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 polymorphims (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

load (p=0.001) while rs919267TT was associated with lower BST-2 mRNA levels (p=0.046), 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 elicite robust immune responses .

Key words

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

62 **Background**

63 Bone marrow stromal antigen 2 (BST-2; also known as CD317, and tetherin) is a host
64 restriction factor that blocks the release of enveloped viruses, including HIV-1, from
65 infected host cells[1] and modulates plasmacytoid dendritic cell (pDC) interferon
66 (INF)-α production [2, 3]. BST-2 is probably a receptor that confers anti-viral antigen
67 presentation properties to pDCs, given the high expression of BST-2 in pDCs following
68 activation [4-6]. Although the tethering capacity of BST-2 was first described HIV-1 [1],
69 it was subsequently shown to occur for other enveloped viruses [7]. While BST-2 can
70 be found in the trans-Golgi network and in vesicular compartments, it is a
71 transmembrane protein primarily located on the apical membrane (reviewed in [8]).
72 The acute "murine AIDS" (LP-BM5) and/or Moloney murine leukaemia virus (Mo-
73 MuLV) replication was not different between the wild type (WT) and BST-2 knockout
74 (KO) mice [6, 9]. However, a different study reported that even though WT and BST-
75 2 KO mice had similar acute LP-BM5 replication levels, BST-2 KO mice had higher
76 replication levels during later time points, when adaptive immune responses operate
77 [10]. Another study reported that BST-2 KO mice had higher acute mouse mammary
78 tumour virus (MMTV) replication levels [11].

79

80 The BST-2 protein is encoded by *bst-2*, an interferon simulated gene located in
81 chromosome 19p13.2 [12]. The gene is 2.69 kb long and comprises of four functional
82 exons [13, 14]. A previous study reported that *bst*-2 gene variants rs3217318, a 19-
83 base-pair insertion/deletion polymorphism in the promoter region and rs10415893, a
84 tag single-nucleotide polymorphism (SNP) in the 3′ untranslated region were
85 associated with HIV-1 disease progression in a Spanish cohort [15]. A subsequent
86 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

112 ADCC [24]. Overall, the aforementioned studies suggest that higher expression of
113 BST-2 may promote cell mediated antiviral activity *in vivo* and attenuate disease
114 outcome.
115
116 However, the influence of *bst*-2 SNPs on expression levels, ADCC and/or ADCP
117 activity has not been determined. We hypothesised that BST-2 SNPs may mediate
118 BST-2 expression levels, ADCC and/or ADCP activity and disease outcome in a cohort
119 of antiretroviral therapy naive, HIV-1 subtype C chronically infected individuals.
120 Therefore, we investigated the effect of select *bst-2* SNPs rs919266, rs919267 and
121 rs9576 on endogenous BST-2 expression levels, anti-HIV ADCC and/or ADCP activity
122 and HIV-1 clinical outcomes.
123
124 **RESULTS**
125 **Association of endogenous BST-2 expression with HIV-1 status**
126 A previous study showed an increased surface BST-2 protein expression on PBMCs,
127 including $CD4^+$ T cells from only 3 HIV-1 subtype B acutely infected patients [25]. Thus,
128 we hypothesized that BST-2 expression levels will be lower HIV-1 subtype C infection,
129 in a cross-sectional study including 100 HIV-1 chronically infected participants (median
130 viral load: 4.23 log_{10} HIV-RNA copies/ml, interquartile range [IQR] 2.97–5.03; median
131 CD4 count: 480 cells/mm^3, IQR 329.75-670.00) and 20 HIV-1 uninfected participants
132 (median CD4 count: 652 cells/mm^3, IQR 451.25-817.50) were characterized for BST-
133 2 expression levels (Table 1). Our findings show a trend towards higher BST-2 mRNA
134 levels in PBMCs from HIV-1 uninfected compared to infected participants (p=0.075),
135 despite heterogeneous expression in both groups (Figure 1A). Next, we investigated
136 whether endogenous BST-2 mRNA expression levels in PBMCs translated to BST-2

137 protein expression levels on the surface of $CD4^+$ T cells, the main target cells of HIV-
138 1 infection *in vivo*. Randomly selected samples from 15 HIV-1 uninfected and 8 HIV-1
139 infected participants were analysed for BST-2 protein expression on $CD4^+$ T cells
140 using flow cytometry (Figure 1B). $CD4^+$ T cells from HIV-1 uninfected participants
141 displayed significantly higher BST-2 protein expression as shown by higher median
142 florescence intensity (MFI) compared to those from HIV-1 infected participants
143 ($p=0.005$) (Figure 1C). Taken together, our data suggest that BST-2 is
144 heterogeneously expressed in both HIV-1 uninfected and infected persons, with HIV-
145 1 infection resulting in significantly lower BST-2 protein expression on $CD4^+$ T cells.
146

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

149 Next we hypothesized that heterogenous BST-2 expression levels are at least partially
150 mediated by its genetic variation. To test this hypothesis, select SNPs rs919267,
151 rs9576 and rs919266 previously reported have a preventative role during HIV-1
152 infection [16, 17] were genotyped in a total of 20 HIV-1 uninfected and 123 HIV-1
153 infected participants. SNP frequencies were analyzed in and described for the HIV-1
154 infected participants only, since the number (n=20) of HIV-1 uninfected participants
155 was small due to sample availability. All polymorphisms analysed satisfied the Hardy-
156 Weinberg equilibrium for allele frequencies (Table 2). The frequency of the wild type
157 genotype (CC) for SNP rs919267 (rs919267CC) in HIV-1 infected participants was
158 41.5% while the heterogenous rs919267CT and homozygous rs919267TT mutants
159 were 39.8% and 18.7%, respectively. The frequency of wild type genotype (CC) for
160 SNP rs9576 (rs9576CC) was 79.5% whereas the heterozygous mutant rs9576CA was
161 20.5% among HIV-1 infected participants while there were no participants harbouring

162 a homozygous rs9576AA mutant. SNP rs919266 was infrequent with the minor allele
163 (rs919266T) frequency of 1.2% among HIV-1 infected participants and was therefore
164 excluded from any further analysis.
165

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

168 Next we explored whether heterogenous BST-2 expression was associated with *bst*-
169 2 genetic variation for the polymorphic *bst*-2 SNPs rs919267 and rs9576. Remarkably,
170 both the heterozygous mutant genotype rs919267CT (p=0.042) and homozygous
171 mutant rs919267TT (p=0.045) were associated with significantly lower BST-2 mRNA
172 median expression levels compared to the wild type genotype rs919267CC among
173 HIV-1 uninfected participants (Figure 2A). Our data further show that BST-2 mRNA
174 expression levels differed across the rs919267 variants such that although similar
175 expression was noted between the rs919267CT versus rs919267CC variants
176 (p=0.669), the rs919267TT homozygous mutant genotype was associated with
177 significantly lower BST-2 mRNA median expression levels compared to rs919267CC
178 among HIV-1 infected participants (p=0.046) (Figure 2B).
179

180 BST-2 mRNA median expression levels of the mutant rs9576CT genotype were not
181 significantly different from the wild type rs9576CC genotype (p=0.359) among the HIV-
182 1 uninfected participants (Figure 2C). In contrast, rs9576CA associated with
183 significantly higher BST-2 mRNA median expression levels compared to rs9576CC
184 (p=0.027) among HIV-1 infected participants (Figure 2D). Taken together, our data
185 suggest that *bst*-2 SNPs rs919267 and rs9576 may regulate BST-2 expression levels
186 following infection.

