Association of BST-2 Gene Variants With HIV Disease Progression Underscores the Role of BST-2 in HIV Type 1 Infection

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We tested bone marrow stromal cell antigen 2 (BST-2) gene variants rs3217318, a 19-base-pair insertion/deletion polymorphism in the promoter region, and rs10415893, a tag single-nucleotide polymorphism in the 3′ untranslated region, for their association with human immunodeficiency virus type 1 (HIV-1) infection and disease progression. The study included 356 subjects exposed to HIV-1 (185 with and 171 without infection) and 188 controls. The first decrease in the CD4+ T-cell count to <200 cells/μL was used as the primary outcome, whereas the primary outcome plus initiation of any antiretroviral treatment was used as a secondary composite outcome. Association with progression was found for both rs3217318 and rs10415893, following an overdominant model. Diplotype analysis revealed faster progression to both outcomes for subjects carrying the Δ19_G/i19_A diplotype. Luciferase assay showed that a promoter sequence containing the i19 allele had the lowest expression levels, suggesting that i19 allele carriers could have less BST-2 expression, reducing their capability to retain viral particles. These results point to the relevance of BST-2 as a host genetic factor modifying HIV-1 disease progression.

Keywords. BST-2; tetherin; CD317; rs10415893; rs3217318; HIV-1 infection; HIV-1 disease progression.

Bone marrow stromal cell antigen 2 (BST-2), also known as tetherin, HM1.24, and CD317, is a 30–36-kDa, 180–amino acid, heterogeneously glycosylated, dimeric type II transmembrane protein [1]. As a newly identified component of the innate immune response to enveloped viruses, BST-2 has been described as a host restriction factor for human immunodeficiency virus type 1 (HIV-1) [2, 3]. BST-2 inhibits the release of viral particles from infected cells by tethering them to the cell surface and to other retained viral particles. In addition, it has been suggested that BST-2 may abrogate the infectivity of released HIV-1 particles [4]. The HIV-1 accessory protein Vpu counteracts BST-2 antiviral activity by sequestering it from the cell surface, as well as by promoting its subsequent degradation, leading to increased viral release [3, 5–7].

The BST-2 gene, located on chromosome 19 and covering a region of 2.71 kb, encodes BST-2/tetherin [8]. The BST-2 gene is constitutively expressed in B cells, pancreas, liver, lung, and heart [8, 9], but there is controversy about its expression in T cells, monocytes, macrophages, and plasmacytoid dendritic cells; it can be induced by interferon in other cell types [9, 10].

The molecular mechanisms involved in BST-2 restriction of viral egress and the countermeasures used by various viruses have been comprehensively characterized [11–13]. Additional host restriction factors have been described, such as cytidine deaminase apolipoprotein B messenger RNA–editing, enzyme-catalytic, polypeptide-like 3G (APOBEC3G); tripartite motif-containing protein 5 alpha isoform (TRIM5α);
and the newly described SAMHD1 [14, 15]. Data supporting their role in HIV infection comes from both experimental trials [16] and association studies of known gene variants [17, 18]. The action of BST-2 in restricting viral egress from the cell surface mainly suggests the association of BST-2 gene variants with progression rates but also suggests a potential role in susceptibility to infection. Following this hypothesis, the present study tested BST-2 gene variants for their association with HIV-1 infection and disease progression. The analysis included HIV-1-infected subjects, HIV-1–exposed, noninfected subjects, and healthy subjects. Our results support the involvement of BST-2 in HIV-1 disease progression.

