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Protective role of *BST2* polymorphisms in mother-to-child transmission of HIV-1 and adult AIDS progression

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Abstract

BST-2/Tetherin is a restriction factor that prevents Human immunodeficiency virus type 1 (HIV-1) release from infected cells and mediates pro-inflammatory cytokine production. This study investigated the risk conferred by single nucleotide polymorphisms (SNPs; rs919266, rs9192677 and rs9576) at BST-2 coding gene (*BST2*) in HIV-1 mother-to-child transmission and in disease progression. Initially, 101 HIV-1+ pregnant women and 331 neonates exposed to HIV-1 from Zambia were enrolled. Additional *BST2* SNPs analyses were performed in two cohorts with acquired immunodeficiency syndrome (AIDS) progression: an adult Brazilian cohort (37 rapid, 30 chronic and 21 long-term non-progressors) and an Italian pediatric cohort (21 rapid and 67 slow progressors). The rs9576A allele was nominally associated with protection during breastfeeding (p=0.019), and individuals carrying rs919266GA showed slower progression to AIDS (p=0.033). Despite the influence of rs919266 and rs9576 in *BST2* expression is still undetermined, a preventive role by *BST2* polymorphisms was found during HIV-1 infection.

Keywords

HIV-1; BST-2; SNPs; Mother-to-child transmission of HIV-1; AIDS progression

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Conflict of Interest

The authors declare no competing financial interests.

Introduction

Rate of mother-to-child transmission (MTCT) of Human immunodeficiency virus type 1 (HIV-1) ranges from 5-10% during pregnancy, 20-30% during delivery and 10-20% via breastfeeding in the absence of treatment, while it is reduced to less than 2% with antiretroviral therapy during gestation[1]. Since the majority of newborns from infected mothers do not become infected, several host factors have been investigated by clinical follow-up of untreated pregnant women, mainly in countries with limited antiretroviral regimen coverage. Despite obstetric, nutritional, socio-demographic and viral factors are involved in natural prevention to MTCT[2], innate immunity have been recently highlighted as a modulator of early antiviral response at the fetal-maternal interface[3]. “Bone marrow stromal cell antigen-2” (BST-2/Tetherin) transmembrane protein prevents HIV-1 particles release by virion retention at membranes of infected cells [4]. HIV-1 tethering leads to BST-2 cytoplasmic tail phosphorylation, which triggers proinflammatory cytokines production mediated by NF-κB signalling[5], and may also facilitate HIV-1 endocytosis followed by type I interferon (IFN) expression[6]. Although BST-2 constitutive expression was found at various immune cells (macrophages, monocytes, plasmacytoid dendritic cells (pDCs), B and T-lymphocytes)[5,7,8], tissues (liver, lung, cord blood and decidua)[9–11] and secretions (colostrum and semen)[11,12], the influence of BST-2 in early HIV-1 prevention and pathogenesis is still underestimated.

Recent studies investigated the role of BST-2 genetic background in differential response to HIV-1 infection. An insertion/deletion polymorphism at the promoter region (rs3217318) and a single nucleotide polymorphism (SNP, rs10415893) of *BST2* gene (located at 19p13.1) were associated with lower transcriptional levels of *BST2* and faster disease progression in a Spanish cohort[13], while rs113189798 SNP was described as a risk factor to HIV-1 acquisition in North American seroprevalent drug users[14].

Thus, this study evaluated the role of *BST2* SNPs in susceptibility to MTCT of HIV-1 in a cohort of 331 infants and 101 infected mothers. Additional analyses were performed in 88 pediatric patients and 88 adults to investigate the influence of *BST2* SNPs in HIV-1-associated disease progression.

