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Molecular and Contextual Markers of Hepatitis C Virus and Drug Abuse

Paul Shapshak^{1,2}, Charurut Somboonwit^{1,3}, Lydia N. Drumright⁴, Simon D.W. Frost⁵, Deborah Commins⁶, Timothy L. Tellinghuisen⁷, William K. Scott⁸, Robert Duncan⁹, Clyde McCoy⁹, J. Bryan Page¹⁰, Brian Giunta², Francisco Fernandez², Elyse Singer¹¹, Andrew Levine¹¹, Alireza Minagar¹², Oluwadayo Oluwadara^{13,14}, Taiwo Kotila¹⁵, Francesco Chiappelli¹³, and John T. Sinnott^{1,3}

¹Division of Infectious Disease and International Medicine, Department of Internal Medicine, Tampa General Hospital, University of South Florida, College of Medicine, Tampa, Florida, USA

²Department of Psychiatry and Behavioral Medicine, University of South Florida, College of Medicine, Tampa, Florida, USA

³Clinical Research Unit, Hillsborough Health Department, Tampa, Florida, USA

⁴Division of International Health, Department of Medicine, University of California, San Diego, La Jolla, California, USA

⁵Department of Veterinary Medicine, University of Cambridge, Cambridge, UK

⁶Department of Pathology, University of Southern California Keck School of Medicine, Los Angeles, California, USA

⁷Scripps Research Institute, Jupiter, Florida, USA

⁸Miami Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, Florida, USA

⁹Department of Epidemiology, Comprehensive Drug Research Center, University of Miami Miller School of Medicine, Miami, Florida, USA

¹⁰Department of Anthropology, University of Miami, Coral Gables, and Department of Psychiatry and Behavioral Sciences Comprehensive Drug Research Center, University of Miami Miller School of Medicine, Miami, Florida, USA

¹¹Department of Neurology and National Neurological AIDS Bank, UCLA School of Medicine, Westwood, California, USA

¹²Department of Neurology, Louisiana State University Health Sciences Center, Shreveport, Louisiana, USA

¹³Division of Oral Biology and Medicine, UCLA School of Dentistry, Los Angeles, California, USA

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Correspondence: *Paul Shapshak*, PhD, Division of Infectious Disease, University of South Florida Health, Tampa General Hospital, 2 Columbia Drive, Tampa, FL 33606, USA. pshapshak@gmail.com.

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¹⁴Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria

¹⁵Department of Hematology, College of Medicine and University College Hospital, University of Ibadan, Ibadan, Nigeria

Abstract

The spread of hepatitis C virus (HCV) infection involves a complex interplay of social risks, and molecular factors of both virus and host. Injection drug abuse is the most powerful risk factor for HCV infection, followed by sexual transmission and additional non-injection drug abuse factors such as co-infection with other viruses and barriers to treatment. It is clearly important to understand the wider context in which the factors related to HCV infection occur. This understanding is required for a comprehensive approach leading to the successful prevention, diagnosis, and treatment of HCV. An additional consideration is that current treatments and advanced molecular methods are generally unavailable to socially disadvantaged patients. Thus, the recognition of behavioral/social, viral, and host factors as components of an integrated approach to HCV is important to help this vulnerable group. Equally important, this approach is key to the development of personalized patient treatment – a significant goal in global healthcare.

In this review, we discuss recent findings concerning the impact of drug abuse, epidemiology, social behavior, virology, immunopathology, and genetics on HCV infection and the course of disease.

1. Social Markers of Hepatitis C Virus (HCV) Infection and Drug Abuse

Risks for becoming infected with hepatitis C virus (HCV) and the subsequent course of the disease involve complex interactions among sociocultural context, ethnicity, volitional behaviors, genetics of pathogen and host, and the natural history of infection. These domains impact specific factors that include reduction of HCV load and spread of HCV disease and also have the potential to explain treatment outcomes. In order to improve the health prospects of people with HCV infection, it is crucial to characterize further behavioral markers of drug abuse and HCV infection. Examining the role of drug abuse in the HCV epidemic is critical. Not only is injection drug abuse the most important risk factor for acquiring HCV infection but drug abuse also affects treatment outcome. Injection drug users (IDUs) are more likely to face barriers to treatment access due to economic issues and because physicians frequently will not utilize antiviral chemotherapy for IDUs because of the lower adherence rates of IDUs and increased recidivism of injection drug abuse.^[1,2] Consequently, HCV-infected IDUs are likely to have a poorer outcome. Understanding risks and susceptibility to infection may help ameliorate these problems to the benefit of this large and growing group of at-risk individuals.

1.1 Epidemiology and HCV Disease

HCV is the most common blood-borne pathogen currently identified^[3] and its characteristics of high infectivity and long latency combine to make it especially difficult to control. Prime risk markers for HCV infection include re-use and sharing of needles/syringes (N/S)¹ and injection paraphernalia (that may be contaminated), plus sexual risk factors. Past or current injection drug use constitute the largest group of persons infected

with HCV in the US.^[1,4] Re-use of N/S in the therapeutic setting also is a risk marker for HCV infection. HCV infection can have a major negative impact on the infected individual and a significant cost to society due to loss of health, productivity, and possibly life. The HCV epidemic has followed a course in some ways similar to that of HIV due to parallel risk behaviors, although the two viruses show different viral properties and clinical outcomes.^[5–7]

HCV incidence rates among IDUs generally range between 10% and 20% per year,^[8–10] and this translates into a growing prevalence of HCV. A mean estimate for the prevalence of HCV based on a number of reports indicates that 180 million people have been infected with HCV worldwide.^[5,11–13] Because it is more infectious than HIV and is likely to have been present longer in human populations, HCV is five times more widespread worldwide than HIV.^[7,14] Estimates of HCV prevalence range from 3% globally to 6% in Central Africa.^[12] Approximately 1.6–1.8% of the US population (about 4 million people) are infected with HCV, and this is anticipated to rise.^[15–18] There is little agreement among different studies as to the prevalence of HCV infection, probably because of variations in prevalence in different geographical areas and over time, due to factors including population growth, population migrations, improvement in treatment, and changes in long-term survival. It is certain, however, that the HCV epidemic is large and expanding. A likely major factor driving this growth is injection drug use, and it is of great concern in view of the ease with which HCV infection spreads parenterally and the elevated levels of virus load attained.^[6,19] For example, in Miami, Florida, HCV prevalence among IDUs ranges from 36% to a staggering 90%.^[6] In other US major metropolitan areas that have cohorts of IDUs recruited from the street or from methadone-maintenance treatment programs (e.g. New York City and San Francisco), the prevalence of HCV approaches 95%. The Vancouver experience, monitored since 1994, indicates a prevalence of 82% as of 2001 among street-recruited IDUs. In addition, ambulatory and hospitalized veterans in the US have HCV prevalence rates that reach 35%.^[20–26] In Iran, HCV spread among injection and non-injection drug abusers has a prevalence rate of 45.4%.^[27] The Republic of Georgia has recently experienced an HCV epidemic, with 68.8% prevalence of HCV among IDUs.^[28] Interestingly in this setting, HCV transmission correlated with drug and N/S sharing behaviors and HIV transmission correlated with sexual risk behaviors. In many such largely epidemiological studies, additional laboratory markers of the spread of HCV would be useful to know, including the extent to which footprints of HCV infection such as HCV RNA and proteins, are found in re-used and shared N/S, cottons, cookers, and wash-waters.²

The incidence of HCV infection from 1997 to 2009 was 10–35% annually.^[8,9,29–32]

Although some studies report a slowing of HCV spread in the US between 1994 and 2004,^[32,33] this has not occurred in Canada (British Columbia) among IDUs.^[34] A factor related to these findings is that viral strains change over time (see discussion on evolution and genetics of viral strains in sections 2.4 and 2.5). It is puzzling that there are indications of increased HCV prevalence in the context of decreased HIV prevalence, in spite of the

¹The term ‘N/S’ refers to needles and syringes that are detachable or not detachable, used in venues of parenteral exposure.

²Prior to injection, cookers are used to dissolve the drugs and the cottons are then placed on the cookers to filter drugs. ‘Wash-waters’ refers to the water used to dissolve the drugs as well as that used to rinse N/S between use.

apparent similarity of the practices involved in the spread of both viruses.^[10,35,36] Harm-reduction methods tend to be less effective for HCV than for HIV. HCV and HIV are disparate viruses with different molecular characteristics and infectivity, making the task of preventing HCV infection distinct from preventing HIV infection. These differences may contribute to the sustained divergent outcomes of infection by these viruses. However, although these differences may be determined at the molecular-virological level, molecular studies at this level of the relationship between viral load and infectivity have been done for HIV1B, but not as yet for HCV.

