Bone marrow stromal antigen 2 (BST-2) genetic variants influence expression levels and disease outcome in HIV-1 chronically infected patients

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ABSTRACT

Background:
Bone marrow stromal antigen 2 (BST-2) also known as Tetherin (CD317/HM1.24), is a host restriction factor that blocks the release of HIV-1 virions from infected cells. Previous studies reported that BST-2 genetic variants or single nucleotide polymorphisms (SNPs) have a preventative role during HIV-1 infection. However, the influence of BST-2 SNPs on expression levels remains unknown. In this study, we investigated the influence of BST-2 SNPs on expression levels and disease outcome in HIV-1 subtype C chronically infected antiretroviral therapy naïve individuals.

Results:
We quantified BST-2 mRNA levels in peripheral blood mononuclear cells (PBMCs), determined BST-2 protein expression on the surface of CD4⁺ T cells using flow cytometry and genotyped single nucleotide polymorphisms (SNPs) rs919267, rs919266 and rs9576 using TaqMan assays from HIV-1 uninfected and infected participants. Determined the ability of plasma antibody levels to mediate antibody-dependent cellular phagocytosis (ADCP) using gp120 consensus C and p24 subtype B/C protein. Fc receptor-mediated NK cell degranulation was evaluated as a surrogate for ADCC activity using plasma from HIV-1 positive participants. BST-2 mRNA expression levels in PBMCs and protein levels on CD4⁺ T cells were lower in HIV-1 infected compared to uninfected participants (p=0.075 and p=0.005, respectively). rs919267CT (p=0.042) and rs919267TT (p=0.045) were associated with lower BST-2 mRNA expression levels compared to rs919267CC in HIV-1 uninfected participants. In HIV-1 infected participants, rs919267CT associated with lower CD4 count, (p=0.003), gp120-IgG1 (p=0.040), gp120-IgG3 (p=0.016) levels and but higher viral
while rs919267TT was associated with lower BST-2 mRNA levels (p=0.001) and CD4 counts (p=0.001), gp120-IgG1 levels (p=0.033) but higher plasma viral load (p=0.007). Conversely, rs9576CA was associated with higher BST-2 mRNA expression levels (p=0.027), CD4 counts (p=0.079), gp120-IgG1 (p=0.009), -IgG3 (p=0.039) levels and but with lower viral load (p=0.037). However, there was no correlation between BST-2 SNPs, p24-IgG subclass, ADCC and ADCP activity.

Conclusion:

Our findings show that bst-2 SNPs mediate BST-2 expression and disease outcome, correlate with gp120-IgG1, gp120-IgG3 levels but p24-IgG levels, ADCC and ADCP activity. Future studies should investigate the influence of BST-2 polymorphic variants on the ability of antigen presentation dendritic cells (DCs) to elicit robust immune responses.

Key words

BST-2, SNPs, expression levels, gp120- and p24-IgG levels, viral loads
Background

Bone marrow stromal antigen 2 (BST-2; also known as CD317, and tetherin) is a host restriction factor that blocks the release of enveloped viruses, including HIV-1, from infected host cells[1] and modulates plasmacytoid dendritic cell (pDC) interferon (INF)-α production [2, 3]. BST-2 is probably a receptor that confers anti-viral antigen presentation properties to pDCs, given the high expression of BST-2 in pDCs following activation [4-6]. Although the tethering capacity of BST-2 was first described HIV-1 [1], it was subsequently shown to occur for other enveloped viruses [7]. While BST-2 can be found in the trans-Golgi network and in vesicular compartments, it is a transmembrane protein primarily located on the apical membrane (reviewed in [8]).

The acute “murine AIDS” (LP-BM5) and/or Moloney murine leukaemia virus (Mo-MuLV) replication was not different between the wild type (WT) and BST-2 knockout (KO) mice [6, 9]. However, a different study reported that even though WT and BST-2 KO mice had similar acute LP-BM5 replication levels, BST-2 KO mice had higher replication levels during later time points, when adaptive immune responses operate [10]. Another study reported that BST-2 KO mice had higher acute mouse mammary tumour virus (MMTV) replication levels [11].

The BST-2 protein is encoded by bst-2, an interferon simulated gene located in chromosome 19p13.2 [12]. The gene is 2.69 kb long and comprises of four functional exons [13, 14]. A previous study reported that bst-2 gene variants rs3217318, a 19-base-pair insertion/deletion polymorphism in the promoter region and rs10415893, a tag single-nucleotide polymorphism (SNP) in the 3’ untranslated region were associated with HIV-1 disease progression in a Spanish cohort [15]. A subsequent study showed that SNPs rs9576 and rs919266 were associated with disease outcome,
with rs9576 marginally associated with protection against mother-to-child HIV-1 transmission in a Zambian cohort while SNP rs919266 was associated with slower progression to AIDS in Brazilian and Italian cohorts [16]. A different study showed that SNP rs919267 was associated with faster HIV-1 disease progression in an Indian cohort [17]. Collectively, these data suggest that bst-2 genetic variants may have a preventative or modifying role in HIV-1 infection or disease progression. However, the influence of bst-2 SNPs on endogenous BST-2 expression or other underlying mechanisms remain to be determined.

