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A Genetic Variant Creating a Novel Interferon Analog (IFNAN) Protein is Associated with Impaired Clearance of Hepatitis C Virus

Supplementary Materials and Methods

Cells

Primary human hepatocytes (PHH) from liver donors not infected with HCV were purchased from Lonza (Walkersville, MD) or Celsis (Chicago, IL). Information about age, sex, BMI, cause of death and viral infection status (CMV, HCV, HIV) was available for each donor. The cells were received attached in 6-well collagen-coated plates or in suspension, within 24-48 hours of isolation from the livers. Upon receiving, cell counts and viability were evaluated for each sample. PHH shipped in suspension were centrifuged at 50g for 5 min and plated in *InVitro*GRO CP medium (Celsis) in collagen-coated plates or chamber slides (BD Biosciences, San Jose, CA). After 6 hours, the medium was replaced with *InVitro*GRO HI medium supplemented with *Torpedo* antibiotic mix (Celsis); the cells were treated after overnight incubation according to specific protocols.

Hepatoma HepG2 cells, cervical carcinoma HeLa and embryonic kidney 293T cells were obtained from ATCC (Manassas, VA) and cultured in DMEM (Cellgro, Manassas, VA) with 10% FBS (Life Technologies, Grand Island, NY) without antibiotics. Hepatoma Huh7-Lunet cells harboring a subgenomic, luciferase-expressing HCV JFH1 replicon[1] were cultured in DMEM media (Cellgro) containing 1g glucose, 10% FCS, L-Glu, Penicillin-Streptomycin antibiotic mix and 1% non-essential amino acids.

Cell treatments

PHH and/or cell lines were treated with PolyI:C (Imgenex, San Diego, CA) or recombinant purified proteins: IFN-α (PBL Interferon Source, Piscataway, NJ), IL28B (custom) and IFNAN (custom). PHH were grown on collagen-coated plates (BD Biosciences) or chamber slides (Thermo Scientific, Rochester, NY); cell lines were grown on regular cell-culture plates. The cells were treated by adding PolyI:C directly to the cell media to a final concentration of 50 μg/ml and processed for confocal imaging or

harvested for RNA and protein analysis 0, 1, 2, 4, 8 or 24 hours post-treatment. PHH were also treated for 24 hours with 100 ng/ml of IFN-α or IL28B and cell lines were treated with 10 ng/ml of IFN-α, IL28B or IFNAN.

Transfection

All transfections were performed using a Lipofectamine™ LTX Reagent (Invitrogen, Carlsbad) with Opti-MEM medium (Life Technologies), using a standard protocol. Amounts of cells, plasmids and the transfection reagents were used as suggested by protocols for 96-, 48-, 24, 12- or 6-well plates by manufacturer (Life Technologies).

HCV infection of PHH

PHH were cultured in collagen-coated 24-well plates or chamber slides ($0.35x10^5$/well) overnight, and then infected with HCV (JFH1 strain)[2] at a multiplicity of infection (M.O.I.) of 2. Cells were processed for RNA extraction or confocal imaging at 0, 6 or 24 hours post-infection.

Antiviral assays

Huh7-Lunet cells harboring a subgenomic, luciferase-expressing HCV JFH1 replicon[1] were transfected with 0.2 ug of expressing constructs for IFNAN, p131 or p107 or an empty GFP vector (mock) in a 48-well plate. Luciferase expression was measured 48 hours post-transfection, with the Luciferase Assay System (Promega, Madison, WI) using a POLARstar Omega instrument (BMG Labtech, Cary, NC).

Extraction of DNA, RNA and protein

DNA and RNA from all samples were prepared using QiaCube (Qiagen, Germantown, MD) - DNA was prepared with a DNeasy kit and DNase-I treated RNA was prepared with an RNeasy kit (Qiagen). The quality and quantity of DNA and RNA was evaluated with NanoDrop 8000 (Thermo Scientific, Wilmington, DE) and BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA). Protein was prepared by lysing cells in RIPA buffer (Sigma Aldrich, St.Louis, MO), complemented with Complete protease inhibitor (Roche, Indianapolis, Indiana). Cell media from transfected or treated cells was concentrated 10x and 100 x using the 9K MWCO protein concentrator tubes (Thermo Scientific).

