Original Article

CCR2, *CX3CR1*, *RANTES* and *SDF1* genetic polymorphisms influence HIV infection in a Zimbabwean pediatric population

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Abstract

Introduction: There is growing evidence that polymorphisms in chemokine and chemokine receptor genes influence susceptibility to HIV infection and disease progression. However, not much is documented about the prevalence and effects of chemokine and chemokine receptor gene variations in the Zimbabwean population despite the high burden of HIV/AIDS in the country. This study therefore describes polymorphisms in *CCR2*, *CX3CR1*, *SDF1* and *RANTES* genes in a Zimbabwean pediatric population and their effects on HIV infection in children born to HIV-infected mothers.

Methodology: A total of 106 children between seven and nine years of age comprising 70 perinatally exposed to HIV (34 born infected [EI] and 36 born uninfected [EU]) and 36 unexposed and uninfected (UEUI) controls were recruited. Six allelic variants in four genes were genotyped using PCR-RFLP and sequencing.

Results: Frequencies for minor alleles in the HIV uninfected groups (EU and UEUI) were *CCR2* 190A (16%), *SDF1* 801A (2%), *CX3CR1* 745A (9%), *CX3CR1* 839T (0%), *RANTES* In 1.1C (20%), and *RANTES* -403A (44%). There were significant differences between the EI and EU groups in the distribution of *CCR2* 190G/A genotype (15% *versus* 39%, respectively, p = 0.02) and *CCR2* 190G/A-*CX3CR1* 745G/G genotype combination (0% *versus* 33%, respectively, p = 0.002).

Conclusions: Our findings suggest that chemokine and chemokine receptor gene variants seem to play an important role in the dynamics of HIV infection and could be used as drug or vaccine targets.

Key words: HIV/AIDS; chemokine; genetic polymorphism; Zimbabwe; perinatal.

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Introduction

Chemokines and chemokine receptors are involved in modulation of innate and adaptive immune processes, which include immune surveillance and inflammatory cell recruitment [1]. Chemokine receptors also act as co-receptors to HIV, aiding viral attachment and entry into host cells [2]. Polymorphisms in genes encoding chemokines such as stromal cell-derived factor 1 (SDF1), regulated on activation normal T-cell expressed and secreted (RANTES), and chemokine receptors such as C-C motif chemokine receptor 2 (CCR2) and C-X3-C chemokine receptor 1 (CX3CR1), have therefore been implicated in differential outcomes of HIV infection and disease progression [3-5]. Identifying genetic susceptibility variants could help in predicting disease course, guiding therapy, and providing potential therapeutic targets [6].

Several studies have been carried out among Caucasian populations, but there are limited reports on African populations. Observations about Caucasian populations cannot be easily extrapolated to African populations because Caucasians are predominantly infected by HIV subtype B, whereas sub-Saharan African populations mostly present with HIV subtype C [7]. HIV subtypes differ in their virulence and infectivity [8], so host antiviral defences could have varying effects on HIV infection and disease progression in different populations. It is therefore important to investigate host genetic determinants of

HIV infection and disease progression in African populations.

In the current study, genetic variation was investigated in four genes: two chemokine receptor genes (*CCR2* and *CX3CR1*) and two chemokine coding genes (*SDF1* and *RANTES*). CCR2 and CX3CR1, in addition to acting as HIV co-receptors, are also actively involved in innate immune antiviral activities [9]. SDF1 and RANTES are ligands to the main HIV co-receptors CXCR4 and CCR5, respectively, and therefore can inhibit HIV infection of host cells by competitively binding to the co-receptors [10]. The distribution and nature of antiviral host gene variants differs among populations, making it imperative to study the less characterised African populations. Therefore, we report on the distribution of genetic variants in four genes – *CX3CR1*, *CCR2*, *SDF1* and *RANTES* – in a Zimbabwean population group and the effects of these genes on differential susceptibility to HIV infection.