187

188 **Association of *bst2* SNPs with CD4 count and viral load**

189 A previous study reported that rs9576 was nominally associated with protection
190 against infection during breastfeeding in a Zambian cohort [16] while rs919267 was
191 associated with the risk of faster disease progression in an Indian cohort [17].
192 Therefore, we hypothesized that SNPs rs919267 and rs9576 may be associated with
193 markers of disease progression such as viral loads and CD4 count at baseline. Our
194 data show that rs919267CT (p=0.003) and rs919267TT (p=0.001) were associated
195 with significantly lower CD4 counts compared to rs919267CC, respectively (Figure
196 3A). Conversely, rs919267CT (p=0.003) and rs919267TT (p=0.007) were associated
197 with significantly higher viral loads compared to rs919267CC (Figure 3B). On the other
198 hand, rs9576CA (p=0.079) was associated with a non-significant trend towards higher
199 CD4 counts compared to rs9576CC (Figure 3C). In contrast, rs9576CA (p=0.037) was
200 associated with significantly lower viral loads compared to rs9576CC (Figure 3D).
201 Taken together, our data suggest that *bst*-2 SNPs rs919267 and rs9576 mediate
202 endogenous BST-2 expression and disease outcome.

203

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

206 Previous studies reported that p24- but not gp120-specific antibody levels are a
207 prognostic marker of disease progression in subtype B infection [26-28]. Consistently,
208 the data from our group showed that p24-specific IgG3 responses are associated with
209 poor viral control while p24-specific IgG1 responses may be a marker of viral control
210 in HIV-1 subtype C infection [22]. However, the underlying mechanisms responsible
211 for the lack of association of gp120-specific antibody responses with markers of

212 disease progression remained to be determined. BST-2 is a restriction factor that
213 tethers HIV-1 virions thus resulting in increased expression of viral Envelop (gp120)
214 on the surface of infected cells and killing of infected cells [1, 20]. Therefore, we
215 hypothesized that endogenous BST-2 expression levels, regulated by *bst*-2 SNPs
216 rs919267 and rs9576 may be correlated with gp120 IgG levels, ADCC and ADCP
217 activity in plasma of HIV-1 infected individuals. To test this possibility, we investigated
218 the association of rs919267 and rs9576 genotypes with titres of gp120 IgG
219 subclasses, ADCC and ADCP activity of HIV-1 infected patients.
220
221 Interestingly, rs919267CT (p=0.040) and rs919267TT (p=0.033) were associated with
222 significantly lower gp120 IgG1 levels compared to rs919267CC (Figure 4A). In
223 addition, rs919267CT was associated with significantly lower gp120 IgG3 levels
224 (p=0.016) while there was no difference in gp120 IgG3 levels for rs919267TT
225 (p=0.374) compared to rs919267CC (Figure 4B). On the other hand, rs9576CA was
226 associated with significantly higher gp120 IgG1 levels (p=0.009) (Figure 4C) and
227 gp120 IgG3 levels (p=0.039) compared to rs9576CC (Figure 4D). However, rs919267
228 and rs9576 did not corelated with p24-IgG subclasses (Additional file 1), gp120
229 phagoscore and gp120 CD107α as a surrogate marker for ADCP and degranulation
230 or ADCC respectively (Additional file 2). These data suggest that *bst*-2 SNPs rs919267
231 and rs9576 may mediate gp120-IgG1 and -IgG3 levels but p24-IgG subclass levels,
232 ADCC and ADCP activity.
233
234
235 **DISCUSSION**

236 BST-2 blocks the release of new HIV-1 virions from infected cells *in vitro* [1, 12]. In
237 this study, we investigated the association of select *bst*-2 SNPs rs919266, rs919267
238 and rs9576 with endogenous BST-2 expression levels, HIV-1 disease outcome, ADCC
239 and ADCP activity in two South African cohorts. Surface BST-2 protein expression
240 levels on PBMCs and $CD4^+$ T cells were shown to be enhanced during HIV-1 acute
241 infection [25]. However, our findings from the current study show that HIV-1 chronic
242 infection is associated with heterogenous BST-2 expression that exhibited a trend
243 towards lower BST-2 mRNA median expression in PBMCs from HIV-1 infected
244 compared to uninfected participants. Consistently, we further found that median BST-
245 2 surface protein expression on $CD4^+$ T cells was significantly lower in HIV-1
246 chronically infected compared to uninfected participants. Significantly reduced BST-2
247 surface protein expression on $CD4^+$ T cells may be facilitated by the HIV-1 accessory
248 viral protein U (Vpu), which interacts with BST-2 and leads to the degradation of BST-
249 2 via the lysosomal pathway [29]. Importantly, targeting of BST-2 by Vpu occurs at the
250 plasma membrane followed by the active internalization of BST-2 independent of
251 constitutive endocytosis [29]. The contradictory data between the previous report and
252 the current study could be attributed to studying difference phases of HIV-1 infection.
253 The previous study investigating BST-2 expression during acute infection where the
254 effect of Vpu may not be as pronounced as in chronic infection [25], whereas in the
255 current study we investigated BST-2 expression during the chronic phase of infection.
256 However, it is noteworthy that despite lower BST-2 median expression levels among
257 HIV-1 infected compared to uninfected participants in the current study, BST-2 was
258 heterogeneously expressed.
259

