Polymorphisms in *CAMKK2* may predict sensory neuropathy in African HIV patients

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Abstract

HIV-associated sensory neuropathy (HIV-SN) is the most common neurological condition associated with HIV. HIV-SN has characteristics of an inflammatory pathology caused by the virus itself and/or by antiretroviral treatment (ART). Here we assess the impact of single nucleotide polymorphisms (SNPs) in a cluster of three genes that affect inflammation and neuronal repair*: P2X7R, P2X4R* and *CAMKK2.* HIV-SN status was assessed using the Brief Peripheral Neuropathy Screening tool, with SN defined by bilateral symptoms and signs. Forty-five SNPs in *P2X7R, P2X4R* and *CAMKK2* were genotyped using TaqMan fluorescent probes, in DNA samples from 153 HIV^+ black Southern African patients exposed to stavudine. Haplotypes were derived using the fastPHASE algorithm, and SNP genotypes and haplotypes associated with HIV-SN were identified. Optimal logistic regression models included demographics (age and height), with SNPs (model $p<0.0001$; $R^2=0.19$) or haplotypes (model $p<0.0001$; $R^2=0.18$, n=137 excluding patients carrying *CAMKK2* haplotypes perfectly associated with SN). Overall *CAMKK2* exhibited the strongest associations with HIV-SN, with two SNPs and six haplotypes predicting SN status in black Southern Africans. This gene warrants further study.

173 words

Key words: HIV, sensory neuropathy, *P2X7R, P2X4R, CAMKK2*

Introduction

HIV-associated sensory neuropathy (HIV-SN) is the most common neurological complication of HIV infection and antiretroviral treatment (ART), and affects up to 50% of HIV^+ individuals (Kamerman et al, 2012a; Keswani et al, 2002; Wadley et al, 2011). As a length-dependent, predominantly small fibre neuropathy, HIV-SN impairs somatosensory function in a stocking-and-glove pattern. Lower limb symptoms typically predominate. A common symptom is pain (Cherry et al, 2012), which negatively affects patient mobility, ability to work and quality of life (Ellis et al, 2010; Phillips et al, 2014). Established associations with HIV-SN include increasing age and exposure to potentially neurotoxic nucleotide reverse transcription inhibitors (NRTIs): zalcitabine, didanosine and stavudine (Kamerman et al, 2012a; Wadley et al, 2011). While zalcitabine is no longer used clinically, the affordability and availability of stavudine means that it are only now being phased out in resource-limited settings. Although these NRTIs contribute to the development of SN, not all patients receiving these drugs develop neuropathy, and SN remains prevalent in untreated HIV-infected patients and those never exposed to known neurotoxic regimens (Ellis et al, 2010).

Studies of HIV-SN patients who have never received ART demonstrate an inflammatory pathology (Tyor et al, 1995), and more recent studies show similar pathology in patients receiving ART (Kamerman et al, 2012a). Accordingly, we have identified haplotypes spanning a conserved block of genes around *TNFA* (the "*TNF Block")* that influence the risk of HIV-SN in Asian, African and Caucasian individuals (Chew et al, 2011; Wadley et al, 2015). Equivalent studies of other inflammatory genes are lacking. Here we report on a block of three neighbouring genes found on the long arm of chromosome 12: *P2X7R, P2X4R* and *CAMKK2* (Erhardt et al, 2007).

It is biologically plausible that each of these genes may contribute to HIV-SN for the reasons outlined below. Indeed, polymorphisms in these genes have been linked with susceptibility to neurological and/or inflammatory pathologies, including neuropathic pain (Erhardt et al, 2007; Ide et al, 2014; Oyanguren-Desez et al, 2011; Sorge et al, 2012). Roles for each of the genes in neuropathy are supported by data from animal models and cultured neurons and microglia.

Both *P2X7R* and *P2X4R* encode products belonging to the family of purinergic receptors for ATP. As well as being a source of intracellular energy, ATP can act as a potent extracellular messenger. Released from cells following injury or inflammation, ATP modulates a variety of tissue functions via the activation of

P2 receptors. This includes apoptosis, nociception, fast excitatory neurotransmission and astroglial cell function (Burnstock and Williams, 2000). Both P2X4R and P2X7R are expressed mainly, but not exclusively, in immune cells (macrophages, monocytes and microglia), and are known to be associated with inflammatory and nociceptive responses (Arulkumaran et al, 2011). They contribute to neuronal injury initiated synergistically by HIV tat protein and morphine in primary mouse striatal neuron-glia co-cultures.