**METHODS**

**Study Populations**

The study included 356 subjects exposed to HIV-1 (185 with and 171 without HIV-1 infection) and 188 healthy subjects (controls). The main characteristics of the HIV-1–positive cohort have been previously described [19, 20]. Briefly, the Lleida AIDS Cohort is a prospective seroprevalent cohort of HIV-1–seroprevalent injection drug users that is drawn from all HIV-1–seropositive adults enrolled in the AIDS Service of the Hospital Arnau de Vilanova. Only white patients recruited between 1982 and 1991 were included in the cohort. Patients in the cohort had been followed for a median duration of 127.7 months (range, 84–198 months). The observation period for progression status ended in December 1999. Epidemiological and clinical characteristics of the HIV-1–positive cohort are presented in Supplementary Table 1. The main characteristics of the exposed, noninfected subjects, who were enrolled in prospective cohort studies, have been previously described [21]. In the present study, 46 exposed, noninfected subjects were added to our previous cohort, all of whom conformed to recruitment requirements, as described by de la Torre et al [21]. In addition, a group of 188 nonexposed white male subjects who were recruited from among anonymous blood donors and who tested negative for HIV-1 and hepatitis C virus (HCV) was used as a control sample. All participants gave written informed consent. Ethics committees of the participating hospitals approved the study.

**Selection of BST-2 Polymorphic Variants and Genotyping**

Selection of BST-2 candidate polymorphisms followed several different strategies. We calculated the pairwise linkage disequilibrium (LD) D’ for the BST-2 gene region, using Haplovie 4.2 software (http://www.broad.mit.edu/mpg/haplovie), for the HapMap-CEU population [22]. Block structure was considered for marker pairs showing a D’ of >0.8, following the Haplovie “solid-spine” block definition. An LD block covering the entire gene was observed, from which we selected rs10415893 as a tag single-nucleotide polymorphism (SNP). In addition, we included markers annotated at the Single Nucleotide Polymorphism Database (dbSNP), because of their putative functional effect. The selected polymorphisms were rs71694748, a 353-base-pair insertion/deletion polymorphism that covers the fourth intron; rs1804402, a G to T change that causes a nonsynonymous mutation located in the fourth exon; and rs3217318, a 19-base-pair insertion/deletion polymorphism located at position −411. Marker positions are indicated in Figure 1A.

Sample DNA was extracted from fresh peripheral mononuclear blood cells and frozen whole blood. Genotyping was performed following protocols described in Supplementary Table 2. For each polymorphism, assay validation was performed using a minimum of 5 representative individuals whose genotypes had been previously determined by sequencing.

**BST-2 Promoter Cloning, Transient Transfection, and Functional Assay**

The promoter region of BST-2 contains some cis regulatory elements, including a tandem repeat of 3 interleukin 6 (IL-6) response type II element/APRF sites, interferon response elements IRF-1/2, ISGF3, and GAS; several IL-6 response type I element/NF-IL-6 sites; and AP-2 and Sp1 binding sites (Figure 2A) [1]. The rs3217318 insertion allele (rs3217318_i19) modifies the promoter sequence, increasing the number of AP-2 and Sp1 binding sites (Figure 2B). A 680-base-pair fragment of the BST-2 promoter (positions −640 to +40) containing the rs3217318 polymorphism was amplified by polymerase chain reaction (PCR), using the Qiagen Multiplex PCR kit (Qiagen), following the manufacturer’s instructions, from genomic DNA of 2 homozygous individuals harboring Δ19_G and i19_A haplotypes. The primers used were as follows: NotI forward: 5’-ACTGACTAGTGGAGTTTCTGCAGGT GCCAAGAGAC-3’; and Sp1 reverse: 5’-ACTGCGGCCCGC CCAGATCTCCTTTAGGTCTGCCTGGGAG-3’ (restriction enzyme recognition sites are underlined). PCR products were separated by agarose gel electrophoresis, stained with methylene blue, subjected to gel excision, and cloned in the pGEM easy vector system I (Promega), generating PGEM-Δ19 and PGEM-i19 plasmids. The fidelity of PCR amplifications and subcloning was confirmed with DNA sequencing by capillary electrophoresis of plasmid inserts for both strands, using the BigDye Terminator v3.1 Cycle Sequencing Kit in a 3130 Genetic Analyzer (Applied Biosystems). Luciferase (LUC) reporter plasmids were constructed by digestion of PGEM-Δ19 and PGEM-i19 constructions with the restriction enzymes NotI and Spel. The products were directionally ligated to the promoterless PACNV-LUC plasmid [23], obtaining P-Δ19-LUC and P-i19-LUC reporter plasmids.