The HIV-1 accessory protein Vpu counteracts the restriction activity of BST-2 by targeting it for proteasomal and/or lysosomal degradation [12, 18]. Thus Vpu shields HIV-1 infected cells from immune clearance [19]. Studies have reported that treating HIV-1 infected cells with cytokines such as interferon alpha (IFN-α), IFN-β and interleukin 27 (IL-27) reversed the antagonistic effect of Vpu on BST-2 in a dose dependent manner [20, 21]. Specifically, these data showed that anti-HIV-1 neutralizing antibodies (NAbs) 3BNC117, PGT126 and PG9 are capable of mediating antibody-dependent cell-mediated cytotoxicity (ADCC) against HIV-1 infected T cells [20]. In addition to ADCC high p24 specific IgG1 levels and also associated with antibody-mediated cellular phagocytosis (ADCP) responses [22]. However, Vpu that induces BST-2 antagonism that attenuates antibody binding and ADCC responses mediated by NAbs could be reversed by IFNα treatment in a dose dependent manner [20, 21]. Moreover, BST-2 enhanced protective immune responses mediated by cells such as NK cells, CD4+ T cells and CD8+ T cells correlated with decreased infection levels [23]. In addition, the tethering effect of BST-2 enhanced recognition and effective killing of infected cells by antibody-mediated effector functions including...
Overall, the aforementioned studies suggest that higher expression of BST-2 may promote cell mediated antiviral activity \textit{in vivo} and attenuate disease outcome.

However, the influence of \textit{bst}-2 SNPs on expression levels, ADCC and/or ADCP activity has not been determined. We hypothesised that BST-2 SNPs may mediate BST-2 expression levels, ADCC and/or ADCP activity and disease outcome in a cohort of antiretroviral therapy naive, HIV-1 subtype C chronically infected individuals. Therefore, we investigated the effect of select \textit{bst}-2 SNPs rs919266, rs919267 and rs9576 on endogenous BST-2 expression levels, anti-HIV ADCC and/or ADCP activity and HIV-1 clinical outcomes.

\textbf{RESULTS}

\textbf{Association of endogenous BST-2 expression with HIV-1 status}

A previous study showed an increased surface BST-2 protein expression on PBMCs, including CD4$^+$ T cells from only 3 HIV-1 subtype B acutely infected patients [25]. Thus, we hypothesized that BST-2 expression levels will be lower HIV-1 subtype C infection, in a cross-sectional study including 100 HIV-1 chronically infected participants (median viral load: 4.23 log$_{10}$ HIV-RNA copies/ml, interquartile range [IQR] 2.97–5.03; median CD4 count: 480 cells/mm$^3$, IQR 329.75-670.00) and 20 HIV-1 uninfected participants (median CD4 count: 652 cells/mm$^3$, IQR 451.25-817.50) were characterized for BST-2 expression levels (Table 1). Our findings show a trend towards higher BST-2 mRNA levels in PBMCs from HIV-1 uninfected compared to infected participants (p=0.075), despite heterogeneous expression in both groups (Figure 1A). Next, we investigated whether endogenous BST-2 mRNA expression levels in PBMCs translated to BST-2
protein expression levels on the surface of CD4+ T cells, the main target cells of HIV-1 infection in vivo. Randomly selected samples from 15 HIV-1 uninfected and 8 HIV-1 infected participants were analysed for BST-2 protein expression on CD4+ T cells using flow cytometry (Figure 1B). CD4+ T cells from HIV-1 uninfected participants displayed significantly higher BST-2 protein expression as shown by higher median florescence intensity (MFI) compared to those from HIV-1 infected participants (p=0.005) (Figure 1C). Taken together, our data suggest that BST-2 is heterogeneously expressed in both HIV-1 uninfected and infected persons, with HIV-1 infection resulting in significantly lower BST-2 protein expression on CD4+ T cells.

**Frequency of select bst-2 SNPs rs919267, rs9576 and rs919266 in HIV-1 positive and negative South Africans**

Next we hypothesized that heterogenous BST-2 expression levels are at least partially mediated by its genetic variation. To test this hypothesis, select SNPs rs919267, rs9576 and rs919266 previously reported have a preventative role during HIV-1 infection [16, 17] were genotyped in a total of 20 HIV-1 uninfected and 123 HIV-1 infected participants. SNP frequencies were analyzed in and described for the HIV-1 infected participants only, since the number (n=20) of HIV-1 uninfected participants was small due to sample availability. All polymorphisms analysed satisfied the Hardy-Weinberg equilibrium for allele frequencies (Table 2). The frequency of the wild type genotype (CC) for SNP rs919267 (rs919267CC) in HIV-1 infected participants was 41.5% while the heterogenous rs919267CT and homozygous rs919267TT mutants were 39.8% and 18.7%, respectively. The frequency of wild type genotype (CC) for SNP rs9576 (rs9576CC) was 79.5% whereas the heterozygous mutant rs9576CA was 20.5% among HIV-1 infected participants while there were no participants harbouring
a homozygous rs9576AA mutant. SNP rs919266 was infrequent with the minor allele (rs919266T) frequency of 1.2% among HIV-1 infected participants and was therefore excluded from any further analysis.