The use of P2X antagonists and immunohistochemical analyses suggest that P2X4R is responsible for cellular toxicity (Sorrell and Hauser, 2014). HIV tat increases production of chemokine ligand 21 (CCL21) in dorsal root ganglia. CCL21 induces P2X4R expression, triggering persistent tactile allodynia (Biber et al, 2011). In microglia, P2X4R activation can be increased through exposure to lipopolysaccharides (Guo and Schluesener, 2006a) and by activation of toll-like receptors, including TLR2 (Guo et al, 2006b). The lateral mobility of P2X4R in the plasma membranes of resting microglia is increased by ATP activation and calcium translocation, acting via the p38 MAPK pathway (Toulme and Khakh, 2012). The p38 MAPK pathway is implicated in inflammatory disorders, such as inflammatory bowel disease (Hollenbach et al, 2004) and rheumatoid arthritis, and invokes the production of pro-inflammatory cytokines, including TNFα (Campbell et al, 2004). Activation of P2X4R in microglia and macrophages also triggers the production of brain-derived neurotrophic factor (BDNF), which interacts directly with nerves, inducing hyperexcitability of peripheral nociceptive synapses (Trang et al, 2011).

P2X7R also activates pro-inflammatory factors, notably TNFα (Suzuki et al, 2004). Activation is mediated by MyD88 and involves NFκB and caspase 1 (Liu et al, 2011), which in turn activates IL-1 and IL-18 in inflammasomes. Inhibition of P2X7R signaling in rats reduces p38 MAPK activation and neuronal apoptosis after subarachnoid hemorrhage (Chen et al, 2013). The binding of extracellular ATP to P2X7R on HIV-1 infected human macrophages triggers the release of HIV virions without killing the cells (Graziano et al, 2015).

In contrast to the P2X receptors, CAMKK2 is mainly expressed in the nervous system. Part of the Ca^{2+}-calmodulin dependent protein kinase family, it plays a role in neuronal differentiation and migration, neurite outgrowth, and synapse formation (Scott et al, 2015). It is also an upstream activator of AMP-activated protein kinase, an inhibitor of inflammation (Racioppi and Means, 2012). Following injury, the influx of Ca^{2+} into the cell activates calmodulin, inducing the CaM kinase signaling cascade. Activated CaMKK2 phosphorylates and activates protein kinases CaMKI and CaMKIV. Both trigger the production of CREB, a transcription factor that promotes the synthesis of several proteins including BDNF (Kokubo

et al, 2009). CaMKIV also triggers the p38 MAPK cascade and activation factor 1 (AP-1), inducing pro-inflammatory cytokines such as TNFα, IL-1β and IL-6 (Ageta-Ishihara et al, 2009). Different isoforms of CaMKI play a role in synaptogenesis, neural plasticity and neurite outgrowth (Guire et al, 2008; Saneyoshi et al, 2008). Aberrations in CaMKK2/CaMKI activity can thus have deleterious effects on neuronal growth, repair and function, as well as inflammatory responses. CaMKI is also responsible for the recruitment of calcium-dependent AMPA receptors, which mediate rapid excitatory synaptic transmission. AMPARs play a role in central sensitization in chronic pain (Garry et al, 2003).

The complex roles of P2X4R, P2X7R and CAMKK2 in the modulation of inflammation, pain and neuronal physiology/pathology make them viable candidates as mediators of HIV-SN. Alterations of the encoding genes may influence an individual's susceptibility to the disorder. Changes to individual base pairs in the genetic code (single nucleotide polymorphisms or SNPs) may alter the expression or function of the encoded protein. Alternatively through non-random assorting of alleles at adjacent loci, a SNP may mark (tag) a disease-causing polymorphism (Cordell and Clayton, 2005). Hence transmitted groupings of SNPs (known as haplotypes) can provide insights on genetic risk besides that provided by individual SNPs (Cordell and Clayton, 2005). The frequency of the polymorphic allele can vary between populations through genetic drift and natural selection. By convention, the most common allele in the populations is called the "major" allele (denoted 1), while the least common is called the "minor" allele (denoted 2). Here the same nomenclature is used to define haplotypes.

