IL28B Single-Nucleotide Polymorphism rs12979860 Is Associated With Spontaneous HIV Control in White Subjects

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The single-nucleotide polymorphism (SNP) rs12979860 near the IL28B gene has been associated with the spontaneous clearance of hepatitis C virus. We sought to determine whether this SNP could be associated with the spontaneous control of human immunodeficiency virus (HIV) infection. We studied the prevalence of the IL28B CC genotype among 53 white HIV controllers, compared with the prevalence among 389 HIV-infected noncontrollers. We found that the IL28B CC genotype was independently associated with spontaneous HIV control (odds ratio [OR], 2.669; \(P = .017\)), as were female sex (OR, 7.077; \(P \leq .001\)) and the presence of HLA-B57 and/or B27 (OR, 3.080; \(P = .017\)). This result supports the idea that common host mechanisms are involved in the spontaneous control of these 2 chronic infections.

**Keywords.** HIV controllers; IL28B, and SNP.

The spontaneous control of human immunodeficiency virus (HIV) infection is observed in a rare group of individuals who have become known as HIV controllers. These individuals are able to maintain undetectable or very low viral loads in the absence of antiretroviral treatment over a long period [1]. Several genetic factors have been associated with this control, including the overrepresentation of different HLA antigens, such as HLA-B57 [1]. Curiously, the presence of HLA-B57 has also been related to the spontaneous clearance of hepatitis C virus (HCV) infection [2], suggesting shared mechanisms of viral control for both infections. We have previously reported the ability of white HIV controllers to maintain lower HCV loads than other noncontroller HIV-infected subjects, as well as an association of HLA-B57 with lower HCV levels in HIV controllers [3]. Recently, a single nucleotide polymorphism (SNP) near the IL28B gene, which codes for interferon λ3 (IFN-λ3), has been associated with the spontaneous clearance of HCV [4] and with sustained virological response (SVR) after HCV-specific treatment [5]. Although it is still unknown how this SNP affects the antiviral activity of IFN-λ, several studies have demonstrated the antiviral activity of IFN-λ against different viruses, including HIV [6]. However, no association was found between these protective alleles and spontaneous HIV control in African American individuals [7, 8]. We wondered whether this IL28B SNP would be overrepresented in white HIV controllers, and, thus, our aim was to analyze the association of the IL28B SNP with the spontaneous control of HIV infection.

**METHODS**

All patients included in this study came from Spain and were white. The HIV controllers included in this study came from the HIV-infected patient cohort of the Infectious Diseases Service of Virgen Del Rocio University Hospital (Seville) and from a National Multicenter Cohort from the HIV Controllers Consortium of the AIDS Spanish Network (some of these subjects came from HIV-Infected Long-Term Nonprogressors Cohort and the prospective HIV-Infected Patients Cohort, both from the AIDS Spanish Network). The sample included 53 HIV controllers, defined as subjects who tested positive for antibodies against HIV had a plasma HIV-1 load of <1000 HIV-1 RNA copies/mL in the absence of antiretroviral therapy (ART) for at least 12 months, and had at least 3 plasma HIV-1 load determinations during this period. Thirty-four of these HIV controllers were naïve to ART; the remaining 19 patients had received ART, with a median interval of 4.42 years (range, 2.76–7.83 years) between their most-recent use of ART and enrollment in the study. The HIV controllers...
were compared with 389 HIV-infected noncontrollers from our cohort who consecutively visited our unit (Virgen Del Rocio University Hospital, Seville) until the study was completed. These patients had had a confirmed plasma HIV-1 load of $>1000$ HIV-1 RNA copies/mL at $\geq 2$ follow-up visit since entrance into the cohort. HIV type 2–infected subjects were excluded. We also studied in a prospective manner 61 HIV-infected noncontrollers who had data available on the primary infection. The time of HIV-1 infection was estimated as the midpoint between the last negative and the first positive HIV antibody test result, with maximum periods of 12 months between the HIV antibody tests and 15 days before onset of acute retroviral symptoms. The virus set point was defined as the median of at least 2 HIV-1 load determinations between months 3 and 6 after the estimated date of infection. All of these patients were asymptomatic at the time of the study. Samples were kindly provided by the HIV Biobank integrated within the Spanish AIDS Research Network. All of the patients participating in the study gave their informed consent, and the protocols were approved by the institutional ethics committees.

