Development of Tropical Spastic Paraparesis in Human T-Lymphotropic Virus Type 1 Carriers Is Influenced by *Interleukin 28B* Gene Polymorphisms

Ana Treviño,[1] Mariola Lopez,[1] Eugenia Vispo,[1] Antonio Aguilera,[2] Jose M. Ramos,[3] Rafael Benito,[4] Lourdes Roc,[5] Jose M. Eiros,[6] Carmen de Mendoza,[1] and Vincent Soriano,[1] on behalf of the HTLV Spanish Study Group

[1]Hospital Carlos III, Madrid; [2]Hospital Conxo-CHUS, Santiago; [3]Hospital General Universitario de Elche; [4]Hospital Clínico Universitario Lozano Blesa, and [5]Hospital Miguel Servet, Zaragoza; and [6]Hospital Clínico Universitario, Valladolid, Spain

Interleukin 28B (*IL28B*) rs12979860 polymorphisms were examined in 41 individuals with human T-lymphotrophic virus type 1 (HTLV-1). The alleles CT/TT were more frequent in 12 individuals with HTLV-1-associated myelopathy/tropical spastic paraparesis than in 29 asymptomatic carriers (80% vs 20%; *P* = .03), and median HTLV-1 proviral load was greater in CT/TT than CC carriers (*P* = .01). Thus, *IL28B* testing and closer follow-up of HTLV-1 asymptomatic CT/TT carriers is warranted.

Human T-lymphotrophic virus type 1 (HTLV-1) was the first identified human retrovirus [1]. Approximately 15–20 million people are infected with HTLV-1 worldwide, with the presence of highly endemic foci on all continents [2, 3]. Only 5%–10% of persons infected with HTLV-1 develop clinical manifestations lifelong, with adult T-cell leukemia/lymphoma (ATLL) and HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) the 2 most serious complications [4–6]. Although predictors of disease development have not been well established, a high HTLV-1 proviral load has been associated with clinical manifestations [7–9].

Immune-mediated mechanisms are involved in the pathogenesis of HAM/TSP, which typically manifests in middle-aged women [10, 11]. Disease development is in part attributed to failure of the innate and adaptive immune system to control HTLV-1 spread [10]. In other chronic viral diseases, such as in hepatitis C virus (HCV) infection, liver damage also occurs through immune mechanisms. Interestingly, a single nucleotide polymorphism near the *interleukin 28B* (*IL28B*) gene that codes for interferon λ3 was recently shown to strongly influence HCV natural history and treatment outcomes [12, 13]. Based on this observation, we assessed whether *IL28B* gene polymorphisms could also play a role in the development of HAM/TSP in HTLV-1 carriers.

METHODS

The Spanish HTLV register records all reported cases of HTLV-1 and HTLV-2 infections in Spain since 1989. Up to January 2012, a total of 199 individuals with HTLV-1 infection had been reported in Spain. Twenty-five (13%) had been diagnosed with HAM/TSP using well-defined criteria [9]. For the purpose of this study, only patients who had frozen peripheral blood mononuclear cells (PBMCs) were chosen.

The *IL28B* rs12979860 allelic variants were examined on DNA extracted from stored PBMCs drawn from patients belonging to the Spanish HTLV-1 register. *IL28B* gene polymorphisms were characterized using allele specific TaqMan probes (ABI TaqMan allelic discrimination kit) [14].

The HTLV-1 proviral DNA was quantified by real-time polymerase chain reaction using primers and probes targeting the *pol* gene, which have been reported elsewhere [15]. Briefly, DNA was extracted from 1×10^6 PBMCs. TaqMan amplification was carried out in a reaction with a final volume of 25 μL using Taqman Universal Master Mix II (Applied Biosystems). Thermal cycling conditions consisted first of an initial step of 2 minutes at 50°C and an activation step at 95°C for 10 minutes, followed by 45 cycles at 95°C for 15 seconds and 60°C for 1 minute. For each run, a standard curve was generated using $10–10^6$ copies of a recombinant HTLV-1 plasmid DNA that contains one HTLV-1 *pol* fragment (198 base pair) [16]. The HTLV-1 copy number in each clinical sample was estimated by interpolation from the plasmid regression curve. To determine the proviral load, the HTLV-1 DNA copy number was normalized to the amount of cellular DNA by quantifying in parallel the human albumin gene [15]. All samples were

run in duplicate. Results were expressed as HTLV-1 DNA copies per 10^4 PBMCs.

