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High Prevalence of ITPA Alleles Associated with Ribavirin-Induced Hemolytic Anemia Among Mexican Population

Karina Gonzalez-Aldaco,*,** Arturo Panduro,*,** João R. Rebello Pinho,***
Erika Martinez-Lopez,*,** Ketti Gleyzer,*** Nora A. Fierro,*,** Sonia Roman*,**

* Department of Molecular Biology in Medicine, Civil Hospital of Guadalajara, "Fray Antonio Alcalde", Guadalajara, Jalisco, Mexico.
** Health Sciences Center, University of Guadalajara, Guadalajara, Jalisco, Mexico.
*** Departamento de Patologia Clínica e Anatomia Patológica, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil.

ABSTRACT

Background. The prevalence of two functional polymorphisms (rs1127354 and rs7270101) of the inosine triphosphatase (*ITPA*) gene associated with ribavirin-induced hemolytic anemia (RIHA) during antiviral therapy for hepatitis C virus (HCV) infection varies by ethnicity. In Mexico, the distribution of these polymorphisms among Native Amerindians (NA) and admixed population (Mestizos) is unknown. This study aimed to determine the prevalence of the *ITPA* polymorphisms among healthy NA and Mestizos, as well as in HCV patients from West Mexico. **Material and methods.** In a cross-sectional study, 600 unrelated subjects (322 Mestizos, 100 NA, and 178 treatment-naïve, HCV-infected Mestizos patients) were enrolled. A medical history was registered. *ITPA* genotype was determined by Real-Time PCR. Fst-values and genetic relatedness between study and reference populations were assessed. **Results.** The frequency of the risk genotypes rs1127354CC and rs7270101AA was higher among NA (98-100%) than in Mestizos (87-92.9%), ($p < 0.05$). The NA presented the highest prevalence of the rs1127354CC genotype reported worldwide. The Fst-values revealed a genetic relatedness among Mexican NA, South Americans and African populations ($p > 0.05$). The frequency of the predicted risk for RIHA was higher among NA (98%) than in Mestizos (80.5%) and HCV-infected patients (81.5%) ($p < 0.01$). The CC/AA alleles were associated with lower values of total bilirubin, aspartate/alanine aminotransferases, and aspartate-to-platelet-ratio-index score among HCV-patients. **Conclusion.** A high prevalence of the *ITPA* polymorphisms associated with RIHA was found in Mexican NA. These polymorphisms could be a useful tool for evaluating potential adverse effects and the risk or benefit of antiviral therapy in Mexicans and other admixed populations.

Key words. Hepatitis C. Direct-acting antivirals. Adverse effects. Native Amerindians.

INTRODUCTION

Hepatitis C virus (HCV) infection is a significant health problem affecting 185 million individuals worldwide.[1] While spontaneous clearance may occur in approximately 30% of individuals infected with HCV, the majority progress to chronic infection and are candidates for antiviral therapy.[2] Also, once chronic infection is established it can advance to liver cirrhosis and hepatocellular carcinoma.[3]

Before 2010, the standard of care for chronic HCV infection was based on pegylated-interferon alpha plus ribavirin (RBV); however, its effectivity was limited.[4] As of 2011, with the approval of the new direct antiviral agents (DAAs) for chronic hepatitis C, such as boceprevir and telaprevir among others, a higher efficacy has been achieved. These new DAAs target the nonstructural proteins involved in the HCV life cycle.[5] In some cases, the DAAs offer an enhanced advantage when prescribed in conjunction with RBV. Thus, with the implementation of double or

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even triple therapy, the use of RBV will continue. RBV is a synthetic purine analogous effective against many DNA and RNA viruses, such as herpesviruses, poxvirus, influenza, measles, adenoviruses and hepatitis viruses.[7] Unfortunately, RBV may not be easily tolerated because it has been associated with RBV-induced hemolytic anemia (RIHA). The development of RIHA during antiviral treatment against HCV infection is responsible for treatment discontinuation, dose reduction and consequently a decrease in the sustained virological response (SVR).[8]

The genetic locus responsible for the RBV-induced hemolytic anemia is found in the inosine triphosphatase (*ITPA*) gene, which encodes an enzyme with the same name.[9] ITPA is a highly conserved enzyme that hydrolyzes deaminated purine (inosine) nucleoside triphosphates (ITP) into monophosphate and pyrophosphate derivates.[9,10] As described by Felly, *et al*., the combination of two validated functional single nucleotide polymorphisms (SNPs) in the *ITPA* gene can predict its phosphatase (ITPase) activity.[11] The ITPase activity predicted by the combination of two SNPs: rs1127354 (C > A) P32T, a missense variant located in exon 2 and the rs7270101 (A > C) a splicing-altering SNP located in the second intron is associated with RIHA.[11] Specifically, the genotypes rs1127354AA and rs7270101CC lead to ITPase activity deficiency, a benign red cell enzymopathy characterized by an intense accumulation of ITP in erythrocytes and associated with protection against RIHA in HCV-infected patients.[12] By contrast, the genotypes rs1127354CC and rs7270101AA that lead to functional ITPase activity have been linked to RIHA, yet the molecular mechanism remains unknown. However, oxidative damage and erythrocyte lysis have been related to the intracellular accumulation of pharmacologically active RBV.[13]