Here we investigate whether SNPs and haplotypes in *P2X7R, P2X4R and CAMKK2* are associated with prevalent HIV-SN in a population of black Southern African adults receiving stavudine-based ART. Associations between demographic variables (greater height and age), *TNF Block* genotypes and HIV-SN have been described in this cohort (Wadley et al, 2011; Wadley et al, 2015).

Materials and Methods

Participants

HIV$^+$ patients were recruited between July 2008 and April 2009 at the Virology Clinic of the Charlotte Maxeke Johannesburg Academic Hospital, South Africa (Wadley et al, 2011). An interpreter fluent in English and local African languages facilitated consent and study procedures. The study was approved by

the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa (protocol: M080220). Participants were screened using the AIDS Clinical Trials Group Brief Peripheral Neuropathy Screen, as it is validated for identifying symptomatic HIV-SN (Cherry et al, 2005). HIV-SN was defined by at least one symptom experienced bilaterally (pain, aching, burning, numbness or pins-and-needles) and at least one bilateral clinical sign (reduced vibration sense or absent ankle reflexes). Demographic and clinical data were collected from medical records. DNA samples were available from 153 HIV^+ patients who had been on stavudine-based ART for at least six months and had no unrelated cause of neuropathy (Wadley et al, 2011).

Selection of genetic markers

Polymorphisms in *P2X7R, P2X4R* and *CAMKK2* were identified using the HapMap databases describing three African groups (YRI – Yoruban in Ibadan, Nigeria; LWK - Luhya in Webuye, Kenya; MKK – Maasai in Kinyawa, Kenya) and one Caucasian (CEU –Northern and Western European ancestry). Fourty-five SNPs were selected (23 in *P2X7R*, 9 in *P2X4R* and 13 in *CAMKK2),* based on (in order) exonic location, published links with inflammatory/neurological diseases, location in proximal untranslated regions and presence in more than one population. Linkage disequilibrium data from HapMap collated in HaploView showed blocks of coinheritance within and between the three genes.

Genotyping and construction of haplotypes

Genotyping was performed using custom TaqMan OpenArray Genotyping Plates (Life Technologies, NY). DNA samples were adjusted to 50ng/uL and diluted at 1:1 in TaqMan OpenArray Genotyping Master Mix for 50 cycles of PCR amplification. The output was viewed using OpenArray™ SNP Genotyping Analysis software and genotypes were allocated manually. SNPs not meeting Hardy-Weinberg Equilibrium (HWE) were excluded. Haplotypes were identified using fastPHASE (Scheet and Stephens, 2006) run 20 times with random seeds. Haplotypes were sampled from the observed genotypes 10,000 times per sample. Phylogenetic networks were created with Network 4.6.1.2 (Fluxus Technology, Suffolk, England).

Statistical analyses

Statistical analyses were performed using Stata11 (StataCorp, TX). Univariate analyses evaluating associations between HIV-SN and clinical/ demographic factors, SNPs and haplotypes were performed using t-tests, Wilcoxon rank sum tests, Chi^2 tests or Fisher's exact tests, as appropriate. As this is the first comprehensive genetic study of *P2X4R, P2X7R* and *CaMKK2,* it is structured as a hypothesis-generating study and correction for multiple comparisons was not undertaken as it was important to avoid false negative findings. Factors potentially associated with HIV-SN were selected using a cut off of $p<0.2$ in univariate analyses, to ensure SNPs and haplotypes from each of the genes of interest were included in logistic regression analyses. These were refined by a step-wise removal process to obtain optimal models.

Results

Age and height associated with HIV-SN

The 153 participants included in our study matched the parent cohort [n=395; (Wadley et al, 2015)] when assessed by the prevalence of HIV-SN - 57% vs 56%, gender - 75% vs 75% female, age - 39±8 and 39±8 years and height - 159±8 and 158±9 cm (resp.). Logistic regression modeling included factors previously linked with HIV-SN, specifically gender (Mehta et al, 2011), anti-tuberculosis drugs (Maritz et al, 2010), nadir $CD4^+$ T cell counts (Ellis et al, 2010) and current $CD4^+$ T cell count, although this has only shown an effect pre-ART (Dubeyu et al, 2013). Here the final model identified only increasing age (OR=1.09; p=0.001; 95% CI 1.04-1.14) and height (OR=1.05; p=0.036; 95% CI=1.00-1.09) as being independently associated with HIV-SN (model p=0.0002, R^2=0.084). These had been associated with HIV-SN in the parent cohort (Wadley et al, 2011), and were included in subsequent multivariable analyses.

