Published in final edited form as:

J Viral Hepat. 2015 September ; 22(9): 708–717. doi:10.1111/jvh.12384.

Factors associated with hepatitis C virus RNA levels in early chronic infection: The InC³ Study

Behzad Hajarizadeh¹, Bart Grady², Kimberly Page³, Arthur Y. Kim⁴, Barbara H. McGovern^{5,6}, Andrea L. Cox⁷, Thomas M. Rice³, Rachel Sacks-Davis^{8,9}, Julie Bruneau¹⁰, Meghan Morris³, Janaki Amin¹, Janke Schinkel¹¹, Tanya Applegate¹, Lisa Maher¹, Margaret Hellard^{8,9}, Andrew R. Lloyd¹², Maria Prins^{2,11}, Ronald B Geskus^{2,11}, Gregory J. Dore¹, and Jason Grebely¹ on behalf of the InC³ Study Group

¹The Kirby Institute, UNSW Australia, Sydney, NSW, Australia ²Cluster Infectious Diseases, GGD Public Health Service of Amsterdam, Amsterdam, The Netherlands ³Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA ⁴Harvard Medical School, Boston, MA, USA ⁵Tufts Medical School, Boston, MA, USA ⁶Abbvie, Chicago, IL, USA ⁷Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD, USA ⁸Burnet Institute, Melbourne, VIC, Australia ⁹Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia ¹⁰CRCHUM, Université de Montréal, Montreal, QC, Canada ¹¹Academic Medical Center, Amsterdam, The Netherlands ¹²Inflammation and Infection Research Centre, School of Medical Sciences, UNSW Australia, Sydney, NSW, Australia

Abstract

Improved understanding of natural history of hepatitis C virus (HCV) RNA levels in chronic infection provides enhanced insights into immunopathogenesis of HCV and has implications for the clinical management of chronic HCV infection. This study assessed factors associated with HCV RNA levels during early chronic infection in a population with well-defined early chronic HCV infection. Data were from an international collaboration of nine prospective cohorts studying acute HCV infection (InC³ Study). Individuals with persistent HCV and detectable HCV RNA during early chronic infection (one year [±4 months] post-infection) were included. Distribution of HCV RNA levels during early chronic infection were compared by selected host and virological factors. A total of 308 individuals were included. Median HCV RNA levels was significantly

Corresponding Author: Behzad Hajarizadeh, The Kirby Institute, UNSW Australia, Wallace Wurth Building, Sydney NSW 2052, Australia; Phone: +61-2-9385 9208; Fax: +61-2-9385 0876; bhajarizadeh@kirby.unsw.edu.au.

InC³ Study Group: Steering Committee: Kimberly Page (Chair, UFO STUDY), Julie Bruneau (HEPCO), Andrea L. Cox (BBAASH), Gregory J. Dore (ATAHC), Jason Grebely (ATAHC), Margaret Hellard (N2), Georg Lauer (BAHSTION), Arthur Y. Kim (BAHSTION), Andrew R. Lloyd (HITS-p), Lisa Maher (HITS-c), Barbara H. McGovern (BAHSTION), Maria Prins (ACS) and Naglaa H. Shoukry (HEPCO).

Coordinating Centre: Meghan Morris (Study Co-ordinator), Judy Hahn (Co-Investigator), Steve Shiboski (Co-Investigator) and Thomas M. Rice (Data Manager)

Site Data Managers: Maryam Alavi (ATAHC), Rachel Bouchard (HEPCO), Jennifer Evans (UFO Study), Bart Grady (ACS), Jasneet Aneja (BAHSTION), Rachel Sacks-Davis (Networks 2), Suzy Teutsch (HITS-p), Bethany White (HITS-c), Brittany Wells (BBAASH) and Geng Zang (HEPCO).