Routinely, the absolute numbers of CD4$^+$ and CD8$^+$ T cells were assayed from fresh whole blood samples, using the Epics XL-MCL flow cytometer (Beckman Coulter). Plasma HIV RNA load was measured in fresh samples by quantitative polymerase chain reaction (PCR; COBAS Ampliprep/COBAS Taqman HIV test; Roche molecular systems), according to the manufacturer’s instructions. The detection limit was 40 HIV RNA copies/mL. Quantitative reverse transcription PCR was performed for plasma HCV RNA amplification (COBAS Amplicor; Roche Diagnosis, Barcelona, Spain), with a detection limit of 15 IU/mL. HCV genotype was determined using a reverse-hybridization assay (InnoLIPA HCV II; Innogenetics, Barcelona, Spain).

The HLA-B group alleles were genotyped using a reverse sequence-specific oligonucleotide bound to a fluorescently coded microsphere system (LABType SSO, RSSO1B, One Lambda, Canoga Park, CA), following the manufacturer’s instructions. The genotyping of the IL28B SNP rs12979860 was performed as previously described [9], using a TaqMan 5′ allelic discrimination assay (Applied Biosystems, Foster City, CA).

The statistical analyses were performed using SPSS software, version 18.0 (SPSS, Chicago, IL). Median and interquartile ranges were used to describe continuous variables and percentages to describe categorical variables. To analyze the association of the IL28B SNP with spontaneous HIV control, in the multivariate analysis, HIV controller status was the dependent variable, and only innate host parameters (ie, sex, HLA-B57 and HLA-B27 alleles, and IL28B CC genotype) were included. All of the differences between the groups in the bivariate and multivariate logistic regression analysis with a $P$ value of $<.05$ were considered statistically significant. The studied population was in Hardy-Weinberg equilibrium in relation to the IL28B CC genotype ($P > .05$).

**RESULTS**

The characteristics of the study subjects are summarized in Table 1. Some of these subjects were included in our previous studies [3, 10]. The frequency of women was significantly greater among HIV controllers, compared with noncontrollers. As expected, having one of the protective alleles (HLA-B57 and/or HLA-B27) was overrepresented in the HIV controllers (odds ratio [OR], 2.631; 95% confidence interval [CI], 1.151–6.015; $P = .022$). The HIV controllers showed lower rates of previous AIDS, compared with noncontrollers (8.8% vs 28.9%).

In relation to HCV coinfection, the spontaneous clearance rates were similar in both study groups, and the HIV controllers displayed a tendency to maintain lower levels of HCV as compared to noncontrollers. As we previously described [3], HCV genotype 3 was overrepresented among HIV controllers as compared to noncontrollers (46.4% vs 20.2%; OR, 3.792; 95% CI, 1.473–9.763; $P = .006$). The association of the IL28B CC genotype with spontaneous clearance of HCV, as previously reported [4], was also observed in our study when all patients were analyzed together (OR, 4.316; 95% CI, 1.723–10.575; $P = .002$). However, this association was not observed in the HIV controllers, most likely because of the HCV genotype heterogeneity observed in this group.