Western blotting

Proteins were resolved on 4-12% tris-glycine gels (Life Technologies) and processed according to standard methods. The protein detection was done using the custom anti-IFNAN mouse and rabbit monoclonal antibodies, rabbit anti-Halo antibody (Promega, Madison, WI), and corresponding secondary goat anti-rabbit (sc-2030) or goat anti-mouse (sc-2031) antibodies with IgG-HRP (Santa Cruz, Santa Cruz, CA). The signals were detected with ECL Plus western blotting detection system (GE Healthcare Life Sciences, Pittsburg, PA).

Analysis of STAT1/STAT2 phosporylation

The levels of activated STAT1 and STAT2 in transiently transfected HepG2 cells were determined by measuring the levels of tyrosine-phosphorylated STAT1 and STAT2 by Western blotting as described previously[3]. HepG2 cells were transfected with corresponding expression constructs for 6 protein isoforms in 6-well plates, using the same standard conditions for all expression constructs, mock (Halo-tag vector) and non-transfected cells. The cells were incubated at 37^{o}C, and then whole cell lysates were prepared 48 hours post-transfection. Treatment of cells for 1 hour at 37^{o}C with 50 ng/ml of recombinant IL28B was used as a positive control. Equal amounts of each protein lysate (50 µg/lane) were resolved by electrophoresis on 8% sodium dodecyl sulfate (SDS)-PAGE gels (Life Technologies) and then transferred to polyvinylidene difluoride (PVDF) membranes. The levels of tyrosine-phosphorylated STAT1 (pY-STAT1) and tyrosine-phosphorylated STAT2 (pY-STAT2) were measured with ECL Plus western blotting detection system using rabbit anti-phospho-Tyr701-STAT1 (Cell Signaling Technology Inc., Beverly, MA) and rabbit anti-phospho-Tyr689-STAT2 (Millipore, Billerica, MA) antibodies, respectively. The blots were subsequently stripped and re-probed with rabbit anti-STAT1 and anti-STAT2 antibodies (Santa Cruz Biotechnology) to measure the levels of total STAT1 and STAT2 proteins, respectively.

RNA-sequencing

Total RNA (1 ug) from PHH or HepG2 cells was used for selection of PolyA mRNA transcripts and library preparation with TruSeq kit (Illumina Inc., San Diego, CA). The libraries were enriched by 12 PCR cycles and sequenced at a concentration of 4.5 pM, using Genome Analyzer (GAII), generating in average 47.2±6.6 million of 107 bp paired-end sequencing reads per sample. The reference human genome was built

based on UCSC hg19 index using Bowtie software. After standard quality control procedure the sequenced reads were processed using Illumina Pipeline OLB 1.9.0 and CASAVA 1.7.0 and aligned to the reference genome using TopHat v1.2.0. A library of all human transcripts from Ensemble database, version GRCh37.61: useast.ensembl.org/info/data/ftp/index was used to generate a library of exon junctions and reconstruct splicing forms. Default TopHat algorithm removes from analysis RNA-seq reads that map to more than one genomic region. Considering the complexity of the region surrounding *IL28B* gene, we implemented a special strategy. To allow non-exclusive mapping of reads to regions of high similarity, such as *IL28A, IL28B,* and *IL29* and *IL28A/B* pseudogene, we changed TopHat settings to allow multiple alignments (up to 10). The mapping identified expression clusters, representing potential exons. Based on the created database of potential splice junctions, previously unmapped reads were re-mapped by TopHat v1.2.0. To detect novel transcripts, the final aligned read files were processed by Cufflinks v0.9.3. The TopHat alignment algorithm brakes sequence reads into 25-bp segments that are independently mapped and reconstructed back into sequence reads if all individual segments are mapped correctly. Relative abundances of transcripts were measured with fragments per kilobase of exon per million fragments mapped algorithm (FPKM). Confidence intervals for FPKM estimates were calculated using a Bayesian inference method. In the presence of splicing forms, the highest expressing form of each gene was assigned a ratio 1, and all other forms expressed at least at 1% level of the main form (ratio >0.01) were used for analysis. To discover possible genetic variants, we allowed 2 or 3 mismatches per each 25-bp segment. Mapping results were visualized using both the UCSC genome browser http://genome.ucsc.edu/ and a local copy of the Integrative Genomics Viewer (IGV) software: broadinstitute.org/igv/. Genetic variants in the *IL28B/IL29* region were visualized by IGV and then examined manually and validated by Sanger sequencing in DNA and cDNA samples.

