Demographics, Clinical Characteristics, IFNL3- and IFNL4-Polymorphisms in a Cohort of Hepatitis C Patients from Puerto Rico

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Objective: To describe the risk factors for infection, complications, treatment received and response in Puerto Ricans with HCV attending gastroenterology clinics at UPR-MSC, and the prevalence of single nucleotide polymorphisms (SNPs) in IFNL3 and IFNL4 in this population.

Methods: After consent, demographic and medical data were obtained and blood samples were drawn from each patient. The QIAamp Blood-Maxi Kit was employed for DNA extraction. The TaqMan allelic discrimination assay was employed for SNP genotyping. HCV-RNA was measured by branched-chain DNA assay. Frequency distributions were used to describe the study population and the prevalence of SNPs. The UPR Medical Sciences Campus IRB approved the study.

Results: Of 259 patients recruited, 64% were men. Genotype 1 was found in 112/136 (82%). Of 150 subjects treated, 19% had sustained virological response (SVR), 40% received treatment with pegylated interferon plus ribavirin. The SNP frequencies (n = 239) of IFNL3 locus rs12979860 were 27% (C/C), 50% (C/T), and 23% (T/T), and for rs8099917 were 46% (T/T), 47% (T/G), and 7% (G/G). SNP frequencies of IFNL4 locus ss469415590 were 26% (TT/TT), 48% (TT/ΔG), and 26% (ΔG/ΔG).

Conclusion: HCV-infected Hispanics in our sample (all of which were Puerto Rican) were shown to have a low SVR rate of 19%. The demographic characteristics were similar to those of other study groups in the US, except for the annual income. Genotype-1 was the most prevalent in those patients with known HCV genotypes. This study group showed significant differences with frequencies observed in other populations. Lower frequencies of the favorable genotypes were found in our group compared with the populations having European and Asian ancestry. [*P R Health Sci J 2014;33:177-183*]

Key words: Hepatitis C, IFNL3, IFNL4, Single Nucleotide Polymorphisms, Puerto Ricans, Hispanics

Hepatitis C virus (HCV) infection is one of the leading etiologies of liver disease. It is an important cause of acute and chronic hepatitis. The risk factors most strongly associated with the infection are being an injection drug user (IDU) and having received a blood transfusion before 1992. HCV prevalence is about 2 to 3% worldwide. There are an estimated 4 to 5 million people infected in the United States of America (US) and approximately 5 million infected in Western Europe (1, 2, 3).

In San Juan, Puerto Rico, the prevalence of HCV in adults has been estimated at 6.3% in a study by Pérez et al. (4). An increased prevalence was found in adults 21 to 64 years old, living in San Juan, in comparison with that prevalence found in the overall adult US population. An island-wide survey estimated an overall prevalence of 2.3% in the adult population of Puerto Rico; however, this prevalence increased to 2.7% in each municipality in which a high incidence of AIDS among IDUs was found (5). Recently, it was found that for 2 groups of US-Hispanic adults, the prevalence of HCV was high in those of Puerto Rican origin, relative to those of South American, Mexican, Dominican, Cuban, or Central American origin (6). The research in which this finding was made was conducted in 3,210 individuals who took part in the National Health and Nutrition Examination Survey (NHANES) 2007–2010 and 11,964 who took part

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The authors have no conflicts of interest to disclose.

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in the Hispanic Community Health Study/Study of Latinos. These 2 studies concluded independently that HCV prevalence among the several Hispanic groups varies depending on their background subgroups.

Translating research discoveries into efficient treatment for HCV-infected patients is a challenging task in clinical practice. For interferon-based therapies, the difference in treatment efficacy favors clinical-trial versus clinical practice settings in the general population. The dual-therapy of pegylated-interferon (Peg-IFN) and ribavirin (RBV) can attain SVR in about 56% of cases (7). In contrast, in "real-world" settings of patient care, SVRs of 16 to 19% relative to all HCV-infected patients who started treatment were reported by North CS et al. (8). They found that fewer than half (39–41%) of all patients were eligible for treatment, and only 19 to 21% actually started treatment. They concluded that medical and psychosocial problems were the major factors negatively affecting treatment eligibility.