260 There are many factors that could contribute to differential immune gene expression
261 in a population including immunity status, gender, age, environment and genetics in
262 addition to virus production and IFN secretion (reviewed in [30]). In the present study
263 we undertook to investigate the impact of select genetic variants, *bst*-2 SNPs
264 rs919267, rs919266 and rs9576 on gene expression since previous studies reported
265 that these SNPs are associated with disease outcome in different cohorts [16, 17].
266 However, the effect of these SNPs on BST-2 expression levels was not determined in
267 the previous studies. In the current study we show that rs919267CT and rs919267TT
268 are associated with significantly lower BST-2 mRNA median expression levels
269 compared to rs919267CC in PBMCs from HIV-1 uninfected participants. The
270 heterozygous variant rs919267CT exhibited heterogenous BST-2 mRNA expression
271 but similar expression levels to rs919267CC, whereas rs919267TT was associated
272 with significantly lower median mRNA expression levels compared to rs919267CC in
273 PBMCs from HIV-1 infected participants.
274
275 Although the mRNA expression levels were similar between rs9576CC and rs9576CA
276 in PBMCs from HIV-1 negative participants, rs9576CA was associated with
277 significantly higher BST-2 mRNA median expression levels compared to rs9596CC in
278 HIV-1 positive participants suggesting protection against HIV-1 disease progression.
279 Our data are consistent with a report showing that rs9576A allele was nominally
280 associated with protection during breastfeeding in a cohort of Africans from Zambia
281 [16]. Although SNP rs919266A was previously reported to be associated with slower
282 progression to AIDS [16], it was uncommon in this study population occurring only in
283 1.2% of HIV-1 infected and 5% of HIV-1 uninfected participants and therefore, it was
284 excluded from further analysis. The data from the present study suggest that *bst*-2

285 SNPs rs919267 and rs9576 modulate BST-2 expression levels, which could correlate
286 with disease with disease outcome.
287
288 Next, we investigated the association of SNPs rs919267 and rs9576 with viral loads
289 and CD4 counts. We found that rs919267CA and rs919267TT were also associated
290 with lower CD4 counts but higher viral loads compared rs919267CC. These findings
291 are in line with a previous study that was conducted in the Indian cohort, which
292 reported that rs919267CT associated with higher risk to HIV-1 disease progression
293 [17]. On the other hand rs9576CA associated with a trended towards higher CD4
294 counts and lower viral loads and compared to rs9576CC. These data are also
295 consistent with the findings reported by Kamada *et al.* where rs9576CA was
296 associated with protection in mother-to-child transmission in a Zambian cohort [16].
297 Collectively, our data suggest that increased BST-2 expression levels *in vivo* correlate
298 with better disease outcome, reduced HIV-1 viral loads and higher CD4 counts.
299
300 Lastly, we investigated the mechanisms by which rs919267 and rs9576 mediated
301 BST-2 expression modulate disease outcome. High levels of IgG1 and IgG3
302 antibodies are generally indicative of superior ability to provide a “first line of defense”
303 against infections, including neutralization of viruses, increased ADCC and ADCP
304 killing of virus-infected cells [31, 32]. HIV-1-specific IgG antibody responses have been
305 shown to contribute to the control of HIV-1 infection [32]. Consistent with their
306 association with lower BST-2 mRNA median expression, both rs919267CT and
307 rs919267TT were associated with significantly lower Gp120 IgG1 levels compared to
308 rs919267CC. rs919267CT was further associated with significantly lower Gp120-IgG3
309 levels while rs919267TT exhibited similar Gp120-IgG3 levels compared to

310 rs919267CC. On the other hand, consistent with its association with increased mRNA
311 median expression levels, rs9576CA was associated with higher Gp120-IgG1 and -
312 IgG3 levels compared to rs9576CC. However, the genotypes of both SNPs rs919267
313 and rs9576 were not associated with the levels of p24-IgG subclasses, probably due
314 to the fact that p24 is an internal viral protein. A previous report by Pharm *et al*. showed
315 that increased *ex vivo* BST-2 levels results in efficient tethering of the virus on the
316 surface of the infected cell, envelope recognition, binding of antibodies and ADCC
317 killing by NK cells [20]. Therefore, data from the current study show that despite their
318 association with BST-2 expression and Gp120-IgG1 and -IgG3 levels, rs919267 and
319 rs9576 are not correlated with Gp120 phagoscore and Gp120 CD107α and meaning
320 they do not modulate ADCP and ADCC activity.
321
322 Monoclonal antibodies carrying an antigen targeted to specific DCs surface markers
323 is an effective strategy for eliciting protective immune responses (reviewed in [33]). A
324 number of DC surface molecules that have been targeted to date, includes BST-2.
325 However, the relative efficiency of unique DC receptors that are expressed by different
326 DC subsets has not been properly defined. Factors that determine the outcome of DC
327 targeting include a specific DC subset that is targeted, activation status of DC, antigen
328 load, the trafficking route accessed by the targeted molecule (reviewed in [33]).
329 Targeting BST-2 elicited a robust antibody response compared to targeting Siglec-H
330 suggesting divergent outcomes depending on targeted DC surface marker [4].
331 Furthermore, a different study showed that BST-2-targeted activated conventional
332 DCs present antigen more efficiently compared to steady state pDC [34]. However,
333 the effect of BST-2 genetics variants and expression on DCs function has not been
334 determined.

335

336 **CONCLUSION**

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

347

348 **Materials and Methods**

349 **Study design, participants and sample processing:** The Sinikithemba cohort
350 comprised of 450 antiretroviral naïve, HIV-1 subtype C chronically adults enrolled from
351 McCord Hospital (Durban, South Africa) from August 2003 to 2008 and followed up
352 longitudinally as previously described [35-37]. The time of infection for these
353 participants is unknown. Sociodemographic characteristics, plasma viral load and CD4
354 cell count measurements were obtained at baseline. CD4 counts and viral load were
355 measured every 3 and 6 months from enrolment, respectively. Viral loads were
356 determined using automated Cobas Amplicor HIV-1 Monitor test version 1.5 (Roche
357 Diagnostics, Rotkreuz, Switzerland) and $CD4^{+}$ T cells were enumerated using the
358 Multitest kit CD4/CD3/CD8/CD45 on a FACSCalibur flow cytometer (Becton
359 Dickinson). The Masibambisane cohort comprised of HIV negative women recruited

360 from antenatal clinics in Durban [38]. Peripheral blood mononuclear cells (PBMCs)
361 from participants were isolated by Ficoll-Histopaque (Sigma) density gradient
362 centrifugation from blood within 6 hours of phlebotomy and frozen in liquid nitrogen
363 until use.
364
365 **Characterization of BST-2 mRNA expression levels in PBMCs from HIV-1**
366 **infected and uninfected participants**
367 RNA extraction and cDNA synthesis
368 Cryopreserved PBMC samples from 20 HIV-1 uninfected and 123 HIV-1 infected
369 participants from the Masibambisane and Sinikithemba cohorts respectively were
370 available. Only 100 of 123 PMBC samples from HIV-1 infected participants were
371 available for mRNA quantification.
372
373 Total RNA was extracted immediately after thawing and counting of PBMCs without
374 stimulation. Total RNA was extracted from 2 x 10^6 PBMCs using the RNeasy Mini kit
375 (Qaigen, Hilden, Germany), according to the manufacturer's protocol. Extracted RNA
376 was quantified using the NanoDrop 2000 Spectrophotometer (ThemoFisher Scientific,
377 Wilmington, USA) and samples were used only if their OD_{260}/OD_{280} ratio was 1.90 or
378 greater. All RNA samples were DNase treated. Approximately, 1 μg of total RNA from
379 each of the 20 HIV-1 uninfected and 100 HIV-1 infected (a total of 120) samples was
380 reverse transcribed using the iScript cDNA synthesis kit as per the manufacturer's
381 instruction (BioRad Laboratories, Inc, Berkeley, CA).
382
383 Real-time PCR RNA quantitation

