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ORIGINAL ARTICLE

Distribution of genetic polymorphisms associated to hepatitis C virus (HCV) antiviral response in a multiethnic and admixed population

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The prevalence of genetic polymorphisms identified as predictors of therapeutic-induced hepatitis c virus (HCV) clearance differs among ethnic groups. However, there is a paucity of information about their prevalence in South American populations, whose genetic background is highly admixed. Hence, single-nucleotide polymorphisms rs12979860, rs1127354 and rs7270101 were characterized in 1350 healthy individuals, and ethnicity was assessed in 259 randomly selected samples. The frequency of rs12979860CC, associated to HCV treatment response, and rs1127354nonCC, related to protection against hemolytic anemia, were significantly higher among individuals with maternal and paternal Non-native American haplogroups (64.5% and 24.2%), intermediate among admixed samples (44.1% and 20.4%) and the lowest for individuals with Native American ancestry (30.4% and 6.5%). This is the first systematic study focused on analyzing HCV predictors of antiviral response and ethnicity in South American populations. The characterization of these variants is critical to evaluate the risk–benefit of antiviral treatment according to the patient ancestry in admixed populations.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major health problem affecting 130 million individuals worldwide.[1] Although approximately 25% of HCV-exposed individuals can clear the virus spontaneously, the majority becomes persistently infected.[2] Chronic HCV infection leads to progressive liver damage over a period of years or decades and even liver cancer.[2,3]

During the past decade, the standard of care for the treatment of chronic hepatitis C has been the combination of pegylated-interferon-alpha (PEG-IFN)-2a or -2b and ribavirin (RBV).[4] These semi-synthetic protein–polymer conjugates of IFN with a polyethylene glycol (PEG) chain protect the protein from degradation; reduce the immunogenicity; and prolong exposure to drug by a sustained absorption, restricted volume of distribution and sustained high serum concentration. On the other hand, RBV is a guanosine ribonucleoside analog with minimal antiviral activity against HCV. However, it demonstrates significant clinical synergism when administered in combination with IFN.[4]

In addition to its limited effectiveness, this therapy is associated with major adverse effects that warrant dose reduction or treatment discontinuation, with a consequent decrease of response rates.[5]

All factors mentioned above increased the need for new therapeutic options. In this regard >50 new drugs, collectively termed direct-acting antivirals (DAA), have been developed to act against essential proteins HCV uses for replication.[5] In 2011, the US Food and Drug Administration approved boceprevir and telaprevir and triple combination therapy, including either of these two DAA, is currently recommended for patients infected with HCV genotype 1.[5]

Recent genome-wide association studies have identified single-nucleotide polymorphism (SNP) rs12979860, on chromosome 19q13.13 near the interleukin 28B (IL28B; or IFNL3) gene, as an important predictor of either spontaneous or therapeutic-induced HCV clearance following PEG-IFN/RBV therapy.[6–9]

The correlation of rs12979860 with therapeutic response was validated by several groups and across different HCV genotypes[10–13] as well as in individuals co-infected with HIV.[14,15] IL28B genotyping has become an important diagnostic tool for the management of HCV-infected patients and will continue to be important for new treatment regimens using DAA in combination with IFN[16] and even in IFN-free regimens.[17]

HCV therapy is difficult to tolerate with significant associated morbidity. Among the important adverse effects that may compromise the effectiveness of HCV therapy, RBV-induced hemolytic anemia often forces dose adjustments, drug discontinuations and/or use of erythropoietin, all of which complicates management and ultimately leads to reduced responses.[18,19] Therefore, the possibility of predictive markers of response to treatment allows both patients and clinicians to make more appropriate decisions regarding the risk–benefit of the treatment and the likelihood of success for each individual.

