The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Brief communication

Prevalence of *IFNL3* gene polymorphism among blood donors and its relation to genomic profile of ancestry in Brazil

Silvia Renata Cornelio Parolin Rizzo[a,b], *Diana Gazito*[a], *Henrique Pott-Junior*[b], *Flavia Roche Moreira Latini*[a], *Adauto Castelo*[b,*]

[a] *Associação Beneficente de Coleta de Sangue (Colsan), São Paulo, SP, Brazil*
[b] *Universidade Federal de São Paulo (Unifesp), Departamento de Medicina, São Paulo, SP, Brazil*

ARTICLE INFO

Article history:
Received 3 January 2016
Accepted 13 October 2016
Available online 25 October 2016

Keywords:
Ancestry informative markers
Chronic hepatitis C virus infection
Direct-acting antivirals
Single nucleotide polymorphisms

ABSTRACT

The recent development of interferon-free regimens based on direct-acting antivirals for the treatment of chronic hepatitis C virus infection has benefited many but not all patients. Some patients still experience treatment failure, possibly attributed to unknown host and viral factors, such as *IFNL3* gene polymorphism. The present study assessed the prevalence of rs12979860-CC, rs12979860-CT, and rs12979860-TT genotypes of the *IFNL3* gene, and its relationship with ancestry informative markers in 949 adult Brazilian healthy blood donors. Race was analyzed using ancestry informative markers as a surrogate for ancestry. *IFNL3* gene was genotyped using the ABI TaqMan single nucleotide polymorphisms genotyping assays. The overall frequency of rs12979860-CC genotype was 36.9%. The contribution of African ancestry was significantly higher among donors from the northeast region in relation to southeast donors, whereas the influence of European ancestry was significantly higher in southeast donors. Donors with rs12979860-CC and rs12979860-CT genotypes had similar ancestry background. The contribution of African ancestry was higher among rs12979860-TT genotype donors in comparison to both rs12979860-CC and rs12979860-CT genotypes. The prevalence of rs12979860-CC genotype is similar to that found in the US, despite the Brazilian ancestry informative markers admixture. However, in terms of ancestry, rs12979860-CT genotype was much closer to rs12979860-CC individuals than to rs12979860-TT.

* *Corresponding author.*
E-mail address: acastelof42@gmail.com (A. Castelo).

http://dx.doi.org/10.1016/j.bjid.2016.10.002

Introduction

Chronic hepatitis C (CHC) virus infection is a global disease that imposes a major global health burden. In a recently published study, the world prevalence of anti-HCV was estimated at 1.6% (1.3–2.1%), corresponding to 115 (92–149) million infections.[1]

Evidence suggests that the ability to control viral replication is related to viral and host factors. Several studies have demonstrated that a strong initial innate and adaptive immune response against hepatitis C virus (HCV) favors viral clearance.[2–4] Thus, variation in genes involved in the immune response may contribute to the ability to clear the virus.[2]

In 2009, single nucleotide polymorphisms (SNP) of the *IFNL3* gene (rs12979860) were related to natural clearance of HCV.[3,5] According to this genome-wide study, rs12979860-CC genotype was associated with spontaneous HCV infection resolution among individuals of both European and African ancestry. Further investigations have implicated *IFNL3* gene polymorphisms in the development of spontaneous resolution of acute infection and therapeutic cure of HCV-infected patients treated with interferon-based regimens.[6]

Before 2011, interferon-based therapy was the standard-of-care for treating HCV. Recently, interferon-free regimens based on direct-acting antivirals (DAAs) have revolutionized the treatment of CHC. Compared to conventional interferon-based regimens, DAAs have demonstrated greater sustained virological response (SVR) rates, shorter treatment duration, increased tolerability, and lower incidence of adverse events.[7] Nonetheless, some patients still experience treatment failure due to on-treatment viral breakthrough or relapse after cessation of therapy, possibly attributed to unknown host and viral factors, such as *IFNL3* gene polymorphism.

