Role of chronic hepatitis C virus (HCV) infection and host genetic variants in risk of myocardial infarction among people living with human immunodeficiency virus (HIV)

Jessica Williams-Nguyen

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Reading Committee

Joseph A Delaney, Chair

Sara Lindström

Stephen E Hawes

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Abstract

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Jessica Williams-Nguyen

Chair of the Supervisory Committee:
Joseph A Delaney
Research Associate Professor
Department of Epidemiology

With the advent of highly effective antiretroviral therapy in the last 20 years, survival of people living with human immunodeficiency virus (HIV) (PLWH) has improved dramatically. Over the same period, morbidity and mortality from non-AIDS-defining illnesses, including cardiovascular disease (CVD), have increased. PLWH are at higher risk of myocardial infarction (MI) compared to the general population, and more MI events in this group are Type 2 MI (T2MI) rather than Type 1 MI (T1MI). In contrast to classical atheroembolic T1MI, T2MI results from myocardial oxygen demand-supply mismatch, a scenario that occurs secondary to a variety of conditions, including sepsis and stimulant-induced vasospasm.

A large body of literature has linked CVD to chronic infection with hepatitis C virus (HCV), a common coinfection in PLWH. However, evidence is not conclusive regarding the association between HCV and MI in PLWH, and no research has addressed whether such an association may differ by MI type. We examined the association between HCV and MI in the Center for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS), a multi-center clinical cohort of PLWH, using centrally adjudicated MI ascertainment based on the Universal MI definition. We estimated the association between chronic HCV and time to MI accounting for demographic characteristics, cardiovascular risk factors, clinical characteristics and substance use. We found that, among 23,407 PLWH aged ≥18, HCV was associated with a 68% greater risk of T2MI (adjusted hazard ratio (aHR) 1.68, 95% CI: 1.22, 2.30) but not T1MI (aHR 0.96, 95% CI: 0.63, 1.45). In a cause-specific sensitivity analysis of T2MI, HCV was associated with a 2-fold greater risk of T2MI attributed to sepsis (aHR 2.26, 95% CI: 1.34, 3.81).

In a subsequent analysis, we assessed whether variants in the genomic region coding for immune regulators, interferon lambda (IFNL) 3 and IFNL4, were associated with MI events by type. We found that selected variants were not individually associated with T2MI or the subgroup of T2MI attributed to sepsis, but that 3 of 4 selected variants were associated with a 23 to 30% higher odds of atheroembolic T1MI.

We leveraged these four variants as instrumental variables, in a Mendelian randomization analysis, to test for evidence of causality in the observed association of HCV with T2MI. Based on these four variants, Mendelian randomization analyses did not

provide evidence that chronic HCV causally increases the risk of T2MI (Ratio of odds ratios (ORR) 0.78, 95% CI: 0.33, 1.83) or T2MI attributed to sepsis (ORR 1.13, 95% CI: 0.54, 2.36) in PLWH.

Taken together, our findings indicate that, while HCV and T2MI are observationally associated in this cohort of PLWH, this association is unlikely to be causal but instead may result from bias due to unmeasured or misclassified confounding factors. This finding highlights the importance of using the full set of epidemiological methods to explore large and unexpected observational associations to verify their robustness. However, we also found that 3 of 4 selected variants in the *IFNL3/4* genomic region were associated with both chronic HCV and T1MI in this cohort. The association of these variants with T1MI has not been previously reported. This raises the question of whether a common genetic background may play a role in observations from numerous sources that HCV is associated with subclinical and clinical CVD and opens a new line of inquiry that may ultimately inform research and clinical care priorities for PLWH who are coinfected with HCV.

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Abbreviations

aHR, adjusted hazard ratio

aOR, adjusted odds ratio

AIDS, acquired immunodeficiency syndrome

ART, antiretroviral therapy

CFAR, Centers for AIDS Research

CI, confidence interval

CNICS, CFAR Network of Integrated Clinical Systems

CVD, cardiovascular disease

FIB-4, Fibrosis-4 score

GWAS, genome wide association study

HCV, hepatitis C virus

HIV, human immunodeficiency virus

IFNL, interferon lambda

LDL, low density lipoprotein

MI, myocardial infarction

PLWH, people living with HIV

RNA, ribonucleic acid

SD, standard deviation

SNP, single nucleotide polymorphism

T1MI, Type 1 myocardial infarction

T2MI, Type 2 myocardial infarction

US, United States

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Chapter 1: Introduction

Introduction

With the advent of highly effective antiretroviral therapy (ART) for the treatment of human immune deficiency virus (HIV) and continuous improvement in clinical care, the survival of people living with HIV (PLWH) has improved dramatically in the last 20 years [1-5], however challenges remain. Cardiovascular disease (CVD) and chronic infection with hepatitis C virus (HCV) represent two important health risks faced by PLWH in the United States (US) [6, 7], and the extent to which these chronic conditions may intertwine to increase morbidity is an open scientific question [8]. The goal of this dissertation project is to improve understanding of the relation between chronic HCV and myocardial infarction (MI) in PLWH.

Cardiovascular disease in people living with HIV

Over half of those living with HIV in the US are now 50 years of age or older, and by 2030 this figure will reach 70% [9]. Diseases of aging, such as CVD, are therefore now an important source of morbidity in this population [10, 11]. In addition, PLWH are at higher risk of coronary artery disease (CAD) and MI than the general population after accounting for traditional CVD risk factors such as diabetes, hypertension, blood lipids and smoking [12, 13]. Evidence suggests that systemic immune activation in PLWH initiates a cascade of arterial inflammation and endothelial dysfunction (reviewed in [14]). Compared to the general population, a higher proportion of atherosclerotic plaques in PLWH are of the uncalcified, high-risk type that are prone to rupture [15-17].

The frequency of non-atherosclerotic cardiovascular disease may also differ in PLWH. The Third Universal Definition of Myocardial Infarction [18] classifies MI into types. Type 1 MI (T1MI) are classical, arethrothrombotic events wherein a plaque ruptures and creates an embolus that reduces blood flow to the myocardium leading to necrosis of myocardial tissue, while Type 2 MI is defined as myocardial necrosis resulting from a non-CAD-related mismatch in oxygen demand and supply, a scenario that may result from a variety of precipitating conditions, including sepsis, stimulant-induced vasospasm, decompensated heart failure, and hypotension (**Figure 1**) [18]. Recent work has shown that T2MI account for half of MI in PLWH [19], in contrast to the general population where atheroembolic T1MI predominates [20-28]. The reasons for this difference are not known, however Crane and colleagues [19] found large numbers of T2MI secondary to sepsis and stimulant-induced vasospasm. This suggests that populations of PLWH may have different profile of risk factors relevant to T2MI compared to the general population.

Given the higher risk of CAD and the increasing age in PLWH as well as differences in the frequency of CVD secondary to other medical conditions, improved understanding of MI in PLWH is important for clinical care of this vulnerable patient population into the future.

Hepatitis C virus in people living with HIV

HCV is a bloodborne viral pathogen that establishes chronic infection in most of those infected and is cleared spontaneously in the remaining one-quarter to one-third [29, 30]. If left untreated, chronic infection causes serious sequelae over the course of years, including cirrhosis, hepatocellular carcinoma and, ultimately, death [31]. Due to common routes of exposure, particularly injection drug use [32, 33], HIV and HCV constitute overlapping public health epidemics, in the US [34, 35] and throughout the world [36]. HCV chronically infects over 100 million people worldwide, representing approximately 2% of the world's population [37], with over 3 million of these people residing in the US [38]. More than 30 million people globally and over 1 million people in the US are living with HIV [39, 40]. Between 7 and 30% of PLWH in the US are coinfected with HCV [35, 36, 41-44]. HCV-associated liver disease causes an increasing proportion of mortality in PLWH [6, 45], and extrahepatic manifestations of HCV, such as lymphoproliferative disorders, renal disease and metabolic disorders, are also associated with considerable morbidity [46, 47].

The recent development of new direct-acting antiviral agents (DAAs) for HCV treatment is transforming HCV care. DAAs are safer and more effective with a shorter treatment duration that prior pharmaceutical therapies for HCV [48], however they are not yet uniformly available for all chronically infected patients [49]. It is, therefore, important to establish the health risks associated with chronic HCV infection in order for medical providers and payers to accurately weigh the cost and benefits of early treatment.

Relation between chronic hepatitis C virus and cardiovascular disease

Research over the last two decades has raised the question of whether HCV is an independent risk factor for atherosclerotic CVD. In 2002, Ishizaka and colleagues [50] found an association of HCV seropositivity with carotid artery plaque and carotid intima-media thickening, an indicator of subclinical atherosclerosis. Since that time, a large body of evidence has evaluated the role of HCV in atherosclerosis and coronary artery disease. In a recent review, Babiker and colleagues [8] concluded that the preponderance of evidence in the general population as well as PLWH supports an association between HCV and subclinical CVD as well as clinical CVD outcomes in composite (defined as two or more of CAD, MI, unstable angina, cerebrovascular accident, transient ischemic attack, peripheral artery disease and congestive heart failure). Restricting to studies that focus only on MI, these have generally not supported an association with HCV [51-54]. One recent study found a higher risk of MI in those with HCV but only among the subgroup with high levels of total and low density lipoprotein (LDL) cholesterol [55]. Among PLWH, only two studies have assessed the risk of MI associated with HCV. Bedimo and colleagues [56] reported a 25% higher risk of acute MI in US veterans infected with HCV and HIV compared to those infected with HIV alone, however this association did not reach the level of statistical significance, while the D:A:D study group found no association between HCV seropositivity and MI [57]. Thus, evidence is not conclusive as to the risk of MI associated with chronic HCV among PLWH.

Importance of host genetic polymorphisms for hepatitis C virus clearance

Germline genetic variation, and particularly polymorphisms affecting immune regulators such the interferon lambda (IFNL) family of proteins, has been shown to influence the risk of HCV spontaneous clearance, response to therapy, and progression to liver fibrosis [58, 59]. The first genome wide association study (GWAS) of response to HCV therapy, published in 2009, showed strong associations with single nucleotide polymorphisms (SNPs) in the *INFL3/4* genomic region in a multi-ethnic cohort composed of European, African and Hispanic Americans [60]. Within one month, two additional GWAS were published replicating these findings in individuals of European [61] and Asian ancestry [62]. The following year, a GWAS in individuals from Switzerland showed that the associations of *INFL3/4* variants with HCV treatment response were consistent between HCV mono-infected and HIV/HCV coinfected individuals [63]. Since that time, a large number of GWAS and candidate gene studies have confirmed these findings, with meta-analysis estimating that risk alleles of *INFL3/4* variants are associated an approximately 3 to 4-fold higher risk of failure to spontaneously clear HCV infection or respond to treatment across ethnicities [58]. While the exact mechanisms by which *IFNL3/4* variants influence HCV outcomes remain to be discovered, it is known that viral infection of the liver induces IFNL responses [64]. Interferon response, generally, is a component of innate immunity wherein inflammation is triggered to fight virus infection. Given the important role of inflammation in atherosclerotic CVD processes [65, 66], this raises the question of how immunogenetic variation may influence both HCV and MI in PLWH, a population with chronically elevated inflammation [67, 68].

Contribution of this project

The goal of this dissertation is to investigate the role of chronic infection with HCV and HCV-associated genetic variants associated with risk of MI (**Figure 2**) among PLWH enrolled in the Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS). In chapter 2, I present the estimated observational association of chronic HCV with T1MI and T2MI in a cohort of 23.407 PLWH in the US accounting for potential confounders and considering potential mediation by liver fibrosis. In chapter 3, I present the observed associations of four HCV-related genetic variants with T1MI and

T2MI in a subset of 6,116 PLWH. Also in chapter 3, I present a Mendelian randomization analysis that leverages genetic information to estimate the causal effect of HCV on MI in an effort to validate findings from Chapter 2.

The results of these analyses will provide insight into the relation between chronic HCV and MI in PLWH, while also making available novel information about the contribution of immunogenetic variation to MI risk in the context of coinfection with two common viral pathogens. Such knowledge may ultimately provide valuable information for clinical decision-making regarding care and treatment for HCV-coinfected PLWH. In the process of completing this dissertation, I also coordinated quality control activities between laboratory and clinical sites to create one of the largest datasets of genetic markers available in PLWH. In addition to the findings of this work, the genetic data produced will provide an enduring resource for future studies of the impact of genetic polymorphisms in PLWH.