Identification and cloning of novel splicing forms

Rapid amplification of cDNA ends (5' and 3'RACE) was performed with SMARTer RACE cDNA kit (Clontech, Mountain View, CA) using a pool of DNAse-I treated RNA samples from PolyI:C-treated PHHs from several liver donors. The full-length open reading frames for all transcripts were PCR-amplified with specific cloning

primers (Supplementary Table 11) from cDNA of PolyI:C–treated PHHs. PCR reactions were performed with AmpliTaq Gold 360 Master Mix (Life Technologies) and 360 GC Enhancer (Life Technologies) using the touchdown PCR program with initial denaturation step with 10 minutes at 95 ^{o}C, followed by 20 cycles (30 seconds at 95 ^{o}C, 45 seconds for 2 cycles at each temperature from 70 through 60 ^{o}C decreasing by 1 ^{o}C at each step, 45 seconds at 72 ^{o}C); 25 additional cycles (30 seconds at 95 ^{o}C, 45 seconds at 60 ^{o}C and 45 seconds at 72 ^{o}C); and final extension time of 7 minutes at 72 ^{o}C. Gel-purified PCR fragments were cloned into a C-terminal pFC14A–Halo tag expression vector (Promega) and sequenced for validation. A full-length expression construct IL28B-Halo was generated using the same approach. p179 was also cloned into a pcDNA3.1-FLAG-tagged expression vector.

Production of recombinant proteins

Full-length open reading frames for p179 and IL28B were recloned from the pFC14A-Halo tag constructs into a pFastBac C-terminal His-tag vector (Life Technologies) to generate Bacmids which were transfected into a sf9 baculoviral strain. Using the anti-His-tag antibody (Sigma), expression of IL28B was detected in cell media, while expression of p179 was detected only in cell pellet. Thus, IL28B was purified from cell media and p179 from the cell pellet after cultivation of cells for 3-5 days in 2 liters of SF-900 III medium (Life Technologies). Proteins were first purified on HisTrap 5-ml nickel column followed by size-exclusion chromatography preparative column TSK G3000pw of 21.5 mm x 60 cm (Tosoh, Grove City, OH). The purified protein fractions were concentrated and dialyzed into a buffer suitable for downstream applications. High purity (>90%) achieved by several rounds of affinity purification was confirmed by Coomassie staining and Western blot analyses with anti-His antibody (#H1029, Sigma, 1:3000 dilution), anti-IL28B and custom mouse and rabbit monoclonal anti-p179 antibodies. The IL28B and p179 proteins were custom-produced by Protein One (Rockville, MD).

Development of anti-IFNAN antibodies

A mouse monoclonal antibody was custom-developed for a synthetic peptide KALRDRYEEEALSWGQRNCSFRPRRRDSPRPS corresponding to amino acids 44-74 of p179 protein, by Precision Antibody (Columbia, MD). A rabbit monoclonal antibody

was custom-developed for a synthetic peptide PGSSRKVPGAQKRRHKPRRADSPRC corresponding to amino acids 128-152 of p179 protein, by Epitomics (Burlingame, CA). Specific detection using both antibodies was confirmed by Western blot with recombinant purified p179 at several concentrations and with lysates of cells transfected with expression constructs, and confocal imaging of transfected cells and treated PHH. There was no cross-reaction with IFN-α, IL28B or with negative anti-IgG isotype control.

Evaluation of biological activity of novel proteins

Analysis of 45 signaling pathways in HepG2 cells was performed with the Luciferase Cignal 45-Pathway Finder Reporter Arrays according to instructions (Qiagen Inc., Valencia, CA). Full list of pathways is available on the website: http://www.sabiosciences.com/reporter_assay_product/HTML/CCA-901L.html. The arrays represent 96-well transfection-ready plates which contain 45 luciferase reporter assays, in duplicates, and all necessary negative and positive controls. Transfections were performed as follows - HepG2 cells, transfection agent and one expression construct at a time were added to 96-well plates with pre-plated reporter assays; luciferase expression was assayed 48 hours post-transfection. In total, 10x96-well plates were used in this experiment - a plate with cells mock-transfected with an empty vector was used as a reference plate for all transfection experiments; a plate with cells mock-treated with PBS was used as a reference plate for all treatment experiments. Cells were transfected with expression constructs for all full-length proteins, p179, p170, p143, p131, p124 and p107. Cells were also treated with purified recombinant proteins - 10 ng/ml of IFN-α (PBL InterferonSource, Piscataway, NJ) or IL28B (custom-generated) and/or IFNAN-p179 (custom-generated) for 24 h. The luciferase values measured 24 hours post-transfection were first normalized to control reporters on each plate, and then presented as fold difference compared to the reference plates (mock-transfected or mock-treated cells). Validation analysis was performed in cells transfected with an individual interferon-stimulated response element (ISRE) luciferase Cignal reporter, the same assay as included in the panel of the 45 reporters tested (Qiagen). This assay includes the firefly luciferase reporter gene under the control of a minimal (m)CMV promoter. The construct contains tandem repeats of the ISRE, which consists of STAT1/STAT2 binding sites