Interestingly, the IL28B CC genotype was associated with spontaneous HIV control (OR, 2.018; 95% CI, 1.118–3.641; $P = .020$). There was an overrepresentation of the IL28B CC genotype among HIV controllers, compared with noncontrollers (62.3% vs 45.0%). In a multivariate statistical analysis that used HIV controller status as the dependent variable and adjusted for sex, HLA-B57 and/or HLA-B27 positivity, and IL28B CC genotype, the IL28B SNP was still independently associated with the spontaneous control of HIV in white HIV controllers (Table 2). However, when HCV-related parameters (ie, log HCV RNA load and HCV genotype) were included in the multivariate statistical analysis, only the IL28B CC SNP (OR, 4.222; 95% CI, 1.360–13.109; $P = .013$) and female sex (OR, 10.977; 95% CI, 3.261–36.944; $P < .001$) remained independently associated with spontaneous HIV control, showing that this association was not mediated by HCV coinfection. We also sought to determine whether the IL28B CC genotype was related to a lower viral load set point in primary HIV infection. Therefore, we analyzed the prevalence of the IL28B CC genotype among 61 noncontrollers who had available data on primary HIV infection. We compared the median of HIV load set points within the first 12 months since primary infection between subjects with IL28B CC and subjects with IL28B
CT/TT, and we did not observe significant differences between the groups (Supplementary Figure 1).

DISCUSSION

In this study, we demonstrate that the IL28B CC genotype is independently associated with spontaneous HIV control in white individuals. This finding adds to the suggestion of common shared mechanisms involved in the control of 2 persistent infections, HIV infection and HCV infection. In fact, we and others have shown how HIV controllers can control HCV infection in terms of lower HCV loads [3] and higher rates of spontaneous clearance [7], compared with non–HIV controllers.

The interest in the SNP in IL28B arose from its association with the control of HCV infection [4, 5]. However, this study is the first to show that this SNP is independently related to the control of HIV infection. Interestingly, previous work showed that the IL28B SNP was not associated with spontaneous HIV control in African American individuals [7, 8]. Racial differences may explain the discordant results found by the present work in white subjects. Examples of racial differences and associations with HIV disease outcome have been previously shown for SNPs in natural killer cell–related genes, where the association between these SNPs and HIV

Table 1. Characteristics of Controllers and Noncontrollers of Human Immunodeficiency Virus (HIV) Infection

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV Controllers (n = 53)</th>
<th>HIV Noncontrollers (n = 389)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>23 (43.4)</td>
<td>60 (15.4)</td>
<td>&lt;.001</td>
<td>4.204 (2.287–7.729)</td>
</tr>
<tr>
<td>Elite controllers</td>
<td>34 (64.2)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>White</td>
<td>53 (100)</td>
<td>389 (100)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Age, y</td>
<td>43.0 (39.0–46.0)</td>
<td>41.0 (35.0–46.2)</td>
<td>.305</td>
<td>1.016 (0.985–1.049)</td>
</tr>
<tr>
<td>Time since HIV diagnosis, y</td>
<td>15.4 (8.0–20.5)</td>
<td>8.1 (2.0–15.4)</td>
<td>&lt;.001</td>
<td>1.108 (1.059–1.160)</td>
</tr>
<tr>
<td>CD4+ T-cell count, cells/μL</td>
<td>642.0 (462.5–937.5)</td>
<td>415 (239.0–582.0)</td>
<td>&lt;.001</td>
<td>1.003 (1.002–1.004)</td>
</tr>
<tr>
<td>CD8+ T-cell count, cells/μL</td>
<td>726.0 (569.3–1100.0)</td>
<td>747.5 (523.3–1032.8)</td>
<td>.464</td>
<td>1.000 (1.000–1.001)</td>
</tr>
<tr>
<td>Previous AIDS</td>
<td>3/34 (8.8)</td>
<td>109/385 (28.3)</td>
<td>.022</td>
<td>0.245 (0.73–1.818)</td>
</tr>
<tr>
<td>Injection drug use</td>
<td>31 (58.5)</td>
<td>143 (36.9)</td>
<td>.004</td>
<td>2.424 (1.352–4.347)</td>
</tr>
<tr>
<td>Anti-HCV detected</td>
<td>37 (69.8)</td>
<td>158/388 (40.7)</td>
<td>&lt;.001</td>
<td>3.366 (1.810–6.260)</td>
</tr>
<tr>
<td>HCV RNA detecteda</td>
<td>24/36 (66.7)</td>
<td>107/151 (70.9)</td>
<td>&lt;.001</td>
<td>1.108 (1.059–1.160)</td>
</tr>
<tr>
<td>HCV spontaneous clearanceb</td>
<td>7/36 (19.4)</td>
<td>22/151 (14.6)</td>
<td>.469</td>
<td>1.415 (0.552–3.627)</td>
</tr>
<tr>
<td>HCV RNA load, log IU/mL</td>
<td>5.88 (5.0–6.5)</td>
<td>6.2 (5.6–6.8)</td>
<td>.058</td>
<td>0.636 (0.398–1.016)</td>
</tr>
<tr>
<td>IL28B rs12979860</td>
<td>33 (62.3)</td>
<td>175 (45.0)</td>
<td>.020</td>
<td>2.669 (1.188–5.997)</td>
</tr>
<tr>
<td>HLA-B57 and/or HLA-B27 detectedc</td>
<td>13/ 41 (31.7)</td>
<td>18/120 (15.0)</td>
<td>.022</td>
<td>2.631 (1.151–6.015)</td>
</tr>
<tr>
<td>HLA B27 detected</td>
<td>6/41 (14.6)</td>
<td>7/120 (5.8)</td>
<td>.094</td>
<td>2.767 (1.872–8.779)</td>
</tr>
<tr>
<td>HLA B57 detected</td>
<td>8/41 (19.5)</td>
<td>11/121 (9.1)</td>
<td>.080</td>
<td>2.424 (1.901–6.526)</td>
</tr>
</tbody>
</table>