There is also a significant degree of uncertainty relating to how a given patient is going to respond to available treatments. The 2009 hepatitis C guidelines of the American Association for the Study of Liver Diseases (AASLD) recommend that the decision regarding how to treat a patient needs to be made on a case-by-case basis, taking into account the severity of the patient's liver disease, the presence in that patient of any concurrent medical and psychiatric diseases, the likelihood of treatment response and the potential for adverse effects in the patient, and the patient's willingness to undertake therapy (9). In general, patients with more advanced fibrosis (stage 3 or greater on the Batts-Ludwig or METAVIR scoring system), should be strongly considered for therapy, as they are at the greatest risk for hepatic-related morbidity and mortality.

Among the several parameters that physicians consider in their treatment algorithms, host genomic information is relevant to the choice of therapy and monitoring procedures. However, the vast majority of available treatments are based on general population characteristics. The natural history of chronic HCV is different between African-Americans and Caucasians; little comparable data are available for Hispanics (or Latinos). Racial differences have also been observed in the responses to antiviral therapy, with lower responses being seen in African-Americans and Hispanics than in Caucasians. This difference in response is unrelated to viral characteristics. In Puerto Rico, there is limited information about the epidemiology and response to treatment of patients infected with HCV, despite the observed prevalence of HCV in general and high-risk populations (9, 10, 11, 12, 13).

Studies have shown that a combination of basic serum markers such as α-macroglobulin, haptoglobulin, GGT, γ-globulin, total bilirubin, and apolipoprotein is helpful in the evaluation of progression of the disease in patients with HCV. Other important factors are viral genotype, viral load, and IFNL3 (IL28B) genotype. The AASLD and the European Association for the Study of the Liver have included IFNL3 testing in their guidelines (9, 14). Therefore, the identification of genetic markers that predict the course of the disease or the response to therapy might provide clues that would help to explain racial/ethnic differences.

Several genome-wide association studies have concluded that specific variations in the human genetic code near the IFNL3 gene at chromosome 19 could help to predict the spontaneous clearance of HCV infection and an individual's response to interferon plus ribavirin treatment (15, 16, 17, 18). Neither clinical factors nor compliance can completely explain the variable effects of ethnicity on treatment response, suggesting that host genetics also plays an important role (19). In particular, locus rs12979860 has been associated with treatment-induced viral clearance in patients with chronic HCV. It is associated with an approximately 2-fold higher odds ratio in response to the standard treatment of pegylated α-interferon plus ribavirin, both in patients of European ancestry ($p<0.0001$) and in African-Americans ($p<0.0001$). These studies indicate that the virus was eradicated in approximately 80% of homozygote patients with the protective C allele compared to only about 25% of those with genotype T/T, while in those with C/T, the response rate was intermediate. The locus rs8099917 minor alleles (T/G or T/T) were associated with progression to chronic HCV infection and also with the failure to respond to therapy, with the strongest effects in patients with HCV genotype 1, 2, or 4 (20, 21).

The prevalence of "protective" alleles in loci rs12979860 and rs8099917 has been determined for many populations and ethnic groups throughout the world (22, 23, 24, 25). Data reported by Balagopal et al., taken from the International HapMap Project, have shown that Hispanics have the "protective" allele C at locus rs12979860 and allele G at locus rs8099917, as the major variants (0.56:0.44, C/T allele frequencies, and 0.69:0.31, T/G allele frequencies, respectively). Most Puerto Ricans have genomes derived from 3 ancestral populations (European, African, and Amerindian), and thus they are considered a genetically admixed population (26, 27, 28). However, the prevalence of the aforementioned alleles is unknown for Hispanics in Puerto Rico.

Recently, Prokunina-Olsson et al. (29) reported the discovery of a new gene involved in the clearance of HCV. Variations of this gene, known as interferon λ4 (IFNL4), at locus ss469415590, were associated with HCV clearance, particularly in those individuals of African ancestry. The TT/TT genotype was found to be a favorable variation for SVR and the spontaneous clearance of HCV. Results showed that IFNL4 protein could be generated in IFNL4-ΔG carriers and not in IFNL4-TT homozygote carriers. Related studies have shown that IFNL4-ΔG is strongly associated with impaired spontaneous HCV clearance (30). Variants of ss469415590 are also unknown for Hispanics in Puerto Rico.

The aims of this study were to describe the demographics and clinical characteristics of as well as the genetic polymorphisms in

IFNL3 (rs12979860 and rs8099917) and IFNL4 (ss469415590) in a cohort of Hispanic patients chronically infected with HCV.