Figures

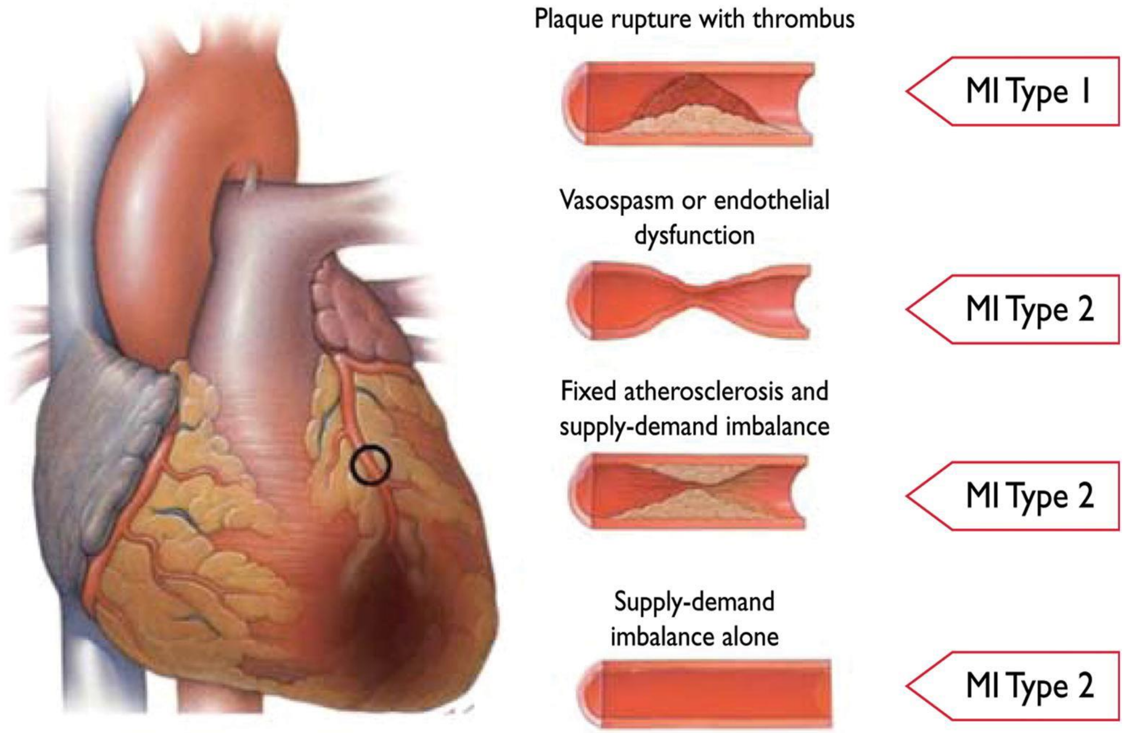

Figure 1. Differentiation between myocardial infarction (MI) types 1 and 2 according to the condition of the coronary arteries [18]. This graphic is licensed under Creative Commons BY-NC-ND 4.0.

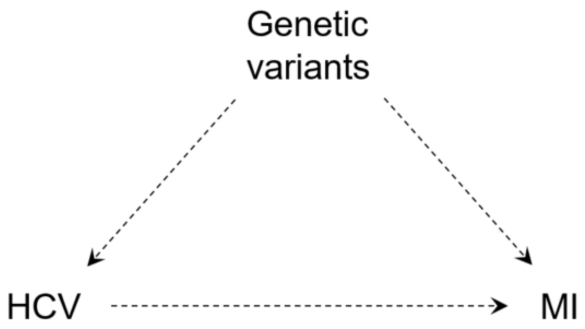

Figure 2. Conceptual model of research objectives

Chapter 2: Association between chronic hepatitis C virus infection and myocardial infarction in people living with HIV in the United States

Association between chronic hepatitis C virus infection and myocardial infarction in people living with HIV in the United States

Authors: Jessica Williams-Nguyen, Stephen E Hawes, Robin M Nance, Sara Lindström, Susan R Heckbert, H Nina Kim, W Chris Mathews, Edward R Cachay, Matt Budoff, Christopher B Hurt, Peter W Hunt, Elvin Geng, Richard D Moore, Michael J Mugavero, Inga Peter, Mari M Kitahata, Michael S Saag, Heidi M Crane, Joseph A Delaney

Author affiliations: Department of Epidemiology, University of Washington, Seattle, Washington (Jessica Williams-Nguyen, Stephen Hawes, Susan R Heckbert, Sara Lindström, and Joseph Delaney); Department of Medicine, University of Washington, Seattle, Washington (Robin M Nance, H Nina Kim, Mari M Kitahata, and Heidi M Crane); Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA (Sara Lindström); Cardiovascular Health Research Unit, University of Washington, Seattle, Washington (Susan R Heckbert and Joseph Delaney); Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York (Inga Peter); Department of Medicine, Los Angeles Biomedical Research Institute at Harbor – University of California at Los Angeles Medical Center, Torrance, California (Matt Budoff); Department of Medicine, University of California San Diego, San Diego, California (W Chris Matthews, Edward R Cachay); Institute for Global Health & Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina (Christopher B Hurt); Division of Experimental Medicine, University of California San Francisco, San Francisco, California (Peter W Hunt and Elvin Geng); Department of Medicine, Johns Hopkins University, Baltimore, Maryland (Richard D Moore); Department of Epidemiology, Johns Hopkins University, Baltimore, Maryland (Richard D Moore); Division of Infectious Disease, University of Alabama at Birmingham, Birmingham, Alabama (Michael J Mugavero and Michael Saag); Center for AIDS Research, University of Washington, Seattle, Washington (H Nina Kim, Mari K Kitahata and Heidi M Crane)

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Abstract

Hepatitis C virus (HCV) is common among people living with HIV (PLWH). The potential for extrahepatic manifestations of HCV, including myocardial infarction (MI), is a topic of active research. MI is classified into types, predominantly atheroembolic Type 1 MI (T1MI) and supply-demand mismatch Type 2 MI (T2MI). We examined the association between HCV and MI in the CFAR Network of Integrated Clinical Systems (CNICS), a multi-center clinical cohort of PLWH. MIs were centrally adjudicated and categorized by type using the Universal MI definition. We estimated the association between chronic HCV (RNA+) and time to MI adjusting for demographic characteristics, cardiovascular risk factors, clinical characteristics and substance use. Among 23,407 PLWH aged ≥18, there were 336 T1MI and 330 T2MI during a median of 4.2 years of follow-up. HCV was associated with a 68% greater risk of T2MI (adjusted hazard ratio (aHR) 1.68, 95% CI: 1.22, 2.30) but not T1MI (aHR 0.96, 95% CI: 0.63, 1.45). In a cause-specific analysis of T2MI, HCV was associated with a 2-fold greater risk of T2MI attributed to sepsis (aHR 2.26, 95% CI: 1.34, 3.81). Extrahepatic manifestations of HCV in this high-risk population are an important area for continued research.

Introduction

Survival of people living with human immunodeficiency virus (HIV) (PLWH) has improved dramatically in the last 20 years, owing to a decline in mortality associated with antiretroviral therapy [1-5]. During the same period, morbidity and mortality from non-AIDS-defining illnesses, including cardiovascular disease (CVD), have increased in this group [7, 45, 69, 70]. PLWH are at higher risk of myocardial infarction (MI) compared to the general population [13]. Recent work has shown that among PLWH approximately half of incident MIs are Type 2 MI (T2MI) [19], whereas in the general population T2MI accounts for a much smaller proportion of MIs [20-28]. In contrast to classical atheroembolic Type 1 MI (T1MI), T2MI results from myocardial oxygen demand-supply mismatch, a scenario that may result from a variety of conditions, including sepsis, illegal stimulant or drug-induced vasospasm, decompensated heart failure, and hypotension [18].

A large body of literature has linked CVD to chronic infection with hepatitis C virus (HCV), a common viral infection impacting an estimated 10 to 30% [36, 41-44] of PLWH in the United States (US). Observational evidence supports an association between HCV and clinically evident CVD, in both the general population [8, 71] and in PLWH [68]. Yet studies in the general population focusing on the role of HCV in coronary artery disease risk are inconclusive [8] and, when restricting to MI only, have generally not supported an association [51-54]. One recent study found a higher risk of MI in those with HCV but only among the subgroup with high levels of total and low density lipoprotein (LDL) cholesterol [55]. Among PLWH, very little research has assessed the association of HCV-coinfection with MI and, to our knowledge, none has examined whether such an association may differ by MI type.

Due to the higher prevalence of MI and HCV infection among PLWH, unclear associations between HCV and MI in the general population, different patterns of MI types among PLWH, and an increasing availability of highly effective treatments for HCV, it is important to establish whether HCV increases the risk of MI for PLWH. In this study, we sought to estimate the relative risk of incident T1MI and T2MI among PLWH who are coinfected with HCV compared to those who are not.

Methods

Study population. The Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort is a population of >32,000 PLWH who have received HIV care at one of 8 sites in the US from 1995 to the present and have consented to participation in CNICS research activities [72]. Included in this study were all participants ≥18 years of age from six CNICS sites with comprehensive access to inpatient and outpatient electronic medical records (Johns Hopkins University; University of Alabama at Birmingham; University of California, San Diego; University of California, San Francisco; University of North Carolina at Chapel Hill; and University of Washington, Seattle). This analysis includes participants who contributed person-time during the years 1998 through 2016; the majority of follow-up occurred from 2010 to 2016. Entry into the study cohort was defined as six months after CNICS cohort entry or the first date of surveillance for MI events at the study site – whichever was later. Participants were followed until nine months after the date of last clinic visit or laboratory result [73], death, or the administrative censoring date for the site. Ultimately, 23,407 participants contributed person-time to this analysis. Institutional review boards at each institution have approved CNICS research activities.

CNICS data repository. Each CNICS site captures demographic, clinical, medication and laboratory data from all outpatient and inpatient encounters, including cardiac biomarkers, medications, diagnoses, and historical clinical information. In addition, participants were invited to complete an assessment of patient reported outcomes (PRO) and measures at 4-6 month intervals, capturing use of tobacco, alcohol and illicit substances as well as other health behaviors [74].

Variables of interest. Ascertainment and adjudication of MI events in this cohort has been described in detail elsewhere [75]. Briefly, identification of potential events was done centrally using multiple criteria, including MI-associated diagnostic codes (*International Classification of Diseases, Ninth Revision*, 410.00, 410.01, and 410.10), having an invasive cardiac procedure (percutaneous coronary intervention or bypass grafting), and elevated cardiac biomarkers. Sites then generated clinical data packets including primary data for all potential MI events. Packets contained chart notes, electrocardiograms, imaging and procedure reports, and laboratory values, but antiretroviral medication data were redacted. Packets were reviewed centrally by two physician experts to adjudicate MIs as definite or probable and to classify them as T1MI or T2MI, based on the Universal MI definition [18, 19, 75]. While the Universal MI definition includes other types (e.g., cardiac procedure-related type 4 MI), these were uncommon (<10 cases) and so are not further discussed. In cases of discrepant findings, the assessment of a third reviewer was used to break ties [75].

Laboratory test results obtained in the course of routine care were used to determine HCV status. Chronic HCV infection was defined as a detectable result (i.e., above the lower limit of detection) for the most recent HCV ribonucleic acid (RNA) test before entry into the study cohort, and all remaining participants in the study cohort were classified as not chronically infected with HCV.

Covariates. Covariates were taken from measurements available at study entry, except for height used to calculate body mass index which could be measured at any time. Diabetes was defined as any one of the following: hemoglobin A1c ≥ 6.5%, a clinical diagnosis of diabetes mellitus type 1 (T1DM) or type 2 (T2DM) and prescription of diabetes-related medication, or prescriptions of a diabetes-specific medication [76]. Treated hypertension was defined as a clinical diagnosis of hypertension and documentation of an antihypertensive medication. Hepatitis B virus infection was defined on the basis of a positive result for hepatitis B e-antigen or surface antigen or deoxyribonucleic acid (DNA). Fibrosis-4 (FIB-4) index, a noninvasive measure of predicted liver fibrosis, was calculated as: [age (years) × aspartate aminotransferase (AST)]/[platelet count × alanine aminotransferase (ALT)$^{1/2}$] [77] and categorized as >3.25 (severe fibrosis), 1.45-3.25 (indeterminate), <1.45 (no severe fibrosis).

History of injection drug use was derived from HIV transmission risk factor reported at CNICS cohort entry and was coded as 1 if the participant reported membership in a transmission risk group that included injection drug use. Ever-smoker was the presence of a tobacco-use diagnosis or self-report of tobacco use. Use of methamphetamine, cocaine/crack, marijuana, opiates (illicit use), cigarettes and alcohol at study cohort entry were available for a subset of participants (approximately 13%) as assessed by the ASSIST questionnaire [78]. Drug and cigarette use categories were current, former or never use. Alcohol use was the AUDIT-C score derived from a short screening questionnaire about the frequency and quantity of alcohol consumed [79]. Values range from 0 (least alcohol use) to 12 (most alcohol use).

Statistical analysis. Approximately 87% of participants were missing information about methamphetamine, cocaine, opiate, marijuana, cigarette and alcohol use (from PROs) at the start of follow-up, as follow-up often began before PRO administration was conducted for these participants. Missing values in all analytic variables were multiply imputed using chained equations with fully conditional specification in the R package mice [80] based on all other covariates yielding 100 complete datasets. For each complete dataset, the association between chronic HCV infection and time to the patient's first MI event was estimated using Cox proportional hazard regression models. Resulting inferences were pooled using Rubin's Rules [81]. Separate models examined T1MI, T2MI, cause-specific T2MI outcomes, and a composite outcome of all MIs. Models were adjusted for potential confounding factors. Minimally adjusted models considered only age and sex, while fully adjusted models included study site, demographic characteristics (age, sex, race/ethnicity, men who have sex with men), clinical characteristics (diabetes, treated hypertension, statin use, body mass index, lipid profile, lowest CD4+ cell count, hepatitis B virus infection, HIV viral load, antiretroviral therapy), history of injection drug use, ever smoker and self-reported substance use (smoking, alcohol use, illicit substance use). Proportionality of hazards was assessed using Schoenfeld residuals for a random subset of imputations, and no consistent deviations were discovered.