1.2 Social and Community Degrees of Risk

There are markers of risk for HCV infection and hepatocarcinoma that are associated with a number of social and community factors, including income, ethnicity, and occupation. Specifically, factors associated with low socioeconomic class such as low income, African American and/or Hispanic ethnicity, and occupation as a street sex worker have been found to carry a significantly increased risk for HCV infection and hepatocellular carcinoma. Unfortunately, these are exactly the people who lack the social support and access to healthcare necessary to treat and diagnose their HCV infections. The continued spread of infection is also associated with younger individuals who are recruited into the drug injection venue. Some studies have found increased or decreased HCV transmission from HIV-infected individuals and, similarly, there are varying results concerning the risk of spread from IDUs to their sexual partners.^[7,37–40] However, extensive travel and/or engaging in sex was shown to further increase the risk of HCV infection already experienced by IDUs.^[31,39,41,42] Additional prospective studies are needed to more precisely quantify the relative contributions of sexual and IDU risks as markers for HCV infection.^[8,9,17,29–32,41–54]

1.3 Injection, Sexual, and Environmental Risks for HCV Infection

There are a number of distinct behaviors involved with the injection of drugs, each with its associated risks for infection. These include sharing and re-use of N/S, cottons, cookers, and wash-waters as well as front-loading and back-loading³ N/S^[6,8,9,30,55–63] Several harm-reduction interventions impact the various markers discussed so far. For example, the interventions include chemical means that have been demonstrated to inactivate HIV1B in N/S; the most successful and widely used method utilizes undiluted bleach. However, there are no actual experimental data regarding the ability of bleach or other chemicals to inactivate HCV in the context of injection drug use.^[55,56,64–67] Another harm-reduction intervention is needle exchange; this method has been crucial and successful to reduce the spread of HIV in those locales where it is allowed. Some studies indicate that needle exchange is up to 60% effective in reducing the use of HIV-contaminated N/S by IDUs. Despite the implementation of needle exchange, however, shared and re-used N/S, cottons, cookers, and wash-waters continue to contribute significantly to the spread of HCV, as well as HIV-1.^[9,30,55,57,68–77] Many social or behavioral markers are identified; however, it is laboratory studies that are needed to translate markers into quantitative laboratory tests.

³During front-loading and back-loading, respectively, the N/S are filled from the front of the syringe barrel or back of the syringe barrel. These are additional risk factors for HCV infection when drugs and paraphernalia are shared.

There are additional markers of HCV infection involving environmental viral contamination. This type of risk for HCV infection occurs when individuals are exposed to HCV coated onto surfaces in the drug abuse and medical venues, and where exposure can result from penetration through breaks in the skin or internalization of the virus by other means. Lankenau and Clatts^[78] showed that crack cocaine inhalation and dual user IDUs have an additional avenue for HCV infection involving contaminated surfaces used in drug aspiration, for example at crack houses. Additional risk situations by which HCV may also be transmitted include needle stick injuries, acupuncture, tattooing, sharing razors, and maternal-fetal transmission when the mother is HCV-infected.^[5,15,78–83] HCV infection also occurs during hemodialysis due to environmental contamination and noncompliance with standard precautions at hemodialysis facilities.^[84] Blood transfusion used to be a frequent means through which HCV infection occurred; however, since 1990, it has become common practice for donated blood to be screened for HCV antibodies. Such screening reduces the risk of transfusion-associated HCV infection to <1 case in 100 000 transfused units.^[33] Finally, although alcohol consumption is considered innocuous by many consumers, there is a high prevalence of HCV infection in alcoholic patients (up to 30%) in the absence of other risk factors.^[85]

The molecular markers of HCV infection discussed in sections 2–4 occur in individuals who carry out risk-associated behaviors within the settings discussed in section 1. Table I highlights key social risks for HCV infection and drug abuse, which should be taken into consideration in a comprehensive approach to prevention.

2. Viral Markers of HCV Infection and Drug Abuse

2.1 HCV Markers of Detection and the Window of HCV Infection

It is clearly important to be able to detect HCV in people whose behaviors include those risks discussed in section 1. Risks that support screening individuals for HCV infection include IDU history, having received clotting factors before 1987, receiving blood transfusions or organ transplants prior to 1990, receiving chronic hemodialysis, having liver diseases, being HIV positive, having tattoos and body piercing, engaging in high risk sexual behavior (especially sex with IDUs), and crack smoking from shared pipes.^[86–89] There are three main diagnostic test categories for the detection of HCV: antibody/antigen tests, nucleic acid assays, and certain liver function enzyme assays (the first two are discussed here and the third in section 4) [table II]. The enzyme-linked immunosorbent assay (ELISA) is widely used for hepatitis C screening generally in serum. It most commonly detects HCV proteins – especially HCV proteins C22, C33, and C100. It has a low cost, is easy to use, and has low variability. Second- and third-generation ELISAs are more sensitive and specific, with sensitivity and positive predictive values that are up to 95% in highly prevalent populations. The third-generation ELISA is used in blood supply screening. Improved assays include HCV viral protein NS5 detection to increase sensitivity.^[103,106,137,138]

The recombinant immunoblot assay (RIBA) is less sensitive but more specific than ELISA and more labor intensive to perform; it is therefore not used as a screening test. Populations with negative RIBA do not generally receive further evaluation. If RIBAs are indeterminate

or positive, then further evaluation includes detecting HCV RNA using polymerase chain reaction (PCR).^[103,104,138–141]

There are two primary methods for nucleic acid assays for HCV RNA detection: PCR and branched DNA (bDNA) assay. HCV PCR detection is highly sensitive and specific, and is used for diagnosis and confirmation of HCV infection (especially in seronegative symptomatic patients) and to guide treatment response. HCV PCR qualitative and quantitative assays are commercially available and are able to detect HCV RNA at levels as low as 100 copies/mL. However, PCR results are often variable between different laboratories, whereas bDNA varies less but is less sensitive than HCV RNA PCR. bDNA assays can detect down to 200 000 genomics equivalent/mL. As a result, 30% of patients who are HCV RNA PCR-positive are bDNA-negative.^[99,100]

When an individual embarks on injecting drugs, HCV seroconversion is likely to occur within 3.4 years (average). The importance of coming to an understanding of this time of vulnerability is that during this period there is the opportunity to reduce the chances of HCV infection through intervention and treatment.^[101,142] The period has two components. The first relates to the infectivity of HCV transmitted by IDU N/S, paraphernalia, and wash-waters due to risk activity. The second relates to the duration between infection and the onset of replication of the virus and subsequent seroconversion, such that the virus or antibodies to HCV proteins are detectable in IDU blood. One estimate of the duration of seroconversion of IDUs is 2–12 months using an ELISA method.^[143] Blood transfusion studies^[97] indicate a window of 12 weeks based on an enzyme immunoassay (EIA) version 1 method and 10 weeks based on an EIA version 2 method. The second-generation ELISA mentioned also reveals a window period of 16–20 weeks. The third-generation ELISA mentioned above has a window period of 14–21 days.^[106,137,138] PCR and bDNA assays indicate an HCV detection window of 15–25 days. However, these assays are not easily used, as they offer difficult protocols and are not readily available in countries and regions where they are most needed.^[143] Thus, to bolster the attack on HCV infection among IDUs, it is not only relevant to understand and optimize paradigms for the abrogation of the spread of HCV in this population but also to foster and use molecular methods of HCV detection with increased sensitivity and specificity that are feasible to use in the locales where the information is needed.

2.2 HCV Load

Viral RNA is the surrogate marker used as a quantifiable indicator of risk for infectious virus in most areas of virology. This is also the case for HCV, although there have not been as extensive studies for HCV as for HIV ^[25,95,102,144] The prevalence of HCV infection, virus load, and strains (see section 2.5 regarding strains and genotypes) in IDUs affects the presence of HCV in their N/S and paraphernalia and thus influence the spread of HCV that results from injection behavior through re-use and sharing of these items. Quantification of both viral RNA and infectious virus is a powerful laboratory method in concert with epidemiological methods for estimating the spread of viral infection and disease. Although it has been difficult to culture and quantify HCV infectious units *ex vivo*, a recent publication describes the ability to culture HCV genotypes 1, 2, 3, and 4 in human hepatocytes cultures,

and this may lead the way to routine culture of patient specimens.^[145] This will then allow correlation of HCV RNA load with HCV infectious viral load since not all viral RNA is generally infectious.^[146] It is understandable that the precise probability of infection estimated for HCV following each IDU's intravenous drug injection is under contention. The estimates range from 1.4% to 9%^[147,148] and even to up to 30%.^[68] Crucial variables that require detailed study are the participant behaviors, the stability and infectivity of the HCV strains, and the virus load of each inoculum at the moment of injection.

2.3 Impact of HCV Extrahepatic Replication on Injection Drug Abusers

During the drug injection process, blood is passed among IDUs when N/S, cottons, cookers, and wash-waters are reused and shared. Yet, HCV present in blood may be consequent to HCV production by the liver as well as by other extrahepatic sites including blood cells. During the last 17 years there have been many reports supporting the possibility of extrahepatic sites of HCV replication in infected patients. These investigations relied on the ability of high sensitivity and specificity PCR to detect negative strand HCV, the obligatory intermediate molecule produced during HCV replication. It should be noted, though, that an early study did not detect any evidence for extrahepatic replication of HCV;^[149] however, other studies did.^[107,108] Replicating HCV was detected in patient-derived hematopoietic cells and bone marrow,^[150–152] macrophage/ monocytes prepared from peripheral blood mononuclear cells (PBMCs),^[116,127–131,153] neutrophils,^[154] CD8-depleted PBMC preparations,^[127] patient CSF and brain,^[109,132,155–157] and in monocytes derived from HCV-infected patients and transplanted into severe combined immunodeficiency (SCID) mice.^[150] HCV replicating in these various cell types may be excreted into the blood and contribute to the infectious quasispecies involved in the spread of the virus by IDUs because, as discussed above, their blood is transferred directly during their injection procedures. Moreover, different virus-harboring cells may vary in their susceptibility to treatment. For example, because antiviral compounds do not easily cross the blood-brain barrier, the brain is frequently intransigent to treatment and could be an additional source of HCV. The quasispecies contributed by these cells to the blood could be markers for virulent and non-responsive HCV infection for IDUs. Additional quasispecies may be involved for other modes of risk of HCV transmission as discussed above. Suffice it to say, the study of specific viral markers and extrahepatic sites needs development because different risk activities may involve different HCV quasispecies derived from different extrahepatic cell types. Consequently, treatment requirements and outcomes may differ.