Methodology

Study participants

Participants were recruited from the Better Health for the African Mother and Child (BHAMC) cohort [11]. This is a longitudinal study of mother-child pairs who have been followed up since 2002 at three Harare peri-urban clinics (Epworth, St Mary′s Chitungwiza, and Seke North Chitungwiza). One hundred and six (n = 106) unrelated children between seven and nine years of age of Bantu African origin were recruited. They included 34 children perinatally infected with HIV (EI) and 72 healthy HIV-uninfected children, comprising 36 exposed to HIV in utero but not infected (EU) children and 36 unexposed and uninfected (UEUI) children recruited as controls. All HIV-infected mothers of children included in the study were administered a 200 mg single dose of nevirapine at delivery to prevent mother-to-child transmission of HIV. Written consent was obtained from the parents/guardians of each child before samples were collected. A blood sample was collected from each child for CD4+ T-cell counts and genotyping purposes. The demographic characteristics of the recruited children were captured from their medical records. The study received ethical clearance from the Medical Research Council of Zimbabwe and the Research Ethics Committee of Faculty of Health Sciences, University of Cape Town.

Genotyping of single nucleotide polymorphisms

Genomic DNA was extracted from blood using the Nucleospin Blood L kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. The study investigated the following six single-nucleotide polymorphisms (SNPs) in four genes: *CCR2* 190G>A (rs17141036), *CX3CR1* 745G>A (rs3732379), *CX3CR1* 839C>T (rs3732378), *RANTES* -403G>A (rs2107538), *RANTES* In1.1T>C (rs2280789), and *SDF1* 801G>A (rs1801157). Genotyping of SNPs was done using polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) except for *RANTES* -403G>A (rs2280789), for which DNA minisequencing (SNaPshot) was used. All enzymes used were purchased from Fermentas (Burlington, Canada). Enzyme restriction products were electrophosed on 2% agarose gel stained with ethidium bromide.

The *CCR2* 190G>A SNP was genotyped according to the method described by Martinson *et al*. [12]. In summary, a 337 base pair (bp) fragment flanking the SNP was amplified in a 25 μL volume containing 10 picomoles of each of the primers (Integrated DNA Technologies, Coralville, USA), 200 μM dNTP mix (Bioline, London, UK), 1X GoTaq Flexi Green buffer (Promega, Madison, USA), 1.5 mM $MgCl_2$ (Fermentas, Burlington, Canada), 1 U *Taq* polymerase, and 50 ng genomic DNA. A 10 μL volume of the PCR product was digested overnight using 2 U of *BseG*I (*Fok*I) restriction enzyme. The *CCR2* 190A allele is susceptible to *BseG*I restriction digestion; the PCR product is therefore cleaved, giving rise to two fragments of 228 bp and 109 bp, while the *CCR2* 190G allele lacks the sequence recognised by *BseG*I, leaving the 337 bp PCR product undigested.

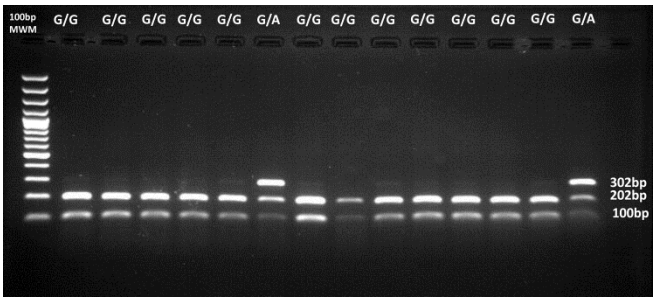
The *CX3CR1* 745G>A genotyping was carried out according to Nassar *et al*. [13]. A 311 bp gene region flanking the *CX3CR1* 745G>A position was amplified in a reaction mixture consisting of 10 picomoles of forward and reverse primers, 200 μM dNTP mixture, 1X GoTaq Flexi Green buffer, 1.5 mM $MgCl_2$, 1 U of *Taq* polymerase enzyme, and 50 ng genomic DNA in a 25 μL volume. Ten μL of PCR fragments were digested using 5 U of *Psp*1406I *(Acl*I*)* restriction enzyme. The enzyme cleaves the PCR product at the *CX3CR1* 745G allele to give two bands of 204 bp and 107 bp. The presence of the *CX3CR1* 745A allele disrupts the cleavage site, leaving the 311 bp PCR fragment undigested.

