	118	23.7%	126	48.4%	0.015
Female	130	49.2%	62	33.9%	68	63.2%	
Baseline viral level(per log10 IU/ml) †							
≤ 6.5	192	45.8%	102	33.3%	90	60.0%	0.009
> 6.5	182	35.7%	78	19.2%	104	48.1%	
Ishak fibrosis score							
0	39	59.0%	18	50.0%	21	66.7%	0.012
1, 2	193	41.5%	97	25.8%	96	57.3%	
3, 4	112	38.4%	54	24.1%	58	51.7%	
5, 6	29	24.1%	10	20.0%	19	26.3%	
Proportion maximum interferon dose taken for first 24 weeks †							
≤ 0.96	185	29.7%	97	21.7%	88	38.6%	<0.0001
>0.96	182	53.3%	77	35.1%	105	66.7%	
ALT(U/L) †							
> 66	187	38.5%	76	21.1%	111	50.5%	0.071
≤ 66	187	43.3%	104	31.7%	83	57.8%	

† cut-off values are medians.

†† race-adjusted Mantel-Haenszel chi-square test

Table 2

Characteristics of HCV cohort 2.

	All (n=228) N	%SVR	AA (n=68) n	%SVR	CA (n=160) n	%SVR	p-value††
Age (years) †							
≤ 49	106	25.5%	17	0%	89	30.3%	0.99
> 49	90	22.2%	29	13.8%	61	26.2%	
Gender (n, %)							
Male	145	33.7%	50	6.0%	95	25.3%	0.02
Female	83	18.6%	18	27.8%	65	35.4%	
Baseline viral level (per log10 IU/ml) †							
≤ 5.8	95	36.8%	29	24.1%	66	42.4%	0.0001
> 5.8	94	12.8%	31	0%	63	19.1%	
Ishak fibrosis score							
0	19	31.6%	3	0%	16	37.5%	0.07
1, 2	64	34.4%	10	0%	54	40.7%	
3, 4	68	16.2%	20	15.0%	48	16.7%	
5, 6	30	13.3%	8	12.5%	22	13.6%	
ALT(U/L) †							
≤ 90	90	22.2%	26	3.9%	64	29.7%	0.9
> 90	90	24.4%	19	15.8%	71	26.8%	

† cut-off values are medians.

†† race-adjusted Mantel-Haenszel chi-square test

Table 3

Allele and carrier frequency analysis of genotyped associated SNPs in combined group adjusting for race.

SNP ID (Alleles)		Allele Frequency n(%)				Carrier	
		AA		CA		RR (95% CI)	*P* Value **
		SVR	NR	SVR	NR		
STAT1	C	36(26.3)	101(73.7)	123(49.2)	127(50.8)		
rs2280234 C/T	T	62(27.8)	161(72.2)	83(61.0)	53(39.0)	0.85(0.64, 1.13)	0.26
STAT1	A	80(25.3)	236(74.7)	191(53.5)	166(46.5)		
rs12693590 A/C	C	18(40.9)	26(59.1)	15(51.7)	14(48.3)	0.80 (0.37–1.72)	0.57
PKR	A	87(27.4)	230(72.6)	206(54.4)	173(45.7)		
rs2307479 A/C	C	11(26.8)	30(73.2)	2(22.2)	7(77.8)	0.54 (0.20–1.47)	0.23
OASL	A	34(28.3)	86(71.7)	84(60.0)	56(40.0)		
rs1169279 A/G	G	64(26.7)	176(73.3)	122(49.6)	124(50.4)	1.30 (1.01– 1.67)	0.04
OASL	C	79(26.2)	223(73.8)	133(50.4)	131(49.6)		
rs3213545 T/C	T	19(32.8)	39(67.2)	73(60.8)	47(39.2)	1.32(1.04,1.67)	0.03
STAT2	A	74(24.8)	225(75.3)	205(53.3)	180(46.8)		
rs2066811 G/A	G	24(39.3)	37(60.7)	1(100.0)	0(0)	1.59(1.02, 2.47)	0.04
OAS2	A	7(13.7)	44(86.3)	92(53.5)	80(46.5)		
rs1293762 A/C	C	91(29.5)	218(70.6)	114(53.3)	100(46.7)	0.82 (0.64– 1.05)	0.12
OAS2	C	8 (20.5)	31(79.5)	64(61.0)	41(39.1)		
rs2010604 C/G	G	90(28.0)	231(72.0)	142(50.5)	139(49.5)	1.09(0.86,1.38)	0.48
IFNaR1	A	72(24.8)	218(75.2)	155(53.3)	136(46.7)		
rs2834202 A/G	G	26(37.1)	44(62.9)	51(53.7)	44(46.3)	0.76 (0.50– 1.16)	0.21
IFNaR2	A	18(25.4)	53(74.7)	81(60.9)	52(39.1)		
rs2834166 A/C	C	80(27.7)	209(72.3)	125(49.4)	128(50.6)	1.20 (0.95– 1.52)	0.13
MX2	T	54(26.6)	149(73.4)	161(51.3)	153(48.7)	1.23(0.96,1.57)	0.10
rs8126663 T/C	C	44(28.0)	113(72.0)	45(62.5)	27(37.5)		