Materials and Methods

The hepatitis C data, serum, and DNA bank study was established in December 2005. HCV-infected patients, ages 21 years old and older, regardless of their treatment or response to treatment, were recruited (December 2005 to April 2012) from a number of University of Puerto Rico clinics—the liver transplant clinic, faculty clinics—and from the Gastroenterology Research Unit. Pregnant women were excluded from the study. All patients signed an informed consent to provide access to their medical records and to agree to participate in the data bank and serum and DNA repository. Each patient was assigned an identification number or code to maintain confidentiality. All identifying and medical record information was kept in password-protected computer files at the Gastroenterology Research Unit. A questionnaire including demographic and medical data was filled out by interviewing the patient and reviewing the medical records. The demographic data for each participant included age, gender, , marital status, ethnicity, education, income, and a relative's contact information. The separate medical data, identified only by the assigned code, included general medical information, risk factors for HCV present in the individual, laboratory data, the etiology of that individual's liver disease, and treatment received for HCV. The database was designed using the computer package Epi-Info 6.04d (CDC, Atlanta, GA). Statistical analysis was performed using the SAS System for Windows, version 9.3 (SAS Institute Inc.; Cary, NC).

All the patients who agreed to participate in the serum and DNA repository project, after filling out the questionnaire, had 10 ml of blood drawn for each bank. These samples were codified, processed, frozen, and properly stored for later studies. For the DNA extraction and purification, the QIAamp Blood-Maxi Kit (Spin Protocol) was employed. DNA was quantified by spectrophotometry and diluted to a concentration of about 12.5 ng/mL before use. For SNP genotyping, the TaqMan allelic discrimination assay was employed.(Applied Biosystems), according to the manufacturer's instructions (26, 27, 28). HCV RNA was measured by a branched-chain DNA assay (29). The HCV antibodies were detected by HCV enzyme immunoassay (30). Genotypic frequencies were obtained by direct counting.

Frequency distributions (frequency and percent) were generated to describe the clinical characteristics and prevalence of IFNL3 and IFNL4 SNPs among HCV-infected patients. The prevalence of IFNL3 SNPs was described according to demographic, laboratory, liver histology, and treatment characteristics of the study group. A single-sample chi-square goodness-of-fit test with an exact p-value was used for the comparison of the frequencies of this study group with the frequencies observed in other populations. The study was approved by the Medical Sciences Campus Institutional Review Board (protocol no. 1250105).

Results

By April 2012, 259 patients had been recruited from the different University of Puerto Rico clinics. All were of Puerto Rican origin. Table 1 shows the characteristics of our study group compared with those of the subjects with HCV in NHANES (36). The majority of the subjects were 50 years old or older (75%), with the highest percentage (48%) in the range of 50 to 59 years old. The majority had a high school or lower level of education (70%) and low annual incomes (70%) (37). The most common risk factors identified for HCV infection were having received a blood transfusion prior to 1992 (36%), being an injection drug user (IDU, 33%) and having a tattoo (27%). Liver-related complications included esophageal varices (44%), ascites (40%), encephalopathy (33%), portal gastropathy (27%), hepatocellular carcinoma (13%), and spontaneous bacterial peritonitis (5%). Ten percent (10%) had had a liver transplant. In the 136 patients who had information on HCV genotype, type 1 was the most common (82%).

The existing information on the METAVIR scores of 112 patients (n = 112) is shown in Table 1. Advanced fibrosis scores (F3/F4) were found in 58% of cases. Of the patients who were biopsied, 64% had no steatosis and 29% had mild steatosis.

More than half of the patients recruited (58%) had received treatment for hepatitis C. Only 19% of them had achieved SVR. The existing treatments consisted of interferon (IFN), pegylated interferon (Peg-IFN), interferon with ribavirin (IFN+RBV), and pegylated interferon with ribavirin (Peg-IFN+RBV). Twenty-one percent (21%) of the patients had received a single therapy of either IFN or Peg-IFN, and 55% had received dual-therapy of IFN+RBV or Peg-IFN+RBV. The Peg-IFN+RBV combination resulted in the highest SVR, with 29%. A second or third treatment was given to 23% of the patients. However, 97% of them did not respond to treatment. Data regarding the duration or completion of therapy was not available in 21% of the cases.