To genotype *CX3CR1* 839C>T SNP, 10 picomoles of each of the primers sense (5′-CATCCAGACGCTGTTTTCCT-3′) and antisense (5′-

TGCTCAGAACACTTCCATGC-3′) were mixed with 200 µM dNTP mixture, 1X GoTaq Flexi Green buffer, 1.5 mM $MgCl_2$, 1 U of *Taq* polymerase enzyme, and 50 ng genomic DNA in a 25 µL volume PCR mix to amplify a 377 bp fragment. In the presence of the 839C allele, *BsmB*I restriction enzyme has two restriction sites, resulting in three fragments of 189 bp, 113 bp and 75 bp, while the *CX3CR1* 839T allele disrupts one of the restriction sites, giving two fragments 113 bp and 264 bp long. Genotyping of *SDF1* 801G>A (rs1801157) was done as described by Balotta *et al*. [14]. Ten picomoles of each of forward and reverse primers were added to a PCR reaction mixture consisting of 200 µM dNTP mixture, 1X GoTaq Flexi Green buffer, 1.0 mM $MgCl_2$, 5% DMSO, 1 U of *Taq* polymerase enzyme, and 50 ng genomic DNA in a 25 µL reaction volume to amplify a 302 bp DNA fragment. *Msp*I (*Hpa*II) restriction enzyme digests the 801G allele into two fragments of 202 bp and 100 bp while *SDF1* 801A variant disrupts the restriction site, leaving the 302 bp fragment undigested as shown in Figure 1.

RANTES In1.1T>C genotyping was done according to a method described by Qian *et al*. [15]. A 343 bp DNA region incorporating the *RANTES* In1.1T>C polymorphism was amplified using a PCR reaction mixture comprising 10 picomoles of each primer, 200 µL of dNTP mixture, 1X GoTaq Flexi Green buffer, 1.0 mM $MgCl_2$, 1 U of *Taq* polymerase enzyme, and 50 ng genomic DNA in a 25 µL volume. The *RANTES* In1.1C allele is cleaved by *Mbo*II restriction enzyme yielding two fragments, 225 bp and 118 bp, while the *RANTES* In1.1T allele is resistant to enzyme activity. PCR-based SNaPshot was the method of choice for *RANTES* -403G>A SNP genotyping. PCR was used to amplify a 527 bp fragment flanking the *RANTES* -403G>A SNP. The reaction consisted of 10 picomoles of each of the forward (5′-CACCTCCTTTGGGGACTGTA-3′) and reverse (5′-CGTGCTGTCTTGATCCTCTG-3′) primers, 200 µM dNTP mixture, 1X GoTaq Flexi Green buffer, 1.5 mM $MgCl_2$, 1 U of Taq polymerase enzyme, and 50 ng genomic DNA in a 25 µL volume. In preparation for SNaPshot, the PCR products were purified using 2U exonuclease and Thermosensitive 1U Alkaline Phosphatase enzymes (FastAP). The SNaPshot reaction mixture was made up of 20 picomoles of primer (5′-CCATAGATGAGGGAAAGGAG-3′), 1 µL of purified PCR product, 1 µL of the SNaPshot multiplex ready reaction mix (Life Technologies, Carlsbad, USA) and made up to 10 µL volume with DNA-free water. The reaction mixture was incubated for 25 cycles of denaturation at 96°C for 10 seconds, primer annealing at 50°C for 5 seconds, and primer extension at 60°C for 30 seconds. The SNaPshot products were then analysed on the ABI PRISM 3130 Genetic Analyzer (Life Technologies-Thermo Fisher Scientific, Carlsbad, Carlifornia, USA). Blue peak on position 34.2 on the electrophoregram was interpreted as *RANTES* -403G allele while a green peak at the same position represented the *RANTES* -403A allele.