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The responsible gene locus for RBV-induced hemolytic anemia is in the inosine triphosphatase (ITPA) gene, which encodes an inosine triphosphate pyrophosphohydrolase (ITPase), a protein that hydrolyses inosine triphosphate (ITP) to its monophosphate derivative.[20] The association signal was entirely explained by two functional variants in the ITPA gene: a missense variant in exon 2 (rs1127354) and a splice-altering SNP in intron 2 (rs7270101) that lead to ITPase deficiency, a benign red cell enzymopathy characterized by accumulation of ITP in erythrocytes and associated with a protective effect against RBV-induced hemolytic anemia in HCV infected patients.[21–24]

It has been suggested that ITPA variants may remain predictive in the setting of HCV/HIV co-infected patients treated with PEG-IFN plus RBV as well as triple combination therapy with telaprevir.[25–27]

In addition, recent studies have shown that the presence of non-CC at rs1127354 (regardless of rs7270101 genotype) is strongly associated with protection from RBV-induced anemia and greatly influence hemoglobin levels as well as the need for RBV dose reduction and erythropoietin use.[28–30]

ITPA deficiency has also been studied in the context of thiopurine toxicity in several diseases, including inflammatory bowel disease, acute lymphocytic leukemia and transplant rejection.[31,32] Although the mechanism is still not fully elucidated, there is a tendency toward consensus that ITPA polymorphisms cause adverse drug reactions in therapy with thiopurine drugs azathioprine and 6-mercaptopurine.[31,32] Therefore, the pharmacogenetic consequences of ITPA seem to be both beneficial and detrimental depending on the involved therapy.

The prevalence of these polymorphisms differs among ethnic groups; for example, the observation that the C allele of SNP rs12979860 is less frequent among individuals of African descent relative to those of European descent might explain in part the discrepancy in the frequency of spontaneous viral clearance in these two ethnic groups, where clearance occurs in 36.4% of HCV infections in non-Africans but only 9.3% in Africans.[8] However, there is a paucity of information about South American populations, whose genetic background is highly admixed, with Native American, European and African contributions.[33]

Hence, the aim of this study was to determine the prevalence of these SNPs in the healthy population of different ethnic groups residing in Argentina.

SUBJECTS AND METHODS

Study population

This study was approved by the Ethics Committee on Research from the Italian Hospital of Buenos Aires (CEPI N° 1701) and conducted according to the Declaration of Helsinki.

After signing an informed consent statement upon enrolment, 1 ml of EDTA blood or buccal swabs were obtained from 1350 unrelated volunteers who exhibited negative serology results for anti-HCV antibodies (AxSYM, Abbott, Chicago, IL, USA). All samples were collected—during the period 2012–2013—by the DNA and blood bank at the Italian Hospital of Buenos Aires, 'Dr José María Vanella' Virology Institute of the Cordoba National University in the city of Cordoba and the blood bank at the 'Sardá' Maternity Hospital in Buenos Aires. The sample size was calculated with a 95% confidence interval and precision level at 0.1 on the basis of the population living in the metropolitan and central area of Argentina (as indicated by the 2010 National Census).[34]

The demographics of the recruited subjects are shown in Table 1. Volunteers were grouped, according to their place of birth, as Argentines, Bolivians, Peruvians and Paraguayans (Table 1).

Table 1. Demographics of the 1350 recruited volunteers

Group	n	*Gender (m:f)*	*Age (mean ± s.d.)*
Argentines	991	503:488	37.7 ± 13.8
Bolivians	185	51:134	29.0 ± 7.2
Peruvians	76	31:45	28.9 ± 7.2
Paraguayans	98	37:61	29.0 ± 7.2

Abbreviations: f, female; m, male.

Isolation of genomic DNA and genotype of IL28B and ITPA

Genomic DNA was extracted from whole blood by using FlexiGene DNA Kit (QIAGEN, GmbH, Hilden, Germany) and from buccal swabs by using QIAamp DNA Blood Mini Kit (QIAGEN) following the manufacturer's protocol.

SNPs rs1127354 (ITPA), rs7270101 (ITPA) and rs12979860 (IL28B) were PCR-amplified from isolated genomic DNA with standard Taq polymerase (Inbio-Highway, Tandil, Argentina).[35,36] The PCR-amplified fragments were bi-directionally sequenced using Big-Dye Termination chemistry system (Applied Biosystems, Life Technologies Corp., CA, USA). The sequencing chromatogram was analyzed by using the BioEdit Sequence Alignment Editor version 7.1.3.0 to discriminate between homozygotes and heterozygotes.