The prevalence of the *ITPA* polymorphisms and the predicted risk for RIHA has not been thoroughly studied across the American continent.[14,15] As in most Latin American countries, the genetic structure of the Mexican population is an admixture of three paternal lineages consisting of Amerindian, European and African ancestry denoted as Mestizos, as well as conserved Native Amerindians (NA).[16,17] Specifically, in Mexico, different degrees of admixture have been estimated among the Mestizos, while, on the other hand, there are nearly 15 million NA, who still maintain their inherited traditions.[18] In consequence, a heterogeneous distribution of polymorphic genes associated with the clinical outcome and SVR of viral hepatitis, including the *ITPA* gene variants may be expected.[19,20] Hence, the genotyping of these variants could be needed to evaluate the possibility of adverse effects and the risk or benefit of antiviral therapy, especially in patients that require extensive periods of treatment with ribavirin, even with the new DAAs. Therefore, the aim of this study was to determine the prevalence of the *ITPA* polymorphisms among healthy NA and Mestizos, as well as in treatment-naïve HCV patients from West Mexico.

MATERIALS AND METHODS

Study population

This study was conducted at the Department of Molecular Biology in Medicine, Civil Hospital of Guadalajara "Fray Antonio Alcalde" in Guadalajara, Jalisco, Mexico. In this cross-sectional study, a total of 600 unrelated subjects from West Mexico were enrolled from January 2010 to December 2013. For the *ITPA* distribution study, 422 healthy individuals were included: 100 NA (50 Huicholes and 50 Nahuas) and 322 Mestizos (240 subjects from Guadalajara, Jalisco; 32 subjects from Villa Purificación, Jalisco and 50 subjects from Tepic, Nayarit). For the association study, 178 treatment-naïve HCV-infected Mestizos patients were included.

The NA were members of the Nahuas and Huicholes ethnic group, spoke their native language and had parents belonging to the ethnic group. The Mestizos subjects were defined as born in Mexico, which spoke Spanish, had Mexican parents and did not belong to any native group.[16,21]

The study protocol was approved by the Ethical Committee of the Hospital Civil of Guadalajara, Guadalajara, Jalisco Mexico and Hospital Israelita Albert Einstein, São Paulo, Brazil and was conducted in compliance with the ethical standards of the 2008 Declaration of Helsinki. All participants signed a written informed consent statement.

Clinical and biochemical evaluation

The healthy subjects were negative for anti-HCV antibodies. All HCV patients were treatment-naïve and negative for hepatitis B virus and human immunodeficiency virus at the time of enrollment. At this stage, a medical history questionnaire was used to register the amount of alcohol intake. Only patients consuming < 20 g of alcohol per occasion for women, and < 40 g alcohol per occasion for men (as recommended to prevent liver damage)[22] were included in the study. Alcohol intake was calculated as previously reported.[23] Anti-HCV antibodies were detected by a third-generation ELISA (AxSYM®, Abbott Laboratories, Illinois, USA). HCV viral load was measured by COBAS® AmpliPrep and COBAS® TaqMan® 48 HCV test (Roche Diagnostics, Pleasanton, CA, USA). Liver enzymes aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined by dry chemistry on a Vitros 250 analyzer (Ortho Clinical Diagnostics, Johnson & Johnson, Rochester, NY, USA). The aspartate ami-

notransferase-to-platelet ratio index (APRI) score was assessed to evaluate the degree of liver damage as previously reported.[24]

Isolation of genomic DNA and *ITPA* genotyping

Genomic DNA was extracted from peripheral whole blood leukocytes by a modified salting-out method.[25] The SNPs rs1127354 and rs7270101 were genotyped using a 5' allelic discrimination method. A Real-Time PCR was carried out using predesigned TaqMan SNP Genotyping Assay (rs1127354 C_27465000_10 and rs7270101 C_29168507_10, Applied Biosystems, Foster, CA, USA). An ABI 7500 Fast Real-Time thermocycler was used for PCR amplification following the standard conditions recommended by the manufacturer. The 7500 software (version 2.0.6.) automatically attributed the sample's genotype. The correct genotype allocation was verified by positive and negative controls. Also, 20% of the samples were rerun, and 100% were concordant. The genotyping experiment was conducted in collaboration with the Albert Einstein Medicina Diagnóstica, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil.

Prediction of risk for RIHA

The prediction of the risk or protection for RIHA was estimated according to the combined effect of the functional *ITPA* SNPs genotypes, as previously described.[11,26] Briefly, high risk was rs1127354CC and rs7270101AA; moderate risk, rs1127354CC and rs7270101AC; mild risk, rs1127354CA and rs7270101AA or rs1127354CC and rs7270101CC; low risk, was rs1127354CA and rs7270101AC or rs1127354AA and rs7270101AA.

Genetic analysis

The genotype frequency of rs1127354 and rs7270101 SNPs was obtained by a direct counting method. The Hardy-Weinberg equilibrium (HWE) expectation was assessed by the exact test. Genetic relatedness between populations based on *ITPA* SNPs was evaluated by pairwise comparisons (exact test) and genetic distances (Fst) using Arlequin software version 3.0.[27] These were additionally represented in a Multidimensional (MDS) scaling plot and in a Neighbor-Joining tree using SPSS software and FigTree version 1.4.2 for Windows (Institute of Evolutionary Biology, University of Edinburgh), respectively.

Statistical analysis

The categorical variables were expressed as frequency and were compared by chi-square or Fisher's exact tests. Quantitative variables were expressed as median ± standard deviation (SD) and were compared with Student's t-test. A p-value < 0.05 was considered as statistically significant. The statistical analysis was performed using SPSS version 21 for Windows (SPSS, Inc, Chicago, IL, USA).