**Association of bst2 SNPs with BST-2 expression levels among HIV-1 uninfected and infected participants**

Next we explored whether heterogenous BST-2 expression was associated with bst-2 genetic variation for the polymorphic bst-2 SNPs rs919267 and rs9576. Remarkably, both the heterozygous mutant genotype rs919267CT (p=0.042) and homozygous mutant rs919267TT (p=0.045) were associated with significantly lower BST-2 mRNA median expression levels compared to the wild type genotype rs919267CC among HIV-1 uninfected participants (Figure 2A). Our data further show that BST-2 mRNA expression levels differed across the rs919267 variants such that although similar expression was noted between the rs919267CT versus rs919267CC variants (p=0.669), the rs919267TT homozygous mutant genotype was associated with significantly lower BST-2 mRNA median expression levels compared to rs919267CC among HIV-1 infected participants (p=0.046) (Figure 2B).

BST-2 mRNA median expression levels of the mutant rs9576CT genotype were not significantly different from the wild type rs9576CC genotype (p=0.359) among the HIV-1 uninfected participants (Figure 2C). In contrast, rs9576CA associated with significantly higher BST-2 mRNA median expression levels compared to rs9576CC (p=0.027) among HIV-1 infected participants (Figure 2D). Taken together, our data suggest that bst-2 SNPs rs919267 and rs9576 may regulate BST-2 expression levels following infection.
Association of bst2 SNPs with CD4 count and viral load

A previous study reported that rs9576 was nominally associated with protection against infection during breastfeeding in a Zambian cohort [16] while rs919267 was associated with the risk of faster disease progression in an Indian cohort [17]. Therefore, we hypothesized that SNPs rs919267 and rs9576 may be associated with markers of disease progression such as viral loads and CD4 count at baseline. Our data show that rs919267CT (p=0.003) and rs919267TT (p=0.001) were associated with significantly lower CD4 counts compared to rs919267CC, respectively (Figure 3A). Conversely, rs919267CT (p=0.003) and rs919267TT (p=0.007) were associated with significantly higher viral loads compared to rs919267CC (Figure 3B). On the other hand, rs9576CA (p=0.079) was associated with a non-significant trend towards higher CD4 counts compared to rs9576CC (Figure 3C). In contrast, rs9576CA (p=0.037) was associated with significantly lower viral loads compared to rs9576CC (Figure 3D).

Taken together, our data suggest that bst-2 SNPs rs919267 and rs9576 mediate endogenous BST-2 expression and disease outcome.

bst-2 SNPs rs919267 and rs9576 correlate gp120 IgG1 and IgG3 levels in HIV-1 infected participants

Previous studies reported that p24- but not gp120-specific antibody levels are a prognostic marker of disease progression in subtype B infection [26-28]. Consistently, the data from our group showed that p24-specific IgG3 responses are associated with poor viral control while p24-specific IgG1 responses may be a marker of viral control in HIV-1 subtype C infection [22]. However, the underlying mechanisms responsible for the lack of association of gp120-specific antibody responses with markers of
disease progression remained to be determined. BST-2 is a restriction factor that
tethers HIV-1 virions thus resulting in increased expression of viral Envelop (gp120)
on the surface of infected cells and killing of infected cells [1, 20]. Therefore, we
hypothesized that endogenous BST-2 expression levels, regulated by bst-2 SNPs
rs919267 and rs9576 may be correlated with gp120 IgG levels, ADCC and ADCP
activity in plasma of HIV-1 infected individuals. To test this possibility, we investigated
the association of rs919267 and rs9576 genotypes with titres of gp120 IgG
subclasses, ADCC and ADCP activity of HIV-1 infected patients.

Interestingly, rs919267CT (p=0.040) and rs919267TT (p=0.033) were associated with
significantly lower gp120 IgG1 levels compared to rs919267CC (Figure 4A). In
addition, rs919267CT was associated with significantly lower gp120 IgG3 levels
(p=0.016) while there was no difference in gp120 IgG3 levels for rs919267TT
(p=0.374) compared to rs919267CC (Figure 4B). On the other hand, rs9576CA was
associated with significantly higher gp120 IgG1 levels (p=0.009) (Figure 4C) and
gp120 IgG3 levels (p=0.039) compared to rs9576CC (Figure 4D). However, rs919267
and rs9576 did not correlate with p24-IgG subclasses (Additional file 1), gp120
phagocore and gp120 CD107α as a surrogate marker for ADCP and degranulation
or ADCC respectively (Additional file 2). These data suggest that bst-2 SNPs rs919267
and rs9576 may mediate gp120-IgG1 and -IgG3 levels but p24-IgG subclass levels,
ADCC and ADCP activity.