384 The levels of BST-2 mRNA were determined by a quantitative real-time PCR (qPCR)
385 assay using SYBR Green chemistry in a LightCycler 480 (Roche). Each PCR reaction
386 consisted of 3 mmol/µL $MgCl_2$, the respective primers at 0.5 pmol/µL, 1 µL Fast Start
387 SYBR Green I (Roche), 1 µg cDNA and water to make up the total reaction volume to
388 10 µL. The BST-2 cDNA was detected using the following primer set, BST-2 F: 5'-
389 AGGTCCGTCCTGCTCGGCTT-3' and BST-2 R: 5'- TCCAGAGGCCCTTCTCCGGC-
390 3' that are designed to specifically and uniquely amplify BST-2 (GenBank accession
391 number NM_004335). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH),
392 (GenBank accession number NM_002046), determined to be the most suitable
393 reference gene based on PCR efficiency in our laboratory [36] was used to correct for
394 differences in the cDNA input. GAPDH cDNA was detected using the following primer
395 set: GAPDH-F: 5'-GTCAACCCCACCGTGTTCTTC-3' and GAPDHR: 5'-
396 TTTCTGCTGTCTTTGGGACCTTG-3' as previously described [35]. The SYBR green
397 qPCR was performed using the following program on the LightCycler 480: (1)
398 preincubation: 95°C for 5 min; (2) amplification: 45 cycles of 95°C for 15 sec, 60°C for
399 15 sec, 72°C for 15 sec; (3) melting curve: 95°C for 5 sec, 65°C for 1 min, 97°C for 0
400 sec with a temperature transition rate of 0.11°C/sec [35]. To control for specificity of
401 the amplification products, a melting curve analysis was performed. There was no
402 amplification of non-specific products observed. The mRNA copy number was
403 calculated from a standard curve, obtained by plotting known input concentrations of
404 four different samples at log dilutions to the PCR cycle number (CP) at which the
405 detected fluorescence intensity reaches a fixed value. The amplification efficiency of
406 the PCR was determined by running log dilutions of standards. The slope of the
407 standard curve was converted to the amplification efficiency.
408

409 **Measurement of BST-2 protein expression on $CD4^{+}$ T cells from HIV-1 infected
410 and uninfected participants by flow cytometry**
411 Cryopreserved cells were rapidly thawed, washed twice in pre-warmed R10 medium
412 (RPMI medium 1640 containing 10% gamma irradiated, heat inactivated fetal bovine
413 serum (FBS) (Gibco, Life Technologies, Carlsbad, California, USA), 1% L-Glutamine,
414 1% penicillin/streptomycin and 1% HEPES buffer (1 molar) (Lonza, Basel, Basel-
415 Stadt, Switzerland) at 500 x g for 8 minutes at room temperature. Cells were rested in
416 R10 medium at 37°C for 2 hours. Sample viability and cell counts were determined by
417 trypan blue exclusion. One million cells were plated in 96-well plates and stained for
418 20 minutes with LIVE/DEAD Fixable Blue Dead Cell Stain (Invitrogen, Carlsbad,
419 California, USA), monoclonal antibodies CD3-brilliant violet 650 (clone OKT3,
420 BioLegend, San Diego, California, USA), CD4-Allophycocyanin (clone SK3,
421 BioLegend), CD8-Fluorescein (clone RPA-T8, BD Biosciences, Franklin Lakes, New
422 Jersey, USA) and BST-2-Phycoerythrin (clone RS38E, BioLegend). Cells were
423 washed with Dulbecco's phosphate-buffered saline, centrifuged at 850 x g for 6
424 minutes and fixed with FIX & PERM Medium A (Invitrogen). Samples were acquired
425 on a BD LSRFortessa, recording at least 250,000 events per sample. Routine
426 instrument QC was performed using Cytometer Setup and Tracking beads (BD
427 Biosciences). Compensation was calculated using the Anti-Mouse Ig, κ/Negative
428 Control Compensation Particles Set (BD Biosciences). FlowJo software version 9
429 (TreeStar, Inc., Ashland, Oregon, USA) was used for sample analysis.
430
431 **Determination of the frequency of *bst*-2 SNPs rs919267, rs919266 and rs9576**
432 The genotypes of select SNPs rs919267, rs919266 and rs9576 located in the promoter
433 region of *bst*-2 gene were analysed in a total of 143 (20 HIV-1 uninfected and 123 HIV-

434 1 infected) samples using TaqMan allelic discrimination assays as previously
435 described [39]. Genomic DNA was isolated from stored buffy coats using the QIAamp
436 DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's
437 instructions. Briefly, 20 µL of QIAGEN Protease, 200 µL of AL Buffer were added to
438 200 µL of buffy coats, mixed by pulse-vortexing for 15s and incubated at 56 °C for 10
439 min. Then 200 µL of ethanol (96-100%) was added to the sample and the mixture
440 transferred to the QIAmp Mini column. Lastly, the QIAamp mini column was placed in
441 a clean 1.5 mL microcentrifuge tube and DNA was eluted using 50 µL of AE Buffer.
442 DNA concentration was standardised at 50 ng/µL with PCR grade water. A cocktail
443 containing Taqman Genotyping master mix (Life Technologies, Carlsbad, California,
444 USA) and probes for the *bst-2* gene (SNP ID: rs919266, rs919267 and rs9576, Applied
445 Biosystems, Foster City, California, USA) was used to amplify target sequence in 50
446 ng genomic DNA by real time PCR in the LightCycler 480 (Roche, Basel, Switzerland)
447 according to the manufacturer's protocol.
448
449 **Association of *bst*-2 SNPs with IgG subclasses levels, ADCP and ADCC activity**
450 **in antiretroviral-naïve HIV-1 subtype C chronic infection cohort**.
451 The IgG tires, ADCP and ADCC data were generated as previously described by
452 Chung *et al*. [22]. Briefly, a 96-well plate was coated overnight at 4°C with 150 ng of
453 recombinant HIV protein per well. 2% bovine serum albumin (BSA) blocked plates
454 were used as antigen controls. The next day the plates were washed 6 times with
455 PBS, 50 µL of plasma from HIV infected participants (diluted 1:100) was added to each
456 well, and incubated at 37°C for 2 hours. HIV negative plasma samples or media alone
457 were used as negative controls, while HIVIG (pooled HIV immunoglobulin G, NIH AIDS
458 Reagents Program) was used as a positive control. The plates were washed and 5 ×

459 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% CO_2. 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.