RESULTS

Prevalence of *ITPA* genotypes and comparative genetic analysis

The prevalence of the *ITPA* genotypes among the West Mexico's populations is shown in Table 1. The genotype frequency of both SNPs differed between the NA and

Table 1. Allelic and genotypic distribution of *ITPA* polymorphisms among Native Amerindians, Mestizos and HCV-infected patients

	Huicholes (n = 50)	Nahuas (n = 50)	Guadalajara (n = 240)	Villa Pur (n = 32)	Nayarit (n = 50)	HCV patients (n = 178)
			% (95% CI)			
rs1127354						
CC	100%*	100%*	92.9% (89.6-96.1)	87.5% (75-100)	96% (90-102)	92.7% (89-97)
CA	-	-	6.7% (3.4-9.8)	12.5% (0.3-24.6)	4% (0.2-10)	6.7% (3-10)
AA	-	-	0.4% (0.4-1.2)	-	-	0.6% (0.01-2.0)
HWE	MPC	MPC	1.0	1.0	1.0	1.0
rs7270101						
AA	98% (93.9-102) **	98% (93.9-102) **	87.9% (83.7-92.0)	84.4% (71.0-97.6)	84% (73.4-94.5)	88.8% (84.0-93.4)
AC	2% (2.0-6.0)	2% (2.0–6.0)	12.1% (7.9-16.2)	15.6% (2.3-28.9)	16% (5.4-26.5)	11.2% (6.5-15.9)
CC	-	-	-	-	-	-
HWE	1.0	1.0	1.0	1.0	1.0	1.0

HWE: Hardy-Weinberg equilibrium. MPC: monomorphic. Villa Pur: Villa Purificación. HCV: hepatitis C virus. * *Huicholes/Nahuas* vs. *Villa Pur* $p < 0.05$. ** *Huicholes/Nahuas* vs. *Guadalajara, Villa Pur, Nayarit and HCV patients* $p < 0.05$

Mestizos. In regards to rs1127354, the wild-type CC genotype associated with risk of RIHA predominated in all the Mestizo populations (> 87.5%), and the Huicholes and Nahuas were monomorphic (CC = 100%). As for rs7270101, the AA genotype associated with risk of RIHA predominated among the Mestizo populations (> 84%). However, the Huicholes and Nahuas had the highest frequency ($p < 0.05$) compared to the Mestizos from Guadalajara, Villa Purificación, and Nayarit. All populations were in HWE for the two SNPs ($p > 0.05$).

Due to the high prevalence of the risk *ITPA* genotypes among NA and Mestizo subjects, their prevalence was compared with reference populations with European, Asian, and African ancestry and other regions of Latin America reported in the 1000 Genomes Project[28] (Figure 1). Furthermore, the genetic relatedness analysis between the study and reference populations (Bolivian, Peruvian, Caucasian, Asian and African) based on the two *ITPA* SNPs revealed two separate clusters. One cluster contained the NA from West Mexico, Bolivian and African individuals while the Mestizos formed a separate cluster and were poorly correlated to the Europeans and Asians ($p > 0.05$). (MDS plot and in Neighbor-Joining tree, Figure 2)

A. rs1127354.

	NA	MTZO	HCV-P	BOL	PER	AFR	SPN	BRI	CHI	JAP
CA/AA	-	7.1	7.3	1.1	2.4	6.7	16.7	11	14.5	25
CC	100	92.9	92.7	98.9	97.6	93.3	83.3	89	85.5	75

B. rs7270101.

	NA	MTZO	HCV-P	BOL	PER	AFR	SPN	BRI	CHI	JAP
AC/CC	2	13	11.2	3.9	9.4	4.7	29.9	35.2	-	-
AA	98	87	88.8	96.1	90.6	95.3	70.1	64.8	100	100