DISCUSSION
BST-2 blocks the release of new HIV-1 virions from infected cells \textit{in vitro} \cite{1, 12}. In this study, we investigated the association of select \textit{bst}-2 SNPs rs919266, rs919267 and rs9576 with endogenous BST-2 expression levels, HIV-1 disease outcome, ADCC and ADCP activity in two South African cohorts. Surface BST-2 protein expression levels on PBMCs and CD4\(^+\) T cells were shown to be enhanced during HIV-1 acute infection \cite{25}. However, our findings from the current study show that HIV-1 chronic infection is associated with heterogenous BST-2 expression that exhibited a trend towards lower BST-2 mRNA median expression in PBMCs from HIV-1 infected compared to uninfected participants. Consistently, we further found that median BST-2 surface protein expression on CD4\(^+\) T cells was significantly lower in HIV-1 chronically infected compared to uninfected participants. Significantly reduced BST-2 surface protein expression on CD4\(^+\) T cells may be facilitated by the HIV-1 accessory viral protein U (Vpu), which interacts with BST-2 and leads to the degradation of BST-2 via the lysosomal pathway \cite{29}. Importantly, targeting of BST-2 by Vpu occurs at the plasma membrane followed by the active internalization of BST-2 independent of constitutive endocytosis \cite{29}. The contradictory data between the previous report and the current study could be attributed to studying difference phases of HIV-1 infection. The previous study investigating BST-2 expression during acute infection where the effect of Vpu may not be as pronounced as in chronic infection \cite{25}, whereas in the current study we investigated BST-2 expression during the chronic phase of infection. However, it is noteworthy that despite lower BST-2 median expression levels among HIV-1 infected compared to uninfected participants in the current study, BST-2 was heterogeneously expressed.
There are many factors that could contribute to differential immune gene expression in a population including immunity status, gender, age, environment and genetics in addition to virus production and IFN secretion (reviewed in [30]). In the present study we undertook to investigate the impact of select genetic variants, bst-2 SNPs rs919267, rs919266 and rs9576 on gene expression since previous studies reported that these SNPs are associated with disease outcome in different cohorts [16, 17]. However, the effect of these SNPs on BST-2 expression levels was not determined in the previous studies. In the current study we show that rs919267CT and rs919267TT are associated with significantly lower BST-2 mRNA median expression levels compared to rs919267CC in PBMCs from HIV-1 uninfected participants. The heterozygous variant rs919267CT exhibited heterogenous BST-2 mRNA expression but similar expression levels to rs919267CC, whereas rs919267TT was associated with significantly lower median mRNA expression levels compared to rs919267CC in PBMCs from HIV-1 infected participants.

Although the mRNA expression levels were similar between rs9576CC and rs9576CA in PBMCs from HIV-1 negative participants, rs9576CA was associated with significantly higher BST-2 mRNA median expression levels compared to rs9596CC in HIV-1 positive participants suggesting protection against HIV-1 disease progression. Our data are consistent with a report showing that rs9576A allele was nominally associated with protection during breastfeeding in a cohort of Africans from Zambia [16]. Although SNP rs919266A was previously reported to be associated with slower progression to AIDS [16], it was uncommon in this study population occurring only in 1.2% of HIV-1 infected and 5% of HIV-1 uninfected participants and therefore, it was excluded from further analysis. The data from the present study suggest that bst-2
SNPs rs919267 and rs9576 modulate BST-2 expression levels, which could correlate with disease with disease outcome.

Next, we investigated the association of SNPs rs919267 and rs9576 with viral loads and CD4 counts. We found that rs919267CA and rs919267TT were also associated with lower CD4 counts but higher viral loads compared rs919267CC. These findings are in line with a previous study that was conducted in the Indian cohort, which reported that rs919267CT associated with higher risk to HIV-1 disease progression [17]. On the other hand rs9576CA associated with a trended towards higher CD4 counts and lower viral loads and compared to rs9576CC. These data are also consistent with the findings reported by Kamada et al. where rs9576CA was associated with protection in mother-to-child transmission in a Zambian cohort [16]. Collectively, our data suggest that increased BST-2 expression levels in vivo correlate with better disease outcome, reduced HIV-1 viral loads and higher CD4 counts.

