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Genetic and molecular characterization of HIV-associated kidney disease in black South Africans

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ABSTRACT

Chronic kidney disease (CKD) due to HIV-associated nephropathy (HIVAN) remains an important cause of morbidity and mortality in African HIV-infected individuals. Genetic susceptibility to HIVAN has been attributed to genetic variations within *MYH9* and *APOL1* on chromosome 22. The expression of apolipoprotein L1 isoforms B and C coupled with high risk variants, play a crucial role in the development of kidney disease. The aims of this study were to determine the prevalence and examine the association of *MYH9* and/or *APOL1* high risk variants in HIVAN compared to other forms of CKD in HIV-infected individuals in a black South African population; to profile the pattern of messenger RNA *N-terminal* apolipoprotein L1 isoform expression in HIVAN, HIV-positive FSGS, other HIV-positive CKD, HIV-negative FSGS, other HIV-negative CKD, HIV-associated immune complex kidney disease (HIVICK) and in normal kidney; and to determine the expression of the *N-terminal* apolipoprotein L1 isoforms in human embryonic kidney (HEK) 293 cells.

Kidney biopsies from Charlotte Maxeke Johannesburg Academic Hospital were obtained from adults (aged ≥18 years) and selected according to histological diagnoses, clinical data were collected and patients were stratified according to age, gender and clinical presentation. Single nucleotide polymorphisms (SNPs) within and flanking *MYH9* and *APOL1* genes were selected by identifying all SNPs documented in 2011 from 1000 base pairs upstream and downstream of the two genes, and with a minor allele frequency of ≥0.05 in African populations based on the *International HapMap* and *1000 Genomes Projects*. SNPs with previous associations to kidney disease and literature SNPs were included, as was a panel of ancestry informative markers (AIMs). Genomic DNA was extracted from formalin fixed paraffin embedded (FFPE) kidney tissues and from whole blood. SNP genotyping was performed using the Illumina BeadXpress SNP genotyping assay and the TaqMan SNP genotyping assay. N-terminal apolipoprotein L1 mRNA expression was performed using

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method. Expression of N-terminal apolipoprotein L1 isoforms in HEK 293 cells was performed using PCR-based cloning and transfection techniques. Data analysis was performed using R program and Stata v11.1. Logic regression, Fisher's exact test, Kruskal Wallis test and Student's *t test* were employed in the data analysis.

A total of 228 samples [38 HIVAN, 41 HIV (+) CKD, 41 HIV (-) CKD, 54 HIV (+) Controls and 54 population controls] were successfully genotyped. Of 96 SNPs genotyped, 77 passed quality control. A total of nine *MYH9* and *APOL1* SNPs were significantly (P< 0.05) associated with HIVAN and not with other forms of CKD including focal segmental glomerulosclerosis (FSGS). However, the risk of *MYH9* SNPs with *P-values* < 0.05 could not be determined. Single *MYH9* E1 haplotype SNPs did not show any association, however, E1 haplotype block C-G-C showed a weaker significant factor with HIVAN (OR 3.45 *P=0.008*). The *APOL1* risk alleles [G1 (0.560), G1 (0.500) and G2 (0.342)] had the highest prevalence among HIVAN individuals. $G1^{GM}$ haplotype was the most frequent in HIVAN (0.530). 19/38 (50.0%) of the HIVAN cases were compound heterozygous for G1 and G2 risk alleles; 11/38 were homozygous for either G1 or G2 risk alleles, 6/38 were heterozygous for a single risk allele and 2/38 were homozygous for non-risk allele. Thus, 30/38 (79.0%) of HIVAN but only 1/54 (2%) of population controls carried two risk alleles. Population allele frequencies were 7.3% for G1 and 11.1% for G2. In a recessive model, individuals carrying two *APOL1* risk alleles had 89.0-fold higher odds 95% CI 17.68, 911.72; $P=1.24x10^{-14}$ for developing HIVAN. *APOL1* risk variants were not significantly associated with other forms of CKD [HIV (+) FSGS (OR=2.13 95% CI 0.03, 44.30; *P=0.48*) and HIVICK (OR=5.60 95% CI 0.4, 86; *P=0.13*) in the HIV (+) CKD group compared to HIV (+) controls] and [Primary FSGS (OR=6.30 95% CI 0.04, 248.70; *P=0.26*) in the HIV (-) CKD group compared to population controls]. Isoform B *N-terminal apolipoprotein L1* mRNA levels were elevated in HIVAN

and HIVICK while isoform C *N-terminal apolipoprotein L1* levels were elevated in normal kidney. We showed that the three isoforms are not only expressed in HEK 293 cells but the expression is variable where isoforms B and C *N-terminal apolipoprotein L1* that has a defective secretory domain and lack the secretory domain respectively, are seen to be more expressed within the HEK 293 cells than isoform A *N-terminal apolipoprotein L1*.

A combination of apolipoprotein L1 isoform B expression with C-terminal risk variants is a major contributor to the development of HIVAN and possibly other kidney diseases. There is a striking increase in the prevalence of *APOL1* risk variants among HIVAN compared to other forms of kidney disease and we estimate that HIV-infected South African blacks with two *APOL1* risk alleles not receiving anti-retroviral therapy (ART) have the greatest risk of developing HIVAN. Further studies are required to elucidate the mechanism of apolipoprotein L1-mediated kidney disease in order to develop effective therapeutic measures.