InC³ Researcher Acknowledgements: ATAHC – Gail Matthews and Barbara Yeung; BAHSTION – Jasneet Aneja and Leslie Erin Prince; HEPCO – Elise Roy and Geng Zang; HITS-c – Anna Bates, Jarliene Enriquez, Sammy Chow, Bethany White; HITS-p – Luke McCredie and Suzy Teutsch; N2 – Campbell Aitken, Joseph Doyle and Tim Spelman; UFO – Jennifer Evans.

Conflict of Interest: The authors do not have any commercial or other association that might pose a conflict of interest

higher among males (vs. females; 5.15 vs. 4.74 log IU/mL; $P<0.01$) and among individuals with HIV co-infection (vs. no HIV; 5.89 vs. 4.86; $P=0.02$). In adjusted logistic regression, male sex (vs. female, adjusted odds ratio [AOR]: 1.93; 95%CI: 1.01, 3.69), interferon lambda 4 (*IFNL4*) rs12979860 CC genotype (vs. TT/CT; AOR: 2.48; 95%CI: 1.42, 4.35), HIV co-infection (vs. no HIV; AOR: 3.27; 95%CI: 1.35, 7.93), and HCV genotype G2 (vs. G3; AOR: 5.40; 95%CI: 1.63, 17.84) were independently associated with high HCV RNA levels (>5.6 log IU/mL=400,000 IU/mL). In conclusion, this study demonstrated that *IFNL4* rs12979860 CC genotype, male sex, HIV co-infection, and HCV genotype G2 are associated with high HCV RNA levels in early chronic infection. These factors exert their role as early as one year following infection.

Keywords

Viral load; *IFNL4* genotype; *IL28B* genotype; sex, HCV genotype; HIV; Cohort study

Introduction

Hepatitis C virus (HCV) infection is an escalating global health problem with increasing morbidity (e.g. cirrhosis and hepatocellular carcinoma) and mortality worldwide (reviewed in (1, 2)). Among people with chronic HCV infection, a pre-treatment high HCV RNA level is predictive of lower sustained virological responses (SVR) following treatment with pegylated interferon and ribavirin, either alone (3-5), or in combination with boceprevir (6, 7), or telaprevir (8). While HCV RNA levels have limited impact on treatment responsiveness of new interferon-free direct-acting antiviral (DAA) therapies (9, 10) and potentially diminishing clinical relevance, they remain informative for HCV immunopathogenesis.

In the setting of chronic HCV infection, previous studies have demonstrated that age, gender, ethnicity, HIV co-infection, HCV genotype, and interferon lambda 4 (*IFNL4*) rs12979860 genotype (formerly known as interferon lambda 3 [*IFNL3*] and interleukin-28B [*IL28B*]) are associated with HCV RNA levels (11-22). However, the majority of studies assessing factors associated with HCV RNA during chronic infection have been cross-sectional. As such, the estimated duration of HCV infection is often unknown or imprecise, making it difficult to measure the longitudinal effect of various factors on HCV RNA levels in chronic infection.

The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts (InC³) Study, is a collaboration of pooled data from nine prospective international cohorts prominently comprised of people who inject drugs (PWID) (23), including a large number of well-defined participants with acute and early chronic HCV infection and longitudinal follow-up. This current study assessed factors associated with HCV RNA levels in early chronic infection (one year post-infection).

Materials & Methods

Study population and design

The InC³ Study, a collaboration of nine prospective cohorts evaluating HIV and HCV infection outcomes, has been previously described (23). All cohorts follow participants at regular intervals using standardized methods. Participants were recruited and followed between 1985 and 2010. The InC³ Study includes both: 1) participants without HCV infection; and 2) participants with documented acute or early chronic HCV infection. All participants provided written informed consent. The study was performed according to the World Medical Association Declaration of Helsinki and the cohort protocols were approved by local ethics committees (23).

For the current study, only individuals with documented acute or early chronic HCV were included. Documented acute or early chronic HCV is defined as either: 1) HCV seroconversion with an HCV antibody (anti-HCV) or HCV RNA positive test within two years of the anti-HCV negative test; or 2) evidence of symptomatic HCV infection. Symptomatic HCV infection was defined as a) a positive anti-HCV/HCV RNA test; b) jaundice or Alanine aminotransferase [ALT] elevation >400 U/L; and c) detection of HCV RNA or history of high-risk exposure within three months of clinical manifestation of acute HCV.