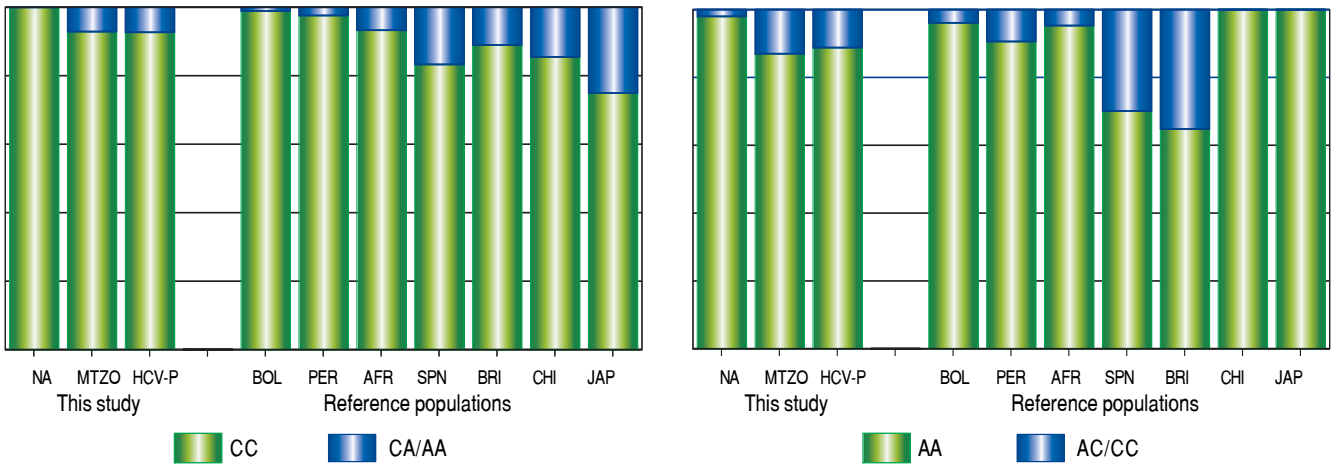

Figure 1. *Prevalence of ITPA rs1127354 and rs7270101 polymorphisms in NA, Mestizos, HCV-infected patients compared with reference populations.* ***A.*** *The prevalence of the risk genotype (CC) was highest in NA than in all groups ($p < 0.05$) except in Bolivians ($p = 0.296$) and Peruvians (PER) ($p = 0.407$). While in Mestizos (MTZO) and HCV-infected patients (HCV-P) the prevalence was similar to all populations except Bolivia (BOL) ($p = 0.002$), China (CHI) ($p = 0.021$) and Japan (JAP) ($p = 1.8 \times 10\text{-}6$).* ***B.*** *The prevalence of the risk genotype (AA) was highest in NA than in all groups ($p < 0.05$) except in BOL ($p = 0.411$), Africans (AFR) ($p = 0.535$), Japan and China ($p = 0.464$). While in MTZO and HCV-P, the prevalence was similar to PER and AFR but different to all others ($p < 0.05$).*

Table 2. Prediction of risk for RIHA in Native Amerindians, Mestizos and HCV patients

rs1127354C>A	rs7270101A>C	Predicted risk for RIHA	Natives (n = 100)	Mestizos (n = 322)	HCV patients (n = 178)	P value
CC	AA	High risk	98 (98)[ab]	259 (80.5)	145 (81.5)	**6.0×10^{-5}**
CC	AC	Moderate risk	2 (2)[cd]	40 (12.4)	20 (11.2)	**0.003**
CA	AA	Mild risk	-	20 (6.2)	12 (6.7)	0.816
CC	CC	Mild risk	-	-	-	-
CA	AC	Low risk	-	2 (0.6)	-	-
AA	AA	Low risk	-	1 (0.3)	1 (0.6)	0.753

The data are expressed as n (%). HCV: hepatitis C virus. RIHA: ribavirin-induced hemolytic anemia. [a] *Natives* vs. *Mestizo* $p = 2 \times 10^{-6}$. [b] *Natives* vs. *HCV-patients* $p = 1.9 \times 10^{-5}$. [c] *Natives* vs. *Mestizos* $p = 0.004$. [d] *Natives* vs. *HCV patients* $p = 0.012$.

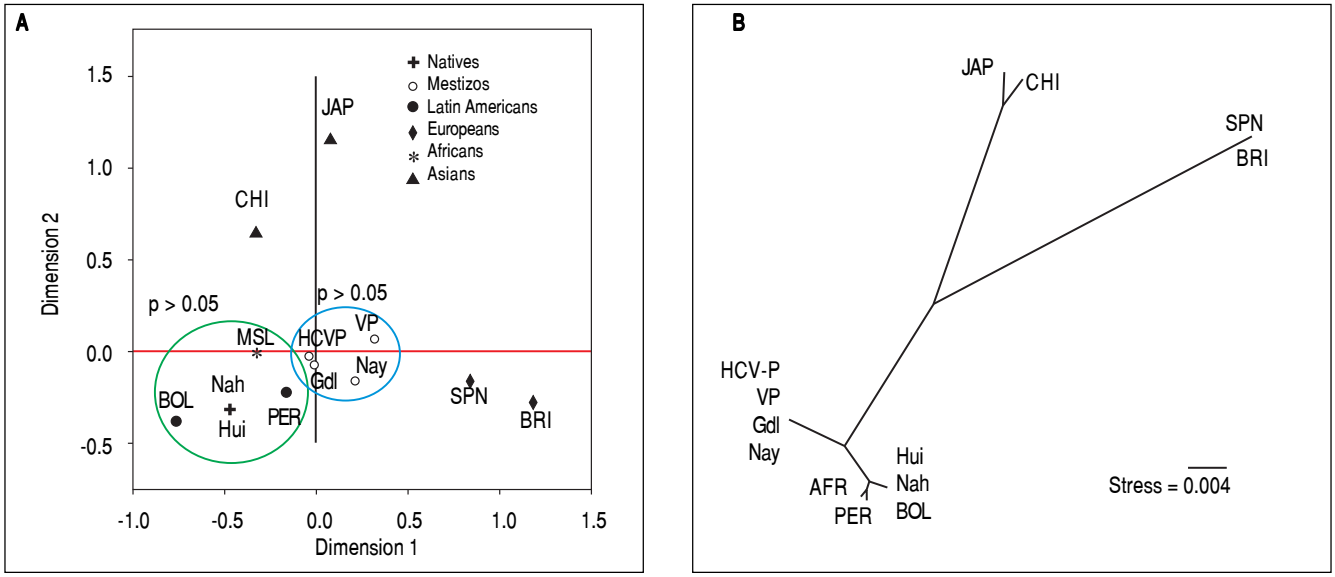

Figure 2. *Genetic relatedness based on the ITPA polymorphisms rs1127354 and rs7270101 between Mexican and reference populations represented on the multidimensional scaling (MDS) plot* ***(A)*** *and the Neighbor-Joining (NJ) tree* ***(B)****. Population clustering depicted in the MDS plot is in agreement with spatial representation in the NJ tree. Hui, Huicholes. Nah, Nahuas. BOL, Bolivians. PER, Peruvians. Gdl, Guadalajara; Nay, Nayarit. VP, Villa Purificación. HCV-P, HCV-infected patients. CHI, China. JAP, Japan. SPN, Spain. BRI, British. MSL, Mende in Sierra Leone, Africa. AFR, Africans.*