Transfected U373 cells were used to evaluate LUC expression from P-Δ19-LUC and P-i19-LUC constructions. U373 cells were washed with cold phosphate-buffered saline (PBS)
and suspended in serum-free Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10 mM dextrose and 0.1 mM dithiothreitol (Gibco). Transient transfection of U373 cells (3 × 10⁶) was performed by electroporation (Gene Pulser II Electroporation System, Bio-Rad) at 260 V, 1200 μF, and ∞Ω with 2 μg of the reporter plasmid per 10⁶ U373 cells, and cotransfection was performed with 625 ng of PSV-β-galactosidase reporter plasmid (Promega). Transfected cells were cultured in 10 mL of RPMI 1640 medium for 24 hours and then cultured in the presence (stimulated) or absence (unstimulated) of 10 ng/mL interferon γ (IFN-γ) for 24 and 48 hours.

**Figure 1.** Exon-intron structure and haplotypes of the BST-2 gene. A, Exon-intron structure of the BST-2 gene, with locations of the rs3217318, rs180402, rs71694748, and rs10415893 genotyped polymorphisms. B, Differences observed between haplotypes of homozygous selected Δ₁₉ G, i₁₉ A, and i₁₉ G individuals at the 680-base-pair cloned fragment and genotyped markers.

**Figure 2.** A, Promoter region of BST-2 gene, including transcription factor binding sites (as described by Ohtomo et al [1]) and the studied polymorphisms. The 680-base-pair cloned region in the promoter is indicated in grey box. Polymorphisms are indicated in bold under their reference single-nucleotide polymorphism code. The sequence at the rs3217318 insertion/deletion polymorphism is represented by the common deletion (Δ₁₉) allele. B, Sequence surrounding rs3217318 insertion/deletion polymorphism that corresponds to insertion (i₁₉) allele, indicating the duplicated transcription factor binding sites, with the inserted sequence in brackets.
LUC activity was measured in a Berthold Sirius V2 luminometer, as previously described [15]. In parallel, cells were washed with PBS and processed for a β-galactosidase chemiluminescent assay (Roche) and Bradford protein assay (Bio-Rad). The transfection efficiency was calculated after normalization by β-galactosidase and protein content. All experiments were repeated a minimum of 3 times. Results are expressed as log-transformed values of normalized LUC activity. Distribution differences across groups were tested by the nonparametric Mann–Whitney U test.

Statistical Analysis
Hardy–Weinberg equilibrium was tested by comparing expected and observed genotype frequencies, using a χ² test. Allele, genotype, haplotype, and diplotype frequencies were estimated and compared using pLink software [24]. Complementary analysis was performed by SPSS-18.0 software.

We explored the relationship between BST-2 polymorphisms and HIV-1 infection by conducting a cohort-based observational analysis comparing the distribution of polymorphisms between healthy controls, exposed, noninfected patients, and HIV-1-positive patients. P values calculated for single-marker association tests were corrected for multiple testing by means of the SNP spectral decomposition approach, using a modified Bonferroni-corrected nominal threshold of $P = 0.05/N$, where $N$ is the effective number of independent marker loci after consideration of LD between markers. $N$ was calculated using the SNP spectral decomposition approach, using the Web-based program SNPSpD (http://gump.qimr.edu.au/general/daleN/SNPSpD/). According to this approach, the experiment-wide significance threshold required to keep the type 1 error rate at 5% was $P < 0.043$. Bonferroni adjustment was performed by dividing the significance level (0.05) by the number of pairwise comparisons, for the genotype association test ($N = 3; P = 0.016$), and by the number of major haplotypes (ie, frequency >5%), for the haplotype association test ($P = 0.016$).