Both the genotype and allele frequencies of ss469415590, rs12979860, and rs8099917 were determined in the majority of the group (n = 239, Table 2). Twenty samples were not suitable for analysis. The frequencies of all 3 SNPs were consistent with the Hardy-Weinberg equilibrium model ($p>0.05$). This analysis showed a prevalence of 27% for the favorable C/C genotype at rs12979860, with a combined prevalence of 73% for the C/T and T/T genotypes (prevalence of 50% and 23%, respectively). The prevalence for rs809917 were 46%, 47%, and 7% for the T/T, T/G, and G/G genotypes, respectively. The newly discovered variant ss469415590 showed frequencies of 26%,

Table 1. Demographics and Clinical Characteristics

Factor	Category	Study Group% (n)	NHANES Study% (n)
Sex	Female	36% (92)	36% (106)
	Male	64% (167)	64% (165)
Age in years	<40	8% (20)	12% (33)
	40-49	16% (42)	33% (89)
	50-59	48% (125)	34% (93)
	60-69	22% (58)	21% (56)[a]
	>70	5% (14)	
Education level	High school or lower	70% (181)	68% (184)
	More than high school	30% (78)	32% (86)
Annual Income	<$20,000	70% (181)	36% (93)[b]
	>$20,000	29% (75)	64% (162)[c]
	Unknown	1% (3)	–
Risk Factors	Blood Transfusion prior to 1992	36% (93)	17% (45/264)
	Drug Use	33% (85)	72% (131/182)
	Tattoos	27% (169)	–
	Multiple Sex Partners	23% (59)	94% (171/181)
	Occupational Exposure	9% (24)	–
	Unknown	5% (13)	–
Serum Markers	HBsAb+	20% (46)	–
	HBV immunization	11% (26)	–
	HBV past exposure	9% (20)	–
	HBsAg + plus HBcAb+	2% (4)	
	HBVc Ab+	20% (46)	–
	HIV+	1% (2)	–
Steatosis (n = 129)	None	64% (82)	–
	Mild	29% (37)	–
	Moderate	5% (7)	–
	Marked	2% (3)	–
METAVIR Score (n = 112)	F0	12% (13)	–
	F1	11% (12)	–
	F2	21% (23)	–
	F3	21% (23)	–
	F4	37% (41)	–
HCV Genotype (n = 259)	1	43% (112)	–
	2	5% (12)	–
	3	4% (10)	–
	4	1% (2)	–
	Unknown	47% (123)	–
Type of Treatment (n = 150)	Interferon	16% (24)	–
	Pegylated Interferon	5% (8)	–
	Interferon + Ribavirin	15% (23)	–
	Pegylated Interferon + Ribavirin	40% (60)	–
	>1 Treatment	23% (35)	–
Response to Treatment (n = 150)	Sustained Virologic Response	19% (28)	–
	No Response	81% (122)	–

a) ≥ 60 years old; b) subjects below the poverty level; c) subjects above the poverty level.

48%, and 26% for TT/TT, TT/ΔG, and ΔG/ΔG, respectively. A frequency of 23% of the favorable genotypes rs12979860 (C/C), rs8099917 (T/T), and ss469415590 (TT/TT) with respect to the combined-risk genotypes with 1 or 2 copies of the T, G, or ΔG alleles for rs12979860, rs8099917, and ss469415590, respectively, was observed.

Figure 1 compares the genotype frequencies of rs12979860 (Figure 1A), rs8099917 (Figure 1B), and ss469415590 (Figure 1C) of our study group with those of European, Asian and African populations. The favorable C/C genotype in rs12979860 was more frequent in Asians (85%) and Europeans (47%) than in our group (27%). Similar findings were observed for the T/T genotype in the rs8099917 genotype (87% in Asians, 68% in Europeans, 88% in African-Americans, and 46% in our group). The TT/TT genotype in ss469415590 was present in 45% of Europeans, 9% of African-Americans, and 26% of our study group (22, 29).

Discussion

The majority of the patients in this study were men who were 50 years old or older; they had lower income and 12 years or fewer of formal education. Most of our study participants had been exposed to one or more common HCV risk factors, such as receiving blood transfusion before 1992 or having used intravenous drugs. These demographic characteristics were similar to those of the members of other study groups in the US except for that of annual income. A direct comparison of this last characteristic cannot be made because in the NHANES study, the parameter was reported as "family income" and was based on the US poverty level. In the US, the poverty level is variable and depends on the persons in the family/household and the territory or state (37). However, the poverty guidelines are not defined for Puerto Rico. In the NHANES study, the majority of the subjects with HCV were reported to have incomes above US poverty level, while more than double the proportion of subjects in our study (compared to NHANES) had incomes below poverty level. This marked difference in poverty rates has been documented for the general population in the US versus that in Puerto Rico (40). A similar SVR rate of 19% was obtained in this study compared to those rates obtained by other studies in the US (8). North CS et al. suggested that improving HCV patient readiness for treatment and, thus, SVR, might be accomplished by the management of medical and psychosocial problems prior to treatment. They reported that about 19 to 21% of all HCV-infected patients started treatment but only