Table 3. Biochemical profile of HCV-infected patients stratified by risk and non-risk ITPase genotypes

Parameter	High risk CC/AA genotype (n = 145)	Non high-risk genotypes* (n = 33)	P value
Subjects (n, %)	145 (81.5)	33 (18.5)	-
Female (n, %)	50 (39.1)	8 (28.6)	0.257
Age (years)	51.3 ± 11.9	50.1 ± 13.3	0.653
Hemoglobin (g/dL)	14.3 ± 1.7	14.03 ± 1.4	0.418
AST (IU/L)	54.6 ± 36.0	89.2 ± 103.2	**0.004**
ALT (IU/L)	62.5 ± 56.8	103.7 ± 120.1	**0.011**
Platelets ($x10^3/\mu L$)	188.2 ± 82.7	187.8 ± 88.8	0.959
HCV load ($x10^6$ IU/mL)	4.13 ± 9.38	4.35 ± 8.64	0.910
APRI score	0.85 ± 0.94	1.5 ± 1.7	**0.014**
Total bilirubin (mg/dL)	0.86 ± 0.51	1.3 ± 1.67	**0.004**
Chronic Infection (n, %)	84 (65.1)	17 (60.7)	0.659

AST*: aspartate aminotransferase.* ***ALT****: alanine aminotransferase.* ***HCV****: hepatitis C virus.* ***APRI****: aspartate aminotransferase-to-platelet ratio index. *Non Risk genotypes: CC/AC; CA/AA; CC/CC; CA/AC; AA/AA combinations.*

Prediction of risk for RIHA among West Mexico's populations

The prediction of the risk for RIHA was assessed using the combination of the two SNPs rs1127354 and rs7270101 (Table 2). Overall, NA subjects presented a high frequency of the risk genotypes (98%) associated with RIHA and were statistically significant compared to Mestizos subjects (80.5%) and HCV-infected patients (81.5%) ($p=6 \times 10^{-5}$). Meanwhile, up to 7.1% of the Mestizos and 7.3% of the HCV-infected patients had mild to low risk for RIHA.

Biochemical profile of HCV patients stratified by risk *ITPA* genotypes

Biochemical variables stratified by *ITPA* genotypes (CC/AA *vs.* all others) were evaluated in HCV-infected patients as shown in Table 3. The risk CC/AA genotypes were associated with lower levels of total bilirubin, AST, ALT and APRI score compared to patients with mild to low-risk genotypes ($p < 0.05$). No differences in hemoglobin and age were observed among these patients.

DISCUSSION

In this study, we reported a high prevalence of *ITPA* polymorphisms and predicted high risk of RIHA among Mestizos, NA and HCV-infected patients residing in West Mexico. In this region, the Mestizos have mainly European ancestry followed by NA and in lesser extent African, as demonstrated by paternal lineages and maternal mtDNA haplogroups.[16,29] However, in this study, the prevalence of the *ITPA* polymorphisms was higher in NA than in Mestizos and HCV-infected patients. Interestingly, the Mestizos and HCV-infected patients also carried the risk genotypes for RIHA, which may be due to the inheritance of the Amerindian component as shown by the comparative genetic analysis. These analyzes revealed that for the rs1127354 SNP, the Mestizos and HCV-infected patients were similar to Spanish and British subjects and different to Bolivian, Chinese and Japanese individuals ($p < 0.05$). In contrast, Huicholes and Nahuas had the highest frequency of the risk genotype (CC) compared to the European, African, and Asian population and they exhibited a similar frequency as in the Bolivian and Peruvian individuals ($p = 0.296$).[15,28] These results are in concordance with previously described frequencies for *ITPA* in these populations from South America, exhibiting high NA ancestry.[15] Also, these data are in agreement with the history of the peopling of Central and South America regions 15 000-25 000 years ago, which according to molecular analysis, share the same ancestors.[30,31] Moreover, in regards to the rs7270101, the risk genotype (AA) had the highest frequency in NA from West Mexico, as well as Bolivians and surprisingly Asians. While, the Mestizos and HCV-infected patients were similar only to Peruvians and Africans. A possible explanation for this result is that NA have their origin in Asians founders who arrived via Beringia to the American continent.[31] Also, rs1127354C and rs7270101A are considered both the ancestral alleles, which may be another reason they were almost fixed in NA and other ancestral populations.

In support of these hypotheses, both the MDS plot and the Neighbor-Joining tree showed the genetic relatedness between the NA from West Mexico, Bolivians, Peruvians, and Africans. However, the relatedness was not evident with the Asians subjects because the multidimensional representation was constructed using both SNPs and their frequencies were similar only in the rs7270101 polymorphism. Moreover, the Mestizos (including HCV-infected patients) were similar to themselves and occupied an intermediate position between NA groups and European populations (Spanish and British).