(TAGTTTCACTTTCCC)n. The cells were also co-transfected with the constitutively expressing renilla luciferase reporter, which serves as an internal control for normalizing transfection efficiencies and monitoring cell viability. This experiment evaluated effects of expression constructs (p179, p131 and p107) or an empty vector (mock construct), transiently transfected in HepG2 cells for 72 hours. All studies were performed using at least 8 biological replicates. Following the specified periods of time, the luciferase and renilla expression levels were measured and luciferase/renilla ratios were calculated in relation to mock-transfected samples. A HepG2 cell line stably expressing the same ISRE-Luc reporter construct was generated by transduction of cells with a Luciferase Cignal Lenti ISRE reporter construct (Qiagen) and selection of positive clones by growth in DMEM + 10% FBS with 1x Antibiotic-Antimycotic (Life Technologies) and 2 ug/mL puromycin. The best HepG2-ISRE-stable clones were identified by testing with purified recombinant IFN-α and IL28B.

Global analysis of transcriptome and pathway analysis

For global analysis of transcriptome, HepG2 cells were mock-transfected with empty Halo-tag vector or with IFNAN-Halo expression construct. Total RNA was prepared from transfected cells (48 hours). Both RNA samples were of high quality (RIN~10) and used for library preparation for sequencing with HiSeq 2000 Sequencing System (Illumina), using one sample on lane, generating 300M reads for IFNAN-transfected cells and 400M reads for mock-transfected cells. To use similar number of reads for both samples, 300 M reads were randomly selected from the mock-transfected cells to match the number of reads generated in IFNAN-transfected cells. Standard analysis identified 535 transcripts which showed change in expression of more than 2-fold and FDR<0.05. Ingenuity Pathway Analysis (IPA) performed on this set of 535 transcripts found to be differentially expressed in mock and IFNAN-transfected samples, nominated a list of pathways and transcripts involved in these pathways. mRNA expression of selected transcripts was evaluated in samples transfected with mock, IFNAN, p107, p131 or/and treated with 10 ng/ml of IFN-α or IL28B, in 4 biological replicates. mRNA expression in all samples was evaluated with pathway-based RT^2 Profiler PCR arrays, according to instructions (Qiagen).

Confocal Imaging in primary human hepatocytes (PHH) treated with PolyI:C or

transfected HepG2 cells

PHH, shipped in suspension, were centrifuged, resuspended in InVitroGRO CP medium (Celsis, Chicago, IL) and plated in collagen coated 6-well ($5x10^5$/well) chamber slides ($2x10^4$/well) (BD Biosciences) for 6 hrs. The media was replaced with InVitroGRO HI media (Celsis) and then cells were cultured overnight. All media were supplemented with antibiotics (Torpedo antibiotic mix; Celsis). Cells were treated with PolyI:C (Imgenex, San Diego, CA) at a concentration of 50μg/ml for different time points – 0 h (untreated), 2 hr, 4 hr, 8 hr and 24 hrs. For analysis of transient recombinant IFNAN expression, the cDNA fragment corresponding to a complete open reading frame of p179 was cloned into a pFC14A (Halo-tag) CMV Flexi vector (Promega) and transfected into HepG2 cells using Lipofectamine 2000 with LTX reagent (Life Technologies). Non-transfected cells (Lipofectamine with LTX only) were used negative control.