Materials and methods

Study group

A cohort of 101 pregnant women living with HIV-1 and 331 infants with confirmed HIV-1 transmission status were selected from a randomized clinical trial “Zambia Exclusive Breastfeeding Study”(ZEBS) in two primary health clinics (George Clinic and Chawama Clinic) from Lusaka (Zambia)(ClinicalTrials.gov Identifier: NCT00310726)[15]. ZEBS evaluated the benefits of short duration exclusive breastfeeding in prevention of MTCT and infant mortality in low resource settings among women that received a single-dose nevirapine regimen. Among 331 infants, 22 were infected via intrauterine transmission (positive HIV-1 Polymerase Chain Reaction (PCR) within two days of birth), 25 were infected during delivery (positive HIV-1 PCR within 42 days of birth), 38 were infected

during breastfeeding (positive HIV-1 PCR after 42 days of birth) and 246 infants did not become infected (Supplementary Digital Content 1 and Supplement Table 1).

A retrospective cohort of 88 adults with clinical progression to AIDS (37 rapid progressors 30 chronic progressors and 21 long-term non-progressors) was established after review of approximately 3300 patient charts from Infectious Diseases Service at Conceição Hospital Group in Porto Alegre (Brazil). The clinical endpoint (AIDS) was determined with highly active antiretroviral therapy start or CD4+ cells count below 350 cells/mm^3. All participants signed an informed consent, answered a standard socio-demographic questionnaire and provided peripheral blood sample. The ethical committee of Conceição Hospital Group approved the study (N. 01-213)(Supplementary Digital Content 2).

A retrospective cohort of 88 infected children with HIV-1-related disease progression from Pediatric Division of IRCCS Burlo Garofolo in Trieste (Italy) was classified according to 1994 CDC AIDS surveillance case definition[16]: 21 rapid progressors (developed severe clinical manifestations within the first two years of infection, defined as “Category C”) and 67 slow progressors (neither progressed to Category C nor developed severe immunosupression beyond eight years of age). Children with clinical symptoms (Categories A, B or C) or immune suppression (categories 2 or 3)[17] started highly active antiretroviral therapy (triple combination of stavudine or zidovudine, lamivudine and ritonavir or nelfinavir or indinavir). The ethical committee of IRCCS Burlo Garofolo approved the study (Prot. L1106).

Sample processing and *BST2* genotyping

DNA extraction from dried blood spot samples of ZEBS cohort was performed using “DNA Extract All Reagents” kit (Life Technologies, Carlsbad, California, U.S.A). Genomic DNA was extracted from peripheral blood samples in Italian and Brazilian cohorts by conventional salting-out procedures[18].

Only 14 out of 37 SNPs (rs112492472, rs12609479, rs73921425, rs28413174, rs28413175, rs28413176, rs11542666, rs34737311, rs113321277, rs2278234, rs919265, rs919266, rs919267 and rs9576) found in public databases (UCSC Genome Browser, NCBI and 1000 Genomes)[19] were selected due to a minor allele frequency (MAF) greater than 5% in Sub-saharan African populations (YRI, LWK, ESN and GWD). Specific regions in BST-2 coding gene were amplified by PCR using KAPA2G Readymix (RESNOVA, Genzano di Roma, Rome, Italy) with a 2720 Thermal Cycler (Life Technologies) and then directly sequenced using ABI PRISM 3130XL sequencer (Life Technologies). The primers used for PCR and sequencing were: **BST2EXON1F** 5’-CTGCCTCTTCAGGTCATAG-3’; **BST2EXON1R** 5’-GAACCTAGGTCCCTTGATG-3’; **BST2EXON2/3F** 5’-GAGGACCCACATGCTTATG-3’; **BST2EXON4F** 5’-GGATAACTTAGCCCCTAGG-3’; **BST2EXON2/3R** 5’-CAGCAGCACAATCAGCAGC-3’; **BST2EXON4R** 5’-CCATAACAACAGGCAGCAC-3’.

The *BST2* gene sequencing was performed in 331 samples from the Zambian cohort, and since only rs9576, rs919266 and rs919267 SNPs showed a MAF greater than 5%, these SNPs were replicated in Brazilian and Italian cohorts with TaqMan® SNP Genotyping Assays (C_11454228_10, C_7493933_10 and C_2931310_10, respectively) and TaqMan®

GTXpress™ Master Mix (Life Technologies) on ABI7500 Real Time PCR (Life Technologies).