The liver is a major source of serum proteins, including inflammatory mediators, and is involved in the maintenance of systemic inflammatory homeostasis [82]. For this reason, liver fibrosis and associated dysfunction were considered to be potential mediators of the relation between HCV and MI, and thus markers of liver fibrosis were not included in the regression models for the main analyses. *Post hoc* assessment of potential mediation by liver fibrosis was conducted using the potential outcomes approach implemented with natural effect models [83] and adapted to the Cox proportional hazards regression setting as described by Lange *et al.* [84]. Analysis was conducted on the multiply imputed datasets described above. Standard errors for model parameters were computed using 1000 bootstrapped samples of each imputed dataset [85]. Inference across imputations was then pooled as described above. The mediator variable was FIB-4, handled as a continuous variable.

Statistical analyses were conducted in R version 3.4.3 (2017-11-30).

Sensitivity analysis. Analyses were repeated to estimate the risk of MI outcomes associated with prior HCV infection in the absence of chronic infection at the time follow-up began. The exposed group for that analysis was those with a positive finding on the most recent HCV antibody test coupled with a negative finding for the most recent RNA test prior to baseline.

Results

Among 23,407 PLWH, we observed 336 T1MI and 330 T2MI (666 MI total) during a median of 4.7 years of follow-up. In all, 2,280 (9.7%) had evidence of chronic HCV infection at the beginning of MI follow-up. The median age of participants was 40, 81% were male, and approximately half were non-white (**Table 1**). Compared with participants without HCV infection, those with HCV tended to be older and were less likely to report being men who have sex with men. Unsurprisingly, a much larger proportion (61.5% vs. 15.0%) had a history of injection drug use (**Table 1**). The crude incidence rate for all MI events was 5.0 (95% CI: 4.7, 5.2) per 1000 person-years, and the rate was 4.6 (95% CI: 4.4, 4.9) among participants without chronic HCV and 8.2 (95% CI: 7.1, 9.4) among those with chronic HCV per 1000 person-years. T2MI events were attributed to a variety of causes (**Table 2**), with sepsis/bacteremia and stimulant-induced vasospasm being the most common.

Using a composite MI outcome, chronic HCV infection was associated with a 36% greater risk of MI after adjusting for demographic characteristics, traditional cardiovascular disease risk factors, clinical characteristics and substance use (adjusted hazard ratio (aHR) 1.36, 95% confidence interval (CI):1.06, 1.73) (**Figure 3**). Considering each MI type, chronic HCV infection was associated with a 68% higher risk of T2MI (aHR 1.68, 95% CI: 1.22, 2.30) after adjusting for potential confounders, but HCV was not associated with T1MI (aHR 0.96, 95% CI: 0.63, 1.45). In further analyses examining adjudicated causes of T2MI, HCV was found to be associated with an approximately 2-fold greater risk of T2MI attributed to sepsis (aHR 2.26, 95% CI: 1.34, 3.81) but was not associated with T2MI attributed to use of a stimulant (aHR 0.96, 95% CI: 0.15, 6.14) or other causes (aHR 1.47, 95% CI: 0.92, 2.34) after covariate adjustment (**Figure 3**). Because there were a small number of T2MI events attributed to use of a stimulant, we assessed the stability of the model for this outcome by estimating a model adjusted for age, sex, and history of injection drug use – which gave relatively consistent results (**Supplementary Table 1**).

The percentage of the HCV-T2MI association mediated by liver fibrosis (FIB-4) was estimated to be 10.6% (95% CI: -16.8, 38.0%) (**Table 3**), meaning that variation in FIB-4 caused by HCV infection could explain approximately 11% of the risk of T2MI. However, this percentage did not differ significantly from zero.

In a sensitivity analysis, we found that there was no association between prior HCV infection without chronic HCV infection (antibody-positive, RNA-negative) and any MI outcome (**Supplementary Figure 1**).

Discussion

In this cohort of PLWH across the US, we found that HCV/HIV-coinfected persons experienced a greater risk of T2MI compared to individuals infected with HIV only, and this was particularly driven by the relation between chronic HCV infection and T2MI attributed to sepsis. In contrast, there was no significant increase in the risk of having T1MI among coinfected persons. To our knowledge, this is the first study to assess the association between HCV infection and MI types, particularly T2MI, among PLWH.

Previous studies in the general population have not found an association between chronic HCV infection and MI [51-54]. Two prior studies in PLWH were inconclusive. R Bedimo and colleagues [56] reported a 25% higher risk of acute MI in U.S. veterans infected with HCV and HIV compared to those infected with HIV alone, however this association did not reach the level of statistical significance, while the D:A:D study group found no association between HCV seropositivity and MI [57]. Although both of these studies ascertained composite MIs without reference to type, both defined MIs using administrative diagnosis codes. The proportion of MIs detected in prior studies that were T1MI versus T2MI is not known, however the ability of administrative codes to detect T2MI seems generally poor [86]. Therefore, prior evidence appears to concur with our finding that chronic HCV is not associated with classical, atheroembolic T1MI.

We observed a greater risk of T2MI associated with HCV and, more specifically, an approximately 2-fold greater risk of T2MI due to sepsis in those coinfected with HCV and HIV compared to those infected with HIV alone. This may be related to consequences of HCV infection, such as chronic inflammation, or differences in sepsis risk factors between HCV-infected and uninfected participants. Risk factors for sepsis include demographic characteristics (being male, older age, non-white race), immunosuppression, genetics, and modifiable risk factors such as cigarette smoking and use of injection drugs [87, 88]. While our results suggest a higher risk of T2MI attributed to sepsis in those with evidence of chronic HCV infection, we did not detect a similar relation when considering prior HCV infection without evidence of viremia. This lends credence to the hypothesis that ongoing chronic infection or associated biological factors (in addition to risk behaviors associated with HCV exposure) may play a role in the risk of T2MI resulting from sepsis or of sepsis, itself. A large study of hemodialysis patients in the US, which found that the risk of bacteremia was greater in those with HCV infection, provides support for HCV as a risk factor for sepsis [89], and the landmark REVEAL study found that HCV seropositivity is associated with an 50% higher risk of mortality due to circulatory diseases [90].

We assessed the extent to which the observed relation between chronic HCV and T2MI was mediated by liver fibrosis and found that in this study approximately 11% of the association could be explained by covariation of HCV with predicted liver fibrosis score (FIB-4), however this percentage could not be statistically differentiated from 0 at 95% confidence. This indicates that the impact of HCV on liver fibrosis plays at most a small role in our observed relation between HCV and T2MI. We note that those with predicted severe fibrosis were uncommon in this cohort, and there were few MI events in that subgroup. Thus, this evidence does not preclude the possibility that liver dysfunction may be an important factor in the relation of HCV and T2MI in those with severe liver disease. In addition to overall replication of our findings, an ideal future study in this area would more comprehensively investigate the role of liver fibrosis in the relation of HCV with cardiovascular disease outcomes, particularly in those with advanced fibrosis. Bacterial infections and sepsis are known complications and major sources of morbidity for patients with cirrhosis [91].

Strengths of this study include adjudicated ascertainment of MI events including typing, extensive follow-up of participants providing a wide range of information about potential confounders, and the CNICS PRO assessment with the use of multiple imputation methodology that permitted inclusion of self-reported substance use in our analytic models. HCV infection status in most studies of HCV and MI was defined on the basis of either seropositivity for anti-HCV antibodies or administrative diagnosis codes for HCV [51-54, 56, 57]. These measures are likely to be less specific for chronic HCV infection compared to ascertainment on the basis of an RNA test as we did here. One study of the general population in an Arkansas medical system found that participants with detectable HCV RNA had a greater incidence of coronary heart disease than those who tested positive for antibodies against HCV but without detectable HCV RNA. In combination with the findings presented here, this supports the use of HCV RNA testing to identify chronic HCV, something that should be highly feasible with the increasing uptake of nucleic acid based tests for HCV in clinical practice [92].

We were unable to differentiate whether HCV is associated with the incidence of sepsis itself, or T2MI as a complication of sepsis. This is an important area for future work. Because adjudication of causes of T2MI events must necessarily rely on expert judgement, some measurement error may have occurred when categorizing these complex medical events. Our study was also not sufficiently powered to assess causes of T2MI other than sepsis and stimulant-induced vasospasm. We also used HCV RNA testing performed in the course of routine clinical care, and therefore potentially missed some cases of chronic HCV in those who did not undergo RNA testing in that setting. Finally, because any study seeking to characterize the effects of HCV infection must be observational, the possibility of unmeasured and residual confounding cannot be completely excluded. In particular, we note that 87% of participants were lacking baseline information for self-reported substance use and time-varying substance use was not included in this analysis, leaving open the possibility of confounding by differences in substance use in those with and without chronic HCV. However, this rich database of clinical and patient-reported information, combined with analytic techniques accommodating missingness in covariates, enabled us to adjust for many important potential confounders.

In this large and diverse multicenter cohort of PLWH in the US, we found that chronic HCV infection at the beginning of follow-up was not associated with incident classical atheroembolic T1MI but was associated with T2MI attributed to sepsis. These findings demonstrate the importance of examining MIs by type among PLWH and support increasing calls for broad-scale efforts to better understand the biological underpinnings of T2MI, and ultimately identify effective management approaches [93]. Further research is needed to replicate these findings, elucidate biological mechanisms and determine whether risk of sepsis and/or T2MI might be an important consideration for HCV treatment decisions in this high-burden population.

Tables and Figures

Table 1. Baseline clinical and demographic characteristics of adults living with HIV in clinical care at 6 CNICS sites across the US in 1998-2016 by chronic hepatitis C virus infection status

Characteristic	**Chronic Hepatitis C**	**No chronic Hepatitis C**	**Everyone**
n	2280	21127	23407
Demographic factors			
Male (%)	1779 (78.0)	17117 (81.0)	18896 (80.7)
Age, years (%)			
<30	108 (4.7)	3666 (17.4)	3774 (16.1)
30-39	511 (22.4)	6626 (31.4)	7137 (30.5)
40-49	931 (40.8)	7072 (33.5)	8003 (34.2)
50-59	619 (27.1)	3062 (14.5)	3681 (15.7)
≥60	111 (4.9)	701 (3.3)	812 (3.5)
Race & ethnicity (%)			
Non-Hispanic, white	981 (43.0)	9096 (43.1)	10077 (43.1)
Non-Hispanic, black	984 (43.2)	8390 (39.7)	9374 (40.0)
Non-Hispanic, all other races	119 (5.2)	1036 (4.9)	1155 (4.9)
Hispanic, all races	196 (8.6)	2605 (12.3)	2801 (12.0)
Men who have sex with men (%)	877 (39.0)	12897 (62.2)	13774 (59.9)
Clinical Characteristics			
Hepatitis B virus (%)	101 (4.4)	1030 (4.9)	1131 (4.8)
Diabetes (%)	163 (7.1)	1030 (4.9)	1193 (5.1)
Hypertension (%)	350 (15.4)	2394 (11.3)	2744 (11.7)
Statin use (%)	97 (4.3)	1422 (6.7)	1519 (6.5)
Body mass index[a] (mean (SD))	25.6 (4.8)	25.9 (5.5)	25.9 (5.4)
Total cholesterol[a] (mean (SD))	159.5 (41.0)	174.9 (45.5)	173.4 (45.3)
HDL cholesterol (mean (SD))	43.5 (17.2)	42.1 (15.2)	42.2 (15.4)
LDL cholesterol (mean (SD))	85.3 (36.2)	99.9 (37.3)	98.4 (37.5)
Triglycerides[a] (mean (SD))	157.2 (124.1)	170.2 (154.0)	168.9 (151.4)
FIB-4 score (%)			
<1.45	1113 (49.5)	16420 (80.5)	17533 (77.5)
1.45-3.25	765 (34.0)	3314 (16.3)	4079 (18.0)
>3.25	370 (16.5)	654 (3.2)	1024 (4.5)
HIV-related factors			
Nadir CD4+ cell count (%)			
<100	556 (24.4)	5330 (25.2)	5886 (25.1)
100-199	421 (18.5)	2972 (14.1)	3393 (14.5)
200-349	559 (24.5)	4956 (23.5)	5515 (23.6)
350-499	389 (17.1)	3732 (17.7)	4121 (17.6)
≥500	355 (15.6)	4137 (19.6)	4492 (19.2)
CD4+ cell count (mean (SD))	416.2 (273.9)	439.1 (285.1)	436.8 (284.1)
CD8+ cell count[a] (mean (SD))	907.8 (503.9)	925.5 (508.8)	923.8 (508.3)
Ratio of CD4+/CD8+ cells[a] (%)			
<0.4	716 (44.5)	6396 (41.7)	7112 (42.0)
0.4–1.0	699 (43.4)	6914 (45.1)	7613 (44.9)
>1.0	194 (12.1)	2031 (13.2)	2225 (13.1)
HIV viral load ≤ 400 copies/mL (%)	1321 (58.1)	12607 (60.4)	13928 (60.2)
Receiving ART at start of follow-up (%)	1911 (83.8)	17954 (85.0)	19865 (84.9)

ART, antiretroviral therapy; FIB-4, Fibrosis-4 score; HDL, high density lipoprotein; HIV, human immunodeficiency virus; LDL, low density lipoprotein; SD, standard deviation

[a] Values of this variable were unknown for greater than 5% of participants using information from the 6 months before initiation of myocardial infarction (MI) study follow-up. Percentages are of non-missing values.