2.4 HCV Superinfection, Reinfection, and Protection

Infection with multiple strains of HCV is known to occur^[117] and has been called 'superinfection', 'dual infection', and 'co-infection'. As discussed below, most of the paradigms in the analysis of HCV were produced in prior work with HIV. Thus, 'superinfection' is a term used when a second infection with the same virus (e.g. HCV or HIV) occurs after the initial infection, while 'co-infection' is used both when the first and second infections occur at approximately the same time or when people are infected with both HIV and HCV. Not only is this confusing due to the different meanings of the term 'co-infection', but the duration of time elapsed between infections used to distinguish 'superinfection' is often ambiguously defined. To avoid confusion, generally the terms used

are ‘HCV-HIV co-infection’, ‘HIV-multiple infection’, and ‘HCV superinfection’ or ‘HCV-multiple infection’ to refer to individuals with both HIV and HCV, those who are infected more than once with different strains of HIV, those infected more than once with different strains of HCV, and those infected with different strains of HCV sequentially in time, respectively.

Less is known about HCV multiple infection or superinfection compared with HIV. Limited data suggest that superinfection occurs at a similar rate as *de novo* infection during early infection, but little is known about occurrence rates in established infections. The frequency of HCV superinfection in a cohort of recently infected young IDUs was 20%, with an HCV incidence of 25%.^[118] However, no long-term HCV seroconverters were available as a comparison group to determine if superinfection was less likely in those with established infection. In a small study of 111 HCV-infected, RNA-positive IDUs in Australia, two different cohorts of superinfected individuals were studied. Changes in the dominant HCV genotype occurred over a range of 215–588 days between studies of three IDUs who reported sharing injection equipment. These results were suggestive of superinfection, which was calculated at a rate of 26.4 changes per 100 person-years.^[158]

Among IDUs with established infection in San Francisco, individuals with resolved infection did not become viremic over the study period (1 year), and individuals with persistent viremia did not change genotype.^[119] That there is some protection from initial HCV infection is suggested by studies demonstrating that surviving, chronically HCV-infected IDUs were HCV RNA-negative after 55 months’ follow-up, with a rate of reinfection of 0–4.1 cases per 100 person-years.^[159–162] Similarly, among IDUs with untreated resolved HCV infection, of whom 42% reported ongoing injection, the reinfection rate was extremely low (0.47 cases per 100 person-years), suggesting some protection occurred due to resolved infection, although risk behavior for reinfection (i.e. continued sharing injection equipment) was not considered in the analysis.^[163] However, reinfection does occur even after successful suppression post-treatment or after self-resolving infection in IDU patients.^[120–124]

Table II shows viral markers of HCV infection and the methods used to detect them.

2.5 HCV Genotypes and Evolution

HCV is an enveloped, single-stranded RNA virus in the Flaviviridae family and *Hepacivirus* genus, which has been classified into six phylogenetically well defined clusters, denoted genotypes (labeled 1 through 6), with about 50 subtypes (e.g. 1a, 1b).^[111,112] The different genotypes differ in biological properties, diversity, and geographic distribution. Additionally, within each host, HCV replication produces large numbers of mutant viruses known as quasispecies, due to lack of proofreading abilities of the polymerase. This creates a major challenge to immune-mediated control of HCV, is associated with variable clinical course, and contributes to the primary difficulties in vaccine development.

At the population level and geographically, genotypes and subtypes can be used to map HCV transmission patterns. Genotypes and subtypes can also be applied to characterization of HCV subtypes in relation to risks for HCV infection, liver disease, as well as prediction

of treatment outcome.^[164] In the US and globally, IDU migratory patterns are likely to contribute to HCV strain variability and serve as markers for differences in risk behaviors.^[60,165–167] Traveling IDUs have additional risk factors for HCV infection, including a larger number of IDU and sexual partners and greater sharing practices in the preparation of drug equipment (compared with non-traveling IDUs).^[31]

It is common to find multiple circulating subtypes of HCV within the same population, and genotypic patterns vary by geographical region and country. Table III summarizes geographic regions where various genotypes/subtypes of HCV were found, and, where possible, indicates predominant genotypes/subtypes reported among IDUs and non-IDUs. Note that the same subtypes can occur in more than one risk classification. However, there has not been uniformity in the study designs of the many reports mentioned.

Here we provide additional details of HCV genotype as markers of the route of infection. Routes of infection may have contributed to strain variation markers over time and geography.^[229] Individuals infected with HCV genotype 1, the most virulent HCV strain, regardless of ethnicity, have poorer response outcomes to interferon- α (IFN α) therapy than those individuals with other genotypes.^[230,231] Baseline viral load, disease characteristics, and medication consumption do not explain these differences.^[232] HCV genotype 1b is most associated with greater resistance to treatment and worse outcome (i.e. particularly severe liver diseases and hepatocellular carcinoma) compared with genotypes 2 and 3. Although genotype 1 appears to be associated with poorer treatment outcomes than all other HCV genotypes, there is also some evidence that genotype 3 may be associated with reduced sustained virologic response (reduction in virus load) compared with genotype 2.^[233] Additionally, HCV-HIV co-infected individuals, regardless of HCV genotype, have lower response rates to therapy.^[231] These differences in virulence, treatment, and disease progression by genotype are likely to have an impact on new vaccine development, as vaccines are likely to be effective only against specific genotypes of HCV.

When analyzed in more detail, different HCV subtypes may also cluster by risk factor, and genotypic clustering with IDUs has been reported in many settings. In Italy, IDUs were more likely to be infected with genotype 1a or 3a, and transfusion-acquired cases were predominantly 1b.^[185] In Poland, chronic liver disease patients with HCV genotype 1 were more likely to have been infected through blood transfusion, while those with genotype 3 were more likely to report injection drug use as their main risk factor.^[193,194] In St Petersburg, Russia, long-term dialysis patients were predominantly infected with subtype 1b, whereas infectious disease clinic patients were more likely to be infected with 3a, and IDUs had equal distributions of 1a and 3a – suggesting bridging or overlap between IDUs and infectious disease clinic patient populations.^[192] In the Netherlands, genotypes 1a and 3a tend to be associated with IDU infection, while those infected through blood transfusions and other medical procedures had much higher prevalence of 1b, but were also infected with 1a, 3a, 2a, 2b, and 4^[54] – suggesting subepidemics of HCV with overlap between risk groups.

IDUs co-infected with HCV and HIV had differences in predominant HCV genotypes compared with monoinfected IDUs. In the Republic of Belarus, co-infected patients had

HCV subtype 3a and monoinfected patients had HCV subtype 1b.^[234] Moreover, co-infected IDUs in Guangxi, China, had a higher proportion of 1a infections than did monoinfected IDUs.^[195] Among IDUs recruited from drug treatment centers in Poland, genotypes 1 (38.7%) and 3 (37.8%) were present and 23.4% were infected with genotype 4. However, 88.5% of those co-infected with HIV and HCV were infected with genotype 4, suggesting that genotype 4 is a marker for HIV-HCV co-infected IDUs in that geographical locale.^[193,194]

Predominant HCV genotypes may change over time, as suggested by the relative dominance of specific subtypes in different age groups.^[185,235,236] Among 747 HCV-infected patients in Germany, younger patients (<40 years) had higher proportions of infection with genotypes 1a, 3a, and 4a than older patients, who were primarily infected with 1b.^[181] A large part, but not all of these differences, could be explained by transmission through injection drug use. In a study of 535 sequences in Southern France, subtype 1b was associated with being older than 60 years of age.^[178]

Change in subtypes may also be mediated by migration of individuals from different countries. In a study of 420 HCV-infected patients in Australia, subtype 1b was associated with foreign-born individuals, subtype 4 was associated with migration from Egypt and 6 with migration from Vietnam.^[225] In Martinique in the Caribbean, genotypes 1a, 3, and 4 were associated with IDUs, while 1b was associated with all other risk categories, with evidence of clustering in genotype 4 with sequences from IDUs in France, suggesting recent migration and introduction of HCV genotype 4 through IDUs.^[195,172] Changes in the predominant subtype among IDUs within a given area have also been observed. Chronic liver disease IDU patients in Poland were more likely to be infected with HCV genotype 3 (57%) than 1 (35%). Subtype 3a has increased among IDUs since 2000, suggesting a shift in the subtype of HCV associated with IDUs.^[193,194]

The use of specific drugs may be linked to subepidemics. Among 101 HCV RNA-positive patients in Prague, Czech Republic, HCV subtype 1b was more common among IDU methamphetamine injectors than infection with 1a,^[133] whereas IDU heroin injectors were equally likely to be infected with HCV subtype 1a and 1b.^[183] Further analysis of 1b specimens for specific mutations in the 5' non-coding region revealed that a molecular variant with T → C transition at position -94 was found only among the IDUs, suggesting a specific network of HCV transmission within this population. In Taiwan, genotypes 1a, 6a, and 3a predominated among HIV-HCV co-infected IDUs; however, the predominant circulating genotype in the general population was 1b.^[196,197,237,238] There were also associations with drug trafficking lanes from China into Taiwan of the three prevalent HCV subtypes 1a, 6a, and 3a.

In some regions there is no apparent difference in the genotype of HCV between different risk groups. 119 patients in a hospital in Northern Brazil were infected with genotypes 1 (46.9%), 3 (34.4%), and 2 (8.3%), and these genotypes were distributed similarly across risk categories.^[173] Similarly, among 156 patients in Iran, IDUs and non-IDUs were equally infected with genotypes 1a and 3a, the two predominant genotypes.^[210] Lack of a difference in genotypes by transmission group may be suggestive of overlap between risk groups, or

long-standing infection with specific genotypes, and little migration of new genotypes into the population.