Toward that end, data from a recently published subgroup analysis of only eight weeks of ledipasvir plus sofosbuvir for genotype 1 HCV showed SVR rates varying significantly according to rs12979860 genotype. In rs12979860-CC genotype patients SVR rate were in excess of 98%, whereas SVR rates were 95.1% and 90.9%, in rs12979860-CT and TT genotype, respectively ($p = 0.02$). Those results prompted discussion on the relationship of polymorphisms near the *IFNL3* gene and SVR to DAA-based regimens.[8]

Patients of European ancestry have a significantly higher probability of being cured with interferon-based regimens than patients of African ancestry. The frequency of rs12979860-CC genotype is substantially greater in European than in African populations. This genetic polymorphism also explains approximately half of the difference in response rates between African-Americans and patients of European ancestry.[5]

Brazilians are one of the most heterogeneous populations in the world, resulting of five centuries of interethnic crosses between European, Africans, and autochthonous Amerindians.[9] A growing number of publications have reported the use of ancestry-informative markers (AIMs) whose allele frequency varies significantly between populations of distinct geographic origins in order to estimate individual admixture and to identify population substructure. In most studies, these AIMs consisted of single-nucleotide polymorphisms (SNPs), but insertion-deletion polymorphisms (INDELs) of small DNA fragments and short tandem repeats have also been used.[10–13]

Thus, the objectives of the present study were to assess the prevalence of rs12979860-CC, rs12979860-CT, and rs12979860-TT genotypes of the *IFNL3* gene and its relationship with ancestry markers in a Brazilian population.

Between January 2nd and July 31st 2011, 949 healthy adult blood donors were selected from the Colsan blood bank network, in São Paulo, Brazil. Of those, 420 were originally from the northeast region and 529 from the southeast region. This study was approved by Research Ethics Committee of the Federal University of São Paulo (UNIFESP).

Samples of peripheral blood from each subject were collected in 0.5 M EDTA tubes, and genomic DNA was extracted from mononuclear cells using DNA blood Mini kit (QIAamp, Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's instructions.

The rs12979860 was genotyped using the ABI TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA). Primers that contemplated polymorphism were IL28B_Foward: 5′ GCCTGTCGTGTACTGAACCA 3′ and IL28B_Reverse: 5′ GCGCGGAGTGCAATTCAAC 3′ (Eurofins MWG Operon, Hunstsville, AL, USA), TaqMan® MGB Probes (Applied Biosystems, Foster City, CA, USA) for each allele which were labeled with a different fluorophore: 5′ TGGTTCGCGC CTTC 3′ – VIC and 5′ CTGGTTCA CGCCTTC 3′ – FAM.[5] The total reaction volume was 8.5 μL containing: 1.1 μL H_2O; genotyping 5 μL TaqMan Master Mix (Applied Biosystems, Foster City, CA); 5 pM primers; 25 pM probe (Applied Biosystems, Foster City, CA) and cycled in PCR machine Real® time 7500 (Applied Biosystems, Foster City, CA, USA) according to standard cycling as follows: denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 92 °C for 15 s, annealing and extension at 60 °C for 1 min. The results were analyzed by using Software System 7500 (Applied Biosystems, Foster City, CA).

Genetic ancestry was determined by analyzing AIMs. DNA samples were typed for 48 biallelic INDELs using three 16-plex PCR reactions as previously described.[10] DNA fragments were separated using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) and analyzed with GeneMapper ID v3.2 software (Applied Biosystems).[10]

Also, at the time of blood donation participants were asked to inform their perceived race, as Caucasian or African-descendent.