** Relative Risk model adjusted for race at the patient level

Table 4

Multiple regression analyses of *OASL* SNP rs1169279, rs3213545, rs2859398, and *STAT2* SNP rs2066811 and clinical factors associated with IFN treatment response.

Variables	*OASL*-rs3213545: Cattier T			*OASL*-rs1169279: Carrier A			*STAT2-rs2066811: Carrier G*			*OASL-rs2859398: Carrier C*		
	RR	95%CI	P-value	RR	95%CI	P-value	RR	95%CI	P-value	RR	95%CI	P-value
Minor allele carrier	1.27	1.03–1.58	0.026	1.32	1.05–1.65	0.016	1.4	0.94–2.10	0.101	1.29	1.04–1.61	0.02
Race(CA)	1.95	1.45–2.63	<0.0001	2.04	1.52–2.73	<0.0001	2.31	1.63–3.28	<0.0001	1.95	1.45–2.62	<0.0001
Baseline viral level* (per log10 IU/ml)	0.58	0.45–0.74	<0.0001	0.56	0.43–0.73	<0.0001	0.58	0.44–0.74	<0.0001	0.57	0.44–0.74	<0.0001
Interaction of CA race with baseline viral level (log_{10} IU/ml)	1.45	1.09–1.93	0.01	1.47	1.10–1.97	0.008	1.45	1.09–1.93	0.011	1.45	1.09–1.93	0.01
Gender (male)	0.73	0.59–0.89	0.002	0.73	0.59–0.90	0.003	0.75	0.61–0.92	0.006	0.73	0.60–0.90	0.003
Ishak fibrosis score (per unit increase)	0.9	0.84–0.97	0.006	0.9	0.83–0.97	0.004	0.9	0.83–0.97	0.006	0.89	0.83–0.96	0.003
Proportion maximum interferon dose taken for first 24 weeks (per 0.1 increase)	1.4	1.19–1.65	<0.0001	1.4	1.19–1.64	<0.0001	1.4	1.19–1.65	<0.0001	1.41	1.20–1.66	<0.0001

* centered at cohort mean baseline viral level (6.3 log_{10})

Table 5

Multiple regression analyses of any minor allele carrier from *OASL* 3 SNPs and clinical factors with IFN treatment response in 2 HCV Cohorts.

	HCV Cohort 1 (Virahep-C Cohort)		HCV Cohort 2 (HCV V Cohort)
Variables	**RR (95%CI)**	**P-value**	**RR (95%CI)**
At lease one allele carrier from rs3213545-C or rs1169279-A or rs2859398-C	1.31 (1.03–1.66)	0.03	2.56 (0.86–7.57)
Race(CA)	2.05 (1.53–2.75)	<0.0001	1.91 (0.61–6.03)
Baseline viral level* (per log10 IU/ml)	0.56 (0.43–0.73)	<0.0001	0.03 (0.004–0.31)
Interaction of CA race with baseline viral level (log10 IU/ml)	1.47 (1.10–1.96)	0.009	12.94 (1.50–111.73)
Gender (male)	0.73 (0.60–0.91)	0.004	0.65 (0.38–1.14)
Ishak fibrosis score (per unit increase)	0.90 (0.83–0.97)	0.004	0.89 (0.73–1.10)
Proportion maximum interferon dose taken for first 24 weeks (per 0.1 increase)	1.40 (1.20–1.64)	<0.0001	

* Centered at cohort mean baseline viral level (6.3 log10)

** Data concerning IFN dose taken were not collected in cohort 2.