Given the reported consistency between the ITPA enzyme activity and the corresponding rs1127354 and rs7270101 *ITPA* alleles, these genetic variants have been proposed as predictors of drug toxicity and RIHA during HCV antiviral treatment in distinct populations,[32,33] thus eliminating the need for measuring ITPA activity in affected patients.[11,26.] In this study, 81.5% of the Mestizo HCV patients may be at high risk of RIHA during antiviral therapy, and only 18.5% presented a predicted low risk. If we take into account that at least 700,000 Mexicans present active viremia, and many of them may have cirrosis,[34] the use of DAAs plus RBV could be a common scheme of therapy in these patients; thus, RIHA and dose adjustment could compromise its effectiveness. However, it would be interesting to further investigate in admixed populations the *ITPA* genotype-phenotype correlation of these polymorphisms and others that have been reported.

Although, ITPA is highly expressed in liver, heart, sex glands, thyroid, and adrenal glands,[35] no adverse effects associated with ITPase activity has been reported in these tissues.[10] However, in this study, patients with the high-risk alleles showed significantly lower values of total bilirubin, ALT, AST, and APRI score compared to patients with moderate to low risk alleles. Interestingly, it has been documented that components of the bilirubin pathway may be altered during viral infection[36] and may play an immune regulatory role in the outcome of HCV infection.[37] Biliverdin is known to inhibit the HCV viral protease, whereas biliverdin reductase upregulation has been observed in chronically HCV-infected patients with an SVR to treatment relative to non-responding patients.[37] Furthermore, the induction of heme oxygenese-1 in HCV infection has been shown to decrease viral replication, as well as protection against oxidative damage.[38] On the other hand, the lack of elevated ALT and AST levels is consistent with retrospective studies of HCV-infected patients who revealed an association between the presence of symptoms, jaundice and spontaneous viral clearance status of serum polymerase chain reaction-negative individuals.[39] Therefore, besides being at risk for RIHA, these patients may have alterations in the progression of HCV infection modulated by this particular clinical profile that requires further study.

Moreover, in conjunction with the *ITPA* polymorphisms, other genetic predictors for an antiviral response in chronic hepatitis C patients are the *interleukin-28B* (*IL28B*) and *interferon lambda-4 (IFNL4)* gene polymorphisms.[40,41] We have recently reported a genetic differentiation of the *IL28B/IFNL4* haplotypes among Huicholes and Nahuas, who are carriers of the risk genotypes for HCV chronic infection and poor SVR.[42] Therefore, if NA people were eventually infected by HCV, they could be at high risk of developing RIHA and/or may not respond to treatment. Likewise, the Mestizos, who presented a high prevalence of the risk alleles, could also have an adverse prognosis for treatment response. Thus, the characterization of *ITPA* and *IL28B/IFNL4* that is currently being used as clinical markers of treatment response in Latin American

countries such as Argentina and Brazil,[43,44] could also be relevant in the Mexican population.[45] Recently, the new DAAs have been incorporated into the health systems in Mexico. However, given the heterogeneous genetic background of the Mexican population for the aforementioned polymorphisms, it would be advisable to test patients to avoid poor responses to antiviral therapy.

Finally, the pharmacogenetic implications of the *ITPA* risk alleles are dependent on the drug therapy involved.[46] These variants have been studied in the context of toxicity to 6-mercaptopurine and azathioprine in several diseases, including acute lymphocytic leukemia,[47] inflammatory bowel disease,[48] and transplant rejection.[49] Thus, the results of this study could have an impact on the prediction of toxicity of drugs implicated in several diseases, as well as in hematological complications of viral infections treated with RBV.[50]

CONCLUSION

This study is the first to report the high prevalence of the *ITPA* risk alleles associated with RIHA in NA, Mestizos and treatment-naïve HCV-infected patients from West Mexico. These findings highlight the importance of pretreatment characterization of the *ITPA* polymorphisms to evaluate potential adverse effects and the risk-benefit of antiviral therapy with RBV, especially in regions where NA ancestry prevails. Further investigations of these variants are necessary to assess the impact on the outcome of HCV antiviral therapy among admixed populations in Latin America and worldwide.

ABBREVIATIONS

- **HCV:** hepatitis C virus.
- **ITP:** inosine triphosphate.
- **ITPA:** inosine triphosphatase.
- **NA:** native Amerindians.
- **peg-IFN:** pegylated interferon.
- **RBV:** ribavirin.
- **RIHA:** ribavirin-induced hemolytic anemia.