To explore the effect of BST-2 variability on HIV-1 disease progression, survival profiles of HIV-1-infected patients grouped according to BST-2 variants were evaluated by Kaplan–Meier survival analysis. The first decrease in the CD4⁺ T-cell count to <200 cells/µL was considered the primary outcome for disease progression. In addition, a secondary composite outcome was used that included the primary outcome plus initiation of any antiretroviral treatment (ART). Survival time ranged from date of the first positive test result to the outcome date or censoring date (defined as the last clinic examination date or as the date of death, if death was not caused by HIV-1 infection). Five patients died after reaching the primary outcome, and 1 patient died of a heroin overdose before reaching the primary outcome. This patient was assumed to be outcome free at the time of death because he had had a CD4⁺ T-cell count of >200 cells/µL for >7 years and had not initiated ART. Differences in survival profiles between groups were compared by the log-rank test. Hazard ratios (HRs) were estimated using a Cox proportional hazard model adjusted for sex, age at first HIV-1-positive test result, and CCR5Δ32 genotype (as previously reported for this cohort [20]). A $P$ value of <.05 was considered statistically significant.

RESULTS

BST-2 Gene Variability and Susceptibility to Infection
After genotyping all individuals, rs71694748 and rs1804402 were found to be monomorphic. In contrast, rs10415893 (tag SNP) and rs3217318 (promoter insertion/deletion) were polymorphic and conformed to Hardy–Weinberg equilibrium in all groups. We evaluated differences in genotype and allele distribution of rs10415893 and rs3217318 polymorphisms between the study groups. No statistically significant differences were observed for the global distribution of genotypes and alleles between the HIV-1-positive group, the exposed and noninfected group, and the control group (Table 1).

Strong LD was observed between rs10415893 and rs3217318 polymorphisms, which permitted haplotype-based analysis. Diploidy imputation was performed by pLink with a probability of imputation of >0.99. Haplotype and diplotype counts for the common Δ19_G/Δ19_G and Δ19_G/i19_A and rare (<5%) diplotypes are shown in Table 1. No statistical differences were observed for the global distribution of haplotypes and diplotypes between the HIV-1-positive group, the exposed and noninfected group, and the control group (Table 1).

BST-2 Gene Variability and Disease Progression Among HIV-1–Positive Patients
Of the 185 HIV-1–positive patients in this study, the CD4⁺ T-cell count during follow-up remained at >200 cells/µL in 110 (59.4%) and decreased to <200 cells/µL in 75 (40.5%). Kaplan–Meier survival analysis for disease progression to the primary outcome according to rs10415893 genotypes under an overdominant model revealed a significantly faster progression rate for patients carrying the rs10415893-G/A genotype (median survival time, 135.3 months [25th percentile, 71.8 months]), compared with rs10415893-(G/G+A/A) homozygotes (median survival time, 171.3 months [25th percentile, 100.7 months]; $P = 0.028$, by the log-rank test; Figure 3A). The Cox proportional adjusted HR for progression among patients with the rs10415893-G/A genotype, compared with rs10415893-(G/G+A/A) homozygotes, remained statistically significant (HR, 1.7 [95% confidence interval {CI}, 1.2–2.8]; $P = 0.034$). In addition, Kaplan–Meier survival analysis for disease progression to the primary outcome according to rs3217318 genotypes under an overdominant model revealed

414 • JID 2013:207 (1 February) • Laplana et al
a trend of a faster progression rate for patients carrying the rs3217318-Δ19/i19 genotype (median survival time, 135.3 months [25th percentile, 71.8 months]), compared with rs3217318-Δ19/Δ19 homozygotes (median survival time, 171.3 months [25th percentile, 100.7 months]; \( P = .046 \), by the log-rank test; Figure 3B). However, the adjusted Cox proportional HR for progression of patients with the rs3217318-Δ19/i19 genotype, compared with rs3217318-Δ19/Δ19 homozygotes, was not statistically significant (HR, 1.6 [95% CI, .96–2.6]; \( P = .070 \)).