Qualitative data are no. (%) of individuals, and quantitative data are median (interquartile range).

Abbreviations: CI, confidence interval; HCV, hepatitis C virus; OR, odds ratio; NA, not applicable.

a Data were not available for 1 HIV controller and 7 noncontrollers.

b Refers to the subjects who cleared HCV infection without HCV-specific treatment. The remaining subjects (5 HIV controllers and 22 noncontrollers) showed a sustained virological response to HCV treatment.

c Data are for HCV RNA–positive patients, including those with HCV clearance on receipt of HCV treatment, for whom the HCV genotype was determined before the start of HCV-specific treatment.

d Associated with the specified HCV genotypes.

Table 2. Association Between IL28B rs12979860 CC and Spontaneous Control of Human Immunodeficiency Virus (HIV) Infection

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>&lt;.001</td>
<td>7.077 (2.893–17.313)</td>
</tr>
<tr>
<td>IL28B rs12979860 CC</td>
<td>.017</td>
<td>2.669 (1.188–5.997)</td>
</tr>
<tr>
<td>HLA-B57 and/or HLA-B27 detection</td>
<td>.017</td>
<td>3.080 (1.225–7.744)</td>
</tr>
</tbody>
</table>

Multivariate logistic regression analysis.

Abbreviations: CI, confidence interval; OR, odds ratio.

CT/TT, and we did not observe significant differences between the groups (Supplementary Figure 1).

DISCUSSION

In this study, we demonstrate that the IL28B CC genotype is independently associated with spontaneous HIV control in white individuals. This finding adds to the suggestion of common shared mechanisms involved in the control of 2 persistent infections, HIV infection and HCV infection. In fact, we and others have shown how HIV controllers can control HCV infection in terms of lower HCV loads [3] and higher rates of spontaneous clearance [7], compared with non–HIV controllers.