The degree of ITPA deficiency—and hence the risk of RBV induced-hemolytic anemia—was calculated according to the combined effect of both ITPA variants on ITPA activity, as described elsewhere.[23] Briefly, ITPA deficiency was 0% for rs11273554C/C + rs7270101A/A, 40% for rs1127354C/C + rs7270101A/C, 70% for rs1127354C/A + rs7270101A/A, 70% for rs1127354C/C + rs7270101C/C, 90% for rs1127354C/A + rs7270101A/C and 90% for rs1127354A/A + rs7270101A/A.

Molecular evaluation of ancestry

In 259 randomly-selected samples (103 from Argentina, 56 from Bolivia, 50 from Peru and 50 from Paraguay), ethnicity was assessed in both maternal and paternal lineages by analysis of haplogroups in mitochondrial DNA (haplogroups A2, B2, C and D1) and Y-SNPs (haplogroups E1b1b, G2a, I, J2, R1b1b2, Q1a3a) using real-time PCR followed by High Resolution Melting as previously described.[37]

Statistical analyses

Fisher's exact test was used for statistical analysis. A P-value of <0.05 was considered as statistically significant. The correlation between the observed number of homozygous and heterozygous individuals and the numbers statistically expected from the Hardy–Weinberg equilibrium was assessed by chi-square goodness-of-fit tests.

RESULTS

Genotype frequency of the polymorphisms

As the aim of this study was to determine the prevalence of these SNPs in the healthy population of different ethnic groups residing in Argentina, 1350 unrelated anti-HCV [−] volunteers were studied.

The data show that the genotype frequency of SNPs rs12979860, rs1127354 and rs7270101 differed greatly between the studied South American populations (Table 2).

With regard to IL28B (rs12979860) polymorphism, CC genotype—related to favorable treatment response—was significantly more prevalent among Argentines than among Bolivians, Peruvians or Paraguayans (Table 2).

With regard to ITPA polymorphisms, the frequency of rs1127354CC genotype—related to the highest risk of RBV-induced hemolytic anemia—was significantly lower for Argentines when compared with the other groups (Table 2). In addition, the frequency of rs7270101AA genotype was significantly lower for Argentines when compared with Bolivians and Peruvians but similar for Paraguayans (Table 2).

No statistical significant difference was observed when the prevalence of these polymorphisms was compared among individuals born in the central (Córdoba, Santa Fe and La Pampa) and metropolitan (Buenos Aires) regions of Argentina (data not shown).

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Table 2. Genotype frequency of SNPs rs12979860 (IL28B gene), rs1127354 and rs7270101 (ITPA gene) among Argentines, Bolivians, Peruvians and Paraguayans

Polymorphisms	*Groups*	*Genotype frequency (%)*		*Comparison between two groups*	P *value*
IL28B					
rs12979860		CC	CT/TT		
	Argentines	51.6	48.4	Argentines: Bolivians	**<0.0001**
				Argentines: Peruvians	**0.0316**
	Bolivians	35.7	64.3	Argentines: Paraguayans	**0.0195**
	Peruvians	38.1	61.9	Bolivians: Peruvians	0.7773
				Bolivians: Paraguayans	0.6071
	Paraguayans	38.8	61.2	Peruvians: Paraguayans	1
ITPA					
rs1127354		CC	CA/AA		
	Argentines	86.5	13.5	Argentines: Bolivians	**<0.0001**
				Argentines: Peruvians	**0.0121**
	Bolivians	98.9	1.1	Argentines: Paraguayans	**0.0387**
	Peruvians	96.1	3.9	Bolivians: Peruvians	0.1495
				Bolivians: Paraguayans	**0.0223**
	Paraguayans	93.9	6.1	Peruvians: Paraguayans	0.7331
rs7270101		AA	AC/CC		
	Argentines	76.7	23.3	Argentines: Bolivians	**<0.0001**
				Argentines: Peruvians	**<0.0001**
	Bolivians	96.2	3.8	Argentines: Paraguayans	0.8028
	Peruvians	94.7	5.3	Bolivians: Peruvians	0.7351
				Bolivians: Paraguayans	**<0.0001**
	Paraguayans	75.5	24.5	Peruvians: Paraguayans	**0.0007**

Abbreviation: ITPA, inosine triphosphatase.