The PHH or HepG2 cells were fixed for 20 min with 4% formaldehyde (Sigma) in PBS, permeabilized with 0.5% TritonX 100 (Sigma) in PBS for 5 min, blocked with 4% BSA (Sigma) in TBS for 1 hour and then incubated with primary antibodies overnight at 4^oC, all at 1:1000 dilution: mouse monoclonal anti-IFNAN ab (custom), rabbit α-tubulin ab (ab-15246, Abcam, Cambridge, MA), rabbit anti-Halo-tag ab (Promega), mouse α-tubulin ab (ab-7291, Abcam). Secondary antibodies were donkey anti-rabbit or anti-mouse Alexa Fluor 594 and donkey anti-rabbit and anti-mouse Alexa Fluor 488, all at 1:1000 dilutions (Life Technologies). Slides were covered with mounting media (Prolong Gold Antifade Reagent with DAPI, Life Technologies). Immmunofluorescent images were obtained with a confocal laser-scanning microscope (LSM 510 META, Carl Zeiss Jena GmbH, Germany).

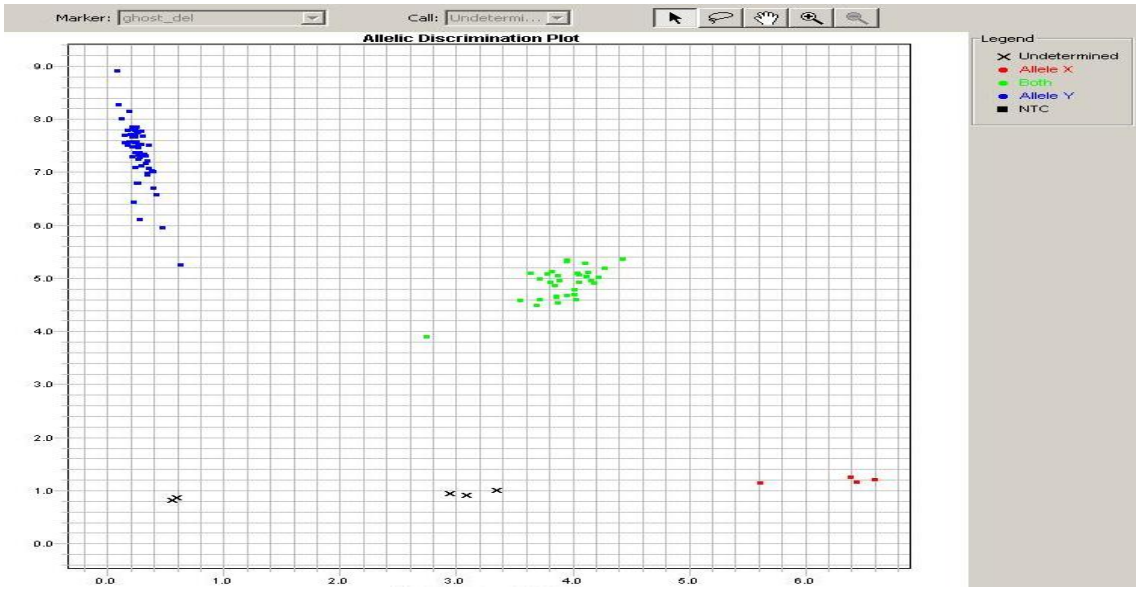
Analysis of *IFNAN* mRNA expression

Information on all expression and genotyping assays is presented in Supplementary Table 11. All custom assays were designed with the Primer Express Software and manufactured by Life Technologies, all pre-developed assays were purchased from the same company. Expression analysis was performed with gene expression master mix (Life Technologies) on DNAseI-treated RNA samples on ABI SDS 7700 instrument. Expression was measured in Ct values (PCR cycle at detection

threshold), which correspond to log 2 scale. Expression was normalized to PPIA (endogenous control) and analyzed according to relative quantification method, as $\Delta Ct = Ct_{PPIA} - Ct_{target}$. Fold difference between any two samples can be calculated as $fold = 2^{(\Delta Ct_1 - \Delta Ct_2)}$.

Genotyping

All genetic variants were initially genotyped by Sanger sequencing in HapMap samples and in subsets of clinical samples. All samples were additionally genotyped by custom-designed TaqMan assays, using Genotype Master Mix (Qiagen), on ABI SDS7700, with standard conditions. Information on all genotyping assays is presented in Supplementary Table 11. There was complete concordance between Sanger sequencing and Taqman genotyping of all markers. Information on ss469415590 amplicon is presented in Supplementary Fig. 13. The assay is designed to specifically target ss469415590 and avoid additional variants located within this amplicon.



Allelic discrimination plot for ss469415590.