Table 1 (continued). Baseline clinical and demographic characteristics of adults living with HIV in clinical care at 6 CNICS sites across the US in 1998-2016 by chronic hepatitis C virus infection status

Characteristic	**Chronic Hepatitis C**	**No chronic Hepatitis C**	**Everyone**
Substance use			
Ever smoker (%)	873 (38.3)	5160 (24.4)	6033 (25.8)
History of injection drug use (%)	1384 (61.5)	3103 (15.0)	4487 (19.5)
Methamphetamine use[b] (%)			
Current	34 (15.9)	262 (9.0)	296 (9.5)
Former	77 (36.0)	704 (24.2)	781 (25.0)
Never	103 (48.1)	1944 (66.8)	2047 (65.5)
Cocaine/crack use[b] (%)			
Current	31 (14.2)	181 (6.2)	212 (6.8)
Former	126 (57.8)	1040 (35.7)	1166 (37.2)
Never	61 (28.0)	1695 (58.1)	1756 (56.0)
Illicit opiate use[b] (%)			
Current	18 (9.0)	67 (2.4)	85 (2.8)
Former	61 (30.5)	288 (10.2)	349 (11.5)
Never	121 (60.5)	2479 (87.5)	2600 (85.7)
Marijuana use[b] (%)			
Current	64 (30.9)	887 (30.9)	951 (30.9)
Former	78 (37.7)	1014 (35.3)	1092 (35.5)
Never	65 (31.4)	969 (33.8)	1034 (33.6)
Cigarette use[b] (%)			
Current	132 (57.4)	1100 (37.3)	1232 (38.7)
Former	52 (22.6)	642 (21.7)	694 (21.8)
Never	46 (20.0)	1210 (41.0)	1256 (39.5)
AUDIT-C score[bc] (mean (SD))	1.84 (2.64)	2.36 (2.54)	2.33 (2.55)

ART, antiretroviral therapy; FIB-4, Fibrosis-4 score; HDL, high density lipoprotein; HIV, human immunodeficiency virus; LDL, low density lipoprotein; SD, standard deviation

[b] Self-reported substance use was collected in a subset of participants because sites added the CNICS clinical assessment starting in 2006 with an additional site starting it every year or two until all CNICS sites included it. Percentages are of the subset of participants from all sites responding to the instrument in the CNICS clinical assessment addressing that domain prior to or at the start of follow-up. Approximately 13% of participants provided information about methamphetamine, cocaine, opiate, marijuana, cigarette and alcohol use.

[c] Measure of alcohol use based on the AUDIT-C questionnaire [79]. Values range from 0 (least use) to 12 (most use).

Table 2. Number of Type 2 myocardial infarctions (T2MI) attributed to causes among adults living with HIV in clinical care at 6 CNICS sites, 1998-2016

Attributed cause	**Number of events (%)**
Sepsis/bacteremia	118 (35.8)
Stimulant-induced vasospasm	37 (11.2)
Hypertensive urgency/emergency	35 (10.6)
Hypoxia	24 (7.3)
Hypotension	20 (6.1)
Arrhythmia	17 (5.2)
Procedure related	14 (4.2)
Gastrointestinal bleed	11 (3.3)
Other rare causes	54 (16.4)
Total	**330 (100.0)**

Table 3. Analysis of mediation by liver fibrosis of the association between hepatitis C virus infection and Type 2 myocardial infarction in adults living with HIV, US, 1998-2016

	aHR	**95% Confidence interval**
Association		
Natural indirect	1.05	0.93, 1.17
Natural direct	1.58	1.40, 1.78
Total	1.65	1.44, 1.89
Percent mediated	10.6%	-16.8, 38.0%

Abbreviations: aHR, adjusted hazard ratio; HIV, human immunodeficiency virus

[*] Adjusted hazard ratios are for incident Type 2 myocardial infarction among those with chronic HVC infection compared to those without evidence of chronic HCV infection. Fully adjusted models took into account age, sex at birth, race, ethnicity, study site, men who have sex with men, ever smoker, history of injection drug use, diabetes, statin use, hypertension, Hepatitis B virus, antiretroviral use, nadir CD4+ cell count, body mass index, total cholesterol, high density lipoprotein cholesterol, triglycerides, alcohol use score, and self-reported use of amphetamines, cocaine, opiates, marijuana, and cigarettes. Hazard ratios were estimated with a natural effects models [83] and adapted to the Cox proportional hazard regression setting as described by Lange et al. [84] considering FIB-4 (as a continuous variable) a mediator. Multiple imputation was used and parameters were pooled using Rubin’s rules.

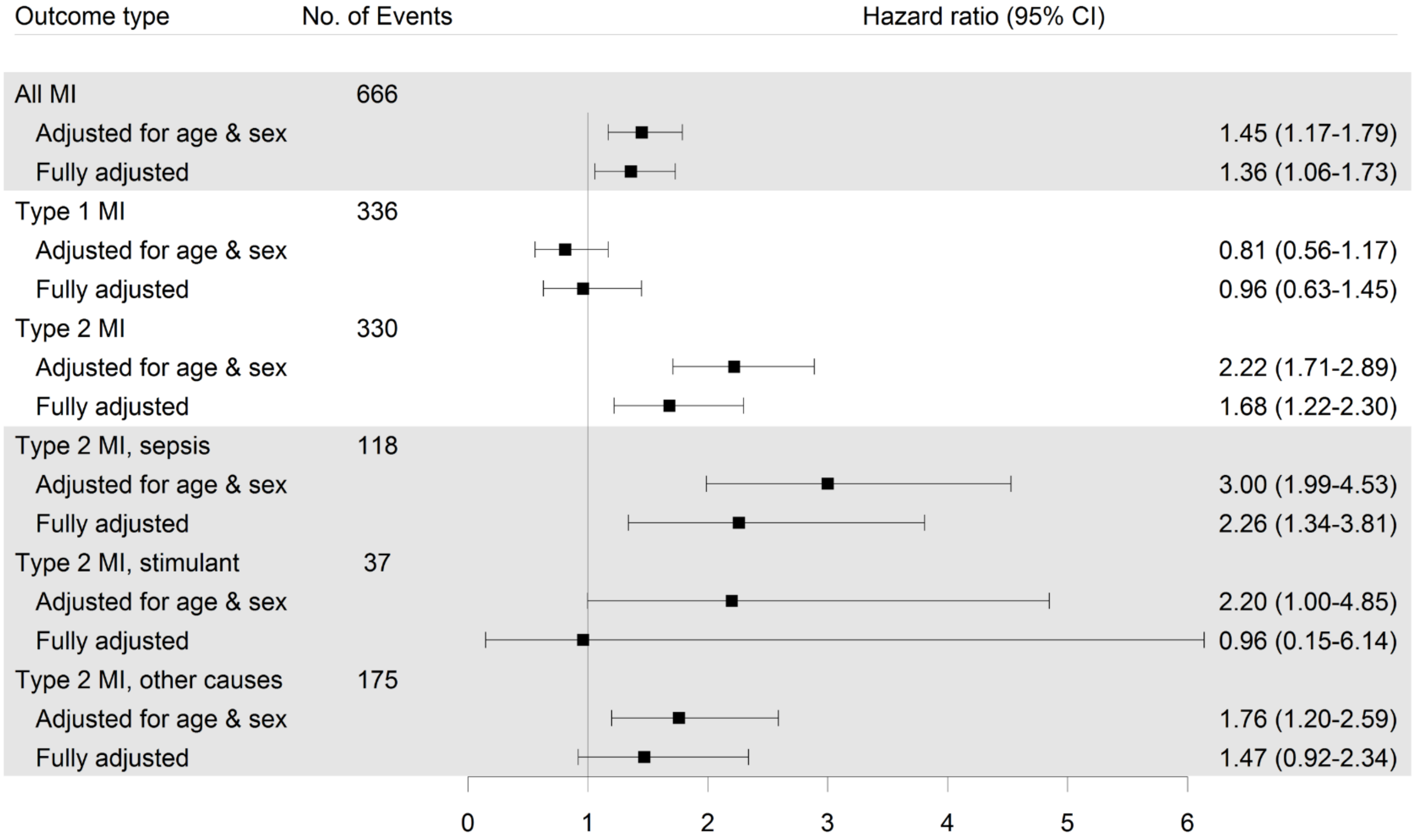

Figure 3. Forest plot of hazard ratios estimating the association of chronic hepatitis C virus infection with myocardial infarction outcomes among people living with HIV at 6 CNICS sites across the US. Fully adjusted models took into account age, sex at birth, race, ethnicity, site, men who have sex with men, ever smoker, history of injection drug use, diabetes, statin use, hypertension, Hepatitis B virus, ART use, nadir CD4+ cell count, HIV viral load, body mass index, total cholesterol, HDL cholesterol, triglycerides, alcohol use score, and self-reported use of amphetamines, cocaine, opiates, marijuana, and cigarettes.

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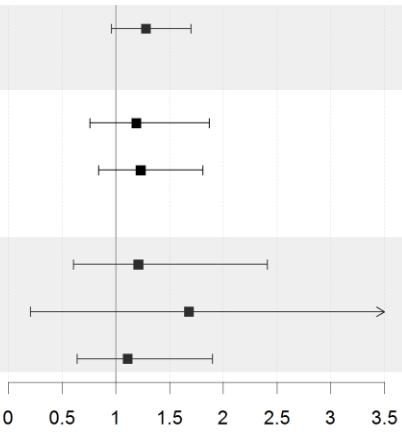
Conflict of interest statement

The authors report no potential conflicts of interest, including relevant financial interests, activities, relationships, and affiliations.

Supplementary Table and Figures



	Number of events	aHR (95% CI)	
MI, all	666	1.28	(0.96-1.70)
T1MI	336	1.19	(0.76-1.87)
T2MI	330	1.23	(0.84-1.81)
T2MI attributed to sepsis	118	1.21	(0.61-2.41)
T2MI attributed to stimulant	37	1.68	(0.21-13.7)
T2MI attributed to other causes	175	1.11	(0.64-1.90)

Supplementary Figure 1. Forest plot of adjusted hazard ratios for incident MI outcomes in those with prior HCV infection without chronic infection compared to those without evidence of prior HCV infection (antibody negative). Prior HCV infection without active infection at the beginning of MI follow-up was defined as a positive result on the most recent HCV antibody test combined with a negative result on the most recent HCV RNA test. Hazard ratios are from Cox proportional hazard regression models adjusted for age, sex at birth, race, ethnicity, site, men who have sex with men, ever smoker, history of injection drug use, diabetes, statin use, hypertension, Hepatitis B virus, ART use, lowest CD4+ cell count, HIV viral load, body mass index, total cholesterol, HDL cholesterol, triglycerides, alcohol use score and self-reported use of amphetamines, cocaine, opiates, marijuana, and cigarettes. Multiple imputation was used and parameters were pooled using Rubin's rules. Abbreviations: aHR, adjusted hazard ratio; CI, confidence interval; MI, myocardial infarction; T1MI, Type 1 MI; T2MI, Type 2 MI

Supplementary Table 1. Hazard ratios describing the association of chronic HCV with T2MI attributed to stimulant use

Adjustment variables	Hazard ratio	95% Confidence interval
None (crude estimate)	**2.60**	**1.19, 5.70**
Age, sex	2.20	1.00, 4.85
Age, sex, history of injection drug use	1.18	0.51, 2.75
All covariates without substance use PROs	1.07	0.45, 2.55
All covariates with substance use PROs	0.96	0.15, 6.14

Hazard ratios are from Cox proportional hazards regression models estimating incident myocardial infarction outcomes in those with chronic HVC infection compared to those without evidence of chronic HCV infection. Covariates were age, sex at birth, race, ethnicity, site, men who have sex with men, ever smoker, history of injection drug use, diabetes, age, sex at birth, race, ethnicity, site, men who have sex with men, ever smoker, history of injection drug use, diabetes, statin use, hypertension, Hepatitis B virus, ART use, lowest CD4+ cell count, HIV viral load, body mass index, total cholesterol, HDL cholesterol, triglycerides, alcohol use score and self-reported use of amphetamines, cocaine, opiates, marijuana, and cigarettes. Multiple imputation was used and parameters were pooled using Rubin's rules. **Boldface indicates significance at α=0.05.**

Chapter 3: Lack of support for a causal association of chronic hepatitis C virus with increased Type 2 myocardial infarction among adults living with HIV in the United States: a Mendelian randomization study

Lack of support for a causal association of chronic hepatitis C virus with increased Type 2 myocardial infarction among adults living with HIV in the United States: a Mendelian randomization study

Authors: Jessica Williams-Nguyen[1], Anshuman Sewda[2], Sara Lindström[1,3], Inga Peter[2], Robin M Nance[1,4], Susan R Heckbert[1,5], H Nina Kim[4,6], Bridget M Whitney[1], W Chris Mathews[7], Edward R Cachay[7], Matt Budoff[8], Christopher Hurt[9], Peter W Hunt[10], Elvin Geng[10], Richard D Moore[11,12], Michael J Mugavero[13], Stephen E Hawes[1], Joseph Eron[9], Bryan Lau[12], Mari M Kitahata[6,7], Michael S Saag[13], Paul K Crane[4], Heidi M Crane[4,6], Joseph A Delaney[1]

Author affiliations: [1]Department of Epidemiology, University of Washington; [2]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai; [3]Public Health Sciences Division, Fred Hutchinson Cancer Research Center; [4]Department of Medicine, University of Washington; [5]Cardiovascular Health Research Unit, University of Washington; [6]Center for AIDS Research, University of Washington; [7]Department of Medicine, University of California San Diego; [8]Department of Medicine, Los Angeles Biomedical Research Institute at Harbor – University of California at Los Angeles Medical Center; [9]Institute for Global Health & Infectious Diseases, University of North Carolina Chapel Hill; [10]Division of Experimental Medicine, University of California San Francisco; [11]Department of Medicine, Johns Hopkins University; [12]Department of Epidemiology, Johns Hopkins University; [13]Division of Infectious Disease, University of Alabama at Birmingham

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Abstract

Background: Chronic hepatitis C virus (HCV), a common coinfection in people living with HIV (PLWH), is thought to be associated with higher rates of cardiovascular disease. Specifically, recent work in PLWH has shown that chronic HCV is associated with supply-demand mismatch Type 2 myocardial infarction (T2MI), but not classical atheroembolic Type 1 MI (T1MI). Biological mechanisms for this relation are not yet understood or proven. The aim of this study was to evaluate the empirical association of HCV and T2MI by (i) testing whether known genetic variants associated with chronic HCV were also associated MI outcomes in PLWH and (ii) using these variants as an instrumental variable to test for a causal association between chronic HCV and T2MI.