While change in predominant genotype and superinfection with multiple genotypes is important in understanding and preventing HCV transmission and ensuring effective HCV treatment among all risk groups, identification of superinfection by a single circulating subtype is also important. Though change in HCV quasispecies within an individual is probably due to superinfection,^[125] one report by Laskus et al. found intraindividual quasispecies stability.^[110] This study of HCV quasispecies in 236 HCV- and HIV-positive patients used single-strand conformation polymorphism (SSCP) analysis of the hypervariable region 1 of the second HCV envelope gene and found HCV quasispecies stability was associated with concurrent elevated HIV and HCV loads. Recombination events among superinfection and prior HCV strains may contribute to variations in quasispecies detection.

Some molecular studies do not report risk factors (e.g. sharing of injection equipment vs non-IDU risks), which is unfortunate because knowing and understanding risk factors is important for developing appropriate interventions and treatment paradigms. This information would greatly assist in the personalized medicine approach.

3. Host Markers of HCV Infection

3.1 Immunopathology of HCV Infection and Drug Abuse

The role of acquired and innate immunity and the effects of abused drugs in HCV-infected individuals are currently under study. The effects of HCV infection on the immune response are complex and operate at several levels. The pathogenesis and outcome of HCV infection are modulated by the host immune response, which is capable of clearing most viruses in the acute phase of infection. HCV, HIV, and other viruses can escape immune surveillance by a variety of mechanisms, including subversion of the innate and acquired immune responses by viral factors, cytotoxic T-cell dysfunction, emergence of viral escape mutations against humoral and T-cell immunity, cytokine production, and establishment of persistent infection. To date, no vaccines exist to combat HCV at any of these levels. Viral strains are important in their interplay with immunity (see section 2). Some of these issues have been reviewed previously;^[239–241] here we discuss the effects of abused drugs on the immune response and on HCV replication because HCV infection occurs predominantly in the context of injection drug abuse. We seek to extricate and delineate key aspects and issues to assist the clinician in identifying possible treatments. Moreover, as complex as HIV studies have been, HCV studies are also very complex.

3.1.1 HCV Infection and Acquired Immune Dysfunction—Immune events that occur in the immediate and early phase post-HCV exposure play a crucial role in determining outcome, and virus-specific T cells have a role in directing the immune response towards a successful outcome.^[11] HCV-specific CD4+ T-cell responses are relatively weak or absent during the course of chronic HCV infection.^[242] This damage consequent to HCV infection also appears in the form of markers of CD8+ T-cell exhaustion and shows specificity for HCV infection. The markers in such studies are termed inhibitory

receptors and up to seven are expressed increasingly with greater HCV load.^[243–246] A comparison of IDUs versus non-IDUs has not yet been reported for these markers; this needs to be done to ascertain if there are IDU-specific CD8+ T-cell markers of exhaustion.

An extensive and detailed study of the HCV-specific immune responses to HCV infection, HCV load, and frequency of HCV exposure in IDUs concluded that those long-term IDUs (after injecting for >10 years) for whom virus load was suppressed were less likely to experience another HCV infection.^[247] Cell-mediated immunity (CMI), assessed by means of enzyme-linked immunospot (ELISPOT), IFN γ secretion, and cytotoxicity assays, correlated with patients who suppressed virus replication. Humoral immunity, assessed by EIAs and *in vitro* neutralization assays, correlated with patients who showed continued viremia. Another more recent study of IDUs showed that both HCV-seropositive and HCV-seronegative IDUs who had injected for 6 years had cytotoxic T lymphocyte (CTL) responses specific for HCV oligopeptides.^[248] The authors concluded that HCV-seronegative IDUs with CMI positive for HCV oligopeptides confirmed their high-risk exposure to HCV.

An important line of immunological defense is provided by natural killer (NK) cells. In African Americans and Caucasians with low HCV viral loads, NK cells were protective. Some human leukocyte antigen (HLA) class I molecules interact as a bridge with killer cell immunoglobulin-like receptors (KIRs) of NK cells modulating HCV infection outcome via regulating immune regulatory cells as well as the molecules they produce.^[249] Marker molecules that helped control HCV included an NK inhibitory receptor-2DL3 (KIR-2DL3) and its ligand, HLA C group 1 (HLA-C1). An inhibition of the KIR complex resulted in increased protection against HCV infection. However, at higher virus load these effects appeared to become vitiated.^[250]

A recently described and defined T-cell phenotype, T-helper (T_h)-17, joins the T_h1 and T_h2 group of T cells. The T_h1 and T_h2 phenotypes served as mechanistic models for immunology in general and specifically for immunity to viral infections, including against HCV, and this group now includes the T_h17 phenotype. The immunological characteristics of the T_h17 phenotype have been reviewed in detail elsewhere.^[251] Key features of this phenotype are that T_h17 cells secrete inter-leukin (IL)-17, IL-21, and IL-22, and are resistant to CD4+CD25+FoxP3+ T-regulatory (T_{reg}) cells. A search of the literature (PubMed and Google) for the possible involvement of T_h17-type CD4+ T cells in susceptibility to HCV infection indicated one publication.^[252] Data in this publication provide a preliminary demonstration that there is some protection against HCV infection by T_h17 cells. However, once the individual is HCV-infected, T_h17 cells are suppressed by IL-10 and transforming growth factor- β (TGF β) production (stimulated by HCV nonstructural protein 4 [NS4]).^[252] One aspect of this finding is puzzling because TGF β is reported to be produced by T_h17 cells in some studies.^[253] Thus, the autocrine and paracrine control of T_h17 cells still requires further investigation in the context of HCV infection.

T_{reg} cells are central in the response to HCV infection and progress of disease. This subpopulation of T (CD3+) lymphocytes is Foxp3+ and mostly CD4+ (CD4+CD25+FoxP3+), which function to inhibit CD4- and CD8-driven cellular immune

responses by regulating *IL2* gene expression. They down-modulate aggressive T-cell responses to HCV infection, decrease autoimmunity,^[254] and play a significant role in relation to the pathogenesis of acute and chronic HCV infection^[255,256] by suppressing HCV-specific T-lymphocyte proliferation and cytokine secretion during chronic HCV infection.^[257] However, the HCV-specific T_{reg} cells in HCV-seropositive patients, though increased in number, are phenotypically and functionally indistinguishable from FoxP3+ T_{reg} cells in uninfected subjects.^[257,258] The fundamental cellular mechanism behind the general lack of effective antiviral immune response in patients with HCV infection is unclear and most likely acts at multiple levels on innate and adaptive cellular immune events.^[259]

The balance between antiviral effector immune cells and T_{reg} cells may contribute to the prognosis of HCV infection and the induction and maintenance of HCV persistence.^[260] Some T_{reg} cells may contribute to HCV pathogenesis by suppressing antiviral immune responses, and other T_{reg} cells may be beneficial and assist in recovery after virus suppression by inhibiting over-activation of the immune response to inhibit continued detrimental inflammation.^[261,262] In this way, HCV-specific CD4+CD25+ T_{reg} cells appear to accompany successful viral clearance^[263] and thus may be associated with reduction of collateral damage once virus is cleared. Some T_{reg} cells are possibly implicated in host immune tolerance during HCV infection.^[257] Finding differences is not an easy task because T_{reg} responses may have personalized patient-specific signatures, since they exhibit subtle differences from patient to patient.^[264,265]

B-lymphocyte malfunction contributes to immune dysregulation in HCV-infected patients. Clinical issues have been reviewed,^[266,267] which include production of autoantibodies, B cell lymphoma, and mixed cryoglobulinemia (a systemic small-vessel vasculitis) in patients with chronic HCV infection.^[161,266,268] HCV may persist by internalization into B cells followed by transmission of the eclipsed or hidden HCV particles into the liver (promoted by CD40 ligand and IL-4).^[269] Moreover, HCV persistence contributes to resistance of the CD5+ B-cell subpopulation to apoptosis leading to the B-cell lymphoma.^[267] These particular B-cell-related immune defects appear to be independent of type of risk for HCV infection and alcoholism,^[270] although more information is needed in relation to other drug abuse risk factors.

Many attempts at HCV vaccine production are in progress and involve several approaches, the details of which are beyond the scope of this review. Potential vaccine strategies touch on CD4- and CD8-induced responses, cross-neutralization of antibodies, CTLs, oligopeptides, vector-based delivery systems for minigene vaccines, and of course the problem of variation due to HCV quasispecies and genotypes.^[98,265,269,271–276] Overall, immune response, intensity, and ability to control and suppress HCV replication may vary across physiological sites as well as among different vaccine types.