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REFERENCES

1. Lavanchy D. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; 17: 107-15.
2. Hoofnagle JH. Course and outcome of hepatitis C. *Hepatology* 2002; 36: S21-29.
3. Westbrook RH, Dusheiko G. Natural history of hepatitis C. *J Hepatol* 2014; 61: S58-68.
4. Feld JJ. Treatment indication and response to standard of care with peginterferon and ribavirin in acute and chronic HCV infection. *Best Pract Res Clin Gastroenterol* 2012; 26: 429-44.
5. Hofmann WP, Zeuzem S. A new standard of care for the treatment of chronic HCV infection. *Nat Rev Gastroenterol Hepatol* 2011; 8: 257-64.
6. Ghany MG, Nelson DR, Strader DB, Thomas DL, Seeff LB. 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, 1433-44.
7. Chang TW, Heel RC. Ribavirin and inosiplex: a review of their present status in viral diseases. *Drugs* 1981; 22, 111-28.
8. Jain MK, Zoellner C. Role of ribavirin in HCV treatment response: now and in the future. *Expert Opin Pharmacother* 2010; 11: 673-83.
9. Sumi S, Marinaki AM, Arenas M, Fairbanks L, Shobowle-Bakre M, Rees DC, Thein SL, et al. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. *Hum Genet* 2002; 111: 360-7.
10. Sakumi K, Abolhassani N, Behmanesh M, Iyama T, Tsuchimoto D, Nakabeppu Y. ITPA protein, an enzyme that eliminates deaminated purine nucleoside triphosphates in cells. Mutat Res 2010; 703: 43-50.
11. Fellay J, Thompson AJ, Ge D, Gumbs CE, Urban TJ, Shianna KV, Little LD, et al. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature* 2010; 464: 405-8.
12. Holmes JA, Roberts SK, Ali RJ, Dore GJ, Sievert W, McCaughan GW, Crawford DH, et al. ITPA genotype protects against anemia during peginterferon and ribavirin therapy but does not influence virological response. *Hepatology* 2014; 59: 2152-60.
13. De Franceschi L, Fattovich G, Turrini F, Ayi K, Brugnara C, Manzato F, Noventa F, et al. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. *Hepatology* 2000; 31: 997-1004.
14. Marsh S, King CR, Ahluwalia R, McLeod HL. Distribution of ITPA P32T alleles in multiple world populations. *J Hum Genet* 2014; 49: 579-81.
15. Trinks J, Hulaniuk ML, Caputo M, Pratx LB, Re V, Fortuny L, Pontoriero A, et al. Distribution of genetic polymorphisms associated with hepatitis C virus (HCV) antiviral response in a multiethnic and admixed population. *Pharmacogenomics J* 2014; 14: 549-54.
16. Martinez-Cortes G, Salazar-Flores J, Fernandez-Rodriguez LG, Rubi-Castellanos R, Rodriguez-Loya C, Velarde-Felix JS, Muñoz-Valle JF, et al. Admixture and population structure in Mexican-Mestizos based on paternal lineages. *J Hum Genet* 2012; 57: 568-74.
17. Ruiz-Linares A, Adhikari K, Acuna-Alonzo V, Quinto-Sanchez M, Jaramillo C, Arias W, Fuentes M, et al. Admixture in Latin America: geographic structure, phenotypic diversity and self-perception of ancestry based on 7,342 individuals. *PLoS Genet* 2014; 10, e1004572.
18. INEGI. Instituto Nacional de Estadística y Geografía. Censo Nacional 2010. Available at: http://www.inegi.org.mx/. Accessed: January 04 2016.
19. Fierro NA, Gonzalez-Aldaco K, Torres-Valadez R, Trujillo-Trujillo ME, Roman S, Trujillo-Ochoa JL, Panduro A. Spontaneous hepatitis C viral clearance and hepatitis C