HCV RNA levels in early chronic infection were assessed among InC³ participants with persistent HCV infection and detectable HCV RNA levels at one year post-infection. A window of ± 4 months around the one year time-point was permitted for the inclusion of HCV RNA levels in this analysis (between 8 and 16 months post-infection). The time period of 12 ± 4 months post-infection was chosen for defining early chronic infection given our initial analysis showed that fluctuations in HCV RNA levels which are the characteristic of acute infection mainly observed during the first eight to ten months following infection and a stable pattern of HCV RNA levels with minor fluctuations was observed after that (24, 25).

From the total population with acute or early chronic HCV infection included in the InC³ study (n=812), individuals with spontaneous HCV clearance (n=173) or unavailable HCV RNA levels between months 8-16 post-infection (n=293) were excluded. Individuals with single undetectable HCV RNA between months 8-16 post-infection (n=28) were also excluded given that enough data was not available to exclude the possibility of spontaneous clearance followed by HCV re-infection. The inclusion of these individuals could have led to misclassification bias. Among individuals receiving HCV antiviral treatment, only HCV RNA data before treatment commencement were included in the analysis. Accordingly, individuals with unknown treatment status (n=5) or unknown treatment commencement date (n=5) were also excluded. As such, factors associated with HCV RNA levels were assessed among 308 people with detectable HCV RNA between months 8-16 post-infection (Figure 1).

The estimated date of HCV infection was calculated based on a hierarchy using all serological (anti-HCV), virological (HCV RNA) and clinical (symptoms and liver function tests) data to arrive at the most precise estimate of infection date:

- a. Among individuals with HCV RNA positive and anti-HCV negative at acute HCV detection, date of infection was four weeks prior to HCV RNA detection (26, 27).
- b. Among individuals with symptomatic acute HCV, date of infection was six weeks prior to its onset (jaundice or ALT >400 IU/L) (28).
- c. Among individuals with a negative anti-HCV test followed by either a positive anti-HCV or HCV RNA test, seroconversion was assumed to occur at the mid-point between the last negative and the first positive test. HCV seroconversion generally occurs about 30-60 days following infection (26, 27, 29). Date of infection in this group was six weeks prior to estimated seroconversion date if the first positive test was anti-HCV test and four weeks prior to estimated seroconversion date if the first positive test was only HCV RNA test.

Laboratory testing

Choice of qualitative and quantitative HCV RNA testing varied by cohort but was consistent at each site. Qualitative HCV RNA testing was performed using the following assays: Versant TMA [Bayer, Australia; <10 IU/ml], COBAS AmpliPrep/COBAS TaqMan (Roche, Branchburg, NJ, USA; <15 IU/ml), COBAS AMPLICOR HCV Test v2.0 (Roche Diagnostics, Mannheim, Germany; <50 IU/ml) or discriminatory HCV transcription-mediated amplification component of the Procleix HIV-1/HCV (Gen-Probe, San Diego, CA, USA; <12 copies/mL). Quantitative HCV RNA testing was performed using the Versant HCV RNA 3.0 (Bayer, Australia; <615 IU/ml), COBAS AMPLICOR HCV MONITOR 2.0 (Roche Diagnostics, Mannheim, Germany; <600 IU/ml), COBAS AmpliPrep/COBAS TaqMan (Roche, Branchburg, NJ, USA; <15 IU/ml) or an in-house PCR (<1000 IU/ml) (30, 31). HCV genotype was determined by line-probe assay (Versant LiPa1/LiPa2, Bayer, Australia) or HCV sequencing at acute HCV detection. Among those with undetectable HCV RNA (no genotype) and available samples, Murex HCV serotyping was performed to determine HCV genotype (Murex Biotech Limited, Dartford, UK). *IFNL4* genotyping was determined by sequencing of the rs12979860 single nucleotide polymorphism, as previously described in (32-35). While rs12979860 has also been referred to as *IFNL3* and *IL28B*, a recent study demonstrated that is located within intron 1 of the *IFNL4* gene (36).