3.1.2 HCV Infection and Innate Immune Dysfunction—Genes of innate immunity associated with HCV infection include Toll-like receptors (TLRs) and cytokines. Mutations in TLRs and cytokine genes can cause immune dysfunction (see section 3.2, related to HCV infection and genes such as IL-1 α , IL-1 β , IFN γ , IL-10, and MHC class II.) The canonical

innate immune response genes are the TLRs, whose ligands include lipoproteins, lipopolysaccharides, peptidoglycans, lipoteichoic acids, fungi, viral glycoproteins, flagellin, single- and double-stranded RNA, unmethylated CpG DNA, and pathogen-associated molecular patterns expressed on infectious agents.^[277,278] A study of all ten TLRs showed that TLR2 and TLR6–10 mRNAs were up-regulated in T cells and monocytes of patients with chronic HCV infection compared with negative controls. TLR4 was up-regulated in their T cells while TLR5 was up-regulated in monocytes.^[279] Another study found TLR4 was the only TLR up-regulated and only in PBMCs.^[280] Elevations of IFN β and IL-6 were thought to be due to the TLR4 elevation in these patients, since small interfering RNA (siRNA) against TLR4 abrogated cytokine production. An examination of all the HCV proteins showed that only NS5A exerted this effect and it was mediated at the level of the TLR4 promoter. In another study,^[281] PBMC expression of TLR2 and TLR4 occurred in HCV-infected patients. Increased circulating TNF α levels and hepatic necroinflammatory disorder was correlated significantly with increased monocyte expression of TLR2 but not TLR4. Another study correlated TLRs with cytokine expression.^[282] TNF α , IL-6, and IL-12 p35 were also found to be increased in PBMCs. In both patients and controls, incubation with HCV core protein resulted in decreased TLR4 and TLR7 whereas TLR2 expression was increased. A further study showed that TLR2 expression was decreased in patient dendritic cells (DCs), and this was correlated with decreased ability of these DCs to proliferate compared with controls.^[283]

TLRs are central to the ability of DCs to sense virus infection.^[284,285] Some work pointed to malfunctions in TLR signaling in HCV-infected patients. TLRs and retinoic acid-inducible gene-I (RIG-I) are both at the base and participate in cascades of immune reactions, including production of inflammatory cytokines and type 1 IFNs. In myeloid DCs from HCV-infected patients, despite increased levels of TLR2, TLR4, and RIG-I, the production of IFN β , TNF α , and IL-12p70 is lower in these patients compared with uninfected controls,^[286] suggesting down-stream damage in these pathways.

There is separate control of expression of TLR/RIG-I and inflammatory cytokines. In DCs, increased TLR2, TLR4, RIG-I, and LGP2 (also known as DHX58) levels were found along with unchanged levels of expression of TLR3 and melanoma-differentiation-associated gene 5 (MDA-5; also known as interferon-induced with helicase C domain protein 1 [IFIH1]). TIR-domain-containing adapter-inducing interferon- β (TRIF) and TNF receptor-associated factor 6 (TRAF6), which are adapter molecules that transmit TLR3- and TLR4-dependent signals, were decreased in the patients versus controls.^[286] Other studies also demonstrated the IFN β interference with HCV replication and increased TLR3 and TLR4 in HCV-infected patients.^[287] The potential damage due to abused drugs is unexpectedly found to relate to TLR4. Hutchinson and colleagues^[288] demonstrated that opioids, including opium (a major IDU drug), morphine, methadone, oxycodone, buprenorphine, fentanyl, and meperidine (pethidine), activate TLR4, and antagonists (e.g. naloxone) specifically inhibit TLR4. The inhibition occurs even in the presence of lipopolysaccharide.

An association was found for single nucleotide polymorphisms (SNPs) in some TLRs with the outcome of liver transplantation in chronic HCV-infected patients.^[289] *TLR2* Arg753Gln, *TLR4* Asp299Gly, and *TLR4* Thr399Ile SNPs were evaluated in 92 HCV+ liver

transplant patients. Kaplan-Meier estimation and Cox proportional hazard analysis determined that 13% of patients had the variant allele *TLR2* Gln753, and 35% had variant *TLR4* alleles Gly299 and/or Ile399. *TLR2* Gln753, when homozygous, was associated with allograft failure and mortality in HCV-infected patients.

3.1.3 Drug Abuse and Immune Dysfunction—IDUs engage in polydrug use by drug injection and non-injection. Polydrug use by IDUs includes ketamines (club drugs), opiates, cocaine, amfetamines, marijuana, and alcohol. This results in a wide range of effects and polydrug interactions that are both physiological and psychological. Moreover, the addition of opiates to the drug mixtures often results in drug overdose.^[78,290–294]

Neuman and colleagues demonstrated effects of drug abuse on the immune response in HCV-infected IDUs.^[2] This population have severe complications due to drug interactions with illegal drugs (e.g. opiates), treatment drugs (e.g. methadone), psychoactive drugs, antipsychotics, and antiretroviral therapy. Dysregulation of cytokine products of T_H1 and T_H2 cells increases susceptibility to HCV infection.^[295] Immune dysfunction occurs in HCV-infected cocaine and heroin ('speedball') injectors.^[296] These individuals over-produce both T_H1 (IL-2, IL-6, IL-12, TNF α , and IFN γ) and T_H2 (IL-4, IL-10, and IL-12) cytokines in their plasma in stimulated peripheral blood T cells, and there is a reduction of lymphoproliferative activity compared with healthy controls. Neuman and colleagues^[2] concluded that combined opioid and cocaine use results in dysregulation of T_H1 and T_H2 cells in HCV-infected individuals. Earlier studies showed HCV infection stimulates cytokine production.^[91] However, with HCV infection and alcohol intake, T_H1 and not T_H2 cytokines were elevated.^[297] Up-regulation of intrahepatic T_H1 cytokines, IL-2 and IFN γ , and down-regulation of T_H2 and in serum, IL-10 increase was detected in prior studies.^[296,298,299] Similarly, in these patients, IL-4 and IL-2 increased and T_H1 IFN γ decreased.^[299] No changes in IL-10 or IFN γ were found in HCV-infected serum in an earlier study.^[300] Developing specific cytokine and chemo-kine profiles and signaling pathways could be useful additional diagnostic markers for HCV therapeutic success or failure, and manipulating cytokines may aid in HCV treatment.^[248,254,301–303]

Morphine can stimulate HCV replicon RNA expression in cultured hepatocytes,^[304] but b-funaltrexamine and naltrexone (opioid receptor antagonists) counteract these effects. Morphine activates the nuclear transcription factor κB (NF κB) promoter; this is inhibited by caffeic acid phenethyl ester. However, morphine withdrawal and naloxone treatment to block opioid receptors also favors HCV replication, *in vivo*, in HCV-infected patients, probably by a mechanism that reduces levels and effectiveness of IFN α as well as stimulating HCV replication.^[305,306] Opiates and cocaine stimulate HIV replication via cytokine growth factor signaling. This is important for IDUs as they co-ingest cocaine and alcohol as part of their polydrug use,^[78,293] and cocaethylene is the by-product of cocaine and alcohol co-ingestion. Moreover, cocaethylene exerts severe pharmacologic effects and stimulates HIV replication.^[307–313] However, the effects of cocaethylene are unknown for IDU HCV-infected individuals who co-ingest cocaine and alcohol, including those IDUs who co-ingest these drugs with opiates. Along these avenues of investigation, a recent study of heroin IDUs in China showed that there was slow clearance of HCV.^[314] As discussed,

this could be due to several factors, including viral genotype, heroin-induced dysregulation of the immune system, and abused drugs that stimulate HCV replication.

3.2 Host Genetics and Susceptibility to HCV Infection

Many studies produced genetic information on HCV-acquired immunity as markers of HCV infection and HCV disease progression. The diagnosis of genetic susceptibility to HCV is increasingly important as the methods become more sophisticated.^[315–318] High throughput SNP detection, sequencing, and bioinformatics technology are essential to address this task. In addition, novel nanotechnology is being developed and could be used in the search for genetic markers.^[319] Several reviews in this field show little overlap, as this is a fast progressing and expanding field.^[239,320–324] However, concomitant with the rapid progress, repeat studies and confirmation of published work are needed. A wide variety of outcomes of HCV infection – including asymptomatic clearance, chronic infection leading to cirrhosis, hepatocellular carcinoma, and liver failure – are associated with host genetic factors. A complication in genetics studies is that ethnic variation causes difficulty in reaching a consensus definition of host-dependent genetic factors that govern the susceptibility and course of HCV disease.^[325] Candidate genes associated with HCV infection and disease include immune response-related genes,^[295,318,326] which are a subset of more than 300 immune deficiency-related genes that appear to be involved in susceptibility to infectious diseases.^[295]

Among candidate immune responses genes, cytokines are associated with susceptibility to disease resulting from HCV infection. For example, IL-1 α and IL-1 β are pro-inflammatory cytokines associated with susceptibility to liver cirrhosis and infection by HCV,^[322] and IL-10 is an anti-inflammatory cytokine associated with HCV infection. Moreover, genetic susceptibility to HCV is influenced by alleles in the *IL10* gene promoter.^[323] IL-12 stimulates IFN γ production and the 1188C allele of the *IL12B* gene is associated with better response to anti-viral combination therapy^[327] and the 1188 CC genotype promotes resistance to severe liver disease in HCV genotype 3-infected patients.^[328] For IDU patients, the *IFNG* gene SNP 764G/C was associated with HCV treatment response (e.g. IFN α -treatment response) and also spontaneous recovery (e.g. spontaneous viral clearance).^[329] Promoter polymorphisms in the gene encoding IL-18, a pro-inflammatory cytokine that is elevated in HCV infection, are implicated in the pathogenesis of chronic HCV infection. IL-18 is an important mediator of the T_H1/T_H2-driven immune response and is elevated in HCV infection (probably more associated with liver infection than blood virus load).^[330] Some studies demonstrated relationships between the *IL18* promoter SNPs – 607C/A and –137G/C with chronic liver disease and hepatocellular carcinoma, and a haplotype containing the –607A and –137C alleles with high HCV clearance. In these patients, chronic liver disease severity was correlated with IL-18 levels.^[331,332]