A secondary composite outcome of first decrease in the CD4+ T-cell count to < 200 cells/μL plus initiation of any ART was considered, with the assumption that ART initiation also reflects important underlying disease progression. Of all 185 HIV-1–positive patients, 30 (16%) never reached the composite outcome, 120 (65%) started ART with CD4+ T-cell counts ranging from 200 to 500 cells/μL, and 35 (19%) started ART after the first decrease in the CD4+ T-cell count to < 200 cells/μL. Kaplan–Meier survival analysis for disease progression to the composite outcome did not reveal statistically significant differences for rs10415893 genotypes (\( P = .085 \), by the log-rank test; Figure 3C). The Cox proportional adjusted HR for progression of patients with the rs10415893-G/A genotype, compared with rs10415893-G/G+A/A homozygotes, did not reach statistical significance (HR, 1.6 [95% CI, .96–2.6]; \( P = .07 \)). In contrast, Kaplan–Meier survival analysis for disease progression to the secondary composite outcome according to rs3217318 genotypes under overdominant model revealed significantly faster progression for patients carrying the rs3217318-Δ19/i19 genotype (median survival time, 73.5

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**Table 1. Genotype, Allele, Haplotype, and Diplotype Distribution of BST-2 Polymorphic Variants rs3217318 and rs10415893 in All Cohorts**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Healthy Control</th>
<th>HIV-1 Exposed, Noninfected</th>
<th>HIV-1 Positive</th>
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<tr>
<td>Genotype</td>
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<tr>
<td>Δ19/Δ19</td>
<td>129 (69)</td>
<td>117 (68.4)</td>
<td>137 (74)</td>
</tr>
<tr>
<td>Δ19/i19</td>
<td>52 (27.8)</td>
<td>49 (28.7)</td>
<td>42 (22.7)</td>
</tr>
<tr>
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<td>6 (3.2)</td>
<td>5 (2.9)</td>
<td>6 (3.3)</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ19</td>
<td>310 (82.8)</td>
<td>283 (82.7)</td>
<td>316 (85.4)</td>
</tr>
<tr>
<td>i19</td>
<td>64 (17.2)</td>
<td>59 (17.3)</td>
<td>54 (14.6)</td>
</tr>
<tr>
<td><strong>rs10415893</strong></td>
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<tr>
<td>Genotype</td>
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</tr>
<tr>
<td>GG</td>
<td>128 (68)</td>
<td>119 (70)</td>
<td>142 (76.8)</td>
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<td>GA</td>
<td>55 (29.3)</td>
<td>47 (27.7)</td>
<td>38 (20.5)</td>
</tr>
<tr>
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<td>4 (2.3)</td>
<td>5 (2.7)</td>
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<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>G</td>
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<td>285 (83.8)</td>
<td>322 (87)</td>
</tr>
<tr>
<td>A</td>
<td>65 (17.3)</td>
<td>55 (16.2)</td>
<td>48 (13)</td>
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<td><strong>rs3217318 and rs10415893</strong></td>
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<td>Haplotype counts</td>
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<tr>
<td>Δ19_G</td>
<td>307 (82.1)</td>
<td>283 (83.2)</td>
<td>310 (85.2)</td>
</tr>
<tr>
<td>i19_A</td>
<td>61 (16.3)</td>
<td>55 (16.2)</td>
<td>48 (13.2)</td>
</tr>
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</tr>
<tr>
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<td>Diplotype counts</td>
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</tr>
<tr>
<td>Δ19_G/i19_G</td>
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<td>117 (68.8)</td>
<td>137 (74)</td>
</tr>
<tr>
<td>Δ19_G/i19_A</td>
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<td>47 (27.7)</td>
<td>47 (20)</td>
</tr>
<tr>
<td>Other (&lt;5%)a</td>
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<td>6 (3.5)</td>
<td>11 (6)</td>
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</table>

The genotype at rs3217318 was missing from 1 healthy control, and the genotype at rs10415893 was missing for 1 HIV-1–exposed, noninfected subject.