Table 3. Prediction of risk of ribavirin-induced hemolytic anemia (RRIHA) among Argentines, Bolivians, Peruvians and Paraguayans

rs1127354C>A	*rs7270101A>C*	*Predicted ITPase activity*	*Predicted RRIHA*	*Argentines (n = 991)*	*Bolivians (n = 185)*	*Peruvians (n = 76)*	*Paraguayans (n = 98)*
CC	AA	100%	+ + +	640 (64.6%)	176 (95.1%)[a]	70 (92.1%)[a]	68 (69.4%)
CC	AC	60%	+ +	199 (20.1%)	6 (3.2%)	3 (4.0%)	23 (23.5%)
CA	AA	30%	+	102 (10.3%)	2 (1.1%)	2 (2.6%)	6 (6.1%)
CC	CC	30%	+	18 (1.8%)	1 (0.6%)	0	1 (1.0%)
CA	AC	10%	−	14 (1.4%)	0	1 (1.3%)	0
AA	AA	<5%	−	18 (1.8%)	0	0	0

Abbreviation: ITPase, inosine triphosphate pyrophosphohydrolase. [a]$P<0.0001$ when comparing Argentines with Bolivians and Peruvians.

The distribution of homozygous and heterozygous carriers was consistent with the expectations of the Hardy–Weinberg equilibrium (chi-square goodness-of-fit test: $P>0.05$ for all polymorphisms in all the groups).

Prediction of RBV-induced hemolytic anemia

ITPA genotypes carrying the minor allele (rs1127354 A, rs7270101 C), either in homozygous or heterozygous condition, were defined as protective genotypes, because they have been associated with ITPA deficiency and minimal risk of RBV-induced anemia. The consideration of both polymorphisms led to prediction of distinct degrees of ITPA deficiency, as follows: overall, 35.4% of Argentines displayed some degree of ITPA deficiency vs 4.9% of Bolivians ($P<0.0001$), 7.9% of Peruvians ($P<0.0001$) and 30.6% of Paraguayans ($P=0.3755$; Table 3).

Molecular evaluation of ancestry

Significant differences exist in the ancestry component of the studied populations. When compared with Bolivians, Peruvians and Paraguayans, the prevalence of Native American maternal and paternal haplogroups was the lowest for Argentines (Figures 1 and 2).

Influence of ethnic components on the genotype distribution

The rs12979860 SNP was unevenly distributed among individuals with Non-native American ancestry compared with those with Native American ancestry (maternal or paternal lineages), being significantly higher the frequency of the rs12979860CC genotype in those with Non-native American haplogroups compared with those with Native American haplogroups (Figures 3 and 4).

Similar results were observed when analyzing the relationship between rs1127354 polymorphism in ITPA gene and ancestry. The frequency of CC genotype was significantly lower among samples with Non-native American maternal or paternal ancestry compared with those with Native American maternal or paternal haplogroups (Figures 3 and 4). However, no statistical difference was found between the prevalence of the AA genotype of rs7270101 among samples with Native American and Non-native American ancestry (Figures 3 and 4).

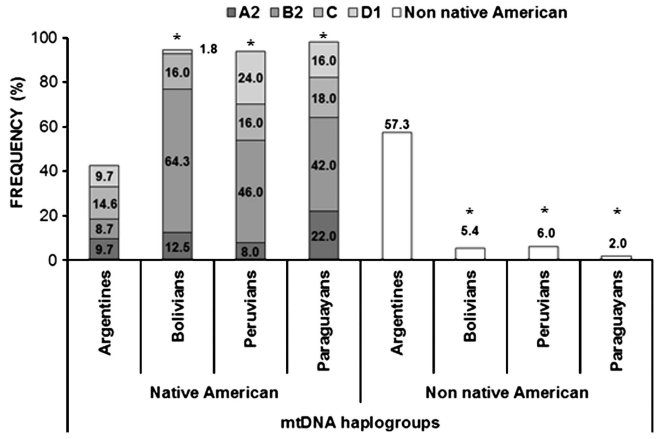

Figure 1. Prevalence of mitochondrial DNA (mtDNA) haplogroups among Argentines, Bolivians, Peruvians and Paraguayans. *$P<0.0001$ when comparing Argentines with the other groups.