Methods: We obtained genome-wide genetic data for 6,116 participants in the CFAR Network of Integrated Clinical Systems (CNICS), a multi-center, multi-ethnic clinical cohort of PLWH. We assessed the association between four genetic variants in the *IFNL3/4* genomic region and MI outcomes using logistic regression. We also used a Mendelian randomization approach to estimate the casual effect of chronic HCV on T2MI.

Results: The selected *IFNL3/4* variants were not individually associated with T2MI or the subgroup of T2MI attributed to sepsis. Based on these four variants, Mendelian randomization analyses did not provide evidence that chronic HCV casually increases the risk of T2MI (Ratio of odds ratios (ORR) 0.78, 95% CI: 0.33, 1.83) or T2MI attributed to sepsis (ORR 1.13, 95% CI: 0.54, 2.36) in PLWH. Three of 4 selected variants were associated with 23 to 30% higher odds of atheroembolic T1MI.

Conclusions: This work does not provide support for a shared genetic association between increased risk for chronic HCV and T2MI, suggesting that confounding is a likely cause of the observed association between chronic HCV and T2MI in PLWH. This finding highlights the importance of using the full set of epidemiological methods to explore large and unexpected

epidemiological associations to verify their robustness. In addition, this study provides novel evidence that selected *IFNL3/4* variants may be associated with risk of T1MI.

Background

People living with human immunodeficiency virus (HIV) (PLWH) have a higher risk of myocardial infarction (MI) [13] and research indicates that the proportion of MI events that are demand-supply mismatch Type 2 MI (T2MI) is greater in PLWH [19] relative to the general population [20-28]. Unlike classical atheroembolic Type 1 myocardial infarction (T1MI), T2MI results from myocardial oxygen demand-supply mismatch secondary to a variety of precipitating conditions, including sepsis, stimulant-induced vasospasm, decompensated heart failure, hypotension and others [18]. Better understanding of the risk factors for T2MI, a serious medical event, in PLWH is important for clinical care of this vulnerable population [19, 94].

Chronic infection with hepatitis C virus (HCV) affects an estimated 10 to 30% [36, 41-44] of PLWH in the US, and observational evidence has shown an association between chronic HCV and clinically evident CVD in PLWH [68] as well as in the general population [8, 71]. Two studies of chronic HCV in populations of PLWH did not find an association with composite MI events [56, 57]. Recent work by our group [95, 96] which was able to investigate MI by type confirmed this finding for T1MI and all MI combined. In contrast, we found that chronic HCV infection was associated with an ~68% higher risk of incident T2MI after adjusting for demographic, clinical and behavioral factors. *Post-hoc* investigation of T2MI suggested that this finding was driven by a strong association between chronic HCV and T2MI attributed to sepsis.

Biological mechanisms for this finding can be hypothesized, including inflammatory effects of chronic viremia or a common host genetic architecture contributing both to the risk of chronic HCV and to risk of sepsis or sepsis-related complications, such as T2MI. However, these hypothesized mechanisms have not yet been confirmed and further, the risk of residual confounding may be high given that some risk factors for both sepsis and HCV, such as intravenous drug use [32, 97], are difficult to measure. As a result, the possibility of residual or unmeasured confounding cannot be excluded as an alternate explanation for the observed associations between chronic HCV and T2MI.

The goal of this study was to assess the causal association between HCV and T2MI leveraging genetic data. Specifically, we aimed to (i) examine whether single nucleotide polymorphisms (SNPs) implicated in chronic HCV are also associated with T1MI and T2MI, and (ii) test the causal relationship of HCV and time to T2MI using a Mendelian randomization analysis.

Methods

CNICS study population and data repository. The Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort is a multi-ethnic population of >32,000 adults (≥18 years of age) living with HIV who have at least once since 1995 received routine clinical HIV care at one of eight sites in the United States [72]. Each CNICS site captures demographic, clinical, medication and laboratory data from all outpatient and inpatient encounters, including items such as cardiac biomarkers, medications, diagnoses, and historical clinical information. CNICS participants from each site are being genotyped for future research purposes. At the time of this analysis, there were 7,825 participants from all eight CNICS sites for whom genotyping information was available after quality control. Of these, 6,116 received care at six CNICS sites where comprehensive MI ascertainment was undertaken and constitute the cohort for these analyses. Institutional review boards at each institution have approved CNICS research activities and written informed consent was obtained from all participants.

Genotyping and data processing. DNA was extracted from Peripheral Blood Mononuclear Cells (PBMC) and Buffy Coat samples. Genotyping was conducted using either Illumina MEGA or MEGA-Ex BeadChips (more than 1.7 million genetic variants), based on availability. Additional detail about sample collection and processing can be found in the Supplementary Materials. Genotypes were called using the GenCall algorithm in GenomeStudio® Genotyping Module v2.0 software (Illumina®, San Diego, California, USA). Rare variants were additionally called using zCall [98].

Standard variant and sample level filtering was performed on the genotyped data using Plink v1.9 [99] as follows. Patients with different inferred sex based on genotype data compared to reported sex at birth were excluded. Duplicate samples were identified through pairwise identity-by-descent and the record with the lower genotyping rate from each duplicate pair was removed. Heterozygosity-based outliers (mean ±3 standard deviations) as well as samples and variants with genotyping rate <95% were excluded (**Supplementary Figure 2**). Principal components analysis was performed using the smartpca function in the EIGENSOFT software package [100], and the genotyped data was plotted against the HapMap v3r2 data as well as 1000 Genomes data to assess the extent of discrepancies between self-identified race/ethnicity and the race inferred through the principal components analyses (**Supplementary Figure 3**). The CNICS patient population includes a number of intersecting subpopulations at risk of stigmatization and identity-related trauma [101-103], including people who inject drugs, men who have sex with men, and racial, ethnic and gender minorities. Due to the complexity of racial self-identification in ethnic minorities who experience trauma [104], participants with discrepant findings were retained.

Genotypes for SNPs that were not directly genotyped were imputed using the Michigan Imputation Server [105] and Haplotype Reference Consortium [106] samples as the reference population. Prior to the imputation, compatibility of the genotyped data was ensured using files containing strand information for each genotyping chip provided by the Wellcome Centre for Human Genetics [107].

Four single nucleotide polymorphisms (SNPs), rs12980275, rs12979860, rs8109886, and rs8099917, in the genomic region of the genes interferon lambda *(IFNL)3* and *IFNL4* were selected for this analysis. These variants have known strong associations with HCV spontaneous clearance and response to therapy, and the relationship of rs1297860 and rs8099917 to HCV spontaneous clearance has been shown to persist within strata of self-identified race in multiethnic cohorts [58]. Two SNPs used in this analysis were imputed (rs8099917 and rs8109886), and dosage values (range 0.000 to 2.000) were used. Information about SNPs selected for this analysis, including imputation information score and distribution of copies of the risk alleles, is provided in **Supplementary Table 2**. Frequencies of the risk allele for each SNP by self-identified race are listed in **Supplementary Table 3**. Pairwise correlations between numbers of risk alleles for the four variants are listed in **Supplementary Table 4.**

Ascertainment of clinical variables. MI events were ascertained from six CNICS sites with comprehensive access to inpatient and outpatient electronic medical records (Johns Hopkins University; University of Alabama at Birmingham; University of California, San Diego; University of California, San Francisco; University of North Carolina at Chapel Hill; and University of Washington, Seattle). Participants were considered to be at risk for MI events six months after CNICS cohort entry (to allow for collection of baseline covariates) or the first date of surveillance for MI events at that study site, whichever was later. Participants were followed for MI until nine months after the date of last clinical activity (visit or laboratory result) [73], death, or the administrative censoring date for that site.

The procedure used to ascertain and adjudicate MI events in this cohort has been described in detail elsewhere [75]. Briefly, potential events were identified centrally using multiple criteria including MI diagnoses, invasive cardiac procedures, or elevated cardiac biomarkers. Sites then generated clinical data packets including primary data for all potential MI events. Packets contained physician notes, electrocardiograms, imaging and procedure reports, and laboratory values. Antiretroviral medication data were redacted to enable blinded review for adjudication. Packets were reviewed centrally by two physician experts to adjudicate MIs as either definite or probable and to classify by type as either T1MI or T2MI based on the Universal MI definition; attributed causes of T2MI were documented [18, 19, 75]. While the Universal MI definition also includes Type 3 (cardiac death due to MI), Type 4 (percutaneous coronary intervention-related) and Type 5 (coronary artery bypass graft-related) MIs [18, 19, 75], these were uncommon (<10 events) and are not further discussed. In cases of discrepant findings, the assessment of a third reviewer was used to break ties [75].

Laboratory test results were obtained in the course of routine care and these tests were used to ascertain HCV status. Chronic HCV infection was defined as a positive result for the most recent ribonucleic acid (RNA) polymerase chain reaction test for HCV at or before the beginning of follow-up for MI events. Those with no record of a positive RNA test prior to the beginning of follow-up were classified as not having chronic HCV infection.

Statistical analysis

For each SNP, separate logistic regression models estimated the relative odds of each MI event type (T1MI, T2MI and T2MI attributed to sepsis) associated with an additional copy of the risk allele under an additive genetic model. Logistic regression models were adjusted for age at the end of follow-up, sex, study site and the top ten principal components. The relative odds of a positive RNA test for HCV (chronic HCV) associated with each additional copy of the risk allele for each SNP under an additive genetic model was estimated using logistic regression adjusting for sex, study site and top ten principal components.

The Mendelian randomization casual effect estimates of chronic HCV on risk of T1MI and T2MI were estimated using the summary parameter estimates and standard errors of the genetic association analyses described above. Estimation was done with weighted generalized linear regression accounting for the correlation between variants using R package, MendelianRandomization [108]. The Cochran Q statistic was used to assess heterogeneity in parameter estimates between variants in the fixed effect meta-analysis.

Potential pleiotropy (the SNPs impact MI outcomes via a non-HCV pathway) was assessed using an online database (PhenoScanner) [109]. SNPs rs12979860 and rs8099917 were found to be related to low density lipoprotein (LDL) and total cholesterol in those chronically infected with HCV [110-114]. To assess average directional pleiotropy of the variants, we estimated MR-Egger regression [115] accounting for correlation between variants in R package, MendelianRandomization [108], and tested whether the intercept term from differed significantly from the null value.

Statistical analyses were conducted in R version 3.4.4 (2018-03-15).

Results

Characteristics of the cohort. Among 6116 PLWH, 498 participants (8.1%) had HCV viremia (positive RNA test) and there were 151 T1MI and 143 T2MI (294 MI total) during a median of 9.1 years of follow-up. Of the 143 T2MI events, 43 of these were attributed to sepsis. Participants averaged 41.2 years at the beginning of MI follow-up and 81% were male (**Table 4**).

Approximately half identified as white (50.4%), 45.6% identified as black, and less than 4% identifies as another race.

Association of genetic variants with MI and HCV. Three of four selected SNPs were significantly associated with an approximately 17 to 32% higher odds of chronic HCV infection (**Table 5**), and the fourth SNP, rs12980275, showed marginal association (p=0.062). The four SNPs jointly explained 4.7% (Nagelkerke R^2) of the variation in chronic HCV in CNICS based on a logistic regression model accounting for study site, sex, and top ten principal components or 0.7% of the variation when modelling the association of SNPs with chronic HCV without adjustment.

Table 6 shows the relative odds of MI outcomes associated with each additional copy of the risk allele for the four genetic variants on Chromosome 19 in the region of type III interferon genes, *IFNL3* and *IFNL4*, after adjusting for age, sex, study site and the top ten principal components. None of the variants were significantly associated with T2MI or the subgroup of T2MI attributed to sepsis. However, three of the four SNPs tested (rs12980275, rs1297980 and rs8109886) were associated with a 23-30% lower odds of T1MI for each copy of the risk allele (**Table 6**). Estimates for unadjusted models are shown in **Supplementary Figure 4**.