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

469

470 **Declarations**

471 **List of abbreviations**

472 **ADCC**: antibody-dependent cell-mediated cytotoxicity

473 **BST-2**: bone marrow stromal antigen 2

474 **HIV-1**: human immunodeficiency virus type 1

475 **SNPs**: Single nucleotides polymorphism

476

477 **Ethics approval and consent to participate**

478 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.

482

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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627 **progression in an African treatment-naive population**. *Cell host & microbe* 2009, **5**(5):413-
628 415.

629

630

631 **Figure legends**

632 **Figure 1.** BST-2 mRNA expression levels in PBMCs of 20 HIV-1 negative and 100
633 HIV-1 chronically infected participants. **A:** BST-2 mRNA levels were compared
634 between uninfected (black *squares*) and HIV-1 chronically infected participants (black
635 *circles*) selected at baseline from the Sinikithemba chronic cohort. **B:** A representative
636 flow cytometry plots showing gating strategy for pure CD^{4+} T cell population. **C:** BST-
637 2 surface staining on randomly selected samples from HIV-1 uninfected (black
638 squares) and infected (Blue circles) participants. Horizontal lines within the clusters
639 are depicting the median. Group comparisons were calculated using either the Mann-
640 Whitney test for PBMCs and $CD4^{+}$ T-cells.

641

642 **Figure 2.** Association of *bst-2* genetic variation (rs919267 and rs9576) with BST-2
643 expression levels. **A:** BST-2 mRNA expression levels in PBMCs obtained from HIV-1
644 uninfected participants with known genotypes, wildtype rs919267CC (black circles),
645 heterozygous mutant rs919267CT (blue squares) and homozygous mutant
646 rs919267TT (red tringles). **B:** BST-2 mRNA expression levels in PBMCs obtained from
647 HIV-1 infected participants with known genotypes, wildtype rs919267CC (black
648 circles), heterozygous mutant rs919267CT (blue squares) and homozygous mutant
649 rs919267TT (red tringles). **C:** BST-2 mRNA expression levels in PBMCs obtained from
650 HIV-1 negative participants with known genotypes, wildtype rs9576CC (black circles),
651 heterozygous mutant rs9576CA (blue squares). **D:** BST-2 mRNA expression levels in
652 PBMCs obtained from HIV-1 positive participants with known genotypes, wildtype
653 rs9576CC (black circles), heterozygous mutant rs9576CA (blue squares). Group
654 comparisons were calculated using either the Mann-Whitney test for PBMCs.

655

656 **Figure 3.** Association of SNP rs919267 and rs9576 with viral load and CD4 cell count
657 in the Sinikithemba Chronic cohort. **A:** Comparison of wildtype genotype rs919267 CC
658 (black circles) with rs919267 CT (blue squares) and rs919267 TT (black triangle) in
659 correlation with Viral load. **B:** Comparison of wildtype genotype rs919267 CC (black
660 circles) with rs919267 CT (blue squares) and rs919267 TT (black triangle) in
661 correlation with Square root of CD4+ T cell count. **C:** Comparison of wildtype genotype
662 rs9576 CC (black circles) with rs9576 CA (blue squares) in correlation with Viral load.
663 **D:** Comparison of wildtype genotype rs9576 CC (black circles) with rs9576 CA (blue
664 squares) in correlation with Square root of CD4+ T cell count. Group comparisons
665 were calculated using either the Mann-Whitney test for PBMCs.
666
667 **Figure 4.** Association of SNP rs919267 and rs9576 with Gp120-IgG1 and -IgG3 levels
668 in the Sinikithemba chronic infection cohort. **A** and **B:** Correlation of wildtype genotype
669 rs919267CC (black circles) with rs919267CT (blue squares) and rs919267TT (red
670 triangle) with Gp120-IgG1 and -IgG3 MFI as percentage positive cells respectively. **C**
671 and **D:** Correlation of wildtype genotype rs9576**CC** (black circles) with rs9576**CA** (blue
672 squares) with Gp120-IgG1 and -IgG3 MFI as percentage positive cells respectively.
673 Group comparisons were calculated using either the Mann-Whitney test for PBMCs.
674
675 **Additional file 1.** Association of rs919267 and rs9576 with p24 IgG1-IgG4 levels in
676 the Sinikithemba Chronic cohort. **A**-**D:** Comparison of wildtype genotype rs919267CC
677 (black circles) with rs919267CT (blue squares) and rs919267TT (red triangle) in
678 correlation with p24 IgG MFI as percentage positive cells. **E-H:** Comparison of wildtype
679 genotype rs9576CC (black circles) with rs9576CA (blue squares) in association with
680 p24 IgG MFI as percentage positive cells.

681

682 **Additional file 2.**

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

696

697

698

699 **Tables**

700 **Table 1**. Demographic, clinical, and analytical characteristics of HIV-1 uninfected

701 and infected participants.

702

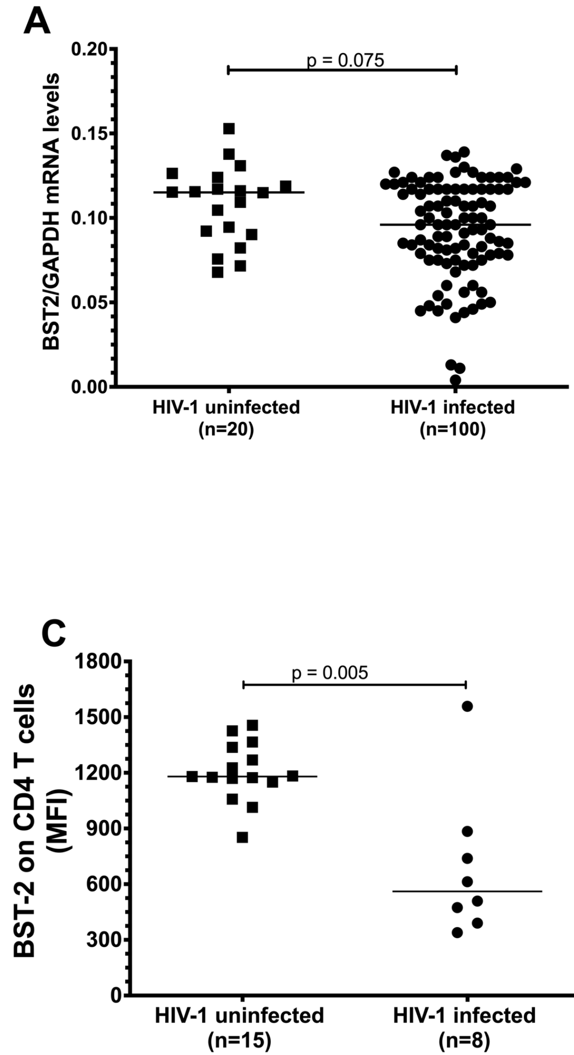
703 **Table 2**. Frequency of BST-2 Polymorphism in HIV-1 subtype C chronically infected

