Title: Identification of HIV-1 *nef* Polymorphisms in HIV-Positive Cardiac Patients

ABSTRACT

Background: Infection with HIV is associated with an increased risk of development of cardiovascular diseases (CVDs), including coronary artery disease (CAD). Potential influential key players involved in the interaction between HIV and CVD include factors such as highly active antiretroviral therapy (HAART) and HIV viral proteins. The HIV-1 Nef viral protein has been associated with increased plasma levels of biomarkers of endothelial dysfunction in HIV-infected subjects, which may be an intermediary process in the development of atherosclerotic plaques. Therefore, given the highly polymorphic nature of the *nef* gene, the aim of this study was to determine if HIV-1 *nef* polymorphisms are associated with endothelial dysfunction and confirmed CAD.

Materials and Methods: Thirty-three (33) HIV-infected subjects with CAD (HIV+/CVD+), of which 31 were on HAART, were recruited alongside 115 HIV-uninfected subjects with CAD (HIV–/CVD+). In addition, 60 HIV-infected subjects on HAART without CAD (HIV+/CVD–) and 60 HIV-uninfected healthy subjects (HIV–/CVD–) were obtained from a previous study. Subjects' demographic information (age, gender, ethnicity), anthropometric data (body mass index; BMI, hip and waist circumference, waist-to-hip ratio), CVD risk factors (diabetes mellitus, CVD and diabetes family history, systolic and diastolic blood pressure, alcohol intake, smoking) and biochemical and immunological factors (triglycerides, total cholesterol, high density lipoprotein- (HDL-C) and low density lipoprotein-cholesterol (LDL-C), and CD4 count were recorded from patients' files. Biomarkers of endothelial dysfunction i.e. intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), von Willebrand factor

(vWF) and thrombomodulin (TM), were quantified by a Human Magnetic Luminex Screening Assay from 33, 38, 33 and 30 subjects of the HIV+/CVD+, HIV+/CVD–, HIV–/CVD+ and HIV–/CVD– groups, respectively. HIV-1 *nef* was sequenced from 25 subjects of the HIV+/CVD+ group and *nef* sequences of the 38 subjects in whom biomarkers of endothelial dysfunction were quantified from the HIV+/CVD– group were obtained from a previous study. The HIV-1 *nef* gene was sequenced by Sanger sequencing and sequences from the HIV+/CVD+ and HIV+/CVD– groups were compared to identify polymorphisms that differed in prevalence between the two groups and associated with plasma levels of ICAM-1, VCAM-1, vWF and thrombomodulin.

Results: The HIV–/CVD+ group had significantly higher plasma levels of ICAM-1 and VCAM-1 versus all the other groups. Significantly higher vWF levels were observed in the HIV+/CVD+ group compared to the HIV+/CVD– and HIV–/CVD–groups while vWF plasma levels of HIV–/CVD+ group were significantly higher in comparison to the HIV+/CVD– group. The HIV+/CVD–group had significantly higher thrombomodulin levels in comparison to the HIV+/CVD+ and HIV–/CVD+ groups, and significantly higher levels of thrombomodulin were observed in the HIV–/CVD–group in comparison to all other study groups. A total of seven HIV-1 *nef* polymorphisms (N51T, E65G, A84G, F86V, R152K, E175D and K185Q) were identified to significantly differ in prevalence between the HIV-infected groups of which N51T, E65G, F86V and K185Q were each associated with higher levels of vWF, while N51T, E175D and K185Q were each associated with lower levels of thrombomodulin, and F86V was associated with a higher level of ICAM-1. Multivariable logistic regression models demonstrated that the significant association of the *nef* polymorphisms with CAD were attenuated after adjusting for levels of vWF, ICAM-1 and thrombomodulin.

Conclusions: This study shows that HIV-1 *nef* polymorphisms are associated with endothelial dysfunction and with CAD which suggests that HIV Nef may play a role in the development of CAD in the HIV-infected population. The study further suggests that endothelial dysfunction may mediate the effect of Nef on CAD development.