Lastly, we investigated the mechanisms by which rs919267 and rs9576 mediated BST-2 expression modulate disease outcome. High levels of IgG1 and IgG3 antibodies are generally indicative of superior ability to provide a “first line of defense” against infections, including neutralization of viruses, increased ADCC and ADCP killing of virus-infected cells [31, 32]. HIV-1-specific IgG antibody responses have been shown to contribute to the control of HIV-1 infection [32]. Consistent with their association with lower BST-2 mRNA median expression, both rs919267CT and rs919267TT were associated with significantly lower Gp120 IgG1 levels compared to rs919267CC. rs919267CT was further associated with significantly lower Gp120-IgG3 levels while rs919267TT exhibited similar Gp120-IgG3 levels compared to
rs919267CC. On the other hand, consistent with its association with increased mRNA median expression levels, rs9576CA was associated with higher Gp120-IgG1 and -IgG3 levels compared to rs9576CC. However, the genotypes of both SNPs rs919267 and rs9576 were not associated with the levels of p24-IgG subclasses, probably due to the fact that p24 is an internal viral protein. A previous report by Pharm et al. showed that increased ex vivo BST-2 levels results in efficient tethering of the virus on the surface of the infected cell, envelope recognition, binding of antibodies and ADCC killing by NK cells [20]. Therefore, data from the current study show that despite their association with BST-2 expression and Gp120-IgG1 and -IgG3 levels, rs919267 and rs9576 are not correlated with Gp120 phagoscore and Gp120 CD107α and meaning they do not modulate ADCP and ADCC activity.

Monoclonal antibodies carrying an antigen targeted to specific DCs surface markers is an effective strategy for eliciting protective immune responses (reviewed in [33]). A number of DC surface molecules that have been targeted to date, includes BST-2. However, the relative efficiency of unique DC receptors that are expressed by different DC subsets has not been properly defined. Factors that determine the outcome of DC targeting include a specific DC subset that is targeted, activation status of DC, antigen load, the trafficking route accessed by the targeted molecule (reviewed in [33]). Targeting BST-2 elicited a robust antibody response compared to targeting Siglec-H suggesting divergent outcomes depending on targeted DC surface marker [4]. Furthermore, a different study showed that BST-2-targeted activated conventional DCs present antigen more efficiently compared to steady state pDC [34]. However, the effect of BST-2 genetics variants and expression on DCs function has not been determined.
CONCLUSION

Taken together, the data from our study suggest that bst-2 SNPs rs919267 and rs9576 mediate BST-2 expression, disease outcome, Gp120-IgG1 and -IgG3 levels in HIV-1 subtype C chronically infected people. However, rs919267 and rs9576 are not associated with p24 IgG levels, ADCC and ADCP activity suggesting that they use other mechanisms to control viral replication. Therefore, future studies should investigate the association of rs919267 and rs9576 with DC antigen presentation and their ability to elicit robust cellular immunity. Better understanding of the molecular mechanisms associated with BST-2 genetic variation to module HIV-1 disease outcome will be valuable for developing enhanced HIV-1 therapeutic and vaccine strategies.

Materials and Methods

Study design, participants and sample processing: The Sinikithemba cohort comprised of 450 antiretroviral naïve, HIV-1 subtype C chronically adults enrolled from McCord Hospital (Durban, South Africa) from August 2003 to 2008 and followed up longitudinally as previously described [35-37]. The time of infection for these participants is unknown. Sociodemographic characteristics, plasma viral load and CD4 cell count measurements were obtained at baseline. CD4 counts and viral load were measured every 3 and 6 months from enrolment, respectively. Viral loads were determined using automated Cobas Amplicor HIV-1 Monitor test version 1.5 (Roche Diagnostics, Rotkreuz, Switzerland) and CD4+ T cells were enumerated using the Multitest kit CD4/CD3/CD8/CD45 on a FACSCalibur flow cytometer (Becton Dickinson). The Masibambisane cohort comprised of HIV negative women recruited
Peripheral blood mononuclear cells (PBMCs) from participants were isolated by Ficoll-Histopaque (Sigma) density gradient centrifugation from blood within 6 hours of phlebotomy and frozen in liquid nitrogen until use.

**Characterization of BST-2 mRNA expression levels in PBMCs from HIV-1 infected and uninfected participants**

**RNA extraction and cDNA synthesis**

Cryopreserved PBMC samples from 20 HIV-1 uninfected and 123 HIV-1 infected participants from the Masibambisane and Sinikithemba cohorts respectively were available. Only 100 of 123 PMBC samples from HIV-1 infected participants were available for mRNA quantification.

Total RNA was extracted immediately after thawing and counting of PBMCs without stimulation. Total RNA was extracted from $2 \times 10^6$ PBMCs using the RNeasy Mini kit (Qaigen, Hilden, Germany), according to the manufacturer’s protocol. Extracted RNA was quantified using the NanoDrop 2000 Spectrophotometer (ThemoFisher Scientific, Wilmington, USA) and samples were used only if their $\text{OD}_{260}/\text{OD}_{280}$ ratio was 1.90 or greater. All RNA samples were DNase treated. Approximately, 1 µg of total RNA from each of the 20 HIV-1 uninfected and 100 HIV-1 infected (a total of 120) samples was reverse transcribed using the iScript cDNA synthesis kit as per the manufacturer’s instruction (BioRad Laboratories, Inc, Berkeley, CA).