The interest in the SNP in IL28B arose from its association with the control of HCV infection [4, 5]. However, this study is the first to show that this SNP is independently related to the control of HIV infection. Interestingly, previous work showed that the IL28B SNP was not associated with spontaneous HIV control in African American individuals [7, 8]. Racial differences may explain the discordant results found by the present work in white subjects. Examples of racial differences and associations with HIV disease outcome have been previously shown for SNPs in natural killer cell–related genes, where the association between these SNPs and HIV
susceptibility were found in African Americans but not in other racial groups [11]. Rallon et al [12] analyzed the IL28B SNP prevalence among white long-term nonprogressors and HIV-exposed seronegative subjects and did not find any association of the IL28B SNP with HIV disease progression or HIV protection. This discrepancy could have arisen because long-term nonprogressors are defined by the maintenance of high levels of CD4+ T cells, in contrast to HIV controllers, who are defined only by their virological control. In addition, a previous study analyzed the association of this SNP with HIV acquisition and AIDS progression, and no association was found [13]. However, the aim of the present study was different because it analyzed the relationship between the IL28B CC genotype and the ability to control HIV viremia.

The association of the IL28B SNP with HIV control was not discussed in a previous genome-wide association study [1], most likely because the IL28B SNP might be in strong linkage disequilibrium with other analyzed SNPs, which could explain the loss of the association with viral control in the statistical analysis. In relation to viral set point establishment, we did not find differences in HIV loads between the IL28B CC and IL28B CT/TT groups. Because this lack may be due to the small number of subjects studied, analysis of a large cohort may contribute to clarifying this issue.

The overrepresentation of HLA-B57 among HIV controllers has been widely documented [1], as has its association with HCV control [2, 3]. This suggests that some common host immunological mechanisms are involved in the response against these 2 viruses, such as the efficient elimination of infected cells by HLA-B57-restricted cytotoxic T lymphocytes that recognize distinct epitopes from HIV or HCV [2]. The association of the IL28B CC genotype with HIV control was observed even when we adjusted for sex and the presence of protective alleles, such as HLA-B57 or HLA-B27. In this regard, sex differences in the course of HIV infection have been already described in several studies, and we had previously observed a higher prevalence of female sex among HIV controllers [3, 10]. In agreement with this observation, lower HIV loads during an early stage of infection [14] have been shown in women. Further studies are necessary to clarify the extent to which female hormones may be implicated in spontaneous HIV control and the mechanisms involved.

In this report, we show for the first time an association between the IL28B rs12979860 SNP and spontaneous HIV control. The mechanistic explanation could be related to the antiviral activity of IFN-λ, which has been demonstrated against different viruses, including HIV [6]. However, the influence of the IL28B SNP on IFN-λ production and activity are still unknown. Further studies are needed to analyze the antiviral properties of IFN-λ in HIV controllers and their possible role in the spontaneous control of HIV infection, as previously shown for IFN-α [15].

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank María del Mar Rodríguez, for providing technical support to the patients; and the patients in this study, for their participation.

Members of the HIV Controllers Consortium of the AIDS Spanish Network are as follows: Ezequiel Ruiz-Mateos, Kawthar Machmach, Sara Ferrando-Martínez, Miguel Genebat, Pompeyo Viciana, Manuel Leal, Jose Miguel Benito, Mariola López, Norma Rallón, Clara Restrepo, Cecilio López-Galán, María Pernas, Concha Casado, Agathe León, Montse Plana, Nuria Climent, Mireia Arnedo, Felipe Garcia and Pepe Alcami.

Financial support. This work was supported by Redes Telemáticas de Investigación Cooperativa en Salud (grant RETICS, 2006, Red de SIDA RD06/0006/0021, 2007–2010 to K.M.); by Consejería Salud Junta de Andalucía (grants CS2008/P10270 and CS2009/P10066); by Fondo de Investigacion Sanitaria (contracts P08/00172 to E. R.-M. and CD10/00382 to S. F.-M and grants PS09/00126 and P106/0915); by Prevencion del SIDA en España (FIPSE) (grant 36624/06) and by a research grant sponsored by Janssen-Cilag. The Biobank of the Spanish AIDS Research Network and HIV-infected Long-Term Non Progressors cohort from the AIDS Spanish Network (LTNP-RIS), and the prospective HIV-infected patients cohort from the AIDS Spanish Network (CoRIS) are supported by Instituto de Salud Carlos III and the Spanish Health Ministry (grant RD06/006/0035).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