HLA genes initiate and regulate immune responses by presenting foreign or self-antigens to T lymphocytes. Immune functional impairments during HCV infection as well as susceptibility have been in part attributed to host genetic factors such as the host HLA genes.^[333] These genes are likely to yield markers of susceptibility to HCV infection.^[323,334] Extended haplotypes including class I *HLA-B54* alleles are closely

associated with the progression of HCV-induced liver injury, whereas haplotypes including the class II alleles *HLA-DRB1*1302-DQB1*0604* are associated with low hepatitis activity in chronic HCV infection.^[335] In a different ethnic sample, *HLA-DR11(5)* [a sub-serotype of DR11] has been associated with less advanced HCV-related liver disease, and the trans-dimer *DQA1*0201-DQB1*0201* may be predisposing to, and the *DRB1*1104*, *DQA1*0501*, *DQB1*0301* haplotype protecting from, chronic hepatitis C.^[336] *HLA-DRB1*11/*12* alleles and *DQB1*0301* are associated with HCV clearance. In contrast, *HLA-DRB1*03* and **07* are associated with HCV susceptibility in multiple ethnic groups.^[249] *HLA-DRB1*03* and **07* may be indicative of non-responsiveness to HCV vaccines still under development.^[249] Cryoglobulinemia is a consequence of B-cell dysfunction that occurs in patients with HCV infections, and *HLA-DQB1*11* and *HLA-DR3* were associated with cryoglobulin production.^[240] *DQB1*0301* and *DRB1*11* are associated with self-limiting HCV infection in Caucasian populations.^[337] Ethnicity influences the outcomes of HLA class II associations with HCV as mentioned above.^[334] For combined ethnic groups, *HLA-DQB1*0301* is associated weakly with viral clearance. This association is stronger in Black subjects, whereas in White subjects *DRB1*0101* and *DQB1*0501* are associated with viral clearance. *DRB1*0301* and *DQB1*0201* are associated with viral persistence in this ethnic group. *HLA-C4BQ0*, a class III gene, is a genetic marker of familial HCV-related liver cirrhosis.^[338] The goal of such studies is to produce combinations of several SNPs that are profiles of disease susceptibility and progression.^[339]

In a population-based study in Japan, individuals were genotyped for SNPs in 103 candidate genes possibly involved in the course of HCV infection. Of these, 20 genes contained SNPs associated with persistent HCV viremia and 15 contained SNPs associated with serum alanine aminotransferase levels in HCV chronic carriers (2 genes, *GRAP2* and *CABIN1*, were common to both groups).^[340] The genes studied were *post hoc* classified as follows: cytokine and immune system-related, viral infection-related, liver-function-related, and cluster designation (CD) series. Genes that relate to infection, persistent virus replication, and immunity to HCV include *IL4*, *IL8RB*, *IL10RA*, *PRL*, *ADA*, *NFKB1*, *GRAP2*, *CABIN1*, *IFNAR2*, *IFI27*, *IFI41*, *TNFRSF1A*, *ALDOB*, *AP1B1*, *SULT2B1*, *EGF*, *EGFR*, *TGFB1*, *LTBP2*, and *CD4*. Other genes related to cell cytotoxicity in HCV carriers were *IL1B*, *IL1RL1*, *IL2RB*, *IL12RB1*, *IL18R1*, *STAT5A*, *GRAP2*, *CABIN1*, *IFNAR1*, *MX1*, *BMP8*, *FGL1*, *LTBP2*, *CD34*, and *CD80*.^[341] For example, two genes that were identified may be directly involved in HCV infectivity of hepatocytes and the virus life-cycle. Firstly, heparan sulfate proteoglycan is a possible receptor for flaviviruses and thus HCV binding and variations in its expression on hepatocytes may regulate their infectability. Secondly, HCV uses clathrin-coated vesicles that interact with membrane surface receptors, and the protein AP1B1 (adaptor-related protein complex 1, $\beta 1$ subunit) interacts with clathrin-coated vesicles. These vesicles are part of the endocytic pathway that is used in the flaviviruses lifecycle. Identification of another gene, *MX1* (myxovirus resistance 1, IFN-inducible protein p78), indicates that SNPs in the *MX1* gene may affect regulation of the HCV response to IFN α therapy.

A similar SNP analysis of Taiwanese chronic HCV patients described a gene profile that may be predictive of responsiveness to standard IFN α /ribavirin treatment. This profile

included SNPs in several genes: adenosine deaminase RNA-specific (*ADAR*), caspase 5, apoptosis-related cysteine peptidase (*CASP5*), fibroblast growth factor 1 (*FGF1*), interferon consensus sequence binding protein 1 (*ICSBP1*), interferon-induced protein 44 (*IFI44*), transporter 2 ATP-binding cassette subfamily B (*TAP2*), and transforming growth factor- β receptor associated protein 1 (*TGFBRAPI*).^[342] Markers on DCs and B cells also relate to HCV infection. DCs express CD209 (DC-SIGN) and endothelial cells express CLEC4M (L-SIGN) in liver sinusoids, and these molecules are implicated in HCV infection.^[343] These various studies did not show similar gene profiles. Prior to attempting to compare or correlate the studies, they need repetition and confirmation.

Table IV summarizes possible immune and gene markers of susceptibility and HCV infection. These studies are suggestive of productive avenues of research which require additional studies and confirmation with a wider selection of risk groups.^[239]

4. Markers of HCV Therapy

Viral and host markers are used to determine therapy for HCV infection.^[164,239] As discussed in section 2.1, methods for HCV detection include ELISA, RIBA, DNA sequencing, and PCR/bDNA assays (see table II), and these methods are used to potentially monitor treatment response and/or guide therapy. The presence of HCV RNA in serum indicates active infection, and virus load decrease indicates response to treatment.^[138,348,349]

Chronic HCV infection develops in 70–80% of people exposed to HCV. Serious liver disease (e.g. liver cancer, cirrhosis) occurs in up to 20% of those with chronic infection, often several decades after initial exposure. Liver failure attributable to HCV infection is the leading cause of liver transplants in the US.^[5,33] Liver biopsy is not used for the diagnosis of HCV and liver disease; rather, it is used to determine the severity of inflammation and prognosis, as it provides a more accurate treatment guide, estimate of the degree of fibrosis, and other concomitant liver pathology.^[239,350] Some liver function enzyme tests are used during treatment of HCV infection. For instance, advanced fibrosis is suggested when levels of aspartate aminotransferase exceed alanine aminotransferase or when there are elevated levels of alkaline phosphatase or gamma glutamyl transpeptidase. Nevertheless, liver enzyme assays alone remain poor surrogate markers for inflammation and fibrosis in patients with HCV compared with biopsy.^[345] However, noninvasive marker panels are used to assess liver fibrosis and include hyaluronic acid, α_2 -macroglobulin, apo-lipoprotein A1, and alpha-2 macroglobulin. When levels are elevated, this suggests advanced fibrosis and differentiates mild from moderate-to-severe fibrosis with a high negative predictive value. Assessing the diagnostic utility of biomarkers is limited though, by variability in methods to quantify fibrosis and poor consensus for histologic staging.^[346,347]

It is recommended clinical practice to consider HLA polymorphism markers in the design of intervention studies to improve treatment options for patients with chronic HCV infection. Insight into viral genetic variation also leads to improved understanding of infection, and both approaches may lead ultimately to improved personalized medicine.^[239,351] Additional host factors – including sex, ethnicity, and overall status of the cellular immune surveillance

mechanisms – greatly influence successful or hampered clearance of HCV. For example, a negative HCV RNA test in a subject with robust immunity in the first symptomatic month represents a predictor of viral clearance, and may serve as a criterion in selecting candidates for early antiviral treatment.^[352] On the one hand CTLs are another potential source of virus-infected cell clearance, but on the other hand CTLs in fact contribute to chronic liver injury.^[353] The diagnosis of acute versus persistent HCV and a lack of T_H1 effector cells within the first few months of HCV symptomatology are predictive markers of HCV viral persistence. In some studies, these criteria are recommended in selecting candidates for early antiviral treatment.^[354]

At the cellular level, an HCV-specific CD4+ T_H1 response, particularly against the nonstructural proteins of the virus (e.g. NS3, NS4), is associated with viral clearance and protection from disease progression. Flow cytometric monitoring of CD4 cellular and cytokine biomarkers now serves as a marker of the course of disease and effectiveness of antiviral therapy, whereas a T_H1 profile promotes cellular effector mechanisms and is more effective in mediating HCV viral clearance. The overall role of these cytokines in mitigating HCV-derived chronic liver disease is less clear.^[242,355]

HCV genotype determines the recommended dose and duration of interferon-based therapy. Patients with HCV genotypes 1 and 4 are treated for 12 months, whereas patients with HCV genotypes 2, 3, 5, and 6 undergo 6 months of treatment.^[103] These treatments are costly and difficult for the patient and the physician, who must deal with confounding host factors, including patient adherence, alcohol and other drug abuse, insulin resistance, age, and sex.^[30,164,356–358] There are often severe adverse effects, including influenza-like symptoms, hematologic abnormalities, interactions between prescribed and abused drugs, anemia, gastrointestinal disturbances, fatigue, thyroid dysfunction, dermatologic effects (e.g. alopecia and pruritus), and neuropsychiatric symptoms (including depression in IDUs).^[2,359–362] Ribavirin increases the mitochondrial toxicity of didanosine, leading to lactic acidosis or pancreatitis.^[94,349] Anemia with ribavirin administration increases risk of further anemia, and often HIV-infected patients already have anemia due to their chronic disease. Using erythropoietin may help improve outcome and overall well-being of the patients. Nonetheless, many HIV-HCV co-infected patients have to discontinue HCV treatment prematurely due to these complications.^[94,349] Clinical pretreatment with standard anti-HCV therapy is generally not carried out for individuals in all risk groups; however, one study with IDUs raised the possibility in the clinic of using antiviral preventative therapy.^[362]