Study outcomes and statistical analyses

The study outcome was HCV RNA levels at one year post-infection (early chronic infection, window 8-16 months). For participants with more than one HCV RNA level during this period (n=220), the median value was used. Nonparametric statistical tests were used for analyses, given that HCV RNA levels (IU/mL) and log₁₀ transformation of HCV RNA levels (log IU/mL) were not normally distributed. The top tertile HCV RNA levels (5.6 log IU/mL equals to 400,000 IU/mL) was defined as a high HCV RNA level. This threshold has also a clinical significance given HCV RNA levels 400,000 is associated with lower response to interferon-based treatment for chronic HCV infection (3).

Factors hypothesized to be associated with HCV RNA levels were determined *a priori* based on factors shown to be associated with HCV RNA levels during chronic HCV infection and included age (11, 17, 18), sex (11, 15, 16, 22), ethnicity (11), *IFNL4* rs12979860 genotype (CC vs. CT/TT) (11-13, 22), HIV co-infection (11, 17, 18), and HCV genotype (11, 14-17, 19, 21).

Median HCV RNA levels were compared between groups using the Wilcoxon-Mann-Whitney (or Kruskal Wallis) test. Logistic regression models were also used to assess factors associated with high HCV RNA levels (> 5.6 log IU/mL). The adjusted model included variables with $P < 0.20$ in unadjusted analyses. To account for potential unmeasured confounders introduced by cohort sites, multivariate regression analysis was performed using mixed modelling, with a random intercept for cohort site. Statistically significant differences were assessed at $P < 0.05$ (P -values are two-sided). All analyses were performed using Stata v12.0 (College Station, TX, United States).

Results

Participant characteristics

HCV RNA levels were assessed among 308 people with detectable HCV RNA in early chronic infection (Figure 1). Background characteristics of study population were summarized in Table 1. The median age was 26 years, 32% were female, 79% were Caucasian, 9% were HIV co-infected, and 95% had a history of injecting drug use. Among those with data on infecting HCV genotype ($n=294$; 96%), 54% had genotype 1. Among those with data on *IFNL4* rs12979860 genotype ($n=285$; 93%), 41% were CC genotype. Background characteristics of InC³ participants not included in the current analysis were summarized in Supplementary Table 1.

HCV RNA levels in early chronic infection

Median HCV RNA levels during early chronic infection was 4.90 log IU/mL (Inter-quartile range [IQR]: 3.99, 5.89) with the top tertile being from 5.59 to 7.35 log IU/mL. Significantly higher median HCV RNA levels were observed among males compared to females (5.15 log IU/mL vs. 4.74 log IU/mL, respectively; $P < 0.01$) and among individuals with HIV co-infection compared to those with no HIV (5.89 log IU/mL vs. 4.86 log IU/mL, respectively; $P = 0.02$). There was no significant difference in median HCV RNA levels by age, ethnicity, *IFNL4* rs12979860 genotype and HCV genotype (Table 2 and Figure 2).

In unadjusted logistic regression analysis (Table 3), factors associated with high HCV RNA levels (> 5.6 log IU/mL) in early chronic infection were sex, *IFNL4* rs12979860 genotype, HIV co-infection, and HCV genotype. In adjusted analysis, male sex (vs. female; AOR: 1.93; 95%CI: 1.01, 3.69; $P = 0.04$), *IFNL4* rs12979860 CC genotype (vs. TT/CT; AOR: 2.48; 95%CI: 1.42, 4.35; $P < 0.01$), HIV co-infection (vs. no HIV; AOR: 3.27; 95%CI: 1.35, 7.93; $P < 0.01$), and HCV genotype 2 (vs. genotype 3; AOR: 5.40; 95%CI: 1.63, 17.84; $P < 0.01$) were independently associated with high HCV RNA levels (> 5.6 log IU/mL). HCV genotype 1 (vs. genotype 3; AOR: 1.87; 95%CI: 0.99, 3.55; $P = 0.05$) and HCV genotype 2 (vs. genotype 1; AOR: 2.88; 95%CI: 0.92, 8.97; $P = 0.07$) trended towards also being

associated with high HCV RNA levels. Interactions between covariates were not statistically significant on the multiplicative scale.