Data analysis

Fisher exact and Mann-Whitney tests were performed respectively in univariate analysis of categorical and quantitative variables with R Software 3.1.0. Bonferroni correction method for multiple tests (*e.g.*: p=0.05/N^{o} of analyzed SNPs) was performed for all analyzed SNPs. All clinical/demographic variables that reached a p-value lower than 0.10 in univariate analysis (Supplementary Digital Content 2) were included as covariates in logistic regression model analysis, using SNPstats[20]. Extensively described risk factors like HIV-1 viral load, CD4+ cell count and *CCR5*Δ32 were included in adjusted analysis. The haplotypes and linkage disequilibrium (LD) of *BST2* SNPs were determined in Haploview v.4.2[21]. Kaplan-Meier survival analysis was performed in IBM SPSS Statistics v.20 to evaluate the influence of *BST2* SNPs in time to adult AIDS progression.

Results

BST2 SNPs in a cohort of mother-to-child transmission of HIV-1

Maternal CD4+ cell counts (p<0.0001) and plasma viral load (p<0.0001) were higher in all groups of HIV-1 infected infants(intrauterine (IU), intrapartum(IP) and postpartum(PP) transmission) than in exposed-uninfected infants(EU), while other features such as maternal(age, BMI and hemoglobin level) and neonate characteristics(sex and weight) were not associated with infection status (Supplement Table 1). Breast milk viral load was also higher in PP infants than EU infants (3.10 [2.81 - 3.24] *vs* 1.69 [1.46 - 1.88] $\log_{10}$ copies/ml, p<0.0001).

rs919266, rs919267 and rs9576 *BST2* SNPs were investigated as candidate markers of natural protection during mother-to-child transmission of HIV-1(Table 1). SNPs frequencies in Zambian infants were all in Hardy-Weinberg equilibrium (HWE). The rs9576A allele was more frequent in EU than in PP (14.85% *vs* 5.26%, OR=0.32 [0.11 - 0.9]; p=0.019) although the difference between allelic frequencies did not reach statistical significance after Bonferroni correction (p>0.017). None of *BST2* SNPs were associated with either IU or IP transmission (p>0.017). The minor allele rs919266A was not found only in the PP infected group, even with a larger sample size than other infected groups. Thus, it was not possible to test the association of rs919266 in transmission of HIV-1 during breastfeeding.

rs919266, rs919267 and rs9576 SNPs were also tested as risk factors to HIV-1 vertical transmission regardless of route of transmission, although none of the *BST2* alleles, genotypes or haplotypes were associated as risk factors (p>0.017, Supplement Table 2). All SNPs but rs919266 in 101 HIV-1 infected mothers were in HWE. HIV-1 infected mothers with rs919266A allele also presented a lower CD4+ cell count during delivery than rs919266G (200.33 ± 43.88 cells/mm^3 (n = 9) *vs* 339.56 ± 21.64 cells/mm^3(n = 92), p=0.008; data not shown), while rs919267 and rs9576SNPs did not influence CD4 cells count and plasma viral load.

BST2 SNPs in adult and pediatric AIDS progression

The difference found in CD4+ cell count between rs919266 variants in HIV-1 infected mothers could indicate a putative role of *BST2* SNPs in the maintenance of CD4+ cells during HIV-1 infection. Since the ZEBS consortium did not cover maternal disease progression, our group investigated *BST2* SNPs as disease risk factors in pediatric and adult cohorts with clinical progression follow-up from two different populations (Trieste (Italy) and Porto Alegre (Brazil) respectively).

The genotype distribution of rs919266, rs919267 and rs9576 were in HWE within the pediatric Italian cohort. The influence of rs919266 and rs9576 in disease progression has not been tested due to the absence of their minor alleles in rapid progressors group (Table 2). None of the *BST2* alleles, genotypes or haplotypes were associated with pediatric disease progression ($p>0.017$).