Results and discussion

Our study evaluated the link between race and polymorphisms near the *IFNL3* (formerly known as IL28B) gene among healthy voluntary blood donors using AIM as a surrogate for ancestry. A total of 949 blood donors were included, 420 (44.25%) from the northeast region and 529 (55.75%) from the southeast region; 328 (34.6%) females and 621 (65.4%) males. The overall frequency of rs12979860-CC genotype was 36.9%, with a tendency for greater prevalence among donors born in the southeast (39.3%) than in the northeast (33.8%; $p = 0.08$) regions. The average AIM of African ancestry was significantly higher among donors from the northeast region (0.279) in

relation to the southeast (0.225; $p < 0.001$), whereas the average AIM of European ancestry was significantly higher in southeast donors. The ancestry background was similar between rs12979860-CC genotype and rs12979860-CT genotype donors. African ancestry was higher among rs12979860-TT genotype donors in comparison to both rs12979860-CC genotype and rs12979860-CTdonors.

In the present study, the prevalence of rs12979860-CC, rs12979860-CT, and rs12979860-TT genotypes were 36.9%, 49.1% and 14%, respectively. The rs12979860-CC genotype in southeastern donors (39.3%) tended to be more frequent than among northeastern donors (33.8%; $p = 0.08$). On the contrary, rs12979860-TT genotype was more prevalent in northeastern donors (17.4%) than in southeastern donors (11.3%; $p = 0.01$). A previous Brazilian study conducted in the northeast city of Salvador, Bahia, evaluated 222 HCV infected patients and found that the prevalence of rs12979860-CC (24.4%) was lower, of rs12979860-TT (20.8%) higher, and of rs12979860-CT (54.7%) was similar to our study findings. The encountered discrepancy of rs12979860-CC and rs12979860-TT prevalence in the two studies could be due to higher representation of blacks in the Salvador study. Indeed, the self-reported prevalence of African-descendants in our study (11.4%) was much lower than that of the Salvador study (36.8%).[9]

A study in 88 Japanese HCV infected patients showed a prevalence of 64.3% of rs12979860-CC and 38.7% of rs12979860-CT genotypes. No rs12979860-TT genotype was encountered.[14] In China, three large studies with hepatitis B infected patients found rates of rs12979860-CC genotype over 85% and rs12979860-TT genotype around 5%.[14–17]

In the United States, where miscegenation remains very low, the prevalence of rs12979860-TT genotype among HCV infected self-reported African descendants is significantly greater than that found in self-reported Caucasians, 37% and 12%, respectively.[18] Interestingly the prevalence of rs12979860-CT genotype in Caucasians (49%) was similar to that of African descendants (51%).

Two studies conducted in Spain evaluated 283 and 119 HCV-infected patients. The rates of rs12979860-CC genotype were 44.7% and 41.1%, respectively.[19,20] These rates are similar to those found in Germany (40.2%),[21] but lower to the prevalence found in Egypt (54%), an intermediary value between rates found among Asians and Caucasians.[22]

Our results show that Brazilian adult healthy blood donors have a prevalence (36.7%) of rs12979860-CC genotype similar to that found in the US, despite its heterogeneous population mix. The prevalence of rs12979860-CT genotype was also similar to that of other western countries. However, in terms of ancestry, rs12979860-CT genotype was much closer to rs12979860-CC individuals than to rs12979860-TT. Accordingly, one could question whether the likelihood of viral clearance among rs12979860-CT Brazilian individuals is closer to that observed in rs12979860-CC than in rs12979860-TT individuals. The same would also apply for the HCV treatment response.

The overall frequency of rs12979860-TT genotype observed in our study was 14.0%, nearly the same as other western countries. In these cases, treatment failure due to on-treatment viral breakthrough or relapse after cessation of therapy may be attributed to an ineffective endogenous interferon response unable to promote viral clearance and, hence, raise the emergence of RAVs.

Despite the large sample size, our study results may not necessarily be representative for the entire Brazilian population.

Finally, the role of CT genotype in viral clearance and treatment response should be further evaluated in populations with significant and prolonged interethnic crosses such as the Brazilian population.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We are in debt to Colsan for the logistic support and laboratory tests.