Figure 1. Agarose gel electrophoresis showing *SDF1* 801G>A (rs1801157) genotyping using *Msp*I enzyme restriction. The 801G allele is digested into two fragments whilst the 801A remains undigested (Method described by Balotta et al., 1999).



Statistical analysis

Genotype and allele frequencies were calculated using Stata version 11.2 (StataCorp LP, Texas, USA) and/or SHEsis online version [16]. Hardy Weinberg Equilibrium (HWE) was tested using Chi-squared (χ^2) or Fisher′s exact with one degree of freedom. Chi-squared (χ^2) and Fisher′s exact tests were used, where appropriate, to test homogeneity in alleles and genotypes among the participant groups. $P < 0.05$ was considered statistically significant for all comparisons except genotype combinations, where $p < 0.025$ was considered significant after correction for multiple testing. Pairwise linkage disequilibrium (LD) analyses between *RANTES* SNPs were carried out using SHEsis. Lewontin's D′ value and r^2 were used to quantify the level of LD ranging from zero for independence to one for complete co-inheritance. Haplotypes were inferred using the expected maximization algorithm.

Results and Discussion

Demographic characteristics

HIV-infected (EI) children presented with significantly lower mean height (cm) of 117.11 ± 6.2 SD ($p = 0.03$) and lower mean weight (kg) of 20.31 ± 1.99 SD ($p = 0.005$) when compared to uninfected children (exposed uninfected + unexposed and

Table 1. Demographic characteristics of study participants

Characteristics	HIV-infected (EI) n = 34	HIV-uninfected (EU and UEUI) n = 72	P value
Mean age in years ± SD (range)	8.22 ± 0.57 (7.25-9.08)	8.43 ± 0.55 (7.5-9.08)	0.28
Mean height in cm ± SD (range)	117.11 ± 6.22 (109-131)	120.37 ± 6.00 (108-134)	0.03
Mean weight in kg ± SD (range)	20.31 ± 1.99 (17-24)	22.35 ± 3.01 (15-31)	0.005
Mean head circum in cm ± SD (range)	51.14 ± 1.57 (49-54)	51.37 ± 1.2 (48-54)	0.48
Sex			
Female	20 (0.59)	36 (0.50)	0.37
Male	14 (0.41)	36 (0.50)	

EI-HIV: exposed infected; EU-HIV: exposed uninfected; UEUI-HIV unexposed uninfected;

Table 2. Comparison of minor allele frequencies between the HIV-uninfected (EUI and UEUI) Zimbabwean group with other populations whose data was extracted from HapMap and NCBI dbSNP databases

Minor allele	ZIM (Current study)	YRI	LWK	MKK	ASW	HCB	CEU
CCR2 190A (rs17141036A)	0.16	0.18	NA	NA	N/A	0.26	0.11
CX3CR1 745A (rs3732379A)	0.09	0.12	0.09	0.11	0.11	0 p = 0.003	0.261 p = 0.001
CX3CR1 839T (rs3732378T)	0	0	NA	0.03	0.03	0	0.15 p = 0.001
SDF1 801A (rs1801157A)	0.02	0.02	0.05	0.08 p = 0.01	0.08	0.24 p < 0.001	0.208 p = 0.001
RANTES In1.1C (rs2280789C)	0.204	0.17	0.23	0.14	0.22	0.34 p = 0.02	0.10.2 p = 0.006
RANTES-403A (rs2107538A)	0.44	0.43	0.48	0.42	0.39	0.35	0.155 p = 0.001

ZIM: Zimbabwe; YRI: Yoruba of Ibadan, Nigeria; LWK: Luhya of Webuye, Kenya; MKK: Masaai of Kinyawa, Kenya; ASW: African ancestry in southwest USA; HCB: Han Chinese in Beijing; CEU: Utah residents with Northern and Western European ancestry. P values are only shown where significant difference were observed when compared to the ZIM population.