New therapeutic approaches include new interferons such as consensus interferon (a synthetic recombinant 166-amino-acid protein) and albuferon (alb-IFN; an 86 kDa recombinant IFN α fused to human albumin). DNA shuffling technology was used to generate R7025, a novel human pegylated (PEG)-IFN α -2a molecule.^[363] Virmidine or taribavirin were used with some success when combined with PEG-IFN α -2a but were less effective at reducing HCV load.^[363] This treatment resulted in a lowered occurrence of anemia compared with ribavirin. Many new therapeutic approaches are being developed, including the use of amantidine, molecular complexes to prevent viral replication, anti-sense RNA, ribozymes, immunomodulation, S-adenosyl methionine and betaine, inhibitors of

nucleoside synthesis, protein translation, NS3/4A protease post-translation, viral assembly and release, binding and cell entry, lipid biosynthesis, and anti-apoptosis inhibition in hepatocytes. Theoretical analyses of cell and virus culture models promote further understanding of their use and clinical application^[363–365]

HCV-HIV co-infection is important, and in the US as of 2004, 25% of HIV-infected persons at outreach centers for drug abuse were HCV co-infected; this can be as high as 90–95%.^[6] Disease progression, complications of treatment, and outcome of HCV is more complex in patients co-infected with HIV, and they have faster progression of hepatic fibrosis than patients infected with HCV infection alone. These patients may also have mental health problems and alcoholism, and should be counseled to cease alcohol consumption since CD4+ cell counts can be improved by drinking cessation alone.^[361,366] All HCV-infected patients should be offered HIV testing. Patients with HIV infection who are on highly active antiretroviral therapy (HAART) are also at risk of liver injury, especially hepatic steatosis from the nucleoside analogs. Whether to treat HCV or HIV first depends upon many factors, but the optimal time to commence HCV treatment is when patients do not require HAART. When HAART is indicated (e.g. CD4 <200 cell/ μ L or HIV viral load >100 000 RNA molecules/mL), drug interaction and medication adverse effects should be monitored closely. There are many countries where patient populations including drug abusers are co-infected with HCV and HIV-1 and receive HAART. HCV co-infection then results in decreased effectiveness of HAART for HIV-1.^[90] There is differing evidence whether HAART – although for the most part keeping HIV-1 infection under control – results in HCV-related disease exacerbation and increased HCV load. When HAART fails for HIV-1 treatment, then HCV load is associated with further HAART failure.^[367]

5. Conclusions

This review discusses the social, viral, and host markers of HCV infection, a consideration of which will enhance strategies for HCV therapy, increase our understanding of the infection, and reduce disease burden. HCV is spread primarily by injection drug abuse and through sexual activity, and is therefore a major public health hazard. Although much work has been done on HCV infection, including gene-related studies, we conclude that more needs to be done. Genetic studies are in their formative stages and most published studies have yet to be replicated and confirmed. This will not only confirm the conclusions of these studies but should also identify genes that can be used for diagnosis and treatment in the clinic.

IDUs are generally individuals with minimal social support, and are unable to afford the cost of diagnosis and treatment of their infections let alone the management of their IDU risk behaviors. The use of advanced genetic technology would benefit IDU and non-IDU individuals alike. The markers developed should be reliably related to their susceptibility, diagnosis, degree of HCV infection, and treatment. Access to modern medical techniques is lacking and the technology is not fully ready for clinical use. Currently, the healthcare system is unable to afford the improvements and modernization needed across the entire population, including the underprivileged.

It is crucial to understand research progress in terms of the reality of funding. The NIH Computer Retrieval of Information on Scientific Projects (CRISP) database (<http://crisp.cit.nih.gov/>), which provides information about funded grants at NIH, showed that, as at October 2008, 346 grants were funded related to HCV; of these, 60 were related to HCV and drug abuse, 30 injection drug abuse, 32 sex, 10 sex and injection drug abuse, 2 needle exchange, 2 paraphernalia, 5 cocaine, and 9 opiates. Many of these topics were also associated with studies on epidemiology and risk, and on HCV and genetics. However, no grants were funded for work related to laboratory studies on inactivation, bleach, needle cleansing, cottons, or cookers. Several research areas need following up, including precise prevalence and incidence studies; the effects of increased age/longevity; how younger people are recruited into injection drug abuse networks; studies on the inactivation of HCV and cleansing IDU paraphernalia (including in the presence of needle exchange); the intensity of the risk activities (e.g. frequencies of these activities over time, quantities of drugs used); the effects of local and global economics and recession; the interactions of drugs, violence, and depression; genetics of HCV susceptibility and disease progression; geography and travel; changes in virus strains over time; time since risk activities commenced, and time of infection. Combining epidemiology and molecular virology in the use of markers for HCV in medical research will greatly enhance our use of therapeutic avenues towards the goal of personalized medicine.

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Table I**Risk factors for hepatitis C virus (HCV) infection**

Risk factors	References
Low income, underprivileged, disadvantaged, needing social support	2,43
IDU current and past	1,6,9,19
IDUs who travel and have multiple sexual partners	31
Female IDU and street sex worker	46
Heterosexual intercourse and MSM	51
Shared and re-used N/S, paraphernalia, and wash-waters by IDUs	8,57–59
Non-injection drug abuse (cocaine inhalation); alcohol	78,80,81,85
Additional non-drug abuse risk: hemodialysis, acupuncture, tattooing, sharing razors, N/S injuries, maternal-fetal transmission when mother is HCV-infected	5,15,82–84

IDU = injection drug user; **MSM** = men who have sex with men; **N/S** = needle/ syringe (this term is used because in some venues, needles may be detachable from the barrels); **paraphernalia** = cottons and cookers; **wash-waters** = water used by IDUs to rinse their N/S and to dissolve drugs.

Table II

Viral markers of hepatitis C virus (HCV) infection and methods of detection

Viral markers	Detection methods	References
HIV infection and HIV VL	EIA, WB, PCR, DNA sequencing	30,50,81,90–94
HBV infection	RIA, EIA, PCR	50,81,95,96
HCV detection and VL	RIBA, ELISA generations 1, 2, and 3, PCR	8,9,97–106
HCV quasispecies	DNA sequencing	7,98,107–115
HCV superinfection	SSCP	116–126
Extrahepatic HCV replicative RNA in blood and brain	SSCP, PCR, and DNA sequencing	116,127–132
HCV genotypes	PCR, branched chain amplification, RFLP, and DNA sequencing	7,133–136

EIA = enzyme-immuno-assay; **ELISA** = enzyme-linked immunosorbent assay; **HBV** = hepatitis B virus; **HCV replicative RNA** = HCV (–) strand RNA; **PCR** = polymerase chain reaction; **RFLP** = restriction fragment length polymorphism; **RIA** = radio-immuno-assay; **RIBA** = recombinant immunoblot assay (WB); **RNA** = HCV (–) strand RNA; **SSCP** = single-strand conformation polymorphism; **VL** = virus load; **WB** = Western blot.

Table III

Hepatitis C virus (HCV) genotype and subtype geography in injection drug users (IDU) versus non-IDUs^{a,b}

Region	HCV genotype by risk group			References
	IDU	non-IDU	unidentified	
North America				
Canada	1, 2a, 3, 4	1, 2, 3, 4	1, 2, 3, 4, 5, 6, 7	136,168
US	1a, 1b	1a, 1b	1a, 1b, 2a, 2b, 3a, 3b, 4, 5, 6	15,169
Mexico		1a, 1b	2a/c, 2b, 3a, 4	170,171
Caribbean				
Martinique	1a, 1b	1a,1b, 3, 4,	2, 3, 4	172
South America				
Brazil	1, 2, 3	1a, 1b, 2, 3, 4, 5	1, 2, 3, 4, 5, 6	173–176
Western, Northern, and Southern Europe				
UK	1a, 3a	1a,1b,3a	1a, 1b, 2a, 2b, 3a, 4, 5a	135,177
France	1a, 3a, 4		1a, 1b, 2a, 2b, 3a, 4, 4a, 4d, 4e, 5	177–179
Luxembourg	3		1, 2, 3, 4, 5	180
Germany	3a	1741b	1a, 1b, 2a/b, 3a, 4a, 5	177,181,182
Austria	1a, 1b, 3a	1a, 3a	1,2,3,4	177,183,184
Spain	3	1b	1, 1a, 2,3, 4	177
Italy	1a, 3a	1b	1a, 1b, 2, 3, 4	177,185
Greece	1, 3	1a, 1b	2, 3, 4	177,186
Finland		1b, 2b, 3a	1a, 1b, 2b, 3a, 4	135
Sweden			1a, 1b, 2, 3,4	177
Netherlands	1a, 3a	1b	1a, 1b, 2a, 2b, 3a, 3b, 3, 4	54,132,187,188
Belgium	1a,3a,4	1	3, 4	189,190
Eastern Europe/Eurasia				
Bulgaria		1b	1a, 1b, 2, 3	191
Czech Republic	2	2	1a, 1b, 3	183,191
Estonia		1b	2, 3	191
Latvia		1a	2,3	191
Lithuania		1b	1a, 2, 3	191
Belarus		1b	1a, 2, 3	191
Moldavia		1b	1a, 3	191
Croatia	3a	1b	1a, 1b, 2, 4	191
Slovenia		1b	1a, 2, 3	191
Hungary		1b	1a, 1b, 3, 4	135,191
Russian Federation	1a, 3a	1b	2a	92,191,192
Poland	3, 4	1, 1b	1, 1a, 2	191,193,194
Asia				
China	1a, 1b, 3a, 3b, 6a, 6e, 6n, 6u	1a, 1b, 2a, 3a, 3b, 6a, 6e		190,195–201
Hong Kong	6a	1b	1a, 1b, 2b, 3a, 3b, 6	135,202