704 cohort and uninfected cohorts of black South Africans

705

706

Figure 1



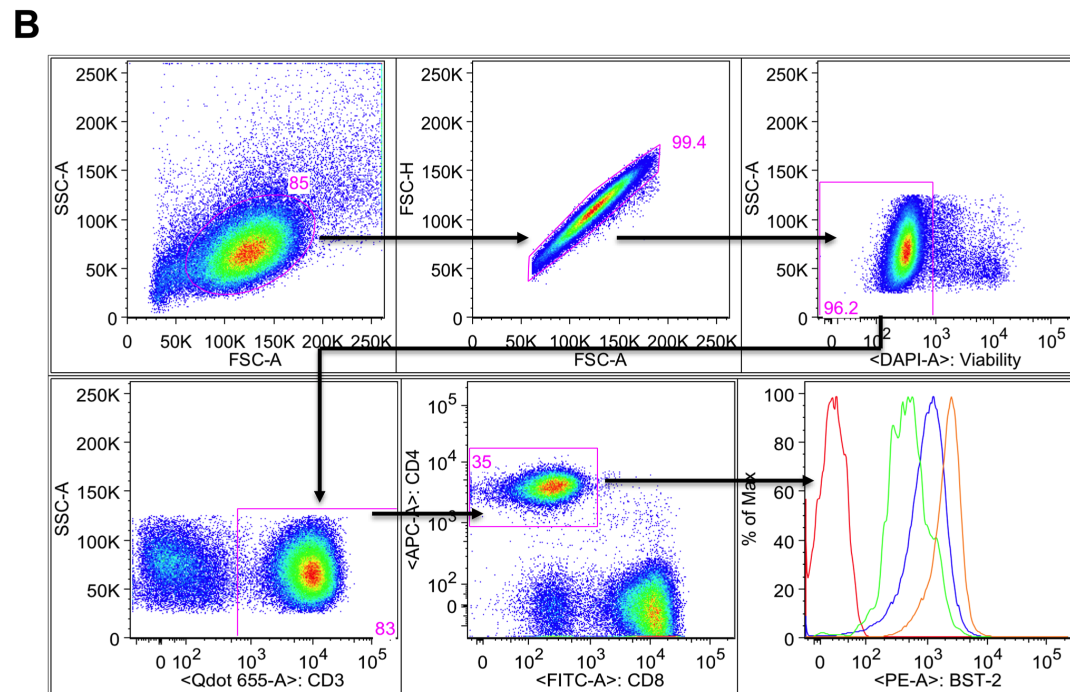


Figure 2

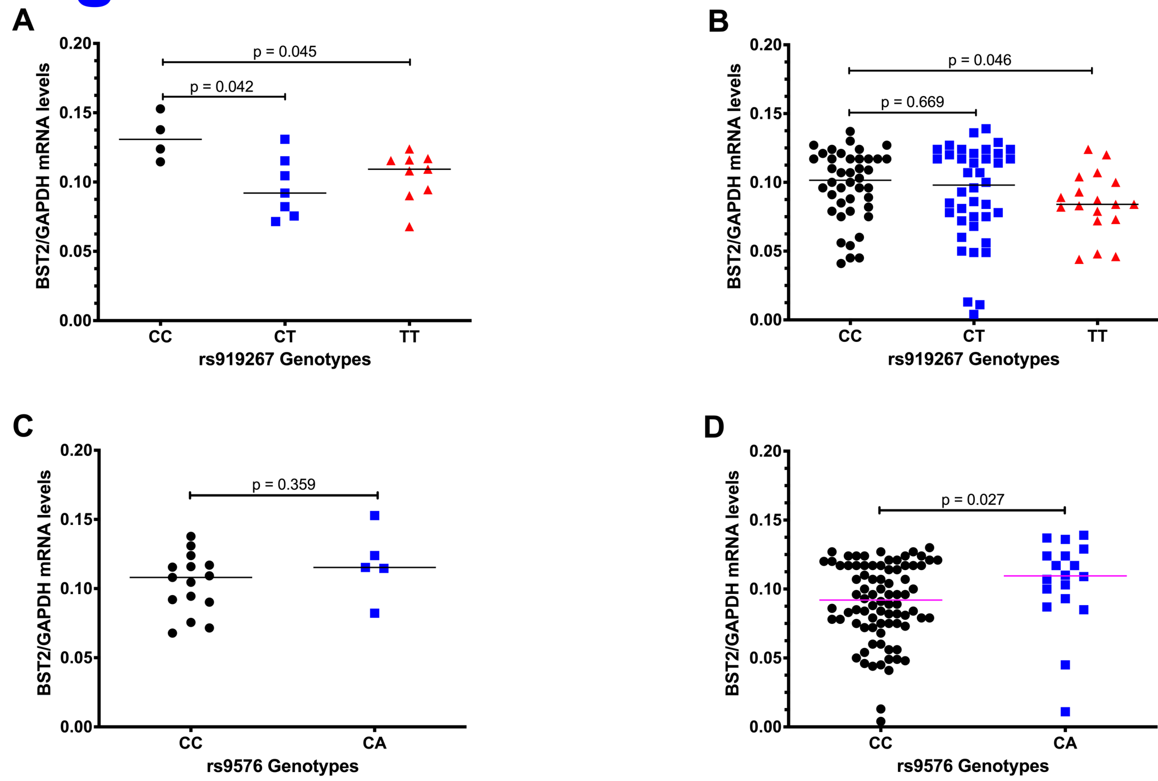