All rs919266, rs919267 and rs9576 SNPs in the Brazilian cohort of AIDS progression were in HWE. The European-descendant Brazilians had lower frequencies of rs919266A (2.75% *vs* 7.46%) and rs9576A (3.70% *vs* 12.50%) than African-descendant individuals, and represented the major ethnic group in this study (56/92 (58.9%); Supplementary Digital Content 2).

rs919266A allele was more frequent in long-term non-progressors than the rapid progressors group ($p=0.025$, OR=0.17 [0.02 - 1.00])(Table 2), while rs919266GA carriers also showed a slower progression to AIDS than rs919266GG carriers (10.33 [7.17 - 13.49] *vs*. 6.56 [5.52 - 7.60] years, log-rank test $p=0.033$), even without statistical significance after Bonferroni correction. rs919267 and rs9576 allele and genotype frequencies did not show statistical differences (Supplementary Digital Content 3).

A lower coefficient of linkage disequilibrium was observed in the Brazilian cohort (D'=0.95) compared to Zambian (D'=0.97) and Italian (D'=0.96) groups, with the occurrence of a novel rs919266G-rs919267C-rs9576A haplotype (Table 2).

Discussion

Tethering of HIV-1 particles and induction of pro-inflammatory cytokines were described as restriction mechanisms by BST-2 during HIV-1 infection[4], despite the strong counteraction by HIV-1 vpu protein that leads to intracellular BST-2 degradation and NF-κB downregulation[22,23]. The protective role of BST-2 was corroborated by recent findings in cohorts of HIV-1 seroprevalent drug users with *BST2* variants that showed a higher *BST2* transcription and slower progression to AIDS by rs3217318 and rs10415893 in a Spanish cohort[13], and also in prevention to horizontal transmission by rs113189798 in a North American cohort [14].

The nominally significant association of rs9576 (located at *BST2* 3'UTR) with prevention of HIV-1 infection during breastfeeding may suggest a novel role of BST-2 in neonatal innate response, even without statistical significance after Bonferroni correction. Although the role of rs9576A allele in *BST2* expression is still unknown, differential expression of BST-2

could lead to an enforcement of HIV-1 restriction by immune cells like macrophages[5] or altering type I IFN production by pDCs[24], since neonatal pDCs already have a lower capacity of IFN-α production than adult pDCs[25]. Despite maternal *BST2* SNPs were not involved as a risk factor during breastfeeding, recent findings demonstrated that colostrum cells from HIV-1-infected mothers presented higher levels of BST-2 expression than healthy mothers but the influence in HIV-1 transmission rate is still unknown[11].

The *BST2* intronic allele rs919266A was associated with a slower progression to AIDS and more frequent in Brazilian long-term non-progressors than in rapid progressors, although rs919266A was associated with lower CD4+ cell count during delivery but did not influence HIV-1 maternal transmission in Zambian cohort. The functional role of rs919266 has not been demonstrated yet, but the linkage disequilibrium with rs10415893 (D'=1.0; Spanish population/IBS from 1000 Genomes), a tag SNP associated with AIDS progression in a Spanish cohort[13], suggests a protective role by the tagged region during AIDS progression. Moreover, it has not been possible to determine the impact of rs919266 SNP on the risk to pediatric disease progression and breastfeeding transmission, since rs919266A allele was not found in rapid pediatric progressors and postpartum-infected infants from Zambia.