Clinical samples

VirahepC

The Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C) was designed to compare response to treatment with pegylated IFN-α/ribavirin in African American patients with chronic hepatitis C to otherwise similar

patients of European ancestry[4]. In Virahep-C, patients with HCV genotype 1 infection who had not undergone previous treatment for chronic hepatitis C received treatment with a standard regimen of pegylated IFN-alfa-2a (180 μg/week) plus ribavirin (1000-1200 mg/day) for up to 48 weeks. Ancestral designation was self-reported. Study end points included: decrease in HCV RNA levels between baseline and various treatment time points; week 24 response (absence of detectable HCV RNA in serum after 24 weeks of therapy); end-of-treatment response (absence of HCV RNA after 48 weeks of therapy); and sustained virologic response (SVR; absence of HCV RNA 24 weeks after treatment was stopped). The protocol was approved by the institutional review boards of the participating institutions and all patients gave informed written consent. Reported results from Virahep-C showed that African-American patients had lower rates of virologic response than European-American patients and that those differences were not explained by differences in patient characteristics, baseline HCV RNA levels or the amount of medication taken during the study[4].

HALT-C

The Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial was a study of patients with advanced chronic hepatitis C who had failed previous interferon-based treatment[5,6]. At enrollment, HALT-C patients had an Ishak fibrosis score >3 by local assessment of liver biopsy, had a Child-Turcotte-Pugh score <7 and had no evidence of hepatocellular carcinoma. Final assessment of fibrosis stage was performed by a panel of hepatopathologists. Patients with other liver diseases, human immunodeficiency virus infection, active illicit drug use or current alcohol abuse were excluded. Ancestral designation was self-reported. During the lead-in phase of HALT-C, patients underwent retreatment with pegylated-interferon-alfa-2a (180 μg/week) plus ribavirin (1000-1200 mg/day). Subjects with undetectable HCV RNA at week 20 remained on combination treatment through week 48 and were followed until week 72. Subjects with undetectable HCV RNA at weeks 48 and 72 were considered to have an SVR. Investigations of human genetics in the HALT-C Trial were conducted in those participants who provided (written) consent for genetic testing. The HALT-C Trial was approved by institutional review boards of the participating institutions.

Urban Health Study (UHS)

As previously described[7], UHS recruited IDUs from street settings in six inner-city San Francisco Bay area neighborhoods from 1986 through 2002, drawing serial cross-sectional samples every six months[8]. Individuals 18 years of age or older were eligible for enrollment if they had injected drugs within the past 30 days or previously enrolled in the UHS study. New participants were screened for visible signs of recent or chronic injection (i.e., venipuncture sites or scars). Interviews were conducted using standardized questionnaires and blood samples were collected from participants who provided written informed consent. The present study included unduplicated IDUs recruited between 1998 and 2000[9]. Participants who were positive for HCV antibody were divided into two groups based on their HCV RNA result: 'chronic' (positive for HCV RNA) or 'cleared' (negative for HCV RNA and positive for antibody). All subjects with cleared infection were included in the study and frequency matched to those with chronic infection (maximum 4:1) on the basis of self-reported ethnicity and age. Information on demographic variables and other potential covariates were assessed through face-to-face personal interviews[9,10]. After being interviewed participants were counseled by trained staff on reducing infection risks and referred to appropriate medical and social services. Participants were not asked about treatment for HCV infection during 1998-2000, but when they were asked during 2001-2002, reports of antiviral treatment were rare[11]; thus it is very likely that the HCV seropositive, HCV RNA negative subjects in this study had recovered spontaneously. For the purpose of genetic investigations, ancestry was ascertained by self report; subjects who reported themselves to be White and not of Latino/Hispanic ethnicity are considered to be of 'European American' ancestry. Study procedures were approved by an Institutional Review Board of the National Cancer Institute and the Committee on Human Subjects Research at the University of California, San Francisco.

ALIVE

The AIDS Link to Intravenous experience (ALIVE) is an ongoing study of injection drug users enrolled in Baltimore, Maryland, from February 1988 through March 1989[12] .HCV infection was established by detection of HCV antibody (anti-HCV) by enzyme immunoassay (EIA) and recombinant immunoblot assay (RIBA [version 3.0]; Novartis). Individuals with cleared HCV infection had anti-HCV (as confirmed by RIBA)

and undetectable HCV RNA in serum or plasma without having received any HCV therapy. Individuals with persistent infection had anti-HCV and HCV RNA in serum or plasma before receiving any HCV therapy. Written informed consent for genetic testing was obtained from all participants. The study was approved by the institutional review board at Johns Hopkins University.