Statistical Analysis

The main characteristics of the study population and the different parameters evaluated are expressed as median (interquartile range). Comparisons between groups were carried out using the chi-square test or the Fisher's exact test, as appropriate. Univariate and multivariate tests were performed to identify independent factors associated to TSP/HAM. All statistical analyses were performed using the SPSS software version 15 (SPSS Inc.). All P values were 2-tailed and considered significant only when <.05.

RESULTS

A total of 41 individuals recorded in the HTLV-1 Spanish register had frozen PBMCs in which examination of HTLV-1 proviral load and *IL28B* testing could be undertaken. Twelve of them (29.3%) had HAM/TSP, and the remaining 29 subjects were asymptomatic HTLV-1 carriers. The median age of the study population was 46 years (range, 4–67), and 56% were women. The regions of origin of the study population were as follows: Latin America ($n = 32$, 78%), native Spaniards ($n = 5$, 12%) and sub–Saharan Africa ($n = 4$, 10%). Table 1 summarizes the main demographics of individuals included in the Spanish database as well as information from the subset of individuals that constituted our study population, with HAM/TSP patients and asymptomatic HTLV-1 carriers considered separately. Overall, no significant differences between groups were recognized.

As shown in Figure 1A, patients with HAM/TSP had a median HTLV-1 proviral load greater than asymptomatic carriers (637 [291–1267] vs 60 [60–469] copies/10^4 PBMCs, respectively; $P = .003$).

The *IL28B* allelic distribution was as follows: CC ($n = 22$, 54%), CT ($n = 16$, 39%) and TT ($n = 3$, 7%). Interestingly, median HTLV-1 proviral load was higher in CT/TT than CC carriers (635 [60–1094] vs 71 [60–230]; $P = .01$) copies per 10^4 PBMCs (Figure 1B). Furthermore, the *IL28B* CC variant was more frequent in asymptomatic carriers than in HAM/TSP patients (62% vs 33%; $P = .1$). When the 3 individuals who had acquired HTLV-1 through solid organ transplantation (thus, involving large HTLV-1 inoculum) were excluded, there was a significantly greater rate of CC variants in asymptomatic HTLV-1 carriers than in HAM/TSP patients (80% vs 20%; $P = .03$).

Factors associated with development of HAM/TSP were finally analyzed using a logistic regression model in which variables known to influence HAM/TSP development were taken into account (Table 2). Analysis was performed excluding the 3 individuals who acquired HTLV-1 following transplantation

Table 1. Main Characteristics of the Study Population

	Total HTLV-1 Spanish Cohort (n = 199)	Asymptomatic HTLV-1 Carriers (n = 29)	HAM/TSP (n = 12)	P
Median age, years	45	44	47	.16
Female gender	120	15	8	.59
Origin				
Latin America	114	24	8	.07
Spain	42	1	4	.04
Africa	30	4	0	.31
Risk group				
Sexual	75	16	6	.7
Vertical	15	6	0	.09
Transplantation	3	0	2	…
Transfusion	7	0	2	.09
Intravenous drug use	12	0	0	.15

Abbreviations: HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; HTLV-1, human T-lymphotopic virus type 1.

of a solid organ from an infected donor. In the univariate analysis, HAM/TSP was significantly more frequent in CT/TT than CC carriers (odds ratio [OR], 6.54; 95% confidence interval [CI], 1.17–36.61; $P = .03$) and in subjects with high HTLV-1 proviral load (>200 DNA copies/10^4 PBMCs) (OR, 17.1; 95% CI, 1.88–154.84; $P = .012$). The final multivariate analysis showed that both factors were strongly linked and consequently did not predict independently HAM/TSP. It should be noted that in this study neither older age nor female gender was significantly associated with HAM/TSP.

DISCUSSION

This study is the first to demonstrate a role for *IL28B* gene polymorphisms in the risk of developing HAM/TSP in HTLV-1 carriers. Individuals with CT/TT variants exhibited approximately a 3-fold increased risk of HAM/TSP than CC carriers. It must be highlighted, however, that this association seemed to be largely mediated by an increased HTLV-1 proviral load in CT/TT carriers. Individuals with CT/TT allelic variants at the *IL28B* rs12979860 gene had nearly 10-fold higher median HTLV-1 proviral loads than CC carriers. Given that a high HTLV-1 proviral load is a well-established risk factor for developing HAM/TSP [7–9], we hypothesize that innate immunity critically involving interferon λ3 might contribute to the control of HTLV-1 replication/expansion in infected persons and, through this mechanism, influence the risk of developing HAM/TSP.