Abbreviation: HIV-1, human immunodeficiency virus type 1.

a Other diplotypes were as follows: for healthy control subjects, Δ19_G/i19_G (n = 2; 1%), Δ19_G/i19_A (n = 3; 1.5%), i19_A/i19_A (n = 5; 2.5%), and i19_G/i19_A (N = 1; 0.5%); for HIV-1–exposed, noninfected subjects, Δ19_G/i19_G (n = 2; 1%) and i19_A/i19_A (n = 4; 2.3%); and for HIV-1–positive subjects, Δ19_G/i19_G (n = 5; 2.7%), i19_A/i19_A (n = 5; 2.7%), and i19_G/i19_A (n = 1; 0.5%).
months [25th percentile, 36.5 months]), compared with rs3217318-Δ19/i19 heterozygotes (median survival time, 91.8 months [25th percentile, 65.5 months]; P = .011, by the log-rank test; Figure 3). The Cox proportional adjusted HR for progression of patients with the rs3217318-Δ19/i19 genotype, compared with rs3217318-Δ19/Δ19 homozygotes, remained statistically significant (HR, 1.5 [95% CI, 1.1–2.2]; P = .019).

According to the strong LD observed between rs10415893 and rs3217318 markers, progression of HIV-1-infected patients was evaluated with respect to BST-2 diplotype. Kaplan–Meier survival analysis for disease progression to the primary outcome revealed a significantly faster progression rate for patients carrying the heterozygote Δ19_G/i19_A diplotype (median survival time, 135 months [25th percentile, 71.7 months]), compared with patients carrying the homozygous Δ19_G/Δ19_G diplotype (median survival time, 171 months [25th percentile, 96.8 months]; P = .026, by the log-rank test; Figure 4). The Cox proportional adjusted HR for progression of patients with the Δ19_G/i19_A diplotype, compared with those with the Δ19_G/Δ19_G diplotype, remained statistically significant (HR, 1.7 [95% CI, 1.1–2.9]; P = .034). In addition, Kaplan–Meier survival analysis for disease progression to the secondary composite outcome revealed a significantly faster progression rate for patients carrying the Δ19_G/i19_A diplotype (median survival time, 79.7 months [25th percentile, 36.5 months]), compared with patients carrying the Δ19_G/Δ19_G diplotype (median survival time, 91.8 months [25th percentile, 65.5 months]; P = .011, by the log-rank test; Figure 4B).

Figure 3. Kaplan–Meier survival analysis, by rs10415893 and rs3217318 genotypes under an overdominant model. A and B, Proportion of patients who did not reach the primary outcome, by rs10415893 genotype (A) and rs3217318 genotype (B). C and D, Proportion of patients who did not reach the secondary composite outcome, by rs10415893 genotype (C) and rs3217318 genotype (D). A first decrease in the CD4+ T-cell count to < 200 cells/µL was used as the primary outcome for progression, while the secondary composite outcome also considered initiation of any antiretroviral treatment. Survival time ranged from date of the first human immunodeficiency virus type 1 (HIV-1)–positive test result to the outcome date or censoring date (defined as the last clinic examination date or as the date of death, if death was not caused by HIV-1 infection).
[25th percentile, 68.5 months]; \( P = .034 \), by the log-rank test; Figure 4B). In addition, the Cox proportional adjusted HR for progression of patients with the \( \Delta_{19}G/i_{19}A \) diplotype, compared with those with the \( \Delta_{19}G/\Delta_{19}G \) diplotype, showed a trend for significance (HR, 1.5 [95% CI, 1–2.2]; \( P = .047 \)).