In sensitivity analysis, analyses were repeated within each stratum of participants who self-identified as black or white race. Results were qualitatively similar and are shown in **Supplementary Table 5**.

Mendelian randomization analysis. We assessed evidence for a causal effect of chronic HCV on MI outcomes using a Mendelian randomization approach. **Table 7** shows the empirical and Mendelian randomization estimates on the basis of 4 genetic variants. There was not a significant estimated causal effect of chronic HCV on T2MI (Odds ratio ratio (ORR) 0.78, 95% confidence interval (CI): 0.33, 1.83) or on T2MI attributed to sepsis (ORR 1.13, 95% CI: 0.54, 2.36).

There was similarly no support for a causal association of chronic HCV on T1MI (ORR 1.11, 95% CI: 0.24, 5.23), however heterogeneity between variants was significant (**Table 7**). Mendelian randomization models on the basis of each variant individually are shown in **Supplementary Table 6**. The MR-Egger intercept test, which allows assessment of average directional pleiotropy, was significant (p = 0.020).

Within the strata of self-identified black and white race, Mendelian randomization estimates similarly indicated no significant causal effect of HCV on T1MI, T2MI or the subset of T2MI attributed to sepsis (**Supplementary Table 7**).

Discussion

In prior work, we found that chronic HCV was empirically associated with a 68% greater risk of oxygen demand-supply mismatch T2MI in PLWH in the US and that this relation was likely driven by the relation between chronic HCV and T2MI attributed to sepsis [95, 96]. We sought to probe the empirical association of chronic HCV with T2MI and found that key variants in the genomic region coding for immune regulators, interferon lambda 3 and 4, were not associated with T2MI or the subgroup of T2MI attributed to sepsis.

We then used these variants as instrumental variables in a Mendelian randomization analysis to test for evidence of a causal effect of chronic HCV on T2MI outcomes and found that support for such an effect was lacking. The estimate of causal effects of chronic HCV on both T2MI and on the subgroup of T2MI that were attributed to sepsis were not significantly different from the null value of one, and point estimates for the causal effect of chronic HCV from Mendelian randomization indicated a lower, rather than a higher, risk of T2MI outcomes. This finding suggests that the strong empirical association of 68% higher risk of T2MI among those with chronic HCV [95, 96] was potentially due to confounding by unmeasured or misclassified factors (**Figure 4**). As a route of exposure for HCV, injection drug use is a factor of considerable concern as a confounder of the HCV-T2MI association. While our prior study accounted for history of injection drug use on the basis of HIV transmission risk factor as well as self-reported use of illicit substances at baseline, we did not have comprehensive measurement of substance use including frequency of or time-updated injection drug use. In addition, only 13% of participants had completed the assessment of substance use at the baseline and data for remaining participants had to be imputed possibly resulting in misclassification. In addition to injection drug use, unmeasured confounding by a variety of other factors could contribute to an observed association between HCV and T2MI, particularly T2MI attributed to sepsis, in PLWH.

The strength of the variant-exposure relationship is a consideration for any Mendelian randomization analysis as weak instruments can contribute to bias in the estimated causal effect [116]. Genetic variants in the genomic region of *IFNL3* and *IFNL4* were selected due to known strong associations with HCV spontaneous clearance and treatment response in diverse cohorts [58]. In our study, the 4 selected SNPs in combination explained only 0.7% of the variation in the odds of chronic HCV. Unlike prior studies of HCV clearance which compare those who are infected with HCV to those who have cleared HCV (either spontaneously or via treatment), the unexposed group for this comparison is an uninfected population wherein some participants may never have been exposed to HCV and as such the difference in risk allele frequency between the two groups would be predicted to be less dramatic. In other words, some in the unexposed group may carry one or more risk allele but have never been challenged with HCV.

However, assessment of the strength of an instrument should consider both variance explained and magnitude of effect [117]. In this study, the variants were associated with between 14 and 32% greater odds of chronic HCV using an additive per allele genetic model. Burgess and colleagues [118] have shown that combining Mendelian randomization estimates from multiple variants using meta-analysis methods, even where those variants are from a single gene region, can reduce bias when using multiple weak instruments.

In addition to the strength of the instrument, potential violations of instrumental variable assumptions, particularly pleiotropy, must also be considered. To our knowledge, there is no published research indicating an association of the selected *INFL3/4* variants with T2MI, and our results also do not support an association between these variants and T2MI or the subset of T2MI attributed to sepsis after accounting for sex, study site and population stratification. An association between one or more of these immune-related variants and sepsis, while possible, has not been reported. In the situation where Mendelian randomization analysis indicates a null finding for the purported casual relation, as we have found here, threats to validity on the basis of pleiotropy are also less concerning as negative and positive biases would need to balance to produce such a result [119].

Prior work in multiethnic cohorts has shown that rs1298760 and rs8099917 remain associated with HCV clearance within race strata [58]. In our cohort which consists predominantly of PLWH in the United States who self-identify as black or white race, we found that genetic associations with HCV and MI outcomes were qualitative similar within strata of self-identified black and white race to the associations estimated in the entire cohort. Adjustment for population stratification, sex and study site did not substantially influence inference regarding associations of rs12980275, rs12979860, or rs8109886 with HCV or T2MI. For rs8099917, adjustment of the logistic regression models resulted in a de-attenuation of the odds ratio for HCV with respect to the unadjusted model, while adjustment resulted in a commensurate attenuation of the estimated odds ratio for T2MI. This indicates that accounting for differences in population stratification in this variant was important. However, inference regarding the causal effect of HCV on T2MI from Mendelian randomization estimates conducted within race strata remained the same as the findings overall, specifically the stratum-specific Mendelian randomization estimates did not support a causal effect of HCV on T2MI or on T1MI.

For classic atheroembolic T1MI, a pleiotropic effect of the selected variants on T1MI is more plausible than in the case of T2MI. Among those with chronic HCV, both rs12979860 and rs8099917 are associated with blood lipids [110-114, 120], an important risk factor for coronary artery disease [121]. Similarly, rs12979860 is associated with prevalence and severity of hepatic steatosis and insulin resistance in chronic HCV [111, 120]. In sensitivity analysis, the intercept from MR-Egger regression differed significantly from zero indicating average directional pleiotropy, i.e. selected variants influence risk of T1MI via a non-HCV pathway. However, this test relies on the assumption that pleiotropic effects of variants on T1MI are independently distributed from the genetic associations with HCV [122]. Significance of the MR-Egger intercept test can also indicate deviation from this assumption whether pleiotropy exists or not.

In this study, we found that three of 4 selected variants were associated with a lower risk of T1MI for each copy of the risk allele after adjustment for potential confounding and for multiple comparisons. The Mendelian randomization analysis concurred with the empirical estimate from our prior work that indicates no association between chronic HCV and T1MI [95, 96], but heterogeneity between variants was significant. Given the serious risk of pleiotropy and some concern as to the strength of the instrument, the inverse variance weighted Mendelian randomization estimates are likely to be biased [116, 123], but a relationship between HCV and T1MI may not be the question of most interest. The more interesting question may ultimately be regarding the role that a common genetic architecture may play as an explanation for the large body of literature that has described an association between chronic HCV infection and markers of subclinical cardiovascular disease [8].

Conclusions

Our findings in this study do not support a causal explanation for prior findings of higher risk of oxygen demand-supply mismatch T2MI among those with chronic HCV. This highlights the importance of using a range of epidemiologic methods to probe large and unexpected exposure-outcome associations for robustness, especially in cases where confounding adjustment is challenging and measurement error of the confounders is plausible.

We also found evidence that selected variants in the *IFNL3/4* genomic region are associated with risk of arethroembolic T1MI after accounting for population stratification, sex and study site. This suggests a possible explanation for observed links between chronic HCV infection and markers of subclinical cardiovascular disease [8].

Declarations

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The authors report no potential conflicts of interest, including relevant financial interests, activities, relationships, and affiliations.

We would like to acknowledge all CNICS study participants and personnel for their contributions to this work.

Tables and Figures

Table 4. Demographic and clinical characteristics of 6116 adults living with HIV in care at CNICS sites across the US at study baseline

Characteristic	Suffered T1MI	Suffered T2MI	Everyone
N	151	143	6116
Age in years at study exit (mean (sd))	51.2 (9.5)	51.2 (9.9)	50.0 (10.5)
Male (%)	123 (81.5)	112 (78.3)	4888 (79.9)
Site (%)			
University of Alabama	35 (23.2)	44 (30.8)	1629 (26.6)
University of California San Diego	22 (14.6)	11 (7.7)	1351 (22.1)
Johns Hopkins	28 (18.5)	31 (21.7)	1074 (17.6)
University of Washington	25 (16.6)	22 (15.4)	1046 (17.1)
University of North Carolina	37 (24.5)	25 (17.5)	674 (11.0)
University of California San Francisco	4 (2.6)	10 (7.0)	342 (5.6)
Self-identified race (%)			
White	75 (49.7)	40 (28.0)	3061 (50.4)
Black	72 (47.7)	99 (69.2)	2766 (45.6)
Asian/Pacific Islander	0 (0.0)	1 (0.7)	75 (1.2)
American Indian	1 (0.7)	0 (0.0)	43 (0.7)
Other	3 (2.0)	3 (2.1)	124 (2.0)
Hispanic (%)	12 (7.9)	6 (4.2)	598 (9.9)
Men who have sex with men (%)	76 (51.0)	66 (47.1)	3478 (57.7)
History of injection drug use (%)	32 (21.5)	50 (35.7)	1279 (21.2)
On ART (%)	60 (39.7)	54 (37.8)	2587 (42.3)
CD4+ cell count below 200 (%)	65 (42.7)	61 (43.0)	2032 (33.2)
On statin	13 (8.6)	5 (3.5)	211 (3.4)
FIB-4 category (%)			
<1.45	92 (60.9)	82 (57.3)	4530 (74.1)
1.45-3.25	48 (31.8)	43 (30.1)	1222 (20.0)
>3.25	11 (7.3)	18 (12.6)	360 (5.9)

ART, antiretroviral therapy; T1MI, Type 1 myocardial infarction; T2MI, Type 2 myocardial infarction
Percentages are of non-missing values. Missingness was less than 2% of participants for all variables. Values are for enrollment in the CNICS cohort unless otherwise noted.

Table 5. Association between SNPs known to be associated with spontaneous clearance and sustained virologic response of chronic hepatitis C virus in adults living with HIV

Gene region	SNP	Risk Allele	aOR, HCV (95% CI; p-value) (n events = 498)
IFNL 3	rs12980275	G	1.14 (0.99-1.30; p=0.062)
IFNL 4	rs12979860	T	**1.25 (1.10-1.44, p=0.001)**
IFNL 4	rs8109886	A	**1.17 (1.01-1.37; p=0.039)**
IFNL 4	rs8099917	G	**1.32 (1.09-1.59; p=0.003)**

aHR, adjusted hazard ratio; HCV, hepatitis C virus; *IFNL*, interferon lambda; SNP, single nucleotide polymorphism
Odds of HCV RNA+ for each additional copy of the risk allele under an additive genetic model was estimated using logistic regression adjusting for sex, study site and top ten principal components. **Boldface indicates significance at α=0.05**.

Table 6. Association between candidate SNPs and Type 1 and Type 2 myocardial infarction in adults living with HIV

			aOR (95% CI; p-value)		
Gene region	SNP	Risk Allele	T1MI (n events = 151)	T2MI (n events = 143)	T2MI attributed to sepsis (n events = 43)
IFNL 3	rs12980275	G	**0.70 (0.55-0.90; p=0.005)**	0.85 (0.66-1.08; p=0.185)	0.97 (0.62-1.49; p=0.878)
IFNL 4	rs12979860	T	**0.77 (0.60-0.98; p=0.035)**	0.89 (0.69-1.14; p=0.344)	1.02 (0.65-1.59; p=0.934)
IFNL 4	rs8109886	A	**0.77 (0.59-1.00; p=0.048)**	0.91 (0.69-1.21; p=0.511)	1.05 (0.64-1.73; p=0.863)
IFNL 4	rs8099917	G	1.10 (0.78-1.52; p=0.569)	0.80 (0.51-1.19; p=0.298)	0.77 (0.35-1.50; p=0.477)

aOR, adjusted odds ratio; CI, confidence interval; *IFNL*, interferon lambda; SNP, single nucleotide polymorphism; T1MI, Type 1 myocardial infarction; T2MI, Type 2 myocardial infarction

Relative odds of myocardial infarction associated with each additional copy of the risk allele under an additive genetic model was estimated with logistic regression adjusting for age, sex, study site and the top ten principal components. **Boldface indicates significance at α=0.05**.