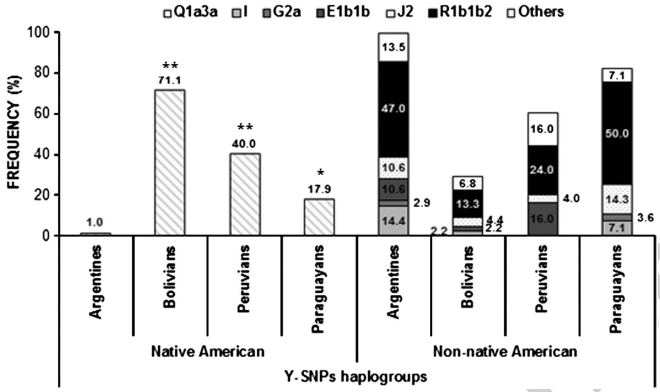

Figure 2. Prevalence of Y-SNPs (Y-chromosome single-nucleotide polymorphisms) haplogroups among Argentines, Bolivians, Peruvians and Paraguayans. *$P<0.01$, **$P<0.0001$ when comparing Argentines with the other groups.

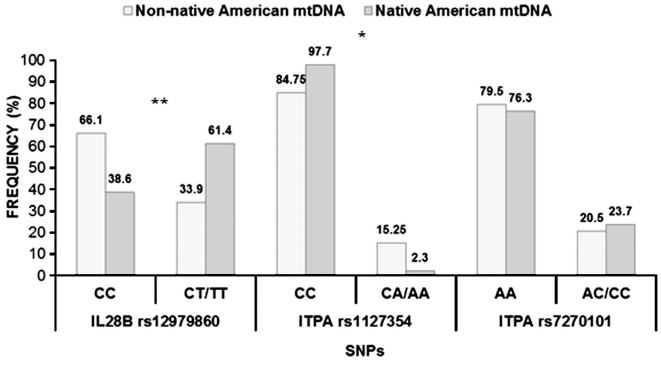

Figure 3. Prevalence of single-nucleotide polymorphism (SNP) rs12979860 (IL28B gene), rs1127354 and rs7270101 (inosine triphosphatase (ITPA) gene) genotypes among samples of Native American and Non-native American maternal ancestry. *$P<0.05$, **$P<0.01$. mtDNA, mitochondrial DNA.

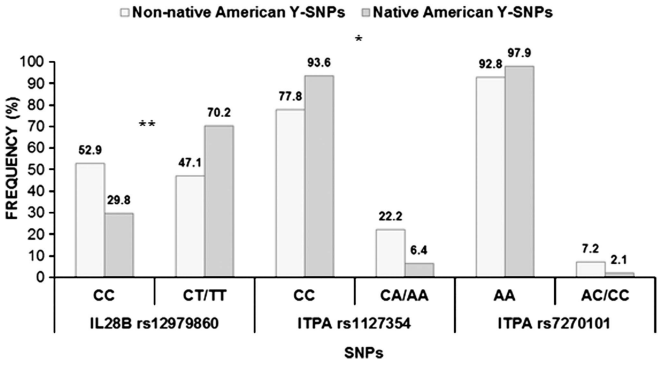

Figure 4. Prevalence of single-nucleotide polymorphism (SNP) rs12979860 (IL28B gene), rs1127354 and rs7270101 (inosine triphosphatase (ITPA) gene) genotypes among samples of Native American and Non-native American paternal ancestry. *$P<0.05$, **$P<0.01$.