Our study supports a protective role by genetic variants of *BST2* in adult AIDS progression and mother-to-child transmission. Despite the limited sample size and ethnic heterogeneity of the cohorts, our findings suggest that BST-2 activity in infants deserves further investigations as an early innate mechanism during breastfeeding and also corroborate previous findings indicating *BST2* SNPs as protective factors during AIDS progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Frequency of *BST2* polymorphisms in a cohort of Zambian infants born from HIV-1 infected mothers

BST2 SNPs		HIV-1 infected infants; n = 85			Exposed-uninfected infants (EU)	IU vs. EU		IP vs. EU		PP vs. EU	
		Intrauterine (IU) [a]	Intrapartum (IP) [b]	Postpartum (PP) [c]		OR [95% CI] [d]	*p*-value	OR [95% CI] [d]	*p*-value	OR [95% CI] [e]	*p*-value
MAF		n = 22 (%)	n = 25 (%)	n = 38 (%)	n = 246 (%)						
rs919266	A	3 (6.82)	4 (8.00)	0 (0.00)	26 (5.28)	1.31 [0.38 - 4.52]	0.934	0.56 [0.52 - 4.66]	0.634	-	
rs919267	T	17 (38.64)	21 (42.00)	21 (27.63)	154 (31.30)	1.38 [0.73 - 2.61]	0.406	1.59 [0.73 - 2.61]	0.167	0.84 [0.49 - 1.43]	0.609
rs9576	A	7 (15.91)	11 (22.00)	4 (5.26)	73 (14.84)	1.09 [0.47 - 2.53]	0.976	1.62 [0.79 - 3.31]	0.259	0.32 [0.11 - 0.9]	**0.019**
Genotypes											
rs919266	G/G	19 (86.36)	21 (84.00)	38 (100.00)	220 (89.43)	Reference	0.932	Reference	0.624	-	
	G/A	3 (13.64)	4 (16.00)	0 (0.00)	26 (10.57)	1.33 [0.37 - 4.82]		0.61 [0.51 - 5.06]			
	A/A	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-		-		-	
rs919267	C/C	8 (36.36)	7 (28.00)	19 (50.00)	117 (47.56)	Reference	0.589	Reference	0.166	Reference	0.632
	C/T	11 (50.00)	15 (60.00)	17 (44.74)	104 (42.28)	1.55 [0.6 - 3.99]		2.41 [0.95 - 6.14]		1.01 [0.5 - 2.04]	
	T/T	3 (13.64)	3 (12.00)	2 (5.26)	25 (10.16)	1.75 [0.43 - 7.08]		2.01 [0.48 - 8.3]		0.49 [0.11 - 2.25]	
rs9576	C/ C	16 (72.73)	15 (60.00)	34 (89.47)	176 (71.54)	Reference	0.437	Reference	0.336	Reference	0.062
	C/A	5 (22.73)	9 (36.00)	4 (10.53)	67 (27.24)	0.82 [0.29 - 2.33]		1.57 [0.66 - 3.77]		0.31 [0.11 - 0.9]	
	A/A	1 (4.55)	1 (4.00)	0 (0.00)	3 (1.22)	3.66 [0.08 - 8.36]		3.91 [0.38 - 39.95]		-	
Haplotypes											
rs919266-rs919267-rs9576 (D′=0.97; R^2=0.29)	G-C-C	27 (61.36)	29 (58.00)	55 (72.36)	333 (68.52)	Reference		Reference		Reference	
	G-T-C	10 (22.72)	10 (20.00)	17 (22.37)	85 (17.49)	1.45 [0.60 - 3.24]	0.39	1.35 [0.56 - 2.98]	0.41	1.21 [0.62 - 2.25]	0.53
	G-T-A	4 (9.09)	7 (14.00)	4 (5.26)	43 (8.85)	1.14 [0.28 - 3.52]	0.77	1.86 [0.65 - 4.70]	0.18	0.56 [0.14 - 1.64]	0.37
	A-T-A	3 (6.81)	4 (8.0)	0 (0.00)	25 (5.14)	1.48 [0.26 - 5.34]	0.47	1.83 [0.43 - 5.85]	0.29	-	-

CI, Confidence interval; SNP, MAF, Minor allele frequency; OR, Odds ratio; SNPs, Single nucleotide polymorphisms.