Our findings have several implications for the management of persons with HTLV-1. First, given its prognostic value,
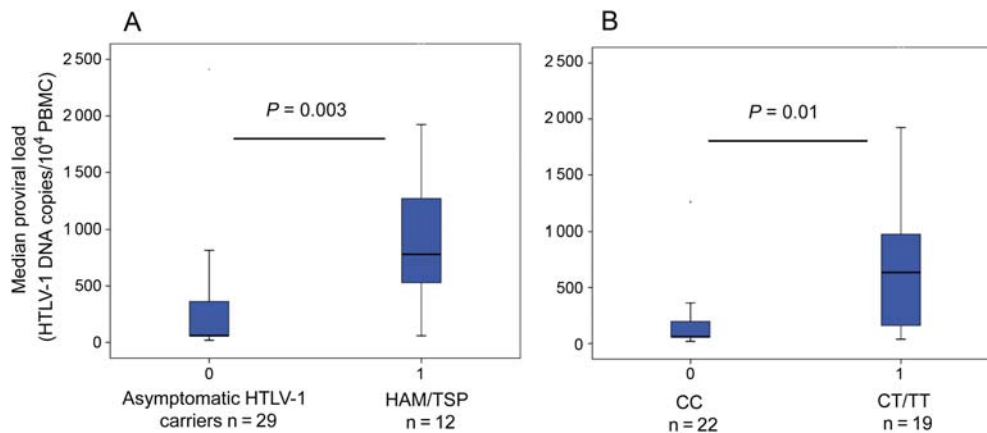
Figure 1. Median human T-lymphotropic virus type 1 (HTLV-1) proviral load in asymptomatic HTLV-1 carriers versus HTLV-1–associated myelopathy/tropical spastic paraparesis patients (*A*) and *interleukin 28B* CC versus CT/TT allelic variants (*B*). Abbreviations: HAM, HTLV-1–associated myelopathy; HTLV-1, human T-lymphotropic virus type 1; TSP, tropical spastic paraparesis.

IL28B testing should be recommended to all asymptomatic HTLV-1 carriers. Second, asymptomatic HTLV-1 carriers harboring CT/TT alleles should be followed more closely because of their increased risk of developing HAM/TSP. In contrast with HTLV-1 proviral load, whose methodology is not well standardized and should be evaluated periodically [17], *IL28B* testing is cheap and commercially available and must be done only once in a lifetime [18].

Several questions may arise from our observation. First, the role of *IL28B* polymorphisms with respect to susceptibility to HTLV-1 infection (and not only risk of disease in carriers) must be examined. As demonstrated for HCV infection [19], CC allelic variants might also protect from establishment of HTLV-1 infection following viral exposure. Second, the role of *IL28B* variants with respect to the risk of developing ATLL should be examined. It would be expected to be relevant as well, given that a high HTLV-1 proviral load has also been found to predict the risk of developing ATLL in subjects infected with HTLV-1 since their childhood [8]. Last, the potential role of interferon λ as therapy for HTLV-1 warrants

consideration. A recombinant interferon λ molecule is currently being tested as treatment for chronic hepatitis C, and preliminary results are quite promising [20]. Treatment options in HTLV-1 patients with ATLL or HAM/TSP are currently very limited [21, 22]. Although interferon λ alone might not improve these conditions once developed, it may help to prevent them by reducing HTLV-1 proviral load in asymptomatic carriers at risk, for example those with *IL28B* CT/TT variants and/or high circulating proviral concentrations.

We acknowledge the small size of our study population as a limitation of this work. Both HAM/TSP patients and asymptomatic HTLV-1 carriers were chosen from the national Spanish registry, and only the subset of individuals with available frozen PBMCs were included in the study. Of note, no significant differences in demographics between our study population and the whole series of cases recorded at the national database registry were found that might account for any bias. Thus, although further studies testing larger HTLV-1 case series of HAM/TSP and asymptomatic HTLV-1 carriers are warranted, we are confident our results are not casual. In

Table 2. Factors Associated With Human T-Lymphotopic Virus Type 1 (HTLV-1)–Associated Myelopathy/Tropical Spastic Paraparesis in HTLV-1 Carriers

	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	*P*	OR	95% CI	*P*
Older age	1.03	.96–1.11	.29	…	…	…
Female gender	2.33	.5–10.91	.28	…	…	…
IL28B CT/TT alleles	6.54	1.17–36.61	.03*	2.80	.40–19.31	.29
High HTLV-1 proviral load (>200 DNA copies/10^4 PBMCs)	17.1	1.88–154.84	.012*	17.1	1.88–154.84	.012*

Abbreviations: CI, confidence interval; OR, odds ratio; PBMCs, peripheral blood mononuclear cells.