**Functional Analysis of BST-2 Promoter Variants**

We cloned and sequenced 680 base pairs of the promoter region from 8 individuals homozygous for the \( \Delta_{19}G \) haplotype, 2 individuals homozygous for the \( i_{19}G \) haplotype, and 6 individuals homozygous for the \( i_{19}A \) haplotype. Differences found between the promoter regions of the individuals bearing these haplotypes are shown in Figure 1B. A cloned 680-base-pair fragment from patients with the \( i_{19}G \) and \( i_{19}A \) haplotypes did not show differences at the sequence level. In contrast, the sequence of the \( \Delta_{19}G \) haplotype differed from that of the \( i_{19}A \) haplotype at SNPs described in the dbSNP (ie, rs12979773, rs12971834, rs12609479, and rs28413174) that showed full linkage with the rs3217318 insertion/deletion polymorphism (Figure 1B). According to this, the 680-base-pair promoter fragments obtained from \( \Delta_{19}G \) and \( i_{19}A \) homozygotes were cloned in the reporter plasmid \( P_{\Delta_{19}}CNV-LUC \). LUC activity measured in U373 transfected cells after 24 and 48 hours of culture showed basal overexpression of the \( P_{\Delta_{19}}-LUC \) construct (\( P = .050 \) and \( P = .127 \), by the Mann–Whitney U test, for \( P_{i_{19}}-LUC \) vs \( P_{\Delta_{19}}-LUC \) at 24 hours and 48 hours, respectively; Figure 5). In an attempt to mimic physiological conditions, we performed our experiments using IFN-\( \gamma \) as a stimulus, because it induces a response that is more strongly mediated by IRF1 [25]. Under these new conditions, the differences between \( P_{i_{19}}-LUC \) and \( P_{\Delta_{19}}-LUC \) constructs were even higher, reaching statistical significance after both 24 hours and 48 hours of stimulation (\( P = .050 \), by the Mann–Whitney U test; Figure 5). In addition, we found that 48-hour stimulus with IFN-\( \gamma \) slightly increased LUC activity in the \( P_{\Delta_{19}}-LUC \) construction (\( P = .127 \), Mann–Whitney U test, for basal vs stimulated at 48 hours) but seemed to have no effect on \( P_{i_{19}}-LUC \) construction.

**DISCUSSION**

Host genetic factors have been identified that affect susceptibility to infection and modulate disease progression rates [26]. In addition, cellular restriction factors that interfere with the virus life cycle have been described. Among them, APOBEC3G,
TRIM5α, and the recently described BST-2 cellular restriction factors have been comprehensively studied [16]. Supporting evidence for the role of APOBEC3G and TRIM5α in HIV-1 infection comes from both experimental and genetic association studies testing genetic variability at each gene locus in relation to the susceptibility to infection and disease progression. In contrast, the experimental evidence that supports BST-2 as an HIV-1 restriction factor has not been complemented with genetic association studies.

In the present study, we evaluated BST-2 gene variability in relation to both susceptibility to HIV-1 infection and disease progression rates. The prevalence of 2 polymorphic BST-2 gene variants, rs10415893 and rs3217318, was determined in 3 previously described cohorts of seroprevalent HIV-positive individuals, HIV-1–exposed, noninfected individuals, and healthy control subjects [19, 20]. The prevalence of BST-2 variants in HIV-positive patients did not differ from that observed in healthy controls and HIV-1–exposed, noninfected subjects, which does not support a putative association between BST-2 polymorphisms and susceptibility to HIV-1 infection. In contrast, survival analysis indicated a potential effect of BST-2 gene variants on progression rates. For this analysis, we evaluated survival profiles of HIV-positive patients according to BST-2 gene variants, using the first decrease in the CD4+ T-cell count to < 200 cells/µL as a primary outcome for progression. In addition, to minimize a possible bias caused by access to different effective treatments before reaching the primary outcome, a secondary composite outcome that includes initiation of any ART was also considered. Statistically significant associations between genotype and progression were found under an overdominant model for rs3217318 heterozygotes, for secondary outcome, and for rs10415893 heterozygotes, for the primary outcome. Furthermore, diploptyle analysis that included rs10415893 and rs3217318 allowed us to detect a statistically significant association between progression and carriage of the Δ19_G/i19_A haplotype diplotype when considering both outcomes.