Table 2. Observed Genotype Frequencies for Each SNP

Locus	Allele	%	Genotype	n	%
rs12979860	C	52%	C/C	64	27%
			C/T	119	50%
	T	48%	T/T	56	23%
rs8099917	T	70%	T/T	111	46%
			T/G	112	47%
	G	30%	G/G	16	7%
ss469415590	TT	50%	TT/TT	61	26%
			TT/ΔG	116	48%
	ΔG	50%	ΔG/ΔG	62	26%
Compounded	–	–	C/C + T/T + TT/TT	54	23%
SNPs[a]	–	–	All others	185	77%

a) Compound SNPs = rs12979860 + rs8099917 + ss469415590

19% of those achieved SVR. In our study, the percentage of patients who started treatment was almost 3 times that of the aforementioned review(58% vs. 19% - 21%), yet we observed the same SVR rate.

Three studies in Puerto Rico have shown that the most frequent genotype found in HCV-infected patients is genotype 1 (38). This genotype is associated with a lower frequency of response to treatment when compared to those frequencies in patients with genotype 2 or 3. In our study, genotype 1 was the most prevalent (82%) in those patients with a known HCV genotype.

Previous studies have shown an association between specific SNPs near IFNL3 or IFNL4 and the viral load exhibited by an HCV-infected patient, the frequency of SVR following completion of treatment, or liver histology (29, 39). The observed genotyping frequencies in our group showed significant differences ($p<0.05$) from the frequencies observed in other populations, using a single-sample chi-square goodness-of-fit test with an exact p-value (Figure 1). Comparing the frequencies of the favorable genotypes in our group with those frequencies in other groups, we determined that rs12979860 was found less frequently in our population than it was in both the European and the Asian populations (27% vs. 47% and 85%, respectively); rs8099917 was also found less frequently in our population than it was in the European and Asian populations (46% vs. 68% and 87%, respectively).

Given the available information for ss469455590, lower frequencies were also observed in our group compared with such frequencies observed in individuals of American–European ancestry (25% versus 45%). When our study group was compared with populations having African ancestry, variable frequency distributions were observed. Lower frequencies of the favorable T/T genotype rs8099917 were observed in our population than were observed in African populations (46% versus 88%), while, compared to the same African populations, higher frequencies for rs12979860 (27% versus 15%) and ss469415590 (26% versus 9%) were observed. Overall, only 23% of the HCV-infected patients were found to have all 3 favorable genotypes (i.e., C/C in rs12979860, T/T in rs8099917, and TT/TT in ss469415590).