**Real-time PCR RNA quantitation**
The levels of BST-2 mRNA were determined by a quantitative real-time PCR (qPCR) assay using SYBR Green chemistry in a LightCycler 480 (Roche). Each PCR reaction consisted of 3 mmol/µL MgCl₂, the respective primers at 0.5 pmol/µL, 1 µL Fast Start SYBR Green I (Roche), 1 µg cDNA and water to make up the total reaction volume to 10 µL. The BST-2 cDNA was detected using the following primer set, BST-2 F: 5’-AGGTCCGTCCTGCTCGGCTT-3’ and BST-2 R: 5’-TCCAGAGGCCCTTCTCCGGC-3’ that are designed to specifically and uniquely amplify BST-2 (GenBank accession number NM_004335). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), (GenBank accession number NM_002046), determined to be the most suitable reference gene based on PCR efficiency in our laboratory [36] was used to correct for differences in the cDNA input. GAPDH cDNA was detected using the following primer set: GAPDH-F: 5’-GTCAACCCCACCGTGTTCTTC-3’ and GAPDHR: 5’-TTTCTGCTGCTTTGACCTTG-3’ as previously described [35]. The SYBR green qPCR was performed using the following program on the LightCycler 480: (1) preincubation: 95°C for 5 min; (2) amplification: 45 cycles of 95°C for 15 sec, 60°C for 15 sec, 72°C for 15 sec; (3) melting curve: 95°C for 5 sec, 65°C for 1 min, 97°C for 0 sec with a temperature transition rate of 0.11°C/sec [35]. To control for specificity of the amplification products, a melting curve analysis was performed. There was no amplification of non-specific products observed. The mRNA copy number was calculated from a standard curve, obtained by plotting known input concentrations of four different samples at log dilutions to the PCR cycle number (CP) at which the detected fluorescence intensity reaches a fixed value. The amplification efficiency of the PCR was determined by running log dilutions of standards. The slope of the standard curve was converted to the amplification efficiency.
Measurement of BST-2 protein expression on CD4+ T cells from HIV-1 infected and uninfected participants by flow cytometry

Cryopreserved cells were rapidly thawed, washed twice in pre-warmed R10 medium (RPMI medium 1640 containing 10% gamma irradiated, heat inactivated fetal bovine serum (FBS) (Gibco, Life Technologies, Carlsbad, California, USA), 1% L-Glutamine, 1% penicillin/streptomycin and 1% HEPES buffer (1 molar) (Lonza, Basel, Basel-Stadt, Switzerland) at 500 x g for 8 minutes at room temperature. Cells were rested in R10 medium at 37°C for 2 hours. Sample viability and cell counts were determined by trypan blue exclusion. One million cells were plated in 96-well plates and stained for 20 minutes with LIVE/DEAD Fixable Blue Dead Cell Stain (Invitrogen, Carlsbad, California, USA), monoclonal antibodies CD3-brilliant violet 650 (clone OKT3, BioLegend, San Diego, California, USA), CD4-Allophycocyanin (clone SK3, BioLegend), CD8-Fluorescein (clone RPA-T8, BD Biosciences, Franklin Lakes, New Jersey, USA) and BST-2-Phycoerythrin (clone RS38E, BioLegend). Cells were washed with Dulbecco's phosphate-buffered saline, centrifuged at 850 x g for 6 minutes and fixed with FIX & PERM Medium A (Invitrogen). Samples were acquired on a BD LSRFortessa, recording at least 250,000 events per sample. Routine instrument QC was performed using Cytometer Setup and Tracking beads (BD Biosciences). Compensation was calculated using the Anti-Mouse Ig, κ/Negative Control Compensation Particles Set (BD Biosciences). FlowJo software version 9 (TreeStar, Inc., Ashland, Oregon, USA) was used for sample analysis.

Determination of the frequency of bst-2 SNPs rs919267, rs919266 and rs9576

The genotypes of select SNPs rs919267, rs919266 and rs9576 located in the promoter region of bst-2 gene were analysed in a total of 143 (20 HIV-1 uninfected and 123 HIV-
1 infected) samples using TaqMan allelic discrimination assays as previously described [39]. Genomic DNA was isolated from stored buffy coats using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Briefly, 20 µL of QIAGEN Protease, 200 µL of AL Buffer were added to 200 µL of buffy coats, mixed by pulse-vortexing for 15s and incubated at 56 °C for 10 min. Then 200 µL of ethanol (96-100%) was added to the sample and the mixture transferred to the QIAmp Mini column. Lastly, the QIAamp mini column was placed in a clean 1.5 mL microcentrifuge tube and DNA was eluted using 50 µL of AE Buffer. DNA concentration was standardised at 50 ng/µL with PCR grade water. A cocktail containing Taqman Genotyping master mix (Life Technologies, Carlsbad, California, USA) and probes for the bst-2 gene (SNP ID: rs919266, rs919267 and rs9576, Applied Biosystems, Foster City, California, USA) was used to amplify target sequence in 50 ng genomic DNA by real time PCR in the LightCycler 480 (Roche, Basel, Switzerland) according to the manufacturer’s protocol.