Table 3. Frequency and distribution of chemokine and chemokine receptor genotypes and their association with HIV status

Genotypes	HIV EI	HIV EU	OR (95% CI)	EI *vs* EU P value
CCR2 190G>A (rs17141036)	n = 34	n = 36		
190G/G	25 (0.74)	21 (0.58)	1.00	
190G/A	5 (0.15)	14 (0.39)	**0.27 (0.07-0.97)**	**0.02**
190A/A	4 (0.11)	1 (0.03)	4.67 (0.42-236)	0.14
CX3CR1 745G>A (rs3732379)	n = 31	n = 36		
745G/G	24 (0.77)	29 (0.81)	1.00	
745G/A	4 (0.13)	6 (0.17)	0.74 (0.14-3.53)	0.67
745A/A	3 (0.10)	1 (0.03)	3.75 (0.28-202)	0.23
CX3CR1 839C>T (rs3732378)	n = 31	n = 36		
839C/C	31 (1.00)	36 (1.00)	N/A	1.00
SDF1 801G>A (rs1801157)	n = 33	n = 36		
801G/G	31 (0.94)	35 (0.97)	1.00	
801G/A	2 (0.06)	1 (0.03)	2.26 (0.11-136)	0.50
RANTES In1.1T>C (rs2280789)	n = 34	n = 36		
In1.1T/T	19 (0.56)	20 (0.56)	1.00	
In1.1T/C	14 (0.41)	16 (0.44)	0.88 (0.30-2.51)	0.78
In1.1C/C	1 (0.03)	0 (0.00)	N/A	0.30
RANTES -403G>A (rs2107538)	n = 33	n = 36		
-403G/G	7 (0.21)	8 (0.22)	1.00	
-403G/A	22 (0.67)	22 (0.61)	1.27 (0.43-3.84)	0.63
-403A/A	4 (0.12)	6 (0.17)	0.74 (0.14-3.5)	0.67

EI-HIV: exposed infected; EU-HIV: exposed uninfected; UEUI-HIV: unexposed uninfected; N/A: not applicable; OR (95%CI): odds ratio (95% confidence interval)

uninfected) with heights and weights of 120.37 ± 6 SD and 22.35 ± 3.01 SD, respectively, as shown in Table 1. This indicated possible slowed growth due to HIV/AIDS-related challenges.

Chemokine receptor polymorphism and their association with HIV infection

Frequencies of variant alleles observed in the HIV-uninfected group (EU and UEUI) in the study population and how they compare to those of other populations are presented in Table 2. The *CCR2* 190A (64I) variant occurred with a frequency of 18%, which is comparable to what has been reported among other African populations (ranging from 10%–30%) [4,17]. These frequencies of the *CCR2* 190A allele in African populations are higher than what has been observed in Caucasians (7%–10%) [17,18], while Asian populations show a wider range (1%–30%) [15,19]. The distribution of the *CCR2* 190A allele among Caucasians, Africans, and Asians does not seem to exhibit trends suggestive of the variant's role in HIV restriction at the population level.

The frequency of the *CCR2* 190G/A genotype was significantly higher in HIV-exposed uninfected children (39%) than in the HIV-exposed infected children (15%) ($p = 0.02$), suggesting a possible protective role for this genotype against HIV infection. A possible mechanism of protection is through the *CCR2* 190A (64I) allele encoding a protein variant of the CCR2 receptor with increased affinity to dimerize with HIV co-receptor CXCR4 compared to the *CCR2* 190G (CCR2 64V) variant [20]. The dimerization reduces the amount of CXCR4 available for HIV binding, therefore reducing the chances of HIV entering the host cell. Surprisingly, neither the homozygous *CCR2* 190A/A genotype nor the *CCR2* 190A allele frequencies were significantly different between the HIV EI and EU children.

It has been suggested that the *CCR2* 190A allele is dominant over the *CCR2* 190G allele [21]; the heterozygous *CCR2* 190G/A exerts effects similar to CCR2 190A/A genotype. Nevertheless, no statistically significant differences between the EI and EU children were found under this dominant model. This could be due to a limitation in the sample collection, where only those children surviving past seven to nine years of age were available to participate in the study. In addition, CCR2 is a minor HIV co-receptor and its chemotactic roles are also executed by alternative pathways, thus *CCR2* 190G>A variation may not be an important player in antiviral activity; its role in HIV infection remains a subject of debate.