Statistical analysis of genetic association

All statistical comparisons of ss469415590 and rs12979860 were limited to subjects whose DNA specimen was successfully genotyped for both variants at the Laboratory of Translational Genomics, National Cancer Institute, blindly to clinical phenotypes.

We used the Kruskal-Wallis test to compare median HCV RNA levels between genotypes for each variant (e.g., ss469415590-TT/TT versus ss469415590-ΔG/ΔG). We compared mean HCV RNA levels in each of the three ss469415590 genotype groups (i.e., ΔG/ΔG, ΔG/TT, TT/TT) with the respective rs12979860 genotype groups (TT, CT, CC). To determine global statistical significance of these three mean differences, we computed the covariance matrix of the mean differences using a bootstrap procedure. We re-sampled individuals in the study with replacement, and then computed the three differences of the mean RNA levels in the three genotype groups in this bootstrap dataset. We repeated this calculation 1000 times, and used the bootstrap replicates to compute the covariance matrix of the mean differences of the original sample. This covariance matrix was used to compute a three degrees-of-freedom Wald statistic to test the null hypothesis that there was no difference in mean HCV RNA decreases for ss469415590 and rs12979860.

For dichotomous outcomes (e.g., sustained virological response, spontaneous clearance) we calculated the odds ratio and accompanying p-value were calculated using proc logistic in SAS 9.2 TS2M3; p-values are Wald chi-square estimates. We determined the area under the receiver operating characteristic curve (AUC)[13]. To test for differences in AUC values we computed a p-value based on a chi-square test (1 df) that used a bootstrap variance estimate computed by resampling subjects with SVR and non-responders with replacement and then repeating the AUC computations for each bootstrap sample.

References:

1. Jo, J. *et al.* Analysis of CD8+ T-cell-mediated inhibition of hepatitis C virus replication using a novel immunological model. *Gastroenterology* **136**, 1391-401 (2009).
2. Wakita, T. *et al.* Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* **11**, 791-6 (2005).
3. Dickensheets, H.L., Venkataraman, C., Schindler, U. & Donnelly, R.P. Interferons inhibit activation of STAT6 by interleukin 4 in human monocytes by inducing SOCS-1 gene expression. *Proc Natl Acad Sci U S A* **96**, 10800-5 (1999).
4. Conjeevaram, H.S. *et al.* Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. *Gastroenterology* **131**, 470-7 (2006).
5. Di Bisceglie, A.M. *et al.* Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* **359**, 2429-41 (2008).
6. Lee, W.M. *et al.* Evolution of the HALT-C Trial: pegylated interferon as maintenance therapy for chronic hepatitis C in previous interferon nonresponders. *Control Clin Trials* **25**, 472-92 (2004).
7. Shebl, F.M. *et al.* IL28B rs12979860 Genotype and Spontaneous Clearance of Hepatitis C Virus in a Multi-Ethnic Cohort of Injection Drug Users: Evidence for a Supra-Additive Association. *J Infect Dis* **204**, 1843-7.
8. Watters, J.K., Bluthenthal, R.N. & Kral, A.H. HIV seroprevalence in injection drug users. *JAMA* **273**, 1178 (1995).
9. Tseng, F.C. *et al.* Seroprevalence of hepatitis C virus and hepatitis B virus among San Francisco injection drug users, 1998 to 2000. *Hepatology* **46**, 666-71 (2007).
10. Kral, A.H. *et al.* Sexual transmission of HIV-1 among injection drug users in San Francisco, USA: risk-factor analysis. *Lancet* **357**, 1397-401 (2001).
11. Seal, K.H. *et al.* Among injection drug users, interest is high, but access low to HCV antiviral therapy. Society of General Internal Medicine, 28th annual meeting, New Orleans, Louisiana, USA, May 11-14, 2005. *J Gen Intern Med* **20 Suppl 1**, 171 (2005).
12. Vlahov, D. *et al.* The ALIVE study, a longitudinal study of HIV-1 infection in intravenous drug users: description of methods and characteristics of participants. *NIDA Res Monogr* **109**, 75-100 (1991).
13. Pepe, M.S. *The Statistical Evaluation of Medical Tests for Classification and Prediction*, (Oxford University Press, Oxford, 2003).