**Association of bst-2 SNPs with IgG subclasses levels, ADCP and ADCC activity in antiretroviral-naïve HIV-1 subtype C chronic infection cohort.**

The IgG tires, ADCP and ADCC data were generated as previously described by Chung *et al.* [22]. Briefly, a 96-well plate was coated overnight at 4°C with 150 ng of recombinant HIV protein per well. 2% bovine serum albumin (BSA) blocked plates were used as antigen controls. The next day the plates were washed 6 times with PBS, 50 µL of plasma from HIV infected participants (diluted 1:100) was added to each well, and incubated at 37°C for 2 hours. HIV negative plasma samples or media alone were used as negative controls, while HIVIG (pooled HIV immunoglobulin G, NIH AIDS Reagents Program) was used as a positive control. The plates were washed and 5 ×
10^4 NK cells enriched via negative selection from healthy blood donors (RosetteSep, Stemcell Technologies,) were added to each well in the presence of Brefeldin A (BioLegend), Golgi stop, and anti-CD107α-PE-Cy5 (BD Biosciences). The plate was incubated for 5 hours at 37°C and 5% CO₂. Following incubation, cells were stained with anti-CD3-AF700, anti-CD56-PE-Cy7, anti-CD16-APC-Cy7 (BD), fixed with Perm A, permeabilized using Perm B (Invitrogen), and stained with anti-IFNγ-APC and anti-MIP1β-PE (BD). The cells were then fixed with 2% paraformaldehyde and analysed by flow cytometry.

Statistical comparisons were examined by two-tailed Mann-Whitney U tests using GraphPad Prism software (GraphPad Inc., La Jolla, California, USA).

Declarations

List of abbreviations

ADCC: antibody-dependent cell-mediated cytotoxicity

BST-2: bone marrow stromal antigen 2

HIV-1: human immunodeficiency virus type 1

SNPs: Single nucleotides polymorphism

Ethics approval and consent to participate

Ethical approval for the present study was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BREC/00002085/2020). Written informed consent was obtained from all study participants at the time of recruitment and patient identity remained confidential.
Consent for publication
Not applicable

Availability of data and materials
All data generated or analysed during this study are included in this published article [and its supplementary information files]. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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Authors’ contributions
HM, TN and PM conceived the study, HM, KN, BN, PM performed the experiments, HM, KN, PM analyzed the data. PM wrote the manuscript. All authors contributed to revision of the draft manuscript and approved the final version.

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**Figure legends**

**Figure 1.** BST-2 mRNA expression levels in PBMCs of 20 HIV-1 negative and 100 HIV-1 chronically infected participants. **A:** BST-2 mRNA levels were compared between uninfected (black *squares*) and HIV-1 chronically infected participants (black *circles*) selected at baseline from the Sinikithemba chronic cohort. **B:** A representative flow cytometry plots showing gating strategy for pure CD4⁺ T cell population. **C:** BST-2 surface staining on randomly selected samples from HIV-1 uninfected (black squares) and infected (Blue circles) participants. Horizontal lines within the clusters are depicting the median. Group comparisons were calculated using either the Mann-Whitney test for PBMCs and CD4⁺ T-cells.

**Figure 2.** Association of *bst-2* genetic variation (rs919267 and rs9576) with BST-2 expression levels. **A:** BST-2 mRNA expression levels in PBMCs obtained from HIV-1 uninfected participants with known genotypes, wildtype rs919267CC (black circles), heterozygous mutant rs919267CT (blue squares) and homozygous mutant rs919267TT (red triangles). **B:** BST-2 mRNA expression levels in PBMCs obtained from HIV-1 infected participants with known genotypes, wildtype rs919267CC (black circles), heterozygous mutant rs919267CT (blue squares) and homozygous mutant rs919267TT (red triangles). **C:** BST-2 mRNA expression levels in PBMCs obtained from HIV-1 negative participants with known genotypes, wildtype rs9576CC (black circles), heterozygous mutant rs9576CA (blue squares). **D:** BST-2 mRNA expression levels in PBMCs obtained from HIV-1 positive participants with known genotypes, wildtype rs9576CC (black circles), heterozygous mutant rs9576CA (blue squares). Group comparisons were calculated using either the Mann-Whitney test for PBMCs.
Figure 3. Association of SNP rs919267 and rs9576 with viral load and CD4 cell count in the Sinikithemba Chronic cohort. A: Comparison of wildtype genotype rs919267 CC (black circles) with rs919267 CT (blue squares) and rs919267 TT (black triangle) in correlation with Viral load. B: Comparison of wildtype genotype rs919267 CC (black circles) with rs919267 CT (blue squares) and rs919267 TT (black triangle) in correlation with Square root of CD4+ T cell count. C: Comparison of wildtype genotype rs9576 CC (black circles) with rs9576 CA (blue squares) in correlation with Viral load. D: Comparison of wildtype genotype rs9576 CC (black circles) with rs9576 CA (blue squares) in correlation with Square root of CD4+ T cell count. Group comparisons were calculated using either the Mann-Whitney test for PBMCs.