Figure 3

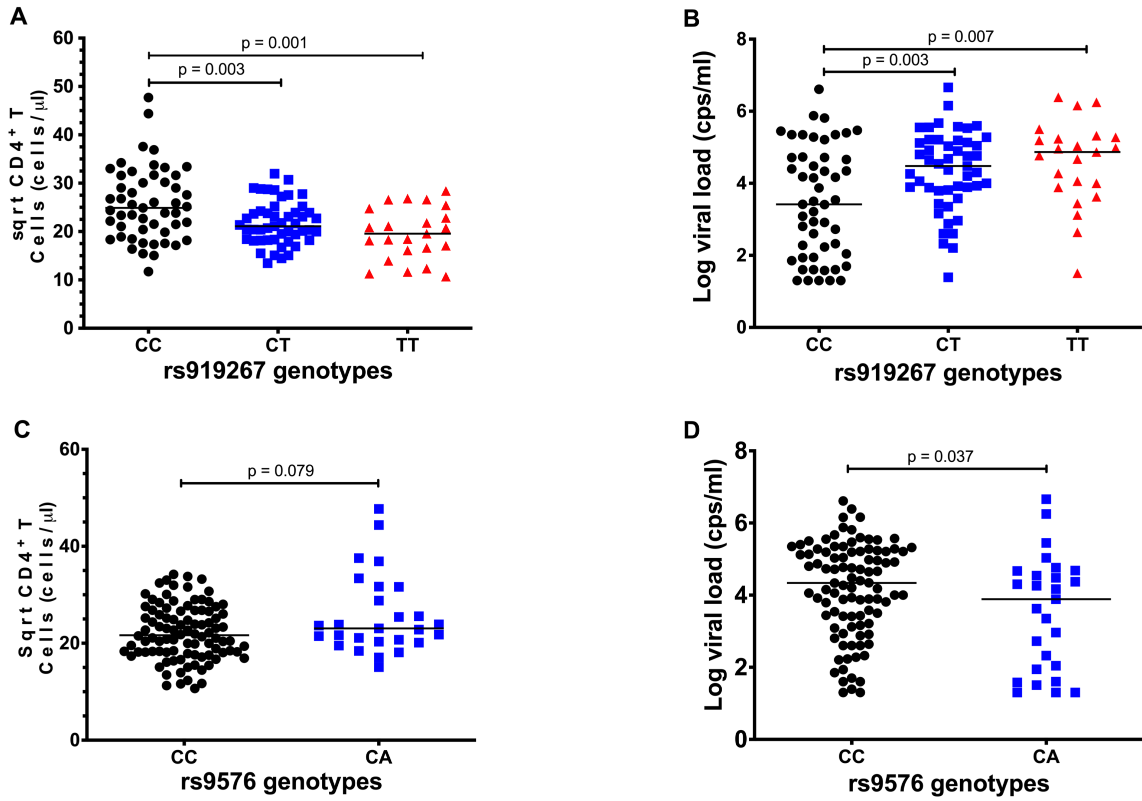
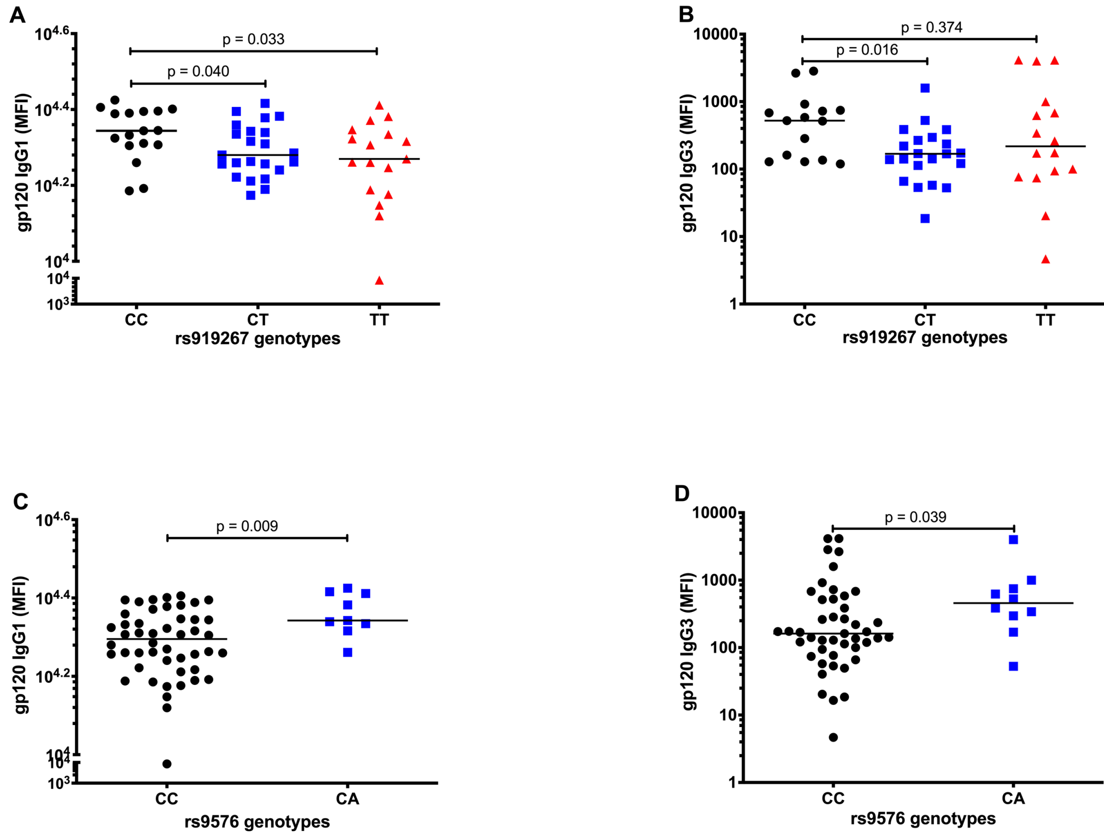
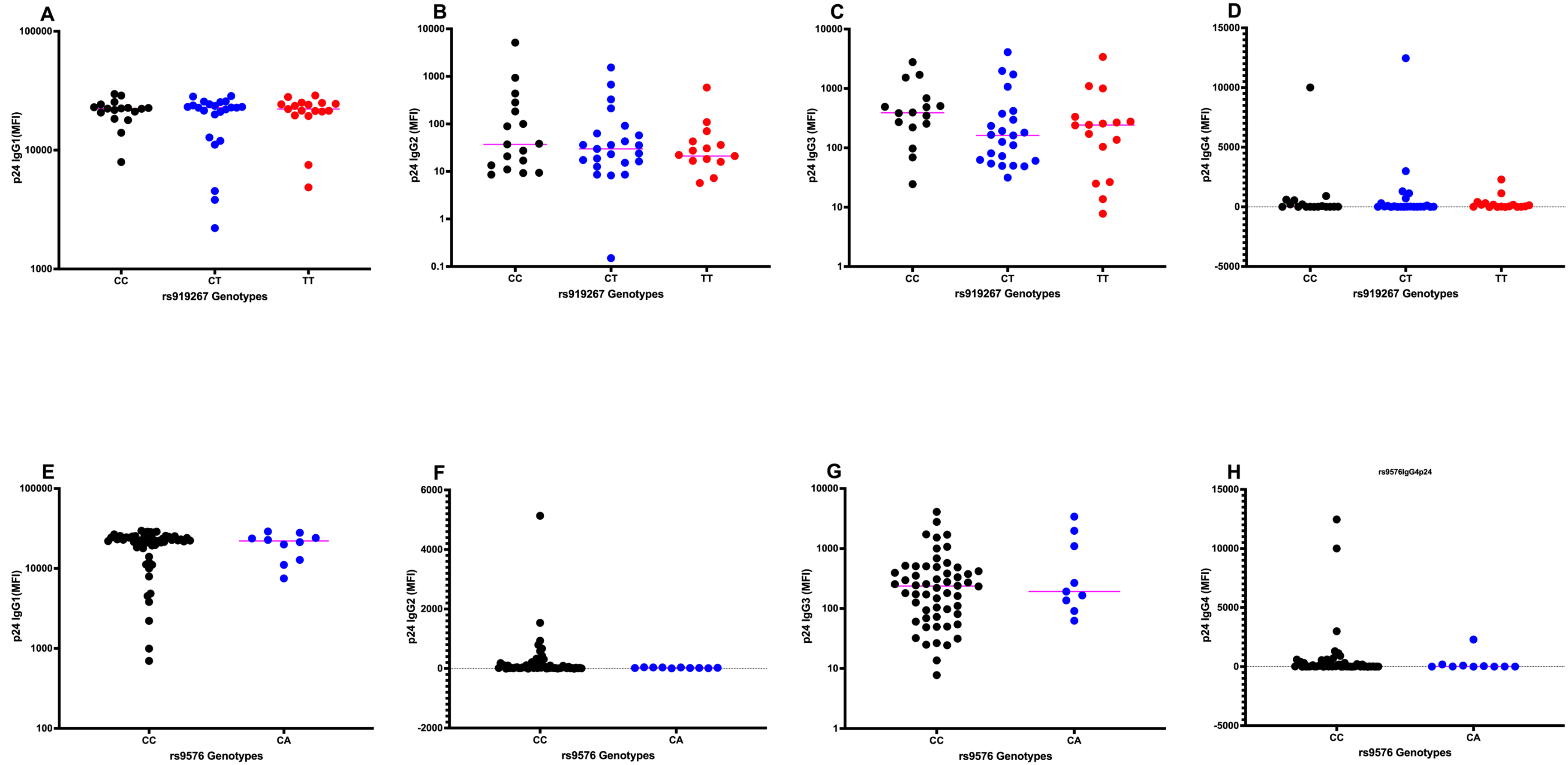