Region	HCV genotype by risk group			References
	IDU	non-IDU	unidentified	
Taiwan	2a, 6a	1b	1a, 1b, 2a, 2b, 3a, 6n	135,196
Korea	1b, 2a, 2c	1b, 2a, 2c	2b, 3a, 3, 4, 6	96,134
Japan		1b	1b, 2a, 2b	135,202
Thailand		1b, 3a, 6	1a, 1b, 2, 3a, 3b, 4, 6m, 6n	135,203
Macau			1a, 1b, 2a, 2b, 3a, 6	135
Singapore			1a, 1b, 2a, 3a	135
Malaysia			1a, 1b, 3a	135
Myanmar		3b, 6a	1a, 2a, 3a, 6m	135,201
Tajikistan		1b	2a, 2c, 3a	204
Turkey		1b	1a, 1b, 2, 3, 4	205
India		3a	1a, 1b, 1c, 2, 3a, 3b, 3g, 4	206,207
Pakistan		1a,1b,1c,2a,2b,2c,3a,3b,3c,4,5a,6a		208,209
Middle East				
Iran	1	1a	1b, 3, 3a, 4	210–212
Saudi Arabia	4	4	1a, 1b, 2b, 3a, 4, 4c/d, 4e, 4h, 5a, 6a	213,214
Kuwait		4	1, 2, 3, 4	215
Lebanon		1a, 1b, 2b, 3a, 4	1a, 1b, 2b, 3c	216
Africa				
Morocco		1a,1b, 2a, 2c	1a, 2a/c	217
Gabon		4c, 4e	4e	218
Egypt		1a, 1b, 4	1b, 1g, 4	219
Sudan			4, 4c, 4d, 4e	220
Madagascar		1b, 2a, 2b, 2k	1a, 1b, 2a, 2k	221
Nigeria		1	1a, 1b, 1c, 4a-4h	222
South Africa		5	1a, 1b, 2a, 3a, 4,5	135
Cameroon		1a, 2f, 4f		223,224
Oceania				
Australia	1a, 3a	1b	1a, 1b, 2a, 2b, 3a, 4, 5, 6	135,225–227
New Zealand	1, 5	1a	1b, 2a, 3a	228

^a Table III was constructed using a combination of peer reviewed published study reports (mentioned in the text, see section 2.5) and available sequences in the Los Alamos HCV Database as of 12 May 2008 (<http://hcv.lanl.gov/content/sequence/HCV/ToolsOutline.html>) Some individual studies describe proportional differences in HCV genotypes and subtypes between IDU and non-IDU risk groups. No statistical analyses were employed for this table (blank cells indicate no information, risk unspecified).

^b The non-IDU group includes sexual risk, vertical transmission, non-injection drug abuse (e.g. cocaine aspiration), hemodialysis, tissue transplant, blood transfusion, hemophiliac, diabetic, body piercing, tattooing, occupational exposure, physician's office, and nosocomial infection (e.g. surgery).

Table IV

Possible immunologic and genetic markers in hepatitis C virus (HCV) infection

Marker	Risk group	Comments	References
CD4	All	HCV infected individuals exhibit a diminished/weak response of their HCV-specific CD4+ T cells for HCV antigens	242
CD8	All	HCV infected individuals exhibit a diminished/weak response of their HCV-specific CD8+ T cells for HCV antigens	243
FoxP3+ T _{reg} cells	U	These cells control CD4 CD8 cellular immunity. The same cells occur in uninfected and infected patients	258
CD4+ CD25+ FoxP3+ T _{reg} cells	All	HCV-specific cells that control CD4 CD8 cellular immunity	254,257,263,264
Autoantibodies	All	These antibodies occur in chronic HCV infection. The patients have B cell lymphoma and mixed cryoglobulinemia	266,268
<i>In vitro</i> neutralization	IDU	Humoral immunity to HCV correlated with patients who showed continued HCV viremia	247
IFN γ , IL-10, and TNF α production	IDU	These are antiviral CTL responses. HCV core, NS3, and NS5 exposure in HCV/HIV co-infected patients vs HCV alone; unchanged response due to ethanol ingestion	344
IFN γ /TNF α production reduced	IDU	Antiviral CTL response for HCV/HIV co-infected patients vs HCV alone	323,344
IFN γ secretion, CTL response; production increased	IDU	CMI antiviral response. For these patients, VL was suppressed	247
IFN γ ; production increased	IDU	CMI antiviral response. Present in HCV seronegative participants; indicates high-risk exposure	248
IL-10 and TGF increased	All	Suppress T _h 17 cell function due to HCV infection. Enhances HCV VL	252
CXCL9, CXCL10, CXCL11 elevated	All	IFN γ -induced proinflammatory cell attractants due to HCV VL	301,302
IL-2 elevated	IDU	In all patients	254,302
TGF α elevated	IDU	In non-cirrhotic patients	254,302
IL-8 elevated	IDU	In inflammation and cirrhotic patients	254,302
TGF β elevated	IDU	In fibrotic patients	254,302
TGF β decreased	IDU	In cirrhotic patients	254,302
MCP-1 elevated	IDU	Shows inflammation	254,302
NAP elevated	IDU	Shows inflammation	254,302
RANTES elevated	IDU	Shows inflammation	254,302
<i>HLA-I.KIR-HLA-C1</i> complex	IDU	Decreased complex expression results in lower VL when at lower VL levels. Modulates HCV infection outcome via regulating NK cells	249,250
Class I HLA-B54	U	Associated with progression of HCV-induced liver injury	335
Class II <i>HLA-DRB1*1302-DQB1*0604</i>	U	Associated with low hepatitis activity in chronic HCV infection	335

Marker	Risk group	Comments	References
<i>HLA-DR11(5)</i>	U	Associated with less advanced HCV-related liver disease	336
<i>HLA-DQA1*0201-DQB1*0201</i>	U	Associated with susceptibility to chronic HCV	336
<i>HLA-DRB1*1104, DQA1*0501, and DQB1*0301</i>	U	Protects from chronic HCV	336
<i>HLA-DRB1*11/*12-DQB1*0301</i>	U	Associated with HCV clearance	249
<i>HLA-DRB1*03-HLA-DRB1*07</i>	U	Associated with HCV susceptibility	249
<i>HLA-DRB1*03-HLA-DRB1*07</i>	U	Associated with non-responsiveness to HCV vaccines that are still under development	249
<i>TLR2, -6, -7, -8, -9, and -10</i> mRNAs in T cells and monocytes	IDU	HCV-positive vs HCV-negative patients	279
<i>TLR4</i> mRNA in T cells	IDU	HCV-positive vs HCV-negative patients	279
<i>TLR5</i> mRNA in monocytes	IDU	HCV-positive vs HCV-negative patients	279
TLR2, TLR1, and TLR6 expression as a result of macrophage activation	IDU	Stimulated by HCV core and NS3 proteins	260
TLR3 expression due to activation by HCV RNA-containing immune complex	IDU	Associated with HCV-associated glomerulonephritis	283
TLR4 activation	U	Associated with use of morphine, methadone, oxycodone, buprenorphine, fentanyl, and meperidine/pethidine. These are opioids and opioid agonists	288
TLR4 suppression	U	Associated with use of naloxone that is an opioid antagonist	288
SNP associations	All	<i>TLR2</i> Arg753Gln, <i>TLR4</i> Asp299Gly, and <i>TLR4</i> Thr399Ile genotypes associated with allograft failure and mortality in HCV-infected patients	289
<i>IL10</i> promoter, MHC class II loci	U	Associated with genetic susceptibility to HCV infection	323
IL12: C/C SNPs in 3'-UTR of <i>IL12B</i> gene	U	Associated with IL-12-stimulated IFN γ production, increased resistance to severe liver disease with HCV genotype 3	328
C4BQ0 allele, MHC class III	U	Associated with familial HCV-related liver cirrhosis	338
IL18 promoter SNPs -607C/A and -137G/C	IDU	Associated with HCV clearance; hemophilia control group	331
IL18 promoter SNPs -607C/A and -137G/C	U	Associated with chronic liver disease; non-disease control group	332
<i>IFNG</i> gene SNP 764G/C	All	Clear HCV	329
SNPs In 20 genes	All	Associated with persistent viremia	340
SNPs In 15 genes	All	Associated with chronic infection	340
SNPs in 7 genes	All	Response to IFN α /ribavirin treatment	342
Standard panel of liver enzymes. Levels of AST > ALT; high levels of ALP or gamma glutamyl transpeptidase	U	Associated with advanced liver fibrosis	345
Elevated levels of α_2 -macroglobulin, hyaluronic acid, apolipoprotein A1, and α -2 macroglobulin (assays performed by FIBROSpect II)	Liver transplant patients	Differentiate mild from moderate-to-severe fibrosis	346,347

AI = IDU and non-IDU risk; **ALP** = alkaline phosphatase; **ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase; **CMI** = cell-mediated immunity; **CTL** = T-cell cytotoxicity; **HLA** = human leukocyte antigen; **IDU** = injection drug user; **IFN** = interferon; **IL** = interleukin; **KIR** = killer cell immunoglobulin-like receptors (of NK cells); **MCP** = monocyte chemoattractant protein; **MHC** = major histocompatibility complex; **NAP** = neutrophil activating peptide; **NK** = natural killer cells; **RANTES** = regulated on activation, normally T-expressed and secreted; **SNP** = single nucleotide polymorphism; **TGF** = transforming growth factor; **T_h** = T helper; **TLR** = Toll-like receptor; **TNF** = tumor necrosis factor; **T_{reg}** = T regulatory cells; **U** = risk group unidentified; **UTR** = untranslated region; **VL** = virus load.

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