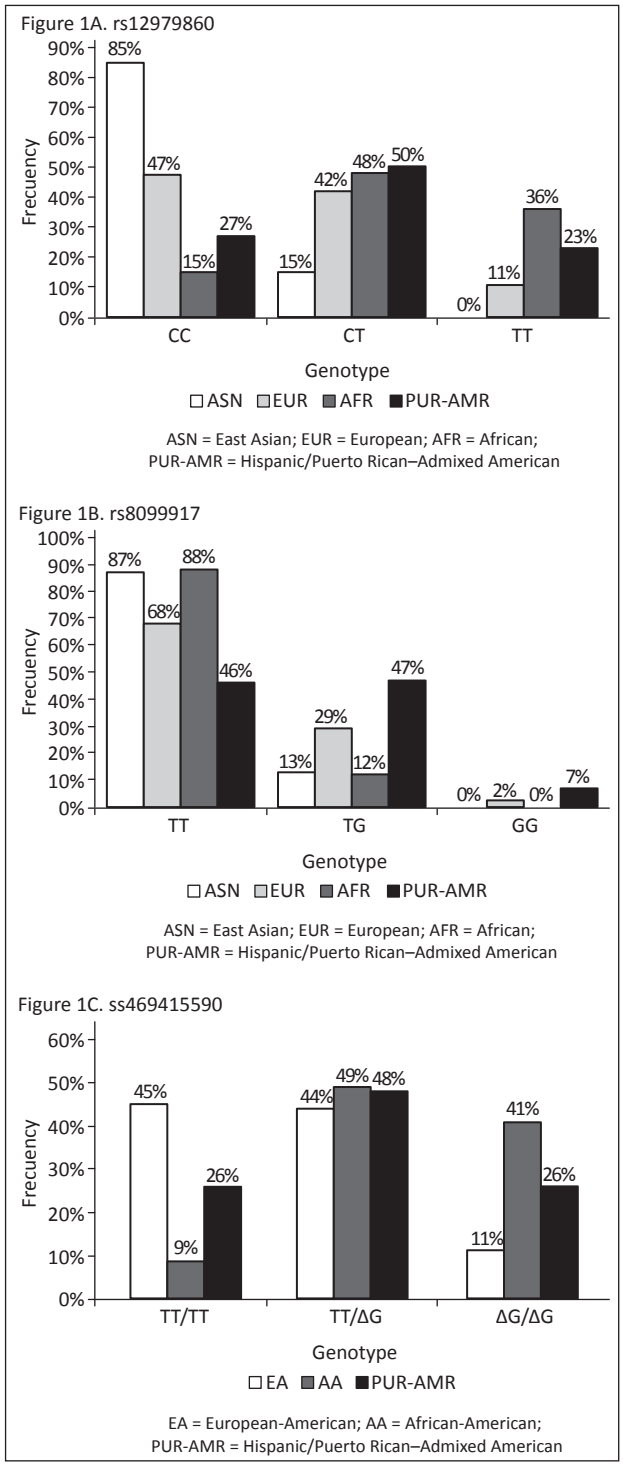

Figure 1. Comparison of SNP Genotype Frequencies Among Several Populations

The relatively low prevalence of favorable host genotypes and the high prevalence of HCV genotype 1 might contribute to the

low number of patients who achieve an SVR after treatment. The findings of this study suggest that host genetic variants may play an important role in treatment outcome, thus accounting for low SVR.

The limitations in this study should be addressed. First, there was a selection bias, since only patients from University of Puerto Rico clinics were included. Second, since patients were only evaluated at the time of the initial interview, some data on genotype and HCV viral load were missing from our patient records. Third, the lack of information on post-treatment HCV RNA titers in some patients limited the scope of our evaluation of the response to therapy in the cohort. Finally, the sample size was relatively small.

In conclusion, this HCV registry must be expanded to collect information on all patients with chronic HCV infection in Puerto Rico. This expanded database could provide a means to better describe the epidemiology of chronic HCV, disease behavior, and evolution, to evaluate the effectiveness of prevention programs and to design better disease management strategies. Future studies in our study group of HCV-infected Hispanics should aim to assess the SVR according to treatment, controlling for IFNL3 and IFNL4 SNPs.

Resumen

Objetivos: El propósito del estudio fue describir factores de riesgo, complicaciones, tratamiento, respuesta y prevalencia de polimorfismos de nucleótidos sencillos (PNS) en IFNL3 e IFNL4 en puertorriqueños con hepatitis C en las clínicas de Gastroenterología- UPR. Métodos: Luego del consentimiento, se obtuvo información médico-demográfica y una muestra de sangre. Para la extracción-purificación del ADN se utilizó el QIAmp Blood-Maxi Kit. Se utilizó metodología TaqMan para los genotipos de los PNS. El ARN del virus se cuantificó con los ensayos de polimerización de las cadenas ADN. Se utilizó la distribución de frecuencias para describir la población y prevalencia de PNS. El Comité Ético de Investigación Clínica del Recinto de Ciencias Médicas aprobó el protocolo. Resultados: De 259 pacientes reclutados, 64% eran hombres. El genotipo 1 estaba presente en 112/136 (82%). De 150 pacientes tratados, 19% mostraron una respuesta viral sostenida (RVS), 40% con interferón pegilado más ribavirina. Las frecuencias (n = 239) para el locus rs12979860 en IFNL3 fueron 27% (C/C), 50% (C/T) y 23% (T/T) y para rs8099917, 46% (T/T), 47% (T/G) y 7% (G/G). Para IFNL4, las frecuencias del locus ss469415590 fueron 26% (TT/TT), 48% (TT/ΔG) y 26% (ΔG/ΔG). Conclusión: Los hispanos de origen puertorriqueño con VHC en nuestra muestra mostraron una RVS baja (19%). Las características demográficas fueron similares a las de grupos de estudio en EE.UU., excepto para los ingresos anuales. El genotipo-1 fue el más frecuente en pacientes con VHC de genotipo conocido. Se encontraron diferencias significativas con frecuencias observadas en otras poblaciones. Menos frecuencias de genotipos favorables fueron encontradas en nuestro grupo en comparación con poblaciones de ascendencia europea y asiática.