A frequency of 9% for the *CX3CR1* 745A variant allele was observed, while the *CX3CR1* 839C>T (rs3732378) SNP was monomorphic in the Zimbabwean population (Table 2). The frequency of the *CX3CR1* 745A allele in the study population was lower than that reported among Caucasian populations (20%) [22] but higher than that reported in Asian populations (2%–5%) [15]. The *CX3CR1* 839T allele, which was not observed in the Zimbabwean population, has also not been reported among the Yoruba of Nigeria, indicating that this variant could have evolved post human migration from Africa. However, this allele has been reported among Caucasian populations (15%) as well as among Asian populations (2%) [23]. The alternate allele, *CX3CR1* 839C, could possibly be associated with increased risk for infections, including HIV, as is seen currently in Africa. It is interesting to note though that the *CX3CR1* 839T has been reported in the Masaai of Kenya, dispelling the notion of being a modern variant (Table 2).

The high frequency of *CX3CR1* 745A (249I) and *CX3CR1* 839T (280M) variants among Caucasians compared to that among Africans and Asians casts doubt on the variants' roles in risk of HIV infection, because the amino acids encoded by these alleles are associated with less efficient binding of CX3CR1 receptors on peripheral blood mononuclear cells to the ligand fractalkine. This reduces fractalkine-induced chemotaxis, hence weakening the immune response mounted through the pathway [24], yet HIV prevalence is much lower in Caucasians where *CX3CR1* 745A and *CX3CR1* 839T alleles are more prevalent than among Africans and Asians.

CX3CR1 variants may be risk factors for some inflammatory diseases that are more common among Caucasians than is HIV/AIDS. For example, *CX3CR1* polymorphisms have been implicated in conditions such as Crohn's disease, multiple sclerosis, and bronchiolitis among Caucasians [25-27]. Further downplaying the possible role of *CX3CR1* genetic polymorphism on HIV infection, no significant differences in *CX3CR1* 745G>A genotype and allele frequencies between HIV EI and EU children were observed (Table 3). *CX3CR1* 745G>A variation alone may not have a significant role in HIV transmission. These observations are supported by a study involving three sub-Saharan countries, South Africa, Uganda and Malawi, which also failed to observe any association between *CX3CR1* 745G>A variation and HIV infection in children born to antiretroviral therapy-naïve mothers [28]. The findings of the above study

[28] may have been distorted by the heterogeneous samples drawn from the three different countries involved. In a separate study, this research group demonstrated differences in distribution of genetic variants among African populations; pooling samples from different countries in genetic association studies, therefore, may introduce bias [29].

CX3CR1 745A and 839T alleles have been shown to be incomplete LD in Caucasian and Chinese populations [15,24]. This is in contrast to observations in the Zimbabwean children, where *CX3CR1* 745A and *CX3CR1* 839T were in complete linkage equilibrium ($r^2 = 0.001$). The two SNPs are therefore not likely to be inherited together in the Zimbabwean population, nor do they form haplotypes that influence HIV-related outcomes. Since chemokine receptors function through activation by chemokine binding, chemokines have also been implicated in HIV infection; therefore, two chemokines – RANTES and SDF1 – also formed part of this study.

Chemokine receptor variants and their association with HIV infection

RANTES and SDF1 are natural ligands of the major HIV co-receptors CCR5 and CXCR4, respectively; they inhibit HIV infection by competitively binding to these receptors [1,10]. A frequency of 2% for the *SDF1* 801A allele in the Zimbabwean population was observed (Table 2). This is comparable to what has been reported among black South Africans (1%) and the Yoruba of Ibadan Nigeria (2%) [17]. In contrast, the *SDF1* 801A allele occurs at frequencies of between 15% and 30% among Caucasians [17,30]. Asian populations exhibit a wider range of *SDF1* 801A allele frequencies, ranging between 10% and 40% [15].