[a] Intrauterine transmission (IU) confirmed with positive HIV-1 PCR within 2 days of birth

[b] Intrapartum transmission (IP) confirmed with positive PCR result within 42 days of birth

[c] Postpartum trnsmission (PP) with positive PCR result at 42 days or older

[d] Adjusted for maternal CD4+ cell count and HIV-1 plasma viral load.

[e] Adjusted for maternal CD4+ cell count, HIV-1 plasma viral load and milk viral load.

Table 2

Frequency of *BST2* polymorphisms in a cohort of pediatric and adult disease progression from Trieste (Italy) and Porto Alegre (Brazil) respectively.

***BST2* SNPs**		**Pediatric disease progression** [a]					**Adult AIDS progression** [b]			
		Rapid progressors	**Slow progressors**	**OR [95% CI]**	***p*-value**		**Rapid progressors**	**Long-term nonprogressors (LTNP)**	**OR [95% CI]**	***p*-value**
MAF		n = 21 (%)	n = 67 (%)				n = 37 (%)	n = 21 (%)		
rs919266	A	0 (0.0)	1 (0.75)	-		A	2 (3.33)	6 (14.29)	0.17 [0.02 - 1.00]	**0.025**
rs919267	T	1 (2.63)	7 (5.38)	2.03 [0.25 - 94.32]	0.69	T	6 (10.00)	7 (16.67)	0.87 [0.28 - 2.91]	0.795
rs9576	A	0 (0.0)	2 (1.49)	-		A	3 (5.00)	7 (16.67)	0.28 [0.06 - 1.22]	0.094
Genotypes[d]										
rs919266	G/G	20 (100.0)	66 (98.51)	-		G/ G	28 (93.33)	15 (71.42)	Reference	
	G/A	0 (0.0)	1 (1.49)			G/A	2 (6.67)	6 (28.58)	0.14 [0.02 - 0.87]	**0.023**
rs919267	C/C	18 (94.74)	60 (89.55)	Reference	0.68	C/ C	24 (80.00)	14 (66.67)	Reference	
	C/T	1 (5.26)	7 (10.45)	2.09 [0.24 - 99.79]		C/T	6 (20.00)	7 (33.33)	1.13 [0.32 - 3.95]	0.85
rs9576	C/C	20 (100.0)	65 (97.02)	-		C/ C	27 (90.00)	14 (66.67)	Reference	
	C/A	0 (0.0)	2 (2.98)			C/A	3 (10.00)	7 (33.33)	0.27 [0.06 - 1.13]	0.068
Haplotypes										
rs919266-rs919267-rs9576 (D′=0.96; R^2=0.49)	G-C-C	40 (95.23)	127 (94.81)	Reference		rs919266-rs919267-rs9576 (D′=0.95; R^2=0.48)	61 (82.43)	34 (80.95)	Reference	
	G-T-C	2 (4.77)	5 (3.73)	1.42 [0.15 -13.04]	0.76		9 (12.17)	0 (0.00)		
	A-T-A	0 (0.0)	1 (0.75)	-			2 (2.70)	6 (14.29)	0.18 [0.02 - 1.13]	0.053
	G-C-A	0 (0.0)	0 (0.0)	-			2 (2.70)	0 (0.00)		
	G-T-A	0 (0.0)	1 (0.75)	-			0 (0.0)	2 (4.76)		

CI, Confidence interval; D', Coefficient of linkage disequilibrium; MAF, Minor allele frequency; OR, Odds ratio; R^2, Correlation coefficient of alleles; SNPs, Single nucleotide polymorphisms.

[c] Adjusted for *CCR5* 32bp deletion (CCR5 Δ32) genotype and ethnicity.

[a] HIV-1-infected children were classified in two groups: RP (developed severe clinical manifestations within the first two years of infection, defined as "Category C" of CDC 1994 AIDS surveillance case definition) and SP (who neither progressed to Category C nor developed severe immunosupression beyond eight years of age).

[b] HIV-1 infected adults from Porto Alegre (Brazil), according to AIDS progression criteria by Casado *et al.* (2010)