Acknowledgments

The project was supported by grant number S21MD001830 from the NIMHD, with partial support by grants from NIMHD-RCMI (G12MD007600), NIMHD-PRCTRC (8U54MD007587-03), and NIGMS-RISE (3R25GM061838-10S1). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The manuscript has not been previously published and is not currently submitted elsewhere. Lilliana Morales-Vásquez and Edgar I. Miranda-Ávalo contributed equally to this work. The authors wish to acknowledge the technical support of Yvonne Casiano, Jessicca Y. Renta, Lilliam R. Villanueva, Vladimir Vélez, Ednalise Santiago, and Nayra Rodríguez.

References

1. Rustgi VK. The epidemiology of hepatitis C infection in the United States. J Gastroenterol 2007;42:513–521.
2. Denniston MM, Jiles RB, Drobeniuc J, Klevens RM, Ward JW, McQuillan GM, Holmberg SD. Chronic hepatitis C virus infection in the United States, National Health and Nutrition Examination Survey 2003 to 2010. Ann Intern Med 2014;160:293–300.
3. Chak E, Talal AH, Sherman KE, Schiff ER, Saab S. Hepatitis C virus infection in USA: an estimate of true prevalence. Liver Int 2011;31:1090–1101.
4. Perez CM, Suarez E, Torres EA, Roman K, Colon V. Seroprevalence of hepatitis C virus and associated risk behaviours: a population-based study in San Juan, Puerto Rico. Int J Epidemiol 2005;34:593–599.
5. Perez CM, Marrero E, Melendez M, Adrovet S, Colon H, Ortiz AP, et al. Seroepidemiology of viral hepatitis, HIV and herpes simplex type 2 in the household population aged 21–64 years in Puerto Rico. BMC Infect Dis 2010;10:76. doi: 10.1186/1471-2334-10–76.
6. Kuniholm MH, Jung M, Everhart JE, Cotler S, Heiss G, McQuillan G, et al. Prevalence of hepatitis C virus infection in US Hispanic/Latino adults: results from the NHANES 2007–2010 and HCHS/SOL studies. J Infect Dis 2014;209:1585–1590.
7. Pearlman BL, Traub N. Sustained virologic response to antiviral therapy for chronic hepatitis C virus infection: a cure and so much more. Clin Infect Dis 2011;52:889–900.
8. North CS, Hong BA, Adewuyi SA, Pollio DE, Jain MK, Devereaux R, et al. Hepatitis C treatment and SVR: the gap between clinical trials and real-world treatment aspirations. Gen Hosp Psychiatry 2013; 35:122–128.
9. Ghany MG, Nelson DR, Strader DB, Thomas DL, Seeff LB; American Association for Study of Liver Diseases. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. Hepatology 2011;54:1433–1444.
10. Cheung RC, Currie S, Shen H, Ho SB, Bini EJ, Anand BS, et al. Chronic hepatitis C in Latinos: natural history, treatment eligibility, acceptance, and outcomes. Am J Gastroenterol 2005;100:2186–2193.
11. Rodriguez-Torres M, Jeffers LJ, Sheikh MY, Rossaro L, Ankoma-Sey V, Hamzeh FM, Martin P; Latino Study Group. Peginterferon alfa-2a and ribavirin in Latino and non-Latino whites with hepatitis C. N Engl J Med 2009;360:257–267.

12. Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. Gastroenterology 2002;122:889–896.
13. Reid AE, Koziel MJ, Aiza I, Jeffers L, Reddy R, Schiff E, et al. Hepatitis C virus genotypes and viremia and hepatocellular carcinoma in the United States. Am J Gastroenterol 1999;94:1619–1626.
14. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. J Hepatol 2011;55:245–264.
15. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009; 461:798–801.
16. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.
17. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100–1104.
18. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–1109.
19. Clark PJ, Thompson AJ, McHutchison JG. IL28B genomic-based treatment paradigms for patients with chronic hepatitis C infection: the future of personalized HCV therapies. Am J Gastroenterol 2011;106:38–45.
20. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 2010;138:1338–45, 1345.e1-7.
21. Kawaoka T, Hayes CN, Ohishi W, Ochi H, Maekawa T, Abe H, et al. Predictive value of the IL28B polymorphism on the effect of interferon therapy in chronic hepatitis C patients with genotypes 2a and 2b. J Hepatol 2011;54:408–414.
22. 1000 Genomes. A Deep Catalog of Human Genetic Variation. Available at: Url: http://browser.1000genomes.org/index.html. Accessed September 12, 2013.
23. Melis R, Fauron C, McMillin G, Lyon E, Shirts B, Hubley LM, Slev PR. Simultaneous genotyping of rs12979860 and rs8099917 variants near the IL28B locus associated with HCV clearance and treatment response. J Mol Diagn 2011;13:446–451.
24. Balagopal A, Thomas DL, Thio CL. IL28B and the control of hepatitis C virus infection. Gastroenterology 2010;139:1865–1876.
25. Shebl FM, Pfeiffer RM, Buckett D, Muchmore B, Chen S, Dotrang M, et al. IL28B rs12979860 genotype and spontaneous clearance of hepatitis C virus in a multi-ethnic cohort of injection drug users: evidence for a supra-additive association. J Infect Dis 2011;204:1843–1847.
26. Tang H, Choudhry S, Mei R, Morgan M, Rodriguez-Cintron W, Burchard EG, Risch NJ. Recent genetic selection in the ancestral admixture of Puerto Ricans. Am J Hum Genet 2007;81:626–633.
27. Parra EJ, Kittles RA, Shriver MD. Implications of correlations between skin color and genetic ancestry for biomedical research. Nat Genet 2004;36(11 Suppl):S54–60.
28. Duconge J, Ruano G. The Emerging Role of Admixture in the Pharmacogenetics of Puerto Rican Hispanics. J Pharmacogenomics Pharmacoproteomics 2010;1.pii: 1000101.
29. Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet 2013;45:164–171.
30. Aka PV, Kuniholm MH, Pfeiffer RM, Wang AS, Tang W, Chen S, et al. Association of the IFNL4-DeltaG Allele With Impaired Spontaneous Clearance of Hepatitis C Virus. J Infect Dis 2013;209:350-354.
31. Sato-Otsubo A, Sanada M, Ogawa S. Single-nucleotide polymorphism array karyotyping in clinical practice: where, when, and how? Semin Oncol 2012;39:13–25.
32. Aparicio E, Parera M, Franco S, Perez-Alvarez N, Tural C, Clotet B, Martinez MA. IL28B SNP rs8099917 is strongly associated with pegylated interferon-alpha and ribavirin therapy treatment failure in HCV/HIV-1 coinfected patients. PLoS One 2010;5:e13771.
33. Halfon P, Bourliere M, Ouzan D, Maor Y, Renou C, Wartelle C, et al. A single IL28B genotype SNP rs12979860 determination predicts treatment response in patients with chronic hepatitis C Genotype 1 virus. Eur J Gastroenterol Hepatol 2011;23:931–935.
34. Anderson JC, Simonetti J, Fisher DG, Williams J, Yamamura Y, Rodriguez N, et al. Comparison of different HCV viral load and genotyping assays. J Clin Virol 2003;28:27–37.
35. Brown EE, Zhang M, Zarin-Pass R, Bernig T, Tseng FC, Xiao N, et al. MBL2 and hepatitis C virus infection among injection drug users. BMC Infect Dis 2008;8:57.
36. Denniston MM, Jiles RB, Drobeniuc J, Klevens RM, Ward JW, McQuillan GM, Holmberg SD. Chronic hepatitis C virus infection in the United States, National Health and Nutrition Examination Survey 2003 to 2010. Ann Intern Med 2014;160:293–300.
37. Office of The Assistant Secretary for Planning and Evaluation. 2014 Poverty Guidelines. Available at: Url: http://aspe.hhs.gov/poverty/14poverty.cfm. Accessed June 20, 2014.
38. Rodriguez-Perez F, Suarez-Perez E, Alvarez-Rohena M, Toro DH. Prevalence of chronic hepatitis C virus genotypes among patients between 21 to 65 years old in Puerto Rico. P R Health Sci J 2004;23(2 Suppl): 49–56.
39. Lin CY, Chen JY, Lin TN, Jeng WJ, Huang CH, Huang CW, et al. IL28B SNP rs12979860 is a critical predictor for on-treatment and sustained virologic response in patients with hepatitis C virus genotype-1 infection. PLoS One 2011;6:e18322.
40. Bishaw A. Poverty: 2000 to 2012 American Community Survey Briefs. U.S. Census Bureau. Available at: Url: http://www.census.gov/prod/2013pubs/acsbr12-01.pdf. Accessed June 24, 2014.