Discussion

This study assessed factors associated with HCV RNA levels during early chronic infection in a large sample of individuals with well-defined early chronic HCV infection, the majority of whom were PWID. Among individuals with persistence and detectable HCV RNA at one year post-infection, male sex, *IFNL4* rs12979860 CC genotype, HIV co-infection, and HCV genotype 2 were independently associated with high HCV RNA levels (>5.6 log IU/mL).

The findings in the current study are consistent with previous studies demonstrating that *IFNL4* CC genotype (11-13, 22), male sex (11, 15, 16, 22), HIV co-infection (11, 17, 18), and HCV genotype 2 infection (11, 16, 19) are associated with higher HCV RNA levels during chronic infection. Although other studies have investigated factors associated with HCV RNA levels during chronic infection, there is considerable uncertainty and heterogeneity with the timing of HCV RNA levels post-infection, given the cross-sectional nature of studies and/or unavailable data on the estimation time of infection (11-21). However, the current study is unique given the well-defined nature of early chronic HCV infection in the InC³ study participants. Our findings suggest that during chronic infection, factors associated with HCV RNA levels exert their roles as early as one year following infection, providing a better understanding of the pathogenesis of HCV infection.

IFNL4 rs12979860 CC genotype was associated with high HCV RNA levels (>5.6 log IU/mL) during early chronic infection in this study. Previous data has demonstrated that *IFNL4* CC genotype is associated with both higher initial HCV RNA levels in acute infection (37, 38) and enhanced spontaneous clearance (32, 33, 35, 39). One hypothesis is that those with *IFNL4* CC genotype who fail to clear virus carry higher viraemia into chronic infection. The somewhat paradoxical association between *IFNL4* CC genotype and both higher HCV RNA levels and enhanced interferon-based treatment responsiveness in chronic infection may be driven by linkages with hepatic interferon-stimulated genes (ISG), which are predictive of treatment response (40, 41). Further, rs12979860 C allele has been shown to be in high linkage disequilibrium with the *IFNL4* rs368234815 TT allele, which results in a non-functional *IFNL4* gene (36). *IFNL4* gene encodes an active type III interferon, named *IFNλ4*, which has HCV antiviral activity mainly through inducing ISGs (42).

Male sex was associated with higher HCV RNA levels during early chronic infection in this study. This finding is consistent with previous data demonstrating higher HCV RNA levels among males compared to females (11, 15, 16, 22), although some studies did not find any association between sex and HCV RNA levels (17, 18). It is also well established that males are more likely to develop chronic HCV infection than females (34, 35, 43, 44). Mechanisms behind the association of sex and HCV RNA levels may be linked to sex-based differences in immunity. Compared to males, females have an increased number and magnitude of immune and inflammatory responses (45), and a higher prevalence of several autoimmune diseases (46). These differences are hypothesized to be a function of

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differential binding of sex steroids to specific receptors expressed in lymphoid tissue cells, macrophages, dendritic cells, and lymphocytes, thereby influencing the function of immune cells (45). However, there are little data on sex-based differences in immunological profiles in those with HCV infection although it has been shown in HIV (47). Another hypothesis to explain the difference in HCV viral control between males and females suggests a role for female hormones, such as estrogens and progesterone in viral control. This hypothesis is supported by the evidence of a lower rate of HCV clearance in postmenopausal women compared to premenopausal women (48, 49), and estrogen receptor alpha promoting HCV replication by interaction with the NS5B protein (50). Further studies should focus on mechanisms explaining the sex-based immune response to HCV infection.