Associations between SNP alleles and HIV-SN.

Four SNPs – all in *CAMKK2* - (rs1560568*A, rs7975295*C, rs2686344*T and rs2686367*A) were associated with HIV-SN ($p<0.05$) on univariate analysis. An additional eight SNPs met our criteria for inclusion in logistic regression modeling (p=0.05-0.2; Table 1). These included four in *P2X7R* (rs1186055*C, rs208307*G, rs10160951*G, rs2230912*G), three in *P2X4R* (rs2686387*C, rs2668252*C, rs1169719*A) and one additional SNP in *CAMKK2* (rs1653587*G).

The *CAMKK2* SNPs rs1560568*A and rs7975295*C were invariably carried by the same people, so only rs1560568 was retained. Logistic regression modeling used 11 SNPs, age and height, with step-wise removal of the weakest associations. The optimal model included age, height and five SNPs representing all three genes (model $p<0.0001$ and $R^2=0.19$; Table 2 upper panel).

We then considered whether these data improved on associations seen with *TNFA* block genotypes. Seven *TNF Block* risk alleles were identified in the parent cohort: rs11796*A, rs3130059*G, rs2071594*C, rs2071592*A, rs2071591*A, rs909253*G and rs10411981*C (Wadley et al, 2015) and associated weakly with SN in our sub-cohort ($p<0.1$). As these were usually carried by the same individuals, rs2071594 was modeled with age, height and the 11 SNPs identified in Table 1. The resulting model ($p<0.0001$; $R^2=0.2$) retained rs2071594 and three SNPs from Table 2; rs1560568 and rs2686367 in *CAMKK2* and rs208307 in *P2X7R*. Hence there was no clear improvement when the *TNF Block* and *P2X4R/P2X7R/CAMKK2* were examined together.

Associations between haplotypes and HIV-SN

The fastPHASE program produced 195 haplotypes spanning the three genes, with many found in only one or two individuals. Consequently, each gene was analysed individually, generating 119 haplotypes in *P2X7R*, 21 in *P2X4R* and 54 in *CAMKK2*. The order of SNPs within the haplotypes matches Table 1. Our statistical analyses were restricted to haplotypes with a frequency of >2%, so there were eight usable haplotypes in *P2X7R,* seven in *P2X4R* and 17 in *CAMKK2.* Usable haplotypes in *P2X7R* accounted for only 34% of the cohort, but those from *P2X4R* accounted for 93% and in *CAMKK2* for 78%. Six *CAMKK2* haplotypes were associated with HIV-SN on univariate analysis ($p<0.05$, Table 3). An additional two *P2X7R* haplotypes, three *P2X4R* haplotypes and three *CAMKK2* haplotypes met our criteria for inclusion in regression modelling ($p=0.05$-0.2, Table 3). The alleles of the SNPs in each haplotype are expressed as being either a 1 (major allele) or a 2 (minor allele), with respect to their frequency in this cohort. They are presented in the sequence corresponding to their chromosomal position (as per Table 1). Three *CAMKK2* haplotypes perfectly predicted the presence (11121221112) or absence (11111111112, 11121212111) of HIV-SN, explaining HIV-SN status in 16/153 (10.5%) of patients (Table 3). Thus 11 haplotypes together with age and height were carried forward in the logistic regression using the

remaining 137 individuals. For these patients, the best model combined age and height with one haplotype of *P2X7R* and three of *CAMKK2* (Table 2 lower panel; $p<0.0001$, $R^2= 0.18$).

We next sought haplotype families that would reduce the number of variants and allow inclusion of rare haplotypes. However, phylogenic networks showed that haplotypes associated with HIV-SN did not form distinct clusters (Figure 1). The only exception is that two *CAMKK2* haplotypes associated with SN (11111111121 and 21111111121) differ only at rs1653587, which associated weakly with HIV-SN ($p=0.12$; Table 1). However their shared minor allele, rs3817190*T showed no association ($p=0.79$). While this allele was carried by 32% of patients, the 11111111121 and 21111111121 haplotypes had frequencies of 5% and 10% (respectively), so the causative allele is not identified.

