Abstract

HIV-1 co-receptors such as CCR5 and CXCR6 have been shown to influence HIV-1 acquisition and disease progression to AIDS. Two *CCR5* (rs746492, rs553615728) and two *CXCR6* (rs2234355, rs2234358) single nucleotide polymorphisms (SNPs) that could potentially serve as protective (rs553615728 and rs2234355) and deleterious (rs746492 and rs2234358) markers in the context of HIV-1 control were reported in Johannesburg (JHB) (Picton *et al*., 2017; Koor *et al*., 2019). To investigate the roles of these variants further, this study was undertaken in Durban (DBN), with the aim of comparing the results of the two cohort studies, given that the epidemic dynamics may differ between the two geographic regions.

The study also describes the screening, recruitment as well as the characteristics and challenges of this process. A total of 287/590 met the eligibility criteria. The majority (73.7%) of the screened participants in this cohort were female with the median age of 29 years. The predominant mode of HIV-1 transmission was heterosexual (98.1%). Intermittent condom usage with regular partners and consistent condom usage with casual partners was reported.

The final cohort recruited was assigned to phenotypic groups based on their $CD4^+$ T-cell counts and viral load (VL) measurements -Progressors (N=200; $CD4^+$ T-cell <200 cells/µl and VL>10,000 RNA copies/ml) and Controllers (N=87; $CD4^+$ T-cell >500 cells/µl and VL <2000 RNA copies/ml). These categories were defined using data from a single time point only at an unknown stage after HIV-1 infection. The Controllers were further sub-grouped into elite controllers (ECs, N=31; VL <50 RNA copies/ml) and viraemic controllers (VCs, N=56; VL >50<2000 RNA copies/ml). The median age was comparable between the Controller and Progressor groups but the number of females was significantly more frequent in the Controllers (both ECs and VCs). For this reason gender was used to stratify some of the analyses below. Data for the same SNPs in a control HIV-1 negative cohort of 87 black South Africans and from HIV-1 infected cohorts from JHB (N=134 and N=113), were obtained from two separate studies for comparative purposes.

Comparison of allelic and genotypic frequencies of the *CCR5* rs746492, *CXCR6* rs2234355 and *CXCR6* rs2234358 SNPs between the HCs and other reference populations revealed some interesting results. Not unexpectedly, all three SNPs showed significantly different allelic representation compared to the EUR population, with *CXCR6* rs2224355 showing the largest difference (P<0.0001) due to the rarity of this SNP in the EUR population group. Compared to

the African populations, the HC group was more similar to the East African population and significantly different to the West African population.

Comparisons of both allelic and genotypic frequencies for all four variants in the DBN cohort revealed no significant differences for both the *CCR5* SNPs and the *CXCR6* rs223455 SNP. However, *CXCR6* rs2234358 minor allele (T) and minor allele homozygosity (TT) showed strong trends of underrepresentation in VCs compared to HCs (P=0.05 for both). When we compared representation of these variants across JHB and DBN cohorts, there were significant differences in the *CCR5* rs746492 allele frequency in the Controller groups. The *CXCR6* rs2234355 genotypic frequency was significantly different between the JHB and DBN cohorts in the Progressor (P=0.03) and VC (P=0.04) groups. The allelic and genotypic frequencies for the *CCR5* and *CXCR6* SNPs in the DBN cohort were more aligned with HCs.

Assessing the effect of these variants on VL in the Progressor group showed no significant associations with the *CCR5* genotypes. Interestingly, the *CXCR6* rs2234358 SNP was significantly associated with higher VL (P=0.0003) with no significant associations of these variants on $CD4^+$ T-cell count.

The *CXCR6* rs2234355 SNP genotypes were significantly differentially distributed between genders in the Controllers, Progressors, and VCs. Given the difference in gender distribution, we assessed the effect on female VCs and Progressors. There was a strong trend of overrepresentation in VCs compared to Progressors in females (P=0.055) as well as the *CXCR6* rs2234355 SNP in dominant mode (GA+AA) (P=0.078).

The combined effect of the two *CXCR6* variants was assessed. The *CXCR6* rs2234355 protective genotype (GA+AA) in the absence of *CXCR6* rs2234358 deleterious genotype (TT) showed a significant higher representation in VCs versus Progressors (P=0.034) and this association was strengthened when females alone were compared between these two study groups (P=0.018). Frequency of 3-SNP genotype combination across *CCR5* and *CXCR6* were not significantly different between VCs and HCs.

In conclusion, the most significant findings in this study centered on CXCR6 and not CCR5, with a stronger effect seen in the female gender. The differences seen between the DBN and JHB cohorts suggest that either ethnic differences, or the epidemic dynamics, or a combination of both may be contributing factors, and that careful consideration needs to be applied in future

analyses going forward with regard to merging data across cohorts. Lastly and importantly, this study has served to yet again highlight an important role for CXCR6 in viraemic and not elite natural HIV-1 control.