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HIV infection was associated with higher HCV RNA levels during early chronic infection in this study. Higher HCV RNA levels in the setting of HIV co-infection has been described previously (11, 17, 18) and may reflect impaired control of HCV replication in the setting of HIV-related immunodeficiency. HIV has been shown to interact directly with hepatocytes and hepatic stellate cells leading to increased HCV replication via transforming growth factor β 1 (TGF- β 1) (51, 52).

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HCV genotype was associated with HCV RNA levels during early chronic infection in this study. Compared to HCV genotype 3, HCV genotype 2 was significantly associated with high levels of HCV RNA (> 5.6 IU/mL). The influence of HCV genotype on HCV RNA levels in chronic infection has been assessed by several studies, but results have been conflicting (11, 14-21). This is partly due to HCV genotype non-1 infections being grouped together, given limited genotypic diversity in some investigated populations (14, 17, 20, 21). These studies reported higher (20, 21) or lower (17) HCV RNA levels in genotype 1 compared to genotype non-1, while one study reported higher HCV RNA levels in genotype 1/4 compared to genotype 2/3 (14). Our data, consistent with most other studies assessing HCV RNA levels among HCV genotypes 1, 2 and 3 separately (11, 16, 19), revealed heterogeneous influence of genotypes non-1 on HCV RNA levels with higher HCV RNA levels being observed in genotype 2. Further research is needed to better understand the impact of viral factors, particularly HCV genotype on the pathogenesis of HCV infection.

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While the current study is unique given the large sample size and well-defined nature of early chronic HCV infection, there are some limitations. Nine cohorts of individuals with acute or early chronic HCV were combined. Participating cohorts bring a range of data types and structures presenting issues surrounding both inconsistent measurement and biological data testing protocols (e.g. HCV RNA assays differed across cohorts with different sensitivity, specificity and lower limit of detection). HCV genotypes 4, 6 and mixed genotype were also grouped together due to small numbers in each category. As such, the current analysis was not able to explore the potential effect of these individual genotypes on HCV RNA levels. The date of HCV infection was estimated based on the best available data, but broad HCV seroconversion intervals among some individuals would have had an impact on some HCV RNA levels included in the analyses.

In conclusion, the current study identified that *IFNL4* rs12979860 CC genotype, male sex, HIV co-infection, and HCV genotype G2 were independently associated with high HCV

RNA levels (5.6 IU/mL) at one year post-infection. These findings enhance our understandings of the immunopathogenesis of HCV during early chronic infection and have implications for the clinical management of chronic HCV infection with interferon-based regimens. Further research is needed to understand the mechanism of *IFNL4* genotype, sex and HCV genotype on HCV replication and whether higher HCV RNA levels are associated with increased HCV-related liver disease progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: The InC³ Study is supported by the National Institute on Drug Abuse Award Number R01DA031056. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Drug Abuse or the National Institutes of Health. The Kirby Institute is funded by the Australian Government Department of Health and Ageing. The views expressed in this publication do not necessarily represent the position of the Australian Government. BH is supported by an Australian Postgraduate PhD Award. JG is supported by a National Health and Medical Research Council (NHMRC) Career Development Fellowship. GD and AL are supported by NHMRC Practitioner Research Fellowships. MH and LM are supported by NHMRC Senior Research Fellowships and MH additionally by a VicHealth Senior Research Fellowship. RSD was supported by an NHMRC postgraduate scholarship and a Centre for Research Excellence into Injecting Drug Use postgraduate top-up scholarship. Other research support includes NIH U19 AI088791 (AC), NIH U19 AI066345 (AK, BM), R01 DA033541 (AK), MOP-103138, MOP-210232 (JB), the Netherlands National Institute for Public Health and the Environment (MP), and NHMRC Project Grant #630483 (LM).