As reflected by the LUC assay, the promoter haplotype containing the rs3217318-i19 allele had lower expression levels and seemed to not respond to IFN-γ stimulus. This result suggests that individuals carrying the i19_A haplotype could have lower BST-2 protein levels and a compromised response to stimulus. Because BST-2 inhibits the release of viral particles from infected cells by tethering them to the cell surface, lower levels of BST-2 could implicate lower retention of viral particles. In accordance with the obtained results, HIV-1–positive patients carrying the heterozygote Δ19_G/i19_A diplotype showed a faster progression to both outcomes, indicating that heterozygosity for the i19_A haplotype could be a risk factor for a faster progression. The standard overdominant model deals with variants affecting the protein structure that confer an advantage (overdominance) or disadvantage (underdominance) to heterozygotes, compared with homozygotes. Nevertheless, regulatory variants affecting protein quantity can also produce an overdominant effect in heterozygotes [27]. Tissue-specific expression of alleles has been described in individuals heterozygous for promoter polymorphisms at the HLA-DQB1 locus. The HLA-DQB1*0301 allele showed higher expression than the HLA-DQB1*0302 allele in primary skin cells, while the expression profile was reversed in other tissues, such as mononuclear cells and B lymphocytes in peripheral blood [28]. This can result in an overdominant behavior in which *0301/*0302 heterozygotes have broad HLA-DQB1 expression, while homozygotes have tissue-restricted expression. On the other hand, we cannot exclude the possibility that the overdominant effect we attributed to the Δ19_G/i19_A haplotype combination could be due to a masked coding variant dragged by the high LD in the region surrounding the BST-2 gene.

Studies of BST-2 antagonism by primate lentiviruses have led to the hypothesis that BST-2 provided a barrier to cross-species transmission of HIV-1 (and HIV-2) to humans. This, together with the apparent lack of a BST-2–counteracting activity in group 0 viruses, has led researchers to consider that BST-2 might tend to inhibit transmission between individuals more than it limits pathogenesis in the individual [29]. Our current study points to the opposite conclusion, wherein BST-2 variants conferring reduced BST-2 levels are associated with HIV-1 pathogenesis without modifying susceptibility to infection.

We acknowledge a number of potential limitations in our study. First, comparisons between blood donors, who composed the healthy reference cohort, and injection drug users, who composed the disease cohort, could have induced bias. To minimize bias related to injection drug use, comparisons were also performed between HIV-1–infected injection drug user and HIV-1–noninfected and exposed intravenous drug users. On the other hand, the main results regarding disease progression were obtained from survival analysis of infected injection drug users, in which bias for injection drug use is not expected. We report the association of the BST-2 rs10415893 tag SNP and the tagged rs3217318 insertion/deletion polymorphism with HIV disease progression, in line with our initial hypothesis that BST-2 gene variants are chiefly associated with progression rates but not with susceptibility to infection. On the basis of our initial screening, we discarded the nonsynonymous rs1804402 polymorphism and the intronic insertion/deletion rs71694748 polymorphism as potential causative variants. Functional assay results suggest that the tagged promoter rs3217318 insertion/deletion polymorphism could modify BST-2 expression levels.

The present study must be considered as an initial approach in the characterization of the genetic component of BST-2 in HIV-1 infection and disease progression that complements
the robust experimental evidence with genetic association data. Replication in larger cohorts will be needed to confirm our findings. In addition, further analysis will be required to identify and characterize other causative variants tagged by the rs10415893 marker that, in addition to rs3217318, could contribute to the association detected.

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

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