Table 7. Comparison of empirical and Mendelian randomization association of chronic hepatitis C virus and myocardial infarction in adults living with HIV in care across the United States

Outcome	Estimation method	Adjusted relative association (95% CI)	Cochrane's Q-statistic (p-value)
T1MI	Empirical [95, 96]	0.96 (0.63, 1.45)	
	Mendelian randomization	1.11 (0.24, 5.23)	11.7 (p = 0.0083)
T2MI	Empirical [95, 96]	1.68 (1.22, 2.30)	
	Mendelian randomization	0.78 (0.33, 1.83)	1.88 (p = 0.5986)
T2MI attributed to sepsis	Empirical [95, 96]	2.26 (1.34, 3.81)	
	Mendelian randomization	1.13 (0.54, 2.36)	0.78 (p = 0.8554)

CI, confidence interval; T1MI, Type 1 myocardial infarction; T2MI, Type 2 myocardial infarction

Empirical associations are relative hazards of each MI outcome in those with chronic HCV (RNA+) compared to those without chronic HCV from Cox proportional hazard regression model adjusting for age, sex at birth, race, ethnicity, site, men who have sex with men, ever smoker, history of injection drug use, diabetes, statin use, hypertension, hepatitis B virus, ART use at baseline, lowest CD4+ cell count at follow-up start, HIV viral load at follow-up start, body mass index, total cholesterol, HDL cholesterol, triglycerides, alcohol use score and self-reported use of amphetamines, cocaine, opiates, and marijuana [95, 96]. Mendelian randomization analyses on summary data was estimated with weighted generalized linear regression accounting for the correlation between variants on the basis of 4 SNPs.

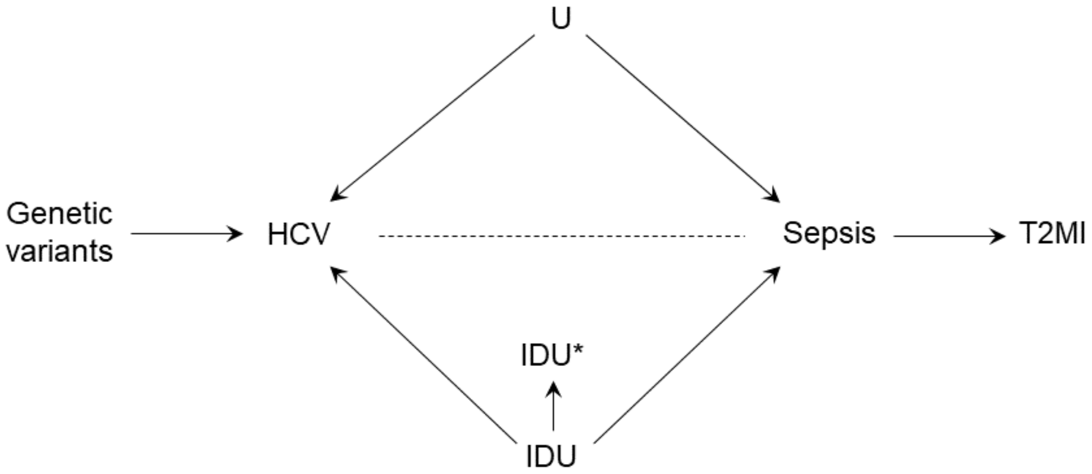


Figure 4. Conceptual model depicting the hypothesized relationships between chronic HCV, sepsis, Type 2 MI (T2MI), the genetic instrumental variable, injection drug use (IDU) and its misclassified counterpart (IDU*) and an unmeasured confounder (U). Dashed line indicates a non-causal statistical association.

Supplementary Methods

Sample collection. Peripheral Blood Mononuclear Cells (PBMC) and Buffy Coat samples were shipped in batches to Mount Sinai. Barcodes were randomly assigned and labeled as samples were input into FreezerPro® software. Samples were sent frozen to the Mount Sinai Institute for Personalized Medicine (IPM) for DNA extraction. The IPM optimized and used the fresh/frozen blood protocol from the FlexiGene DNA kit (Qiagen, #51206) and an automated DNA extraction machine (Autogen).

To prepare samples for DNA extraction, frozen samples were thawed in a 37°C water bath for 5 minutes. 750µl of 1X Phosphate Buffered Saline (PBS) was added to PBMC samples for a volume of 1-2ml, while 3ml of 1X PBS was added to 500ul of buffy coat samples for a final volume of 4ml. Samples were then transferred to the Autogen machine for further processing. Isolation of DNA from both PBMC and Buffy Coat samples began with the lysing of erythrocytes and leukocytes with lysis buffer. Cell nuclei and mitochondria were then centrifuged, and pellets were re-suspended in denaturation buffer for incubation. This denaturation buffer contained chaotropic salt and Qiagen protease to effectively remove any contaminants. Following incubation, DNA was precipitated with isopropanol, centrifuged, and washed with 70% ethanol. Samples were then dried and re-suspended in 10mM Tris-HCl hydration buffer, pH8.5. DNA was quantified by the IPM using their nanodrop equipment. Replacements for samples with a low concentration or poor DNA quality were requested to the individual sites for a second extraction. In total, 8974 samples were extracted at the time of this analysis.

Extracted DNA samples were normalized to a concentration of 75-80ng/µl for genotyping. Any concentrations below 75ng/µl were kept as is, and samples with concentrations above 5ng/µl were sent for genotyping. However for quality control purposes, some samples with concentrations below 5ng/µl, as well as intentional duplicate samples were sent for genotyping for a total of 8650 samples to date. Samples were randomly transferred onto 96-well plates (USA Scientific, #1402-9700) and sent to the Mount Sinai CORE Facility to be genotyped using the Infinium LCG Assay Manual Protocol (Illumina. Part #15023140 Rev A).

Sample processing. Each DNA sample was prepared for amplification through the creation of a MSA6 plate. DNA samples were added to the MSA6 plate after the addition of MA1, then followed by 0.1N NaOH to denature the DNA. RPM reagent was then added to neutralize the DNA, followed by the Multi-Sample Amplification Master Mix (MSM). The MSA6 plate was then incubated for 20-24 hours at 37C. After incubation, FMS was added to each sample to enzymatically fragment the amplified DNA then incubated for 1 hour. PM1 and isopropanol were added to the MSA6 plate for precipitation, with the presence of blue pellets after removal of the supernatant indicating DNA. DNA pellets were then re-suspended in the MSA6 plate with the addition of RA1, and dispensed onto Illumina MEGA or MEGA-Ex BeadChips, based on availability. RA1 was again added to the BeadChips to wash away unhybridized and non-specifically hybridized DNA samples. The EML reagent was added to extend primers hybridized on the BeadChip followed by 95% formamide/1 mM EDTA to remove hybridized DNA. XC3 was then added to neutralize the primers, which then go through a staining process. BeadChips were washed with PB1 and coated with XC4 reagents then dried for 1 hour. BeadChips were scanned into the CORE's system and data files were uploaded to Minerva® for analysis.

Supplementary Tables and Figures

Supplementary Table 2. Information about 4 selected single nucleotide polymorphisms in the *IFNL3/4* genomic region

	rs12980275	rs12979860	rs8109886	rs8099917
Gene	*IFNL3*	*IFNL4*	*IFNL4*	*IFNL4*
Chromosome	19	19	19	19
Position	39731783	39738787	39742762	39743165
Risk allele	G	T	A	G
Alternate allele	A	C	C	T
Genotyping method	Direct	Direct	Imputed	Imputed
Imputation score	-	-	0.887	0.999
Distribution of risk allele[*] (%)				
2 copies	18.6	22.7	35.7	2.7
1 copy	45.3	45.6	43.8	23.7
0 copies	36.1	31.7	20.6	73.6

Nucleotide positions are based on Ensembl genome assembly GRCh37.p13 Annotation release 105. Percentages are of non-missing values. Genotype value was missing at rs12979860, rs8109886, and rs8099917 for 1, 4, and 1 participants. Remaining variants were imputed.

[*] For the purposes of this table, copies of imputed SNPs (rs8109886 and rs8099917) have been classified as 2 copies for dosage values >1.5 to 2, 1 copy for dosage values >0.5 to 1.5 and 0 copies for dosage values 0 to 0.5.

Supplementary Table 3. Frequency of the risk allele for 4 single nucleotide polymorphisms in the *INFL3/4* genomic region by self-identified race

Self-identified race	n	rs12980275	rs12979860	rs8109886	rs8099917
White	3061	0.32	0.34	0.45	0.21
Black	2766	0.52	0.60	0.74	0.07
Asian/Pacific Islander	75	0.27	0.23	0.29	0.15
American Indian	43	0.29	0.35	0.43	0.23
Multiracial	25	0.52	0.52	0.57	0.26
Other	99	0.40	0.39	0.50	0.24
Unknown	47	0.45	0.50	0.58	0.34

Risk allele for each variant is defined in Supplementary Table 2.

Supplementary Table 4. Pairwise Pearson correlation of four genetic variants polymorphisms in the *IFNL3/4* genomic region

	rs12980275	rs12979860	rs8109886	rs8099917
rs12980275	1	0.851	0.695	0.420
rs12979860	0.851	1	0.799	0.371
rs8109886	0.695	0.799	1	0.275
rs8099917	0.420	0.371	0.275	1

Supplementary Table 5. Genetic associations with chronic hepatitis C virus and myocardial infarction in adults living with HIV within strata of self-identified race

	aOR (95% CI; p-value)			
SNP	HCV	T1MI	T2MI	T2MI attributed to sepsis
Black race				
	(n events = 262)	*(n events = 72)*	*(n events = 99)*	*(n events = 24)*
rs12980275	1.06 (p=0.530)	**0.64 (p=0.010)**	0.86 (p=0.292)	0.85 (p=0.570)
rs12979860	**1.24 (p=0.030)**	0.73 (p=0.062)	0.94 (p=0.679)	0.98 (p=0.932)
rs8109886	1.22 (p=0.101)	0.74 (p=0.129)	0.99 (p=0.966)	0.94 (p=0.874)
rs8099917	**1.51 (p=0.014)**	0.81 (p=0.574)	0.86 (p=0.610)	0.62 (p=0.518)
White race				
	(n events = 214)	*(n events = 75)*	*(n events = 40)*	*(n events = 17)*
rs12980275	**1.31 (p=0.010)**	0.80 (p=0.217)	0.87 (p=0.575)	1.28 (p=0.502)
rs12979860	**1.32 (p=0.007)**	0.83 (p=0.297)	0.83 (p=0.472)	1.21 (p=0.597)
rs8109886	1.18 (p=0.122)	0.78 (p=0.166)	0.85 (p=0.520)	1.20 (p=0.628)
rs8099917	**1.27 (p=0.047)**	1.18 (p=0.422)	0.81 (p=0.496)	0.89 (p=0.794)

Risk allele for each variant is defined in Supplementary Table 2. **Boldface indicates significance at α=0.05.**
aOR, adjusted odds ratio; CI, confidence interval; HCV, hepatitis C virus; SNP, single nucleotide polymorphism; T1MI, Type 1 MI; T2MI, Type 2 MI

Supplementary Table 6. Mendelian randomization association of chronic hepatitis C virus and Type 1 myocardial infarction in adults living with HIV in care on the basis of each genetic variant individually

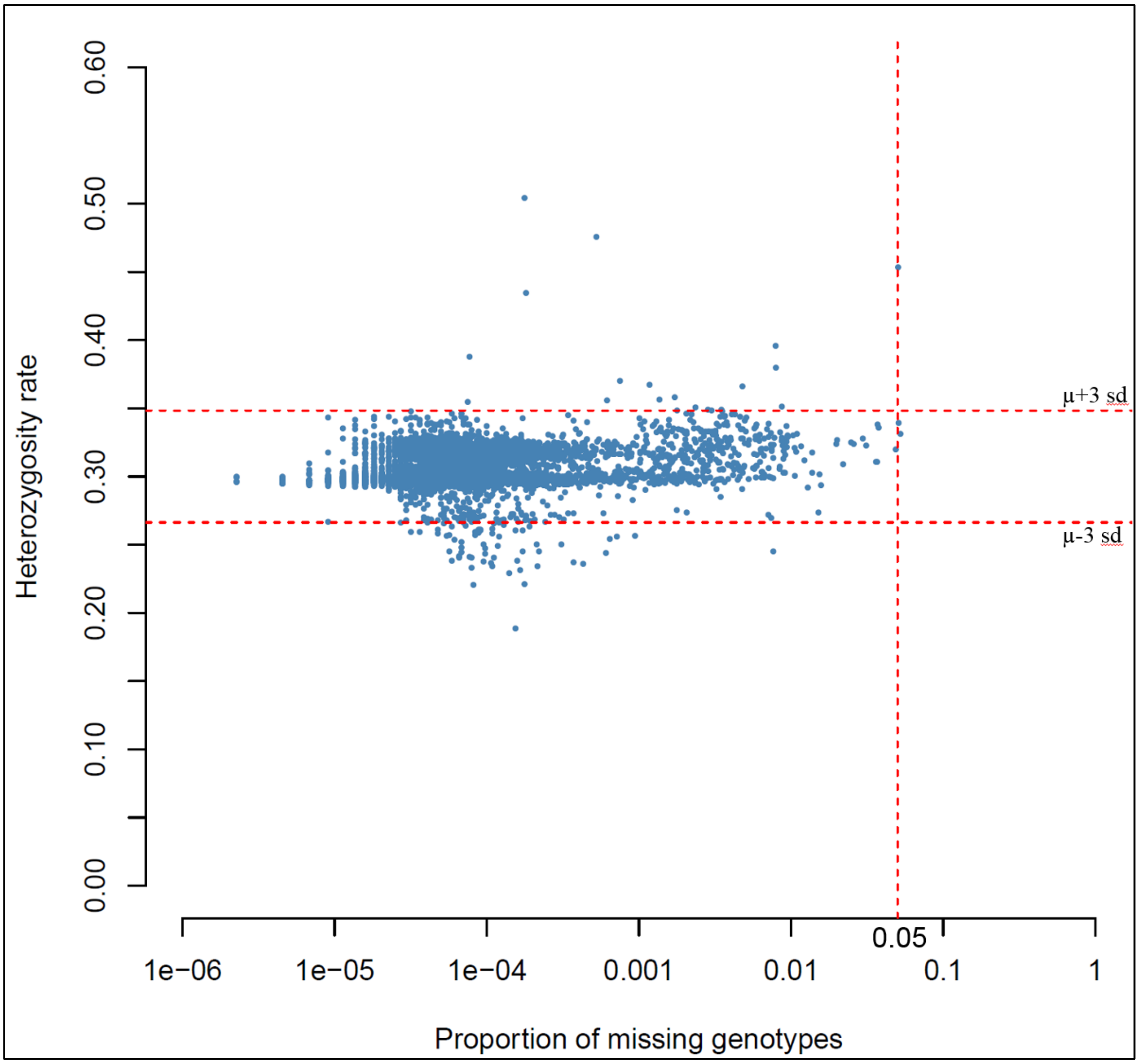
Gene region	SNP	Risk Allele	Mendelian randomization estimate (95% CI)
IFNL 3	rs12980275	G	0.06 (0.01, 0.44)
IFNL 4	rs12979860	T	0.31 (0.11, 0.92)
IFNL 4	rs8109886	A	0.19 (0.04, 0.99)
IFNL 4	rs8099917	G	1.41 (0.43, 4.64)

CI, confidence interval; *IFNL*, interferon lambda; SNP, single nucleotide polymorphism
Odds of HCV RNA+ for each additional copy of the risk allele under an additive genetic model was estimated using logistic regression adjusting for sex, study site and top ten principal components.