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List of abbreviations (in alphabetical order)

ACS	Amsterdam Cohort Studies
ALT	Alanine aminotransferase
anti-HCV	HCV-specific antibodies
AOR	Adjusted odds ratio
ATAHC	Australian Trial in Acute Hepatitis C
BAHSTION	Boston Acute HCV Study, Transmission, Immunity and Outcomes Network
BBAASH	Baltimore Before and After Acute Study of Hepatitis
DAA	Direct-acting antiviral
HCV	Hepatitis C virus
HEPCO	St. Luc Cohort, HEPCO
HITS-c	Hepatitis C Incidence and Transmission Study-community
HITS-p	Hepatitis C Incidence and Transmission Study-prison
HIV	Human immunodeficiency virus
IFNL3	Interferon lambda 3
IFNL4	Interferon lambda 4
IL28B	Interleukin-28B
InC3	The International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts
IQR	Interquartile range
ISGs	Interferon stimulated genes
N2	Networks 2
OR	Odds ratio

PWID	People who inject drugs
SVR	Sustained virological response
UFO	UFO STUDY

Figure 1. Overview of InC³ study population and participants with persistent HCV infection and a detectable HCV RNA in early chronic infection (i.e. between months 8-16 post-infection)

Figure 2.

Distribution of HCV RNA levels during months 8-16 following infection, stratified by *IFNL4* rs12979860 genotype, sex, HCV genotype, and HIV infection status in participants with acute HCV infection in the InC³ study. (A) Stratified by *IFNL4* rs12979860 genotype; (B) Stratified by sex; (C) Stratified by HCV genotype; (D) Stratified by HIV infection status. Horizontal lines represent the medians HCV RNA levels in each subgroup.

Table 1
Background characteristics of participants with detectable HCV RNA between months 8-16 post-infection in the InC³ Study

	Number (%) <i>Total n=308</i>
Site	
ACS (the Netherlands)	23 (7)
ATAHC (Australia)	87 (28)
BAHSTION (United States)	20 (6)
BBAASH (United States)	54 (17)
HEPCO (Canada)	2 (1)
HITS-c (Australia)	6 (2)
HITS-p (Australia)	53 (17)
N2 (Australia)	0 (0)
UFO (United States)	63 (20)
Median age at the time of HCV infection, yrs (IQR)	26 (23, 33)
Sex	
Female	99 (32)
Male	208 (67)
Unknown	1 (<1)
Ethnicity	
Caucasian	243 (79)
Black	15 (5)
Indigenous	18 (6)
Other	23 (7)
Unknown	9 (3)
History of injecting drug use	292 (95)
Symptomatic HCV infection	
No	80 (26)
Yes	36 (12)
Unknown	192 (62)
<i>IFNL4</i> genotype (rs12979860)	
TT	42 (14)
CT	126 (41)
CC	117 (38)
Unknown	23 (7)
HIV infection at the time of HCV infection	
No	264 (86)
Yes	28 (9)

	Number (%) Total n=308
Unknown	16 (5)
HCV genotype	
Genotype 1	159 (52)
Genotype 2	19 (6)
Genotype 3	99 (32)
Genotype 4	6 (2)
Genotype 6	3 (1)
Mixed genotype	8 (3)
Unknown	14 (4)

Abbreviations: ACS: Amsterdam Cohort Studies; ATAC: Australian Trial in Acute Hepatitis C; BAHSTION: Boston Acute HCV Study: Transmission, Immunity and Outcomes Network; BBAASH: Baltimore Before and After Acute Study of Hepatitis; HEPCO: St. Luc Cohort, HEPCO; HITS-c: Hepatitis C Incidence and Transmission Study-Community; HITS-p: Hepatitis C Incidence and Transmission Study-Prison; N2: Networks 2; UFO: UFO STUDY; IQR: Inter-quartile range; *IFNL4*: interferon lambda 4

Table 2
Median HCV RNA levels in early chronic infection by selected demographic and virologic variables in participants with HCV infection in the InC³ study