Figure 4. Association of SNP rs919267 and rs9576 with Gp120-IgG1 and -IgG3 levels in the Sinikithemba chronic infection cohort. A and B: Correlation of wildtype genotype rs919267CC (black circles) with rs919267CT (blue squares) and rs919267TT (red triangle) with Gp120-IgG1 and -IgG3 MFI as percentage positive cells respectively. C and D: Correlation of wildtype genotype rs9576CC (black circles) with rs9576CA (blue squares) with Gp120-IgG1 and -IgG3 MFI as percentage positive cells respectively. Group comparisons were calculated using either the Mann-Whitney test for PBMCs.

Additional file 1. Association of rs919267 and rs9576 with p24 IgG1-IgG4 levels in the Sinikithemba Chronic cohort. A-D: Comparison of wildtype genotype rs919267CC (black circles) with rs919267CT (blue squares) and rs919267TT (red triangle) in correlation with p24 IgG MFI as percentage positive cells. E-H: Comparison of wildtype genotype rs9576CC (black circles) with rs9576CA (blue squares) in association with p24 IgG MFI as percentage positive cells.
Additional file 2.

Association of bst-2 genetic variation (rs919267 and rs9576) with ADCP and ADCC activity. **A:** gp120 phagoschore as a surrogate marker of ADCP activity obtained from HIV-1 infected participants with known genotypes, wildtype rs919267CC (circles), heterozygous mutant rs919267CT (squares) and homozygous mutant rs919267TT (triangles). **B:** gp120 CD107α as a surrogate marker for degranulation obtained from HIV-1 infected participants with known genotypes, wildtype rs919267CC (circles), heterozygous mutant rs919267CT (squares) and homozygous mutant rs919267TT (triangles). **C:** gp120 phagoschore as a surrogate marker of ADCP activity obtained from HIV-1 infected participants with known genotypes, wildtype rs9576CC (circles), heterozygous mutant rs9567CT (squares). **D:** gp120 CD107α as a surrogate marker for degranulation obtained from HIV-1 infected participants with known genotypes, wildtype rs9576CC (circles), heterozygous mutant rs9576CT (squares). Group comparisons were calculated using either the Mann-Whitney test for PBMCs.
Tables

Table 1. Demographic, clinical, and analytical characteristics of HIV-1 uninfected and infected participants.

Table 2. Frequency of BST-2 Polymorphism in HIV-1 subtype C chronically infected cohort and uninfected cohorts of black South Africans
Figure 1

A

\[ p = 0.075 \]

B

\[ p = 0.005 \]
Figure 3

A

B

C

D

rs919267 genotypes

rs919267 genotypes

rs9576 genotypes

rs9576 genotypes
Figure 4
Additional file 1
Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HIV-1 uninfected</th>
<th>HIV-1 infected</th>
<th>p-value</th>
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<tbody>
<tr>
<td>No. of patients (%)</td>
<td>20 (14.1)</td>
<td>123 (85.90)</td>
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<td>Age (yrs) mean ± SD (range)</td>
<td>17.58 ± 1.99</td>
<td>32 ± 13.31</td>
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<td>Gender, male/female ratio</td>
<td>0/20</td>
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<td>Median CD4 counts [IQR]</td>
<td>652 IQR [451.25-817.50]</td>
<td>480 IQR [329.75-670.00]</td>
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<td>Median Viral loads [IQR]</td>
<td>N/A</td>
<td>4.23 log$_{10}$ IQR [2.97-5.03]</td>
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<td>SNP</td>
<td>Study population</td>
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<td>p-value</td>
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<tr>
<td></td>
<td>HIV infected (HI)</td>
<td>HIV uninfected (HU)</td>
<td>OR [95% CI]</td>
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<td>MAF n=123 (%)</td>
<td>n=20 (%)</td>
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</tr>
<tr>
<td>rs919266</td>
<td>A 3 (1.2)</td>
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<tr>
<td></td>
<td>T 95 (38.6)</td>
<td>14 (65.0)</td>
<td>0.29 [10.30 – 9.72]</td>
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