We also considered risk/protective haplotypes identified in the *TNF Block* in studies of the parent cohort (Wadley et al, 2015). One *TNF Block* haplotype remained associated with HIV-SN in our sample (n=153). After inclusion of this haplotype, the optimal model retained all but the 11112121111 *CAMKK2* haplotype ($p<0.0001$; $R^2=0.18$). Hence the modeling was not strengthened by the inclusion of *TNF block* haplotypes.

Discussion

We investigated whether prevalent HIV-SN is associated with genetic polymorphisms in *P2X7R*, *P2X4R* and/or *CAMKK2,* in addition to demographic parameters and *TNF Block* genotypes identified in a Southern African cohort. Our first model included age, height and the alleles of five SNPs spanning the three genes (Table 1). We then analyzed haplotypes, as there was considerable linkage disequilibrium. As there are no equivalent studies of these genes, candidates are discussed individually.

The poor coverage by conserved *P2X7R* haplotypes (34% of the cohort) reflects the extreme heterogeneity of this gene and is an unavoidable limitation of our study. The only *P2X7R* haplotype in

our final haplotype model (2111**2**1211112121111111; Table 2) may be associated with an increased incidence of SN. It contained the minor alleles of five SNPs, where the second (rs1718125*T; intronic, 20bp from a splice site) has been linked to increased cold pain sensitivity (Ide et al, 2014). Of the five minor alleles, only rs1186055*G (3' downstream) showed a weak association with SN on univariate analysis (p=0.17) and none remained in the final SNP model, so individually they do not explain any impact of the haplotype.

The two *P2X4R* alleles in the final SNP model (Table 2) are located 2639 bp apart in the 3' downstream region of the gene. Rs1169719*A associated weakly with protection (p=0.11) and rs2668252*C with risk (p=0.09) for HIV-SN, but any effects are minor and no *P2X4R* haplotypes remained in the final haplotype model. Previous studies of *P2X4R* suggest a role for the gene in pain rather than SN itself (Ide et al, 2014; Sorge et al, 2012; Ulmann et al, 2008). As 94% of patients in this cohort with HIV-SN had pain (Wadley et al, 2011), this may explain any associations seen here. It will be informative to examine *P2X4R* genotypes in cohorts where more patients report painless HIV-SN. For example; in the CHARTER study, 62% of HIV-SN patients reported no neuropathic pain (Ellis et al, 2010).

Two minor alleles in *CAMKK2* remained in the final SNP model (Table 2). The first, rs1560568*A (MAF= 0.33; p=0.03), was consistently co-inherited with rs7975295*C. Both are located in the 3'downstream region of the gene, 1486 bases apart. rs7975295*C also associated with HIV-SN on univariate analysis (Table 1; p=0.007). The second *CAMKK2* SNP associated with SN, rs2686367*A (p=0.01), is located in the 5'UTR. No previous studies have addressed these SNPs.

Three *CAMKK2* haplotypes associated perfectly with either the absence (11111111121, 11121212111) or presence (11121221112) of SN. While the two protective haplotypes share no minor alleles, the single minor allele in 111111111**2**1 (rs3817190*T) was found in another haplotype that may be protective, 211111111**2**1. rs3817190 lies in a coding region and is non-synonymous, so it may affect the structure and function of CAMKK2. The T allele has been associated with increased severity of familial mood disorders such as agoraphobia and anxiety (Erhardt et al, 2007). However, this SNP showed no association with SN on univariate analysis (p=0.79), so its effect may be mediated through co-inherited alleles.

Two minor alleles were common to the second protective *CaMKK2* haplotype (111**2**1**2**12111) and the risk haplotype (111**2**1**2**21112). These alleles, rs1560568*A and rs7975295*C, were invariably co-inherited. While rs1560568*A (and by proxy rs7975295*C) had remained in the final SNP model as a risk allele, its carriage by both the protective and risk haplotype argue against a direct role in SN. The other minor allele in the protective 1112121**2**111 haplotype, rs11837114*G, showed no association with SN in the univariate analysis (p=0.52).

The two remaining unique minor alleles in the *CaMKK2* 111212**2**111**2** risk haplotype were rs7314454*T (3' downstream) and rs2686367*A (5'UTR). Rs2686367*A showed a stronger association with SN on univariate analysis than rs7314454*T (p=0.004 *vs.* p=0.22), and remained in the final SNP model as a risk allele. Hence rs2686367*A warrants consideration as the SNP responsible for the promotion of HIV-SN associated with the 11121221111**2** haplotype. This SNP is discussed below.