	Number Total n=308	Median HCV RNA levels ^a (IQR)	P
Age			0.52
<30 years	193	4.92 (3.90, 5.90)	
30-39 years	54	4.74 (3.56, 5.70)	
40 years	31	5.19 (4.11, 6.09)	
Sex			<0.01
Female	99	4.74 (3.52, 5.46)	
Male	208	5.15 (4.15, 6.03)	
Ethnicity			0.74
Caucasian	243	4.92 (3.90, 5.91)	
Black	15	4.83 (4.26, 5.55)	
Indigenous	18	4.34 (3.14, 6.12)	
Other	23	4.77 (4.35, 5.91)	
<i>IFNL4</i> genotype (rs12979860)			0.17
TT/CT	168	4.88 (4.17, 5.67)	
CC	117	5.26 (3.43, 6.28)	
HIV status			0.02
Negative	264	4.86 (3.84, 5.82)	
Positive	28	5.89 (4.74, 6.24)	
HCV genotype			0.17
Genotype 1	159	5.17 (4.11, 5.91)	
Genotype 2	19	5.71 (3.52, 6.36)	
Genotype 3	99	4.81 (3.63, 5.59)	
Other ^b	17	4.85 (4.12, 6.03)	

Abbreviations: IQR: Inter-quartile range; *IFNL4*: interferon lambda 4

^a log IU/mL

^b Included genotype 4 (n=6), genotype 6 (n=3), and mixed genotype (n=8).

Table 3
Unadjusted and adjusted model assessing factors associated with high HCV RNA levels ($5.6 \log \text{ IU/mL}$) in early chronic infection in the InC³ study

	HCV RNA levels 5.6 log IU/mL n (%) ^a		HCV RNA levels <5.6 log IU/mL n (%) ^a	Unadjusted model		Adjusted model ^b	
	OR (95% CI)	P		OR (95% CI)	P		
Age					0.33		
<30 years	63 (33)		130 (67)	1.00			
30-39 years	16 (30)		38 (70)	0.87 (0.45, 1.68)	0.67		
40 years	14 (45)		17 (55)	1.70 (0.79, 3.66)	0.18		
Sex							
Female	20 (20)		79 (80)	1.00		1.00	
Male	80 (38)		128 (62)	2.47 (1.40, 4.34)	<0.01	1.93 (1.01, 3.69)	
Ethnicity						0.60	
Caucasian	81 (33)		162 (67)	1.00			
Black	3 (20)		12 (80)	0.50 (0.14, 1.82)	0.29		
Indigenous	5 (28)		13 (72)	0.77 (0.26, 2.23)	0.63		
Other	9 (39)		14 (61)	1.28 (0.53, 3.10)	0.57		
IFNL4 genotype (rs12979860)							
TT/CT	45 (27)		123 (73)	1.00		1.00	
CC	52 (44)		65 (56)	2.19 (1.33, 3.60)	<0.01	2.48 (1.42, 4.35)	
HIV status							
Negative	80 (30)		184 (70)	1.00		1.00	
Positive	17 (61)		11 (39)	3.55 (1.59, 7.93)	<0.01	3.27 (1.35, 7.93)	
HCV genotype ^c						0.07	
Genotype 3	24 (24)		75 (76)	1.00		1.00	
Genotype 1	56 (35)		103 (65)	1.70 (0.98, 2.98)	0.06	1.87 (0.99, 3.55)	
Genotype 2	10 (53)		9 (47)	3.47 (1.26, 9.54)	0.02	5.40 (1.63, 17.84)	
Other	5 (29)		12 (71)	1.30 (0.42, 4.07)	0.65	1.40 (0.42, 4.67)	

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Abbreviations: OR: Odds Ratio; CI: Confidence Interval; AOR: Adjusted Odds Ratio; *IFNL4*: interferon lambda 4

^aThe percentages represent row percentages.

^bIncludes 259 participants in the model. The model was adjusted for site using a random intercept model.

^cGenotype 2 vs. genotype 1: AOR: 2.88; 95%CI: 0.92, 8.97; *P*=0.07