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Lack of Duffy Antigen Receptor for Chemokines: No Influence on HIV Disease Progression in an African Treatment Naïve Population

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Dear Editors: He et al recently reported an association between a single-nucleotide polymorphism (SNP, rs2814778, -46T→C) in the erythroid specific promoter region of the Duffy antigen receptor for chemokine (DARC) and HIV-1 acquisition and disease progression in African Americans (He et al., 2008). The authors described a higher frequency of the null homozygous genotype (-46C/C) in HIV-infected (70%) versus non-infected individuals (60%). Based on these findings they argued that the null allele confers susceptibility to infection with HIV-1 and suggested that up to 11% of the HIV-1 burden in sub-Saharan Africa could be attributed to homozygosity for the null allele. Oddly, however, these authors reported that the null genotype associated with *better* outcomes amongst those who became infected, including longer survival and more particularly decelerated loss of CD4+ T-lymphocytes. Another report showed an association between the -46C/C genotype and significantly reduced total white blood cells (WBC) in African Americans explaining ~20% of population variation in WBC (Nalls et al., 2008). The -46C/C genotype is nearly fixed in those sub-Saharan African populations where *Plasmodium vivax* is endemic, apparently because it confers resistance against *P. vivax* malaria, but -46C is virtually absent in individuals of European descent (Tournamille et al., 1995; Hamblin and Di Rienzo, 2000). In those regions of sub-Saharan Africa that are not endemic for *P. vivax*, the -46C allele is not fixed, however, and the wildtype (-46T) allele is frequently observed (Rosenberg et al., 2007). Considering the potential importance of DARC on HIV-1 disease progression in Africans, we studied the effect of the DARC -46 genotype on outcomes to HIV infection in a cohort of untreated black South

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Africans. This region of Africa is not endemic for *P. vivax* and lies outside of the area of 96% mean fixation for -46C; thus, the null allele of DARC is not fixed in our study population and the allele frequency of -46T (the wildtype allele) is about 20%. We assessed the effects of the null (C/C), heterozygous (T/C), and wildtype (T/T) genotypes on rates of CD4 decline and mean HIV-1 viral loads in a group of 381 untreated black South Africans. Since the -46C/C genotype has previously been associated significantly with reduced total WBC (Nalls et al., 2008), we also determined WBC in our cohort in order to verify that our cohort is sufficiently powered to detect an effect of the -46 genotype, where -46C/C is expected to associate with low WBC as previously demonstrated.

HIV-1 positive seroprevalent and antiretroviral therapy naïve subjects (N = 381) were recruited at McCord Hospital in Durban, South Africa from August 2003 to June 2006 and have been followed up to the present time. All subjects belonged to the local black Zulu/Xhosa population. Informed consent was obtained from all participating individuals and the study was approved by institutional review boards at the University of KwaZuluNatal, Massachusetts General Hospital and the University of Oxford. Viral load was measured using the Roche Amplicor version 1.5 assay. The mean viral load of the entire cohort was 4.59 ($\log_{10}$) HIV RNA copies per ml plasma (interquartile range (IQR), 1.69 ($\log_{10}$) − 6.81 ($\log_{10}$)). CD4+ T-cell, CD8+ T-cell, total lymphocyte and total white cell counts were determined by flow cytometry. The median CD4+ T-cell count of all individuals was 350 cells per ul (IQR, 7 - 1161). The DARC T-46C (rs2814778) SNP was genotyped by TaqMan allelic discrimination assays in all subjects and the genotypes were verified by direct sequence analysis of 78 samples, where complete concordance of genotypes between the two assays was observed. Association with rate of CD4 decline was tested using a mixed linear model for longitudinal data assuming random effects. Pairwise comparisons between genotype groups for CD4+ T cell counts and viral load were done with a t test, using the square root of the CD4+ T cell counts and the log of the viral load to give these data approximate normal distributions.