REFERENCES

1. Gower E, Estes CC, Hindman S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus. J Hepatol. 2014;61 1 Suppl:S45–57.
2. Elliot LN, Lloyd AR, Ziegler JB, French RA. Protective immunity against hepatitis C virus infection. Immunol Cell Biol. 2006;84:239–49.
3. Post J, Ratnarajah S, Lloyd AR. Immunological determinants of the outcomes from primary hepatitis C infection. Cell Mol Life Sci. 2009:733–56.
4. Dustin LB, Cashman SB, Laidlaw SM. Immune control and failure in HCV infection-tipping the balance. J Leukoc Biol. 2014;96:1–14.
5. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461:399–401.
6. Rembeck K, Lagging M. Impact of IL28B, ITPA and PNPLA3 genetic variants on therapeutic outcome and progression of hepatitis C virus infection. Pharmacogenomics. 2015;16:1179–88.
7. Stahmeyer JT, Rossol S, Krauth C. Outcomes, costs and cost-effectiveness of treating hepatitis C with direct acting antivirals. J Comp Eff Res. 2015;11:1–11.
8. O'Brien TR, Lang Kuhs KA, Pfeiffer RM. Subgroup differences in response to 8 weeks of ledipasvir/sofosbuvir for chronic hepatitis C. Open forum Infect Dis. 2014;1, ofu110.
9. Pena SDJ, di Pietro G, Fuchshuber-Moraes M, et al. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS ONE. 2011;6.
10. Santos NPC, Ribeiro-Rodrigues EM, Ribeiro-dos-Santos ÂKC, et al. Assessing individual interethnic admixture and population substructure using a 48-insertion-deletion (INSEL) ancestry-informative marker (AIM) panel. Hum Mutat. 2010;31:184–90.
11. Pritchard JK, Stephens M, Rosenberg NA, Donnelly P. Association mapping in structured populations. Am J Hum Genet. 2000;67:170–81.
12. Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 2003;164:1567–87.

13. Pimenta JR, Zuccherato LW, Debes AA, et al. Color and genomic ancestry in Brazilians: a study with forensic microsatellites. Hum Hered. 2006;62:190–5.
14. Asahina Y, Tsuchiya K, Muraoka M, et al. Association of gene expression involving innate immunity and genetic variation in interleukin 28B with antiviral response. Hepatology. 2012;55:20–9.
15. Peng LJ, Guo JS, Zhang Z, Shi H, Wang J, Wang JY. IL28B rs12979860 polymorphism does not influence outcomes of hepatitis B virus infection. Tissue Antigens. 2012;79:302–5.
16. Lee DH, Cho Y, Seo JY, et al. Polymorphisms near interleukin 28B gene are not associated with hepatitis B virus clearance, hepatitis B e antigen clearance and hepatocellular carcinoma occurrence. Intervirology. 2013;56:84–90.
17. Li W, Jiang Y, Jin Q, et al. Expression and gene polymorphisms of interleukin 28B and hepatitis B virus infection in a Chinese Han population. Liver Int. 2011;31:1118–26.
18. Thompson AJ, Muir AJ, Sulkowski MS, et al. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology. 2010;139.
19. Montes-Cano MA, García-Lozano JR, Abad-Molina C, et al. Interleukin-28B genetic variants and hepatitis virus infection by different viral genotypes. Hepatology. 2010;52:33–7.
20. Rivero-Juárez A, Espejo ÁC, Perez-Camacho I, et al. Association between the IL28B genotype and hepatitis C viral kinetics in the early days of treatment with pegylated interferon plus ribavirin in HIV/HCV co-infected patients with genotype 1 or 4. J Antimicrob Chemother. 2012;67:202–5.
21. Sarrazin C, Susser S, Doehring A, et al. Importance of IL28B gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. J Hepatol. 2011;54:415–21.
22. Shaker OG, Sadik NAH. Polymorphisms in interleukin-10 and interleukin-28B genes in Egyptian patients with chronic hepatitis C virus genotype 4 and their effect on the response to pegylated interferon/ribavirin-therapy. J Gastroenterol Hepatol. 2012;27:1842–9.