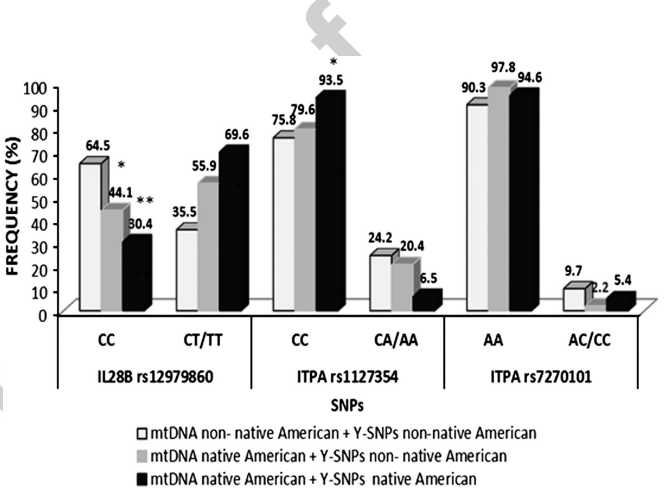

Figura 5. Prevalence of single-nucleotide polymorphisms (SNPs) rs12979860 (IL28B gene), rs1127354 and rs7270101 (inosine triphosphatase (ITPA) gene) among samples of maternal and paternal Non-native American ancestry, maternal and paternal Native American ancestry and maternal Native American and paternal Non-native American lineages (admixed samples). *$P<0.05$; **$P<0.01$ when comparing samples of maternal and paternal Non-native American lineages with the other groups. mtDNA, mitochondrial DNA.

When the prevalence of these predictive polymorphisms was analyzed according to the combined ancestry of the samples (both maternal and paternal lineages), the 'favorable' CC genotype of rs12979860 (IL28B gene) was significantly higher among samples with maternal and paternal Non-native American haplogroups, intermediate among admixed samples and the lowest for samples with Native American ancestry (Figure 5).

Similar results were found when rs1127354 polymorphism in ITPA gene was analyzed. The frequency of CC genotype was significantly lower among samples with maternal and paternal Non-native American haplogroups, intermediate among admixed samples and the highest for samples with Native American ancestry (Figure 5). However, no statistical difference was found when the analysis was carried out for the prevalence of the AA genotype of rs7270101 (Figure 5).

DISCUSSION

This is the first systematic study focused on analyzing the polymorphisms related to antiviral response in HCV infection

and ethnicity in different South American populations. Here, we report a significant bias in the distribution of predictive polymorphisms of response to HCV treatment according to the ancestry.

The prevalence of host genetic factors widely associated with HCV treatment response and protection against RBV induced-hemolytic anemia was higher among Argentines than other South American populations. The complex genetic ancestry picture detected in the population residing in Argentina may help explain the statistical significant differences. When compared with the Bolivian, Peruvian and Paraguayan populations, the frequency of Native American maternal and/or paternal haplogroups was the lowest for the samples collected from central and metropolitan Argentina, as previously reported.[33] In this region of Argentina, the European ancestry component is the most prevalent one for maternal and paternal lineages, followed by Native American and—to a lesser extent—African haplogroups.[33] Therefore, these data suggest that the European component would be predominant among the Argentine samples with Non-native American maternal haplogroups analyzed in this study.

The results presented herein for the Non-native and Native American samples are consistent with previously described frequencies for IL28B and ITPA SNPs in European and Native American populations.[8,38,39]

Regarding IL28B polymorphism, the prevalence of rs12979860 has been reported in 102 healthy subjects from central Argentina, but unfortunately, the ancestry components of this population remain unknown.[40] Moreover, this SNP has been genotyped in 51 worldwide populations ($n = 2371$) from the ALlele FREquency Database (ALFRED).[8,38] However, this thorough study did not include any Argentinean nor Paraguayan samples, and the number of those obtained from Peruvian and Bolivian populations was limited.