chronic infection are associated with distinct cytokine profiles in Mexican patients. *Mem Inst Oswaldo Cruz* 2015; 110: 267-71.
20. Roman S, Jose-Abrego A, Fierro NA, Escobedo-Melendez G, Ojeda-Granados C, Martinez-Lopez E, Panduro A. Hepatitis B virus infection in Latin America: a genomic medicine approach. *World J Gastroenterol* 2014; 20: 7181-96.
21. Aceves D, Ruiz B, Nuno P, Roman S, Zepeda E, Panduro A. Heterogeneity of apolipoprotein E polymorphism in different Mexican populations. *Hum Biol* 2006; 78: 65-75.
22. Roman S, Zepeda-Carrillo EA, Moreno-Luna LE, Panduro A. Alcoholism and liver disease in Mexico: genetic and environmental factors. *World J Gastroenterol* 2013; 19: 7972-82.
23. Ramos-Lopez O, Roman S, Martinez-Lopez E, Gonzalez-Aldaco K, Ojeda-Granados C, Sepulveda-Villegas M, Panduro A. Association of a novel TAS2R38 haplotype with alcohol intake among Mexican-Mestizo population. *Ann Hepatol* 2015; 14: 729-34.
24. Vergara M, Bejarano G, Dalmau B, Gil M, Miquel M, Sanchez-Delgado J, Casas M, et al. Usefulness of indirect noninvasive methods in predicting progression to cirrhosis in chronic hepatitis C. *Eur J Gastroenterol Hepatol* 2015; 27: 826-33.
25. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215.
26. Thompson AJ, Fellay J, Patel K, Tillmann HL, Naggie S, Ge D, Urban T J, et al. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. *Gastroenterology* 2010; 139: 1181-9.
27. Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol Bioinform Online* 2015; 1: 47-50.
28. 1000 genomes project. Available at: http://www.1000genomes.org/. Accessed: October 7, 2015.
29. Martinez-Cortes G, Salazar-Flores J, Haro-Guerrero J, Rubi-Castellanos R, Velarde-Felix JS, Munoz-Valle JF, Lopez-Casamichana M, et al. Maternal admixture and population structure in Mexican-Mestizos based on mtDNA haplogroups. *Am J Phys Anthropol* 2013; 151: 526-37.
30. Schurr TG, Sherry ST. Mitochondrial DNA and Y chromosome diversity and the peopling of the Americas: evolutionary and demographic evidence. *Am J Hum Biol* 2004; 16: 420-39.
31. Tamm E, Kivisild T, Reidla M, Metspalu M, Smith DG, Mulligan CJ, Bravi CM, et al. Beringian standstill and spread of Native American founders. *PLoS One* 2007; 2: e829.
32. Shipkova M, Lorenz K, Oellerich M, Wieland E, von Ahsen N. Measurement of erythrocyte inosine triphosphate pyrophosphohydrolase (ITPA) activity by HPLC and correlation of ITPA genotype-phenotype in a Caucasian population. *Clin Chem* 2006; 52(2): 240-7.
33. Marinaki AM, Duley JA, Arenas M, Ansari A, Sumi S, Lewis CM, Shobowale-Bakre M, et al. Mutation in the ITPA gene predicts intolerance to azathioprine. *Nucleosides Nucleotides Nucleic Acids* 2004; 23(8-9): 1393-7.
34. Panduro A, Escobedo Melendez G, Fierro NA, Ruiz Madrigal B, Zepeda-Carrillo EA, Roman S. [Epidemiology of viral hepatitis in Mexico]. *Salud Publica Mex* 2011; 53(Suppl. 1): S37-45.
35. Lin S, McLennan AG, Ying K, Wang Z, Gu S, Jin H. Wu C, et al. Cloning, expression, and characterization of a human inosine triphosphate pyrophosphatase encoded by the itpa gene. *J Biol Chem* 2001; 276: 18695-701.
36. Zhu Z, Wilson AT, Luxon BA, Brown KE, Mathahs MM, Bandyopadhyay S, McCaffrey AP, et al. Biliverdin inhibits hepatitis C virus nonstructural 3/4A protease activity: mechanism for the antiviral effects of heme oxygenase? *Hepatology* 2010; 52: 1897-905.
37. Corral-Jara KF, Trujillo-Ochoa JL, Realpe M, Panduro A, Roman S, Fierro NA. Rethinking the immune properties of bilirubin in viral hepatitis: from bench to bedside. *Clin Transl Immunology* 2015; 4, e54.
38. Zhu Z, Wilson AT, Mathahs MM, Wen F, Brown KE, Luxon BA, Schmidt WN. Heme oxygenase-1 suppresses hepatitis C virus replication and increases resistance of hepatocytes to oxidant injury. *Hepatology* 2008; 48, 1430-9.
39. Barrett S, Kieran N, Ryan E, O'Keane JC, Crowe J. Intrahepatic hepatitis C viral RNA status of serum polymerase chain reaction-negative individuals with histological changes on liver biopsy. *Hepatology* 2001; 33: 1496-502.
40. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461: 399-401.
41. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; 41: 1100-4.
42. Gonzalez-Aldaco K, Rebello Pinho JR, Roman S, Gleyzer K, Fierro NA, Oyakawa L, Ramos-Lopez O, et al. Association with Spontaneous Hepatitis C Viral Clearance and Genetic Differentiation of IL28B/IFNL4 Haplotypes in Populations from Mexico. *PLoS One* 2016; 11: e0146258.
43. http://www.cibic.com.ar/laboratorios-bioquimicos/polimorfismos-en-el-gen-de-la-itpa-asociados-a-anemia-por-ribavirina-utilizada-en-el-tratamiento-de-la-hepatitis-c-cronica/ Accessed on March 8, 2016.
44. http://www.einstein.br/exames/exames/paginas/exame.aspx?CodExame=20122& Unidade=HIAE. Accessed on March 8, 2016.
45. Cariani, E, Villa E, Rota C, Critelli, Trenti T. Translating pharmacogenetics into clinical practice: interleukin (IL)28B and inosine triphosphatase (ITPA) polymophisms in hepatitis C virus (HCV) infection. *Clin Chem Lab Med* 2011; 49, 1247-56.
46. Bierau J, Lindhout M, Bakker JA. Pharmacogenetic significance of inosine triphosphatase. *Pharmacogenomics* 2007; 8: 1221-8.
47. Azimi F, Esmaeilzadeh A, Ramazani A. Clinical significance of ITPA rs67002563 polymorphism in patients with acute lymphoblastic leukemia treated with 6-mercaptopurine. *Pharmacol Res* 2015; 102: 61-2.
48. Odahara S, Uchiyama K, Kubota T, Ito Z, Takami S, Kobayashi H, Saito K, et al. A Prospective Study Evaluating Metabolic Capacity of Thiopurine and Associated Adverse Reactions in Japanese Patients with Inflammatory Bowel Disease (IBD). *PLoS One* 2015; 10: e0137798.
49. Xiong H, Xin HW, Wu XC, Li Q, Xiong L, Yu AR. Association between inosine triphosphate pyrophosphohydrolase deficiency and azathioprine-related adverse drug reactions in the Chinese kidney transplant recipients. *Fundam Clin Pharmacol* 2010; 24: 393-400.
50. Osinusi A, Naggie S, Poonia S, Trippler M, Hu Z, Fun E, Schalaak J, et al. ITPA gene polymorphisms significantly affect hemoglobin decline and treatment outcomes in patients coinfected with HIV and HCV. *J Med Virol* 2012; 84: 1106-14.

Correspondence and reprint request:
Sonia Roman, Ph.D.
Department of Molecular Biology in Medicine, Civil Hospital of Guadalajara, "Fray Antonio Alcalde", Hospital # 278, Col. Retiro, Guadalajara, Jalisco, 44280, Mexico and Health Sciences Center, University of Guadalajara, Guadalajara, Jalisco, Mexico. Tel/Fax: +52-33-36147743.
E-mail: soniamariaroman@hotmail.com