The effects of *SDF1* 801G>A genotypes on HIV infection in the current study population could not be determined because of the low frequency of the 801A variant. The *SDF1* 801A allele makes the *SDF1* mRNA more stable post transcription, thus up-regulating the synthesis of the SDF1 protein. This makes more SDF1 protein available for competitive binding against HIV [21]. It can therefore be speculated that the lower frequency of the *SDF1* 801A allele among Africans compared to Caucasians may be contributing to the higher HIV prevalence among Africans than among Caucasians. However, the high frequency of *SDF1* 801A in Asians, in whom HIV prevalence is also high, casts doubt on the allele's protective effect against HIV. HIV is a multifactorial condition; there may be other underlying factors that work in concert with the *SDF1* 801A variant to give its differential role in different settings.

In contrast to *SDF1* 801G>A, the two *RANTES* SNPs (In1.1T>C and -403G>A) were highly polymorphic in the Zimbabwean populations with minor allele frequencies of 20% (In1.1C) and 44% (-403A) (Table 2). The frequency of the *RANTES* In1.1C allele in this study population (20%) is lower than what has been observed among Asians (28%) [15], but higher than that among Caucasians (10%) [31]. The occurrence of the *RANTES* -403A allele in Zimbabweans (44%) was higher than that among Caucasians (16%) [32] but was comparable to that observed among Asians (40%) [33]. The *RANTES* In1.1T>C polymorphism is located in an intronic regulatory sequence and the In1.1C variant is associated with down-regulation of RANTES expression [34], resulting in fewer chemokines available to inhibit HIV binding or to internalize CCR5 receptors on T-cells, thus favoring HIV infection. The high frequency of the *RANTES* In1.1C allele in Africans and Asians may argue for similar susceptibilities to HIV infection in the two populations when compared to Caucasians [34].

In contrast to the *RANTES* In1.1C allele, *RANTES* -403A increases expression of *RANTES* [35]; its presence may reverse the down-regulating effect of *RANTES* In1.1C. However, when *RANTES* In1.1T>C and -403G>A genotype frequencies were compared between HIV EI and EU groups, there were no significant differences observed (Table 3). The frequency of *RANTES* haplotypes with respect to In1.1T>C and -403G>A loci also did not show any significant differences between the HIV EI and EU children. This observation suggests the effect of the co-inheritance of *RANTES* In1.1T>C and -403G>A alleles on the risk of HIV infection may be negligible in the population. The *RANTES* In1.1-403 (C-G) haplotype, which carries two *RANTES* down regulating alleles, was observed at very low frequency in the study population (1%), comparable to observations in Caucasians, Indians, and African Americans, where the C-G haplotype was not reported [34]. The C-G haplotype is rare possibly because it carries both *RANTES* down-regulating variants which results in excessively low levels of RANTES protein that might not be able to sustain its multiple roles in immunity and organ development and thus may be aborted during development.

Genotype combinations and HIV infection

HIV/AIDS is a multifactorial condition affected by multiple pathways in the innate and adaptive immune systems. Several antiviral factors work simultaneously in an attempt to mount immunity against HIV. Therefore, co-occurrence of HIV/AIDS restriction gene variants in an individual may have a more pronounced effect compared to the individual SNPs. Given this, genotypes of some SNPs were combined in a pair-wise fashion to determine their possible role in HIV infection (Table 4).

The frequency of *CCR2* 190G/A-*CX3CR*1 745G/G genotype combination was significantly higher in HIV EU children than EI children after controlling for multiple comparisons (33% *versus* 0%, p = 0.0002), suggesting that this genotype combination may have a protective role against HIV infection. This observation and reasoning is supported by the functional roles of alleles in the individual genotypes discussed earlier. The *CCR2* 190A has been reported to confer a protective role by making CXCR4 less available for HIV attachment, while the protein product of *CX3CR1* 745G (249V) results in a stronger chemotactic effect, conferring a more viable immune response. The combined effect of these two protective mechanisms may account for the HIV resistance exhibited by individuals carrying *CCR2* 190G/A-*CX3CR1* 745G/G genotypes.