Regarding ITPA polymorphisms, Marsh *et al.*[39] has studied the distribution of alleles related to ITPA deficiency in multiple worldwide populations. The highest frequency was observed in Asian communities, followed by Caucasian and African populations. In consistency with our results obtained among populations exhibiting high frequency of Native American ancestry, the Mexican and Peruvian samples analyzed by this group showed the lowest prevalence of ITPA deficiency-related alleles.[39]

To the best of our knowledge, this is the first study to report the prevalence of polymorphisms related to antiviral response in HCV infection in an admixed healthy South American population.

The global distribution of allele frequencies for rs12979860 (IL28B gene) shows a striking pattern in which the favorable C allele is nearly fixed throughout East Asia, has an intermediate frequency in Europe and is the minor allele in Africa.[8] Interestingly, the high frequency of the C allele found in North and Eastern Asia is not reflected in its frequency in Native American populations. The fact that a common variant has such a strong impact on HCV infection may indicate that it has actually been under selection pressure.

Looking for evidence of natural selection is an attractive strategy for understanding the relevance of a given gene to host survival by conferring, for example, an increased resistance to infectious diseases.

The dispersal of early humans from Africa to Europe, Asia and the Americas—each with different climates, pathogens and sources of food—varied the selective pressures that challenged human populations. Moreover, the effects of selection could have been accentuated by the marked changes in population size, population density and cultural conditions that accompanied the introduction of agriculture at the beginning of the Neolithic period $\sim$10 000 years ago.[41,42] Today, the functional consequences of the genetic variants that facilitated survival in ancestral human populations might underlie the phenotypic differences between individuals and groups. Therefore, the analysis of genetic variation in populations has become central to understanding the function of genes.[43]

By using an evolutionary genetics approach, which investigates the way in which infections have shaped the variability of host defense genes by natural selection, Manry *et al.*[44] have shown that type III IFN is the only group of IFNs where selective pressures have involved processes of geographically restricted adaptation, revealing that genetic variation at these genes has conferred a selective advantage to specific human populations. Indeed, it has been reported that SNP rs12979860 at IL28B gene has been positively selected in Europeans and Asians, most likely by increasing resistance to viral infection.[44] These authors hypothesized that other ancestral and more virulent flaviviruses are responsible for the selective footprints observed; due to the fact that given the chronic and insidious nature of HCV pathogenesis, it is unlikely that at least the modern form of the virus is really responsible for the selection pressure exerted on IL28B gene. To this end, it will be of interest to determine whether IL28B polymorphisms have been under the effects of natural selection in Native American populations, as well.

Moreover, the high frequency of the ITPA deficiency found in Asia is also not reflected in its frequency in Native American populations. To our knowledge, there is no report indicating whether ITPA gene have been under the effects of natural selection in worldwide populations.

In Argentina, IL28B—but not ITPA—testing has been recommended for all HCV-infected patients before therapy.[45] However, its accessibility is limited to those covered by private health-care insurance. Consequently, this study is likely to have at least two direct implications: (i) it highlights the importance of the previous characterization of these variants to evaluate the risk–benefit of antiviral treatment according to the patient ancestry, particularly in multiethnic and admixed populations; and (ii) it urges the incorporation of testing of host genetic factors in all HCV patient profiles in Argentina to help predict response and personalize therapy and/or dosage before commencement. This prediction will change the 'cost/benefit' of treatment and may help to encourage patients with favorable variants to undergo treatment and to reassure them during a long and often difficult treatment course. As the number of therapeutic options is expanding in coming years, this may help to simplify and optimize treatment algorithms.

Furthermore, in regions where patients with admixed and Native American ancestry—and, therefore, less favorable genetic variants—prevail, therapy efficacy issues must be taken into consideration. The effect of extending PEG-IFN/RBV treatment duration or adding a third or fourth agent on response rates needs to be studied in these particular populations.

In future decades, the number of HCV-infected patients is expected to increase due to the absence of an effective vaccine and the insufficient amount of public awareness and preventive measures, particularly in developing countries.[1,46] As a consequence, the results presented herein imply an unprecedented call of singular relevance for the regional public health, whose genetic background is highly admixed with an important contribution—in some areas and countries—of Native American ancestry components.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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