Figure 4



Additional file 1



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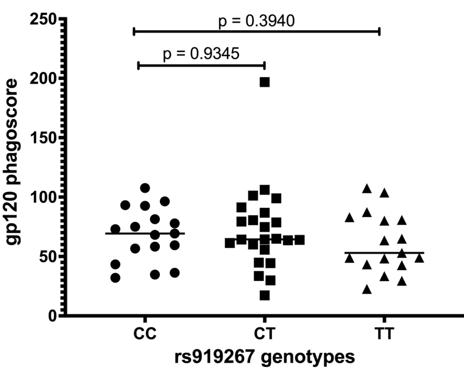

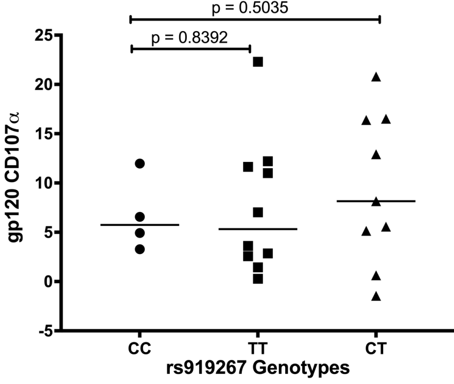

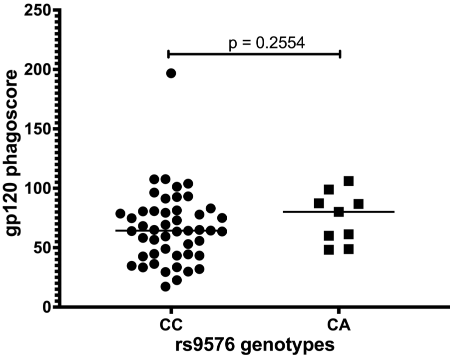

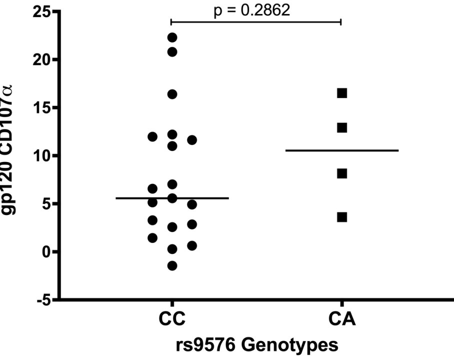

Table 1.

Characteristics	HIV-1 uninfected	HIV-1 infected	p-value
No. of patients (%)	20 (14.1)	123 (85.90)	0.0369
Age (yrs) mean ± SD (rage)	17.58 ± 1.99	32 ± 13.31	0.4011
Gender, male/female ratio	0/20	22/101	N/A
Median CD4 counts [IQR]	652 IQR [451.25-817.50]	480 IQR [329.75-670.00]	0.3668
Median Viral loads [IQR]	N/A	4.23 $\log_{10}$ IQR [2.97-5.03]	N/A

Table 2.

SNP		Study population		HI vs HU	
		HIV infected (HI)	**HIV uninfected (HU)**	**OR [95% CI]**	**p-value**
MAF		n=123 (%)	n=20 (%)		
rs919266	A	3 (1.2)	2 (5.0)	N/A	N/A
rs919267	T	95 (38.6)	14 (65.0)	0.29 [10.30 – 9.72]	<0.0001
rs9576	A	25 (11.0)	4 (10.0)	N/A	N/A
rs919266					
	G/G	120 (97.6)	18 (90.0)		
	G/A	3 (2.4)	2 (10.0)		
	AA	0 (0.0)	0 (0.0)		
rs919267					
	C/C	51 (41.5)	3 (15.0)		
	C/T	49 (39.8)	8 (40.0)		
	TT	23 (18.7)	9 (45.0)		
rs9576					
	C/C	97 (79.5)	16 (80.0)		
	C/A	25 (20.5)	4 (20.0)		
	AA	0 (0.0)	0 (0.0)		