In contrast, the *CX3CR1* 745A/A-*RANTES* In1.1T/T genotype combination was lower in HIV EU children (0%) compared to the EI children (10%) with borderline significance (p = 0.056) (Table 4), suggesting that it might be a risk factor for HIV infection. Rathore *et al*. [5] reported the In1.1T allele to be a risk factor for HIV infection [5]. This study therefore supports Rathore's observation, but on a *CX3CR1* 745A/A genotype background. Paradoxically, *RANTES* In1.1T allele has been described to up-regulate the expression of *RANTES*, therefore making available more of the chemokine for competitive inhibition of HIV binding [34]. Genotype combinations are therefore more likely to have an effect on HIV infection compared to individual SNPs because of the multifactorial nature of anti-HIV immunity.

Conclusions

This is the first study to report the frequencies of the chemokine and chemokine receptor variants in a Zimbabwean population. The distribution of *CCR2* 190G>A, *CX3CR1* 745G>A, *CX3CR1* 839C>T, *RANTES* In1.1T>C, *RANTES* -403G>A and *SDF1* G>A variants in the Zimbabwean population does not differ much from that reported in other populations of African origin. The difference in frequencies of *CX3CR1* 839T, *SDF1* 801A, and *RANTES* In1.1C alleles between African and Caucasian populations are reflective of HIV prevalence and are suggestive of their antiviral activities. It can also be deduced from our findings that host gene variants are more likely than individual SNPs to work together in influencing outcomes of multifactorial conditions such as HIV/AIDS. *RANTES* and *CX3CR1* variants did not show association with HIV infection when analyzed

Table 4. Genotype combinations and their association with HIV status

Genotype combination	HIV EI	HIV EU	OR (95% CI)	P value
*CCR*2 190G>A and *CX3CR1* 745G>A	n = 31	n = 36		
190G/G + 745G/G	22 (0.71)	17 (0.47)	1.00	
190G/G + 745G/A	1 (0.03)	3 (0.08)	0.38 (0.0-5.06)	0.394
190G/G + 745A/A	1 (0.03)	1 (0.03)	1.2 (0.01-96.77)	0.90
190G/A + 745G/G	0	13 (0.33)	N/A	**0.0002**
190G/A + 745G/A	3 (0.10)	2 (0.0.6)	1.86 (0.20-23.68)	0.501
190G/A + 745A/A	2 (0.06)	0	N/A	0.17
190A/A + 745G/G	2 (0.06)	0	N/A	0.17
CX3CR1 745G>A and *RANTES* In1.1T>C	n = 31	n = 36		
745G/G + In1.1T/T	13 (0.42)	17 (0.47)	1.00	
745G/G + In1.1T/C	10 (0.32)	12 (0.33)	0.95 (0.30-2.98)	0.93
745GG + In1.1C/C	1 (0.03)	0	N/A	0.28
745G/A + In1.1T/T	3 (0.10)	3 (0.08)	1.18 (0.15-9.49)	0.85
745G/A + In1.1T/C	0	3 (0.08)	N/A	0.10
745A/A + In1.1T/T	3 (0.10)	0	N/A	0.056
745A/A + In1.1T/C	1 (0.03)	1 (0.03)	1.1 (0.01-94.16)	0.91

EI-HIV: exposed infected; EU-HIV: exposed uninfected; UEUI-HIV: unexposed uninfected; N/A: not applicable; OR (95%CI): odds ratio (95% confidence interval)

individually, but combinations of genotypes that enhance their antiviral activities were more frequent in the HIV-exposed uninfected children than in the infected children, strongly supporting a synergistic antiviral effect. This combinational effect points to critical pathways in HIV infection that may be exploited for therapeutic benefits. We therefore recommend that future work takes advantage of advances in DNA technology such as microarrays and next generation sequencing to carry out genome-wide association studys on HIV and HIV-related outcomes using large sample sizes.

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