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A polymorphism in *IL4* may associate with sensory neuropathy in African HIV patients

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Dear Editor,

HIV-associated sensory neuropathy (HIV-SN) is one of the most common neurological complications of HIV [1] and causes significant suffering and reduced quality of life [2]. It reflects both viral and drug-related toxicity, notably through the effects of stavudine (d4T). Animal and *in vitro* models of HIV-SN suggest a role of inflammation [3], but elucidation of the pathogenic mechanisms is hampered by difficulties accessing affected tissues. Genetic screens can usefully highlight pathways for further study. We have demonstrated the importance of a standardised screening tool that detects signs as well as symptoms of HIV-SN, and demonstrated associations with use of stavudine, patients' age and height [4-6]. In Caucasian, Chinese and Malay HIV-positive patients, the clearest genetic determinant of sensitivity to neuropathy identified was TNF-1031*C [4, 5]. TNF block haplotypes are now under analysis to identify critical single nucleotide polymorphisms (SNPs) in Africans (in preparation).

This study examines other cytokine genotypes that may affect neuropathy in a cohort of HIV-positive Black South Africans (n=342) treated at the Virology Clinic of the Charlotte Maxeke Academic Johannesburg Hospital, South Africa. Inclusion criteria were being Black African, 18 years or older with a confirmed HIV infection and having been on antiretroviral therapy (ART) for at least six months [334 had received stavudine]. The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa (protocol number: M080220), and written informed consent was obtained from all participants. They were screened using the AIDS Clinical Trials Group Brief Neuropathy Screening Tool, and clinical and demographic risk factors were collated [6]. DNA was extracted from blood or saliva samples and Illumina GoldenGate BeadXpress assays were used to genotype candidate SNPs, through the National Health Laboratory Service, (Johannesburg, South Africa). The panel included SNPs selected from published studies of HIV disease and/or neuropathy. SNPs with minor allele frequencies (MAF) <0.1 in our cohorts were discarded as these analyses lacked statistical power. Carriage of alleles in the 190 HIV-SN positive and 152 HIV-SN free individuals were compared.

Associations between alleles of *IL1A, IL1B, IL4, IL10, IL12* and *IL18* and neuropathy are shown in Table 1 Reasons for their inclusion and any associations with HIV-SN are discussed below. *IL1* alleles did not affect neuropathy in our studies of Caucasians [7], but IL1+4845*T associated with a poor virological response to ART. The latter was not evident in African Americans, but we established that haplotypic associations in the *IL1* cluster differ by ethnicity [8]. We found no association between *IL1* alleles and neuropathy in Southern African patients (Table 1).

IL-12 and IL-18 influence the induction of Th1 cytokine responses. Carriage of IL12B (3' UTR)*C was more common in Caucasian HIV-positive patients without neuropathy [9] and without CMV retinitis experienced after commencement of ART [10], suggesting a protective role in both cases. Additionally, this polymorphism and other linked alleles associated with tuberculosis in West Africans [11]. IL18-137*G associated with risk of HIV infection in North Indians [12] and with

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resistance to lipodystrophy in Brazilians [13]. There was no association between *IL12B* or *IL18* alleles and neuropathy in our cohort (Table 1).

The G allele of IL10-1082 (rs1800896) segregated with reduced susceptibility to HIV [14], as well as better survival and slower CD4 T-cell decline amongst African HIV-positive patients [15]. The same allele associated with neuropathy in South Indian patients with type 2 diabetes [16], but not with neuropathy in Caucasian HIV-positive patients [7]. Caucasian carriers of the rs1800872 A allele were at greater risk of HIV neuroretinal disorder as were carriers of haplotypes containing rs1800872*A and rs1800896*A. However, these associations did not exist in African Americans [17]. Here alleles of SNPs in *IL10* did not associate with neuropathy when assayed individually. As haplotypes defined by these alleles were clearer determinants of *ex vivo* IL-10 secretion than the individual alleles [18], the haplotypes were also determined. The haplotypes covered 98% of the population but none showed a significant association with SN (Table 2).

IL4-590*T is associated with high IL-4 levels and hence with Th2–associated conditions, notably asthma [19]. Amongst 24 SNP in immune-related genes assessed in North American HIV-positive adolescents not receiving antiretroviral therapy, the clearest association was between the IL4–590T/T genotype and higher CD4 T-cell counts [20]. Although the allele was not associated with neuropathy in Caucasian HIV-positive patients [7], there was a significant association with increased risk of HIV-SN in the present study. (Table 1). A significant difference remained evident when numbers of individuals with TT, TC or CC were compared (chi2, p=0.04). The association remained after correction for the patients' height and age (logistic regression; p=0.01) but did not withstand correction for multiple comparisons. No associations were found with IL4-590*T and HIV-SN in Caucasian (10/33 SN+ vs. 3/13 SN-; p=0.78), Chinese (13/15 SN+ vs. 44/49 SN-; p=0.86) or Malay (6/6 SN+ vs. 25/26 SN-; p=0.90) cohorts used in our previous study ([7]; unpublished data) suggesting that the SNP may not distinguish critical haplotypes in these populations.

As the association between IL4-590*T and HIV-SN in African patients was unexpected, we sought evidence of a role for the SNP in neuro-inflammation. There are associations between heterozygosity at IL4+33 and multiple sclerosis in a Spanish cohort where there is tight linkage disequilibrium between IL4+33 and IL4-590 [21]. The association of a high IL-4 producing allele with increased risk of HIV-SN may be explained by a response to existing high levels of inflammation as a result of HIV infection.

In conclusion; we have identified a novel association between sensory neuropathy experienced in African HIV-positive patients receiving antiretroviral therapy (including stavudine) and an allele in the promoter of *IL4*. As the allele has clear functional correlates [19], it warrants further investigation.

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Sincerely,
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