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

Ana Treviño,1 Mariola López,1 Eugenia Vispo,1 Antonio Aguilera,2 Jose M. Ramos,3 Rafael Benito,4 Lourdes Roc,5 José M. Eiros,6 Carmen de Mendoza,7 and Vincent Soriano,1 on behalf of the HTLV Spanish Study Group

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

Interleukin 28B (IL28B) rs12979860 polymorphisms were examined in 41 individuals with human T-lymphotropic 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.

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 \( \lambda \)3 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 \( \times 10^6 \) PBMCs. TaqMan amplification was carried out in a reaction with a final volume of 25 \( \mu \)L 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 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 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 $\lambda_3$ 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,
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.

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

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<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
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<tr>
<td></td>
<td>OR 95% CI</td>
<td>P</td>
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<tr>
<td>Older age</td>
<td>1.03 .96–1.11</td>
<td>.29</td>
</tr>
<tr>
<td>Female gender</td>
<td>2.33 .5–10.91</td>
<td>.28</td>
</tr>
<tr>
<td>IL28B CT/TT alleles</td>
<td>6.54 1.17–36.61</td>
<td>.03*</td>
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<tr>
<td>High HTLV-1 proviral load (&gt;200 DNA copies/10⁶ PBMCs)</td>
<td>17.1 1.88–154.84</td>
<td>.012*</td>
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</table>

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

Acknowledgments. HTLV Spanish Study Group: C. Rodríguez and J. del Romero (Centro Sanitario Sandoval, Madrid); C. Tuset, G. Marcaida, and T. Tuset (Hospital General Universitario, Valencia); E. Caballero and I. Molina (Hospital Vall d’Hebron, Barcelona); A. Aguilar, J. J. Rodríguez-Calviño, S. Cortizo, and B. Requeiro (Hospital Conxo-CHUS, Santiago); R. Benito and M. Borrás (Hospital Clínico Universitario Lozano Blesa, Zaragoza); R. Ortiz de Lejarazu and J. M. Eiros (Hospital Clínico Universitario, Valladolid); J. M. Miró, C. Manzanoz, M. M. Gutiérrez, and T. Pumarola (Hospital Clinic-IDIBAPS, Barcelona); J. García and I. Paz (Complejo Hospitalario Universitario, Orense); E. Calderón, F. J. Medrano, and M. Leal (Hospital Virgen del Rocio, Sevilla; CIBER de Epidemiología y Salud Pública); F. Capote (Hospital Puerta del Mar, Cádiz); A. Vallejo, F. Dronda, and S. Moreno (Hospital Ramón y Cajal, Madrid); D. Escudero (Hospital Germans Trias i Pujol, Barcelona); E. Pujol (Hospital Juan Ramón Jiménez, Huelva); M. Trigo, J. Díaz, P. Álvarez, and M. García-Campello (Complejo Hospitalario, Pontevedra); M. Rodríguez-Iglesias (Hospital Universitario Puerta del Mar, Cádiz); A. M. Martin and A. Hernandez-Betancor (Hospital Innsbruck, Las Palmas de Gran Canaria); J. M. Ramos, J. C. Rodríguez, and F. Gutiérrez (Hospital General, Elche); A. Guelar (Hospital del Mar, Barcelona); G. Cilla and E. Pérez-Trallero (Hospital Donostia, San Sebastián); J. López-Aldeguer (Hospital La Fe, Valencia); J. Solà (Hospital de Navarra, Pamplona); L. Fernández-Pereira (Hospital San Pedro de Alcántara, Cáceres); J. Niubió (Ciudad Sanitaria de Bellvitge, Barcelona); S. Veloso (Hospital Universitario, Tarragona); L. A. Arroyo, A. M. López Lirola, and J. L. Gómez Sirvent (Hospital Universitario de Canarias, Santa Cruz de Tenerife); L. Force (Hospital General, Mataró); C. Cifuentes (Hospital Son Llatzer, Palma de Mallorca); J. García (Hospital del Bierzo, Ponferrada); A. González-Praetorius (Hospital Universitario, Guadalajara); A. Mena, J. L. Pérez, and M. Peñaranda (Hospital Son Dureta, Mallorca); J. M. Montejo (Hospital de Cruces, Bilbao); N. Margall, M. Gutiérrez, P. Domingo (Hospital de Sant Pau, Barcelona); L. Roc and A. Martínez Sapiña (Hospital Miguel Servet, Zaragoza); I. Viciana (Hospital Virgen de la Victoria, Málaga); T. Cabezás, A. B. Lozano, and J. M. Fernandez (Hospital de Poniente, Almería); I. García and G. Gaspar (Hospital Universitario de Getafe, Madrid); R. García and M. Gorgolas (Fundación Jiménez Díaz, Madrid); A. Treviño, P. Parra, C. Mendoza, and V. Soriano (Hospital Carlos III, Madrid).

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