*Statistically significant.

this regard, the association we found between *IL28B* allelic variants and HTLV-1 proviral load was further reassuring.

In summary, we found a significant association between *IL28B* allelic variants and HAM/TSP in individuals with HTLV-1 infection. An effect on HTLV-1 proviral load was the most reasonable mechanism explaining this association. Altogether, our results support *IL28B* testing of all asymptomatic HTLV-1 individuals and closer follow-up of *IL28B* CT/TT carriers.

Notes

References

1. Poiesz B, Ruscetti F, Gazdar A, et al. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA **1980**; 77:7415–9.

2. Hlela C, Shepperd S, Khumalo N, Taylor G. The prevalence of human T-cell lymphotropic virus type 1 in the general population is unknown. AIDS Rev **2009**; 11:205–14.

3. Watanabe T. Current status of HTLV-1 infection. Int J Hematol **2011**; 94:430–4.

4. Manns A, Hisada M, La Grenade L. Human T-lymphotropic virus type 1 infection. Lancet **1999**; 353:1951–8.

5. Goncalves D, Proietti F, Ribas J, et al. Epidemiology, treatment and prevention of HTLV-1–associated diseases. Clin Microbiol Rev **2010**; 23:577–89.

6. Proietti F, Carneiro-Proietti A, Catalan-Soares B, Murphy E. Global epidemiology of HTLV-I infection and associated diseases. Oncogene **2005**; 24:6058–68.

7. Nagai M, Usuku K, Matsumoto W, et al. Analysis of HTLV-1 proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-1 carriers: high proviral load strongly predisposes to HAM/TSP. J Neurovirol **1998**; 4:586–93.

8. Iwanaga M, Watanabe T, Utsunomiya A, et al. Human T-cell leukemia virus type 1 (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan. Blood **2010**; 116:1211–9.

9. Grassi M, Olavarria V, Kruschewsky Rde A, et al. Human T cell lymphotropic virus type 1 (HTLV-1) proviral load of HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients according to new diagnostic criteria of HAM/TSP. J Med Virol **2011**; 83:1269–74.

10. Oliere S, Douville R, Sze A, Belnaoui S, Hiscott J. Modulation of innate immune responses during HTLV-1 pathogenesis. Cytokine Growth Factor Rev **2011**; 22:197–210.

11. Martin F, Taylor G. Prospects for the management of human T-cell lymphotropic virus type 1–associated myelopathy. AIDS Rev **2011**; 13:161–70.

12. Ge D, Fellay J, Thompson J, et al. Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance. Nature **2009**; 461: 399–401.

13. Rauch A, Kutalik Z, Descombes P, 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.

14. Livak K. Allelic discrimation using fluorogenic probes and the 5′ nuclease assay. Genet Anal **1999**; 14:143–9.

15. Andrade R, Ribeiro M, Namen-Lopes M, et al. Evaluation of the use of real-time PCR for HTLV-1 and -2 as a confirmatory test in screening for blood donors. Rev Soc Bras Med Trop **2010**; 43:111–5.

16. Toro C, Rodés B, Poveda E, et al. Rapid development of subacute myelopathy in three organ transplant recipients after transmission of human T-cell lymphotropic virus type 1 from a single donor. Transplantation **2003**; 75:102–4.

17. Furtado S, Andrade R, Romanelli L, et al. Monitoring the HTLV-1 proviral load in the peripheral blood of asymptomatic carriers and patients with HTLV-associated myelopathy/tropical spastic paraparesis from a Brazilian cohort: ROC curve analysis to establish the threshold for risk disease. J Med Virol **2012**; 84:664–71.

18. Soriano V, Poveda E, Vispo E, Labarga P, Rallón N, Barreiro P. Pharmacogenetics of hepatitis C. J Antimicrob Chemother **2012**; 67:523–9.

19. Thomas D, Thio C, Martin M, et al. Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus. Nature **2009**; 461:798–801.

20. Donnelly R, Dickensheets H, O'Brien T. Interferon-lambda and therapy for chronic hepatitis C virus infection. Trends Immunol **2011**; 32:443–50.

21. Macchi B, Balestrieri E, Ascolani A, et al. Susceptibility of primary HTLV-1 isolates from patients with HTLV-1-associated myelopathy to reverse transcriptase inhibitors. Viruses **2011**; 3:469–83.

22. Treviño A, Parra P, Bar-Magen T, Garrido C, de Mendoza C, Soriano V. Antiviral effect of raltegravir on HTLV-1 carriers. J Antimicrob Chemother **2012**; 67:218–21.