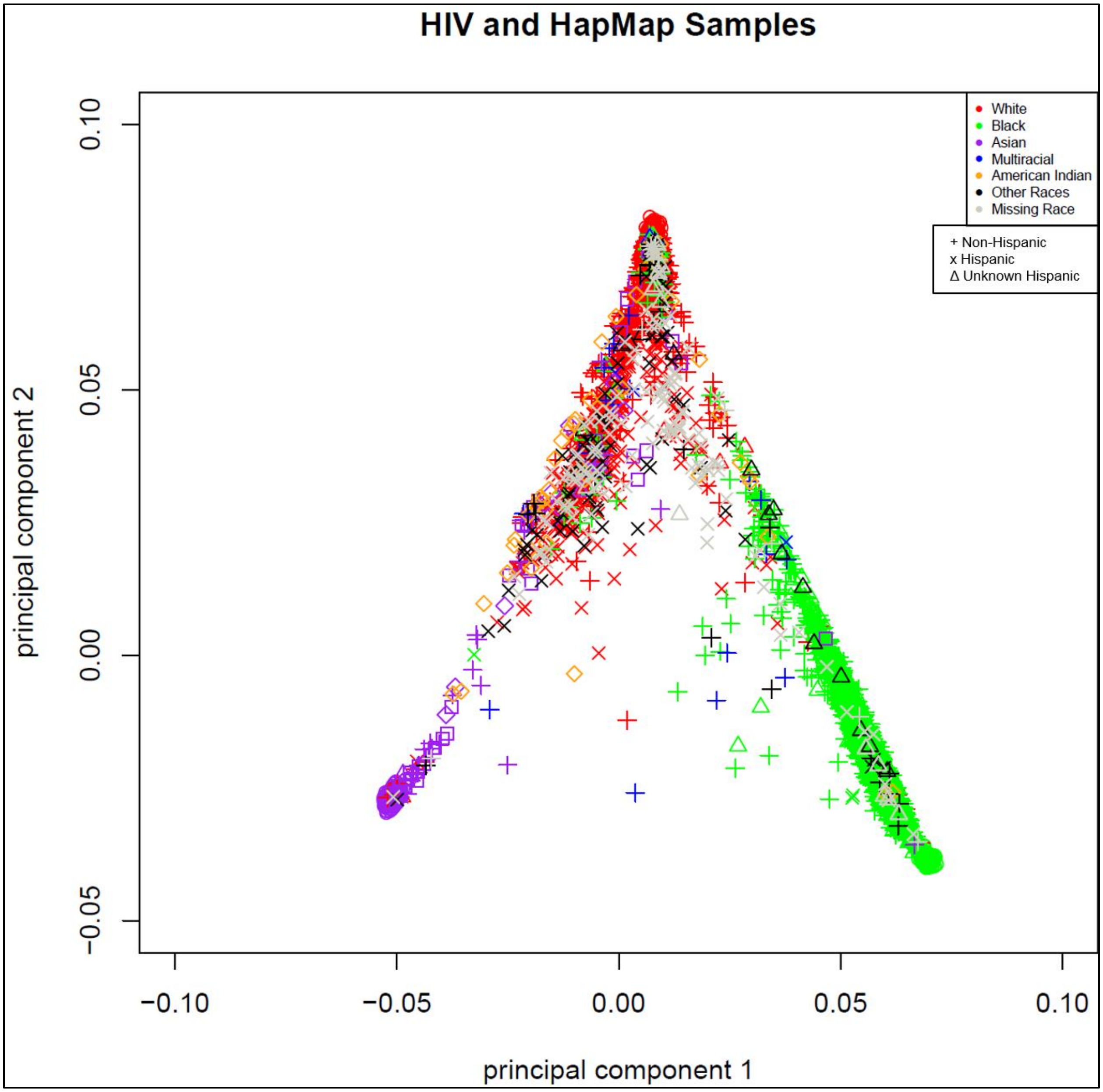
Supplementary Table 7. Mendelian randomization association of chronic hepatitis C virus and myocardial infarction in adults living with HIV within strata of self-identified race

	Black race		**White race**	
Outcome	MR estimate (95% CI)	Cochrane's Q (p-value)	MR estimate (95% CI)	Cochrane's Q (p-value)
T1MI	1.12 (0.28, 5.05)	7.1 (p=0.07)	0.87 (0.23, 3.31)	4.3 (p=0.23)
T2MI	1.26 (0.56, 2.82)	2.0 (p=0.58)	0.57 (0.12, 2.76)	0.3 (p=0.96)
T2MI attributed to sepsis	0.94 (0.38, 2.35)	1.3 (p=0.74)	2.28 (0.83, 6.27)	0.7 (p=0.87)

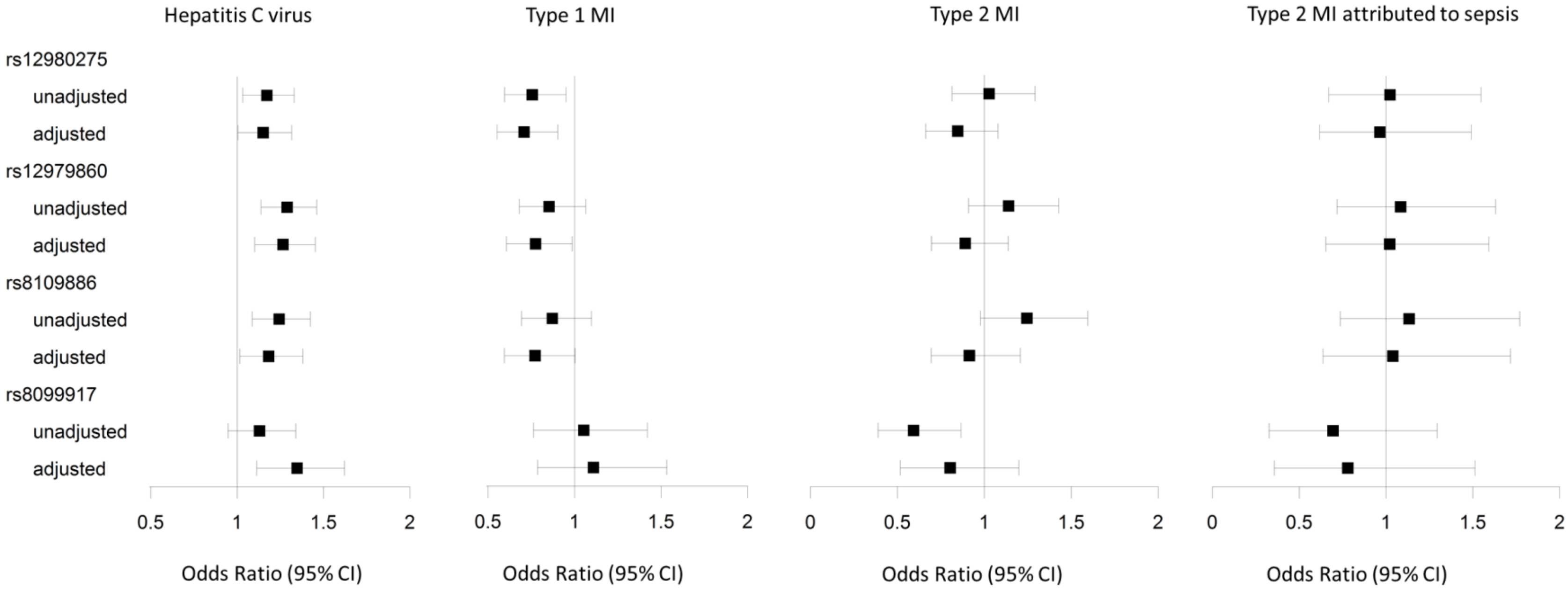
CI, confidence interval; T1MI, Type 1 myocardial infarction; T2MI, Type 2 myocardial infarction
Mendelian randomization analyses on summary data was estimated with weighted generalized linear regression accounting for the correlation between variants on the basis of 4 SNPs.



Supplementary Figure 2. Proportion of samples with missing genotypes versus genotype heterozygosity rate for each sample. Dashed red lines indicate mean heterozygosity rate ± standard deviations (sd) and genotype missingness of 5%.



Supplementary Figure 3. The first versus the second principal component for study participants and reference populations, HapMap v3r2 and 1000 Genomes data.



Supplementary Figure 4. Unadjusted and adjusted relative odds of hepatitis C virus, Type 1 myocardial infarction (T1MI), Type 2 myocardial infarction (T2MI), and the subgroup of T2MI attributed to sepsis for each additional copy of the risk allele for each variant. Adjusted models account for sex, study site and the top ten principal components. MI models additionally account for age at end of end of follow-up.

Chapter 4: Conclusion

Conclusion

This dissertation contributes to our understanding of the role of chronic infection with HCV and HCV-associated genetic variants in risk of MI in PLWH. Prior observational research on this point has not found an association, either in the general population or in PLWH, but has largely been constrained by definitions of MI based on administrative diagnosis codes and has been unable to differentiate MI events by type.

Taken together, our findings do not support a causal association of HCV with T1MI or T2MI. While HCV and T2MI are observationally associated in this cohort of PLWH, a substantial proportion of this association is likely to result from confounding bias due to unmeasured or misclassified factors. This finding highlights the importance of using the full set of epidemiological methods to explore large and unexpected observational associations to verify their robustness. Many studies of HCV and MI, including the ones presented here, have been undertaken in clinical cohorts wherein full ascertainment of sociobehavioral factors may be challenging. In particular, as a transmission route for HCV and a risk factor for sepsis, injection drug use represents an important potential confounder of the HCV-MI relation. Researchers should carefully consider capture of important sociobehavioral factors in future studies of this question. An ideal future study would incorporate complete time-varying measurement of injection drug use over the follow-up period, as drug use in the period immediately preceding the MI may ultimately be the most informative for sepsis risk. Other more feasible studies, such as one restricted to people who inject drugs, could also potentially add important information to confirm our conclusion that confounding is the likely driver of the observed HCV-MI relation as well as potentially shedding light on some reasons for the high incidence of T2MI in this cohort of PLWH.

We also found that three variants (rs12980275, rs12979860 and rs8109886) in the genomic region of immune regulators, interferon lambda (IFNL) 3 and IFNL4, were associated with both chronic HCV and T1MI in this cohort of PLWH. To our knowledge, the association of these variants with T1MI has not been previously reported. This raises the question of whether a common genetic background may play a role in observations from numerous sources that HCV is associated with subclinical and clinical CVD and opens a new line of inquiry that may ultimately inform research and clinical care priorities relevant to PLWH who are coinfected with HCV.

These findings appeared to be robust across strata of self-identified black and white race, but power to detect these associations within the strata was low. Future studies of the relationship between *IFNL3/4* variants and MI will be valuable to replicate this finding, particularly in diverse cohorts with sufficient power to fully assess the influence of population stratification. In addition, several of the variants analyzed here have been associated with serum lipid levels in people with chronic HCV infection [110-114, 120]. Lipid levels, particularly LDL cholesterol, are an important risk factor for MI [121] and, thus, represent a potential biological mechanism for the association between HCV-related *IFNL3/4* variants and arethroembolic T1MI presented here. Assessment of potential mediation by serum lipids, both in a setting of chronic HCV and in those without HCV, would be a valuable addition to scientific understanding of this question.

This dissertation also involved coordination of quality control activities to create one of the largest series of genetic markers for PLWH. This genetic data will provide an enduring resource for future studies of the impact of genetic polymorphisms of PLWH.

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