In total, 247 (64.8%) patients were homozygous for the null allele (C/C), 119 were heterozygous (T/C, 31.2%) and 15 patients were homozygous wildtype (T/T, 3.9%) (table 1). The distribution of genotypes in our cohort was consistent with the Hardy-Weinberg equilibrium (expected numbers: T/T 14.6; T/C 119.9; C/C 246.6; Chi-square value=0.0198, p value=0.888), allowing us to conduct the following comparisons. Furthermore, we performed a power calculation to confirm that we were able to detect significant differences for square root CD4 and log VL values comparing the C/C and T/C genotype; our sample size has 80% power to detect differences of 12% and 7% respectively for the two measures. No significant difference was observed with respect to median CD4 counts and VL among the C/C (sqrtCD4: 17.61; $\log_{10}$VL: 4.63), T/C (sqrtCD4: 18.36; $\log_{10}$VL: 4.51) and T/T (sqrtCD4: 18.25; $\log_{10}$VL: 4.58) subgroups. To investigate the influence of the DARC genotype on disease progression, we compared rates of CD4+ T-cell decline among the three groups. Individuals with the -46C/C genotype showed a CD4 decline of -0.27 sqrtCD4 as compared to -0.21 and -0.09 from patients with -46T/C or -46T/T, respectively. These differences were not statistically different, indicating that the DARC genotype does not have an effect on the rate of CD4 decline in our cohort.

There was also no impact of the DARC SNP on total CD8+ T-cell count or total lymphocyte counts (data not shown). However, consistent with a recent report (Nalls et al., 2008), individuals expressing the -46C/C genotype exhibited significantly lower levels of total white blood cells (4.75×10^9/l) compared to patients with the -46T/C (5.79×10^9/l) or -46T/T (5.84×10^9/l) genotypes ($p=1\times10^{-8}$ and p=0.014, respectively). Limitations to our study include sample size and potential confounding effects by other unknown polymorphisms. However, our data convincingly replicate an effect of the -46 locus on total white cell count, indicating

that our cohort is powered to detect true effects of this locus, but do not support any effect of the -46 variant on outcomes to HIV infection that were tested here.

The profound implications of the He et al report (He et al., 2008) suggesting an effect of the -46 DARC polymorphism on susceptibility to HIV-1 infection and HIV disease progression in African Americans warrant further, careful examination, especially as stratification due to population substructure can create spurious associations between alleles and traits that differ across subpopulations. This applies in particular to African Americans where the DARC null allele may have a particularly large stratification effect. Among the 381 untreated black South Africans studied here, there are no differences in total mean CD4 counts, decline in CD4 counts over time, or mean HIV viral load among the different DARC genotypes. These data indicate that the -46C/C DARC SNP does not play a major role in HIV disease progression in this cohort of Zulu/Xhosa HIV clade C infected South Africans. However, the impact of the SNP on reduced white blood cell counts was clearly replicated in this population, an effect that has now been shown to be attributable to differences in neutrophil numbers rather than to differences in lymphocyte populations (Reich et al., 2009). We conclude that if the DARC -46 polymorphism has an effect on survival among HIV-1 infected people of African descent as previously reported (He et al., 2008), this effect is independent of CD4+ T cell loss and plasma viral load burden.

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Table 1

No effect of the -46 DARC polymorphism on HIV disease outcomes

Genotype	**N**	**mean SqrtCD4**		**p (t test)**
TT	15	18.25	TT vs TC	0.99
TC	119	18.36	TC vs CC	0.26
CC	247	17.61	CC vs TT	0.69
Genotype	**N**	**mean LogVL**		**p (t test)**
TT	15	4.58	TT vs TC	0.79
TC	118	4.51	TC vs CC	0.20
CC	243	4.63	CC vs TT	0.82
Genotype	**N**	**SqrtCD4 change over time**		**p (F test)**
TT	15	-0.09	TT vs TC	0.14
TC	119	-0.21	TC vs CC	0.35
CC	247	-0.27	CC vs TT	0.30
Genotype	**N**	**mean Total WBC ($\times 10^9$/l)**		**p (t test)**
TT	15	5.84	TT vs TC	0.95
TC	117	5.79	TC vs CC	1×10^{-8}
CC	244	4.75	CC vs TT	0.014