Of the 17 *CAMKK2* haplotypes examined, five contained rs2686367*A. Three of these haplotypes (1111111111**2,** 11111112111**2**, and 11121221111**2)** met the criterion for inclusion in the multivariable analyses, but had no other minor alleles in common. The remaining two haplotypes containing rs2686367*A were not associated with SN (p=1) and also shared no other alleles. Thus while rs2686367*A may contribute to SN risk, its effects may be moderated by SNPs not typed in our panel. Rs2686367 lies on the extreme edge of the region studied. We defined this as the edge of a conserved block of genes implicated in neuronal inflammation because linkage disequilibrium broke down in this region. However the neighbouring gene, anaphase-promoting complex unit 5 (*ANAPC5*) encodes a protein that inhibits IL-17, an important mediator of inflammation and immunity (Ho et al, 2013). HapMap data show linkage between rs2686367 and some SNPs in *ANAPC5* in African populations. Further studies should determine whether rs2686367*A marks carriage of critical alleles of *ANAPC5* or is important in its own right. Rs2686367 lies in a promoter region of *CAMKK2* and so may alter production of CAMKK2 and an individual's ability to grow and repair nerves.

Three other *CAMKK2* haplotypes were associated with reduced likelihood of HIV-SN in this cohort (p=0.03-0.05; Table 3). The 111111111**2**1 haplotype contained rs3817190*T, the minor allele in the

perfectly protective 1111111112**1** haplotype. **2**1111111121 was the only haplotype in the final model to contain the rs1653587*G (3'UTR) allele that individually showed a weak negative association with SN (p=0.12). The other two protective haplotypes, 1111**2**111111 and 1111**2**121111 share rs2686344*T. This intronic SNP negatively associated with SN in univariate analyses (p=0.018), but didn't remain in the final SNP model.

P2X4R and *P2X7R* encode products with an established role in inflammation. Similar to *TNFA*, variations in these genes may increase the pro-inflammatory environment surrounding peripheral nerves and so increase SN risk. We began this study expecting associations with one or more of the six non-synonymous exonic SNPs typed in these genes. However no non-synonymous SNP remained in the final SNP model (Table 2) and only two met our criteria for inclusion in the multivariate analyses (Table 1, both in *P2XR7*). Rather our data highlight the need for a careful study of patterns of LD in the target population.

While *CAMKK2* is also involved in inflammatory pathways, it is more clearly implicated in neuronal growth, signaling, plasticity and repair. Polymorphisms impeding the activity of CAMKK2 may hinder recovery from an insult (such as HIV or stavudine exposure) and increase the individual's risk of developing symptomatic HIV-SN. In contrast, variations that promote CAMKK2 activity may alleviate symptoms of neuropathy, or reduce the risk of HIV-SN. This gene warrants further study in relation to sensory neuropathy.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Figure 1: Phylogenic networks linking *P2X4R* and *CAMKK2* haplotypes.

No clear clustering indicative of haplotype families was seen. Node sizes reflect haplotype frequencies. Blue nodes mark haplotypes found in the optimal model. Purple nodes mark haplotypes perfectly associated with the absence or presence of HIV-SN (Table 3). Red nodes mark hypothetical haplotypes inserted by the software to connect existing sequences with maximum parsimony

521 **Table 1:** SNPs in *P2X7R, P2X4R* and *CAMKK2* associated individually with HIV-SN

			P2X7R			
SNP	**Position**	**MA**	**MAF**	**SN free**	**HIV-SN**	**P (Chi^2)**
rs10849849	Intronic	G	0.11	11/65[a] (17%)	21/87 (24%)	0.28
rs11065456	3'down	A	0.32	38/65 (58%)	47/84 (56%)	0.76
rs208288	3'down	C	0.29	33/63 (52%)	42/85 (49%)	0.72
rs17525767	3'down	T	0	1/65 (2%)	0/82 (0%)	0.26
rs1718125	Intronic	T	0.35	38/66 (58%)	47/86 (55%)	0.72
rs208294[b]	*Exonic (non-syn)*	*T*	*0.25*	*23/61 (38%)*	*27/73 (37%)*	*0.93*
rs1169737	Exonic (syn)	T	0	0/65 (0%)	0/86 (0%)	NA
rs1186055[b]	**3'down**	**C**	**0.48**	**46/66 (70%)**	**69/87 (79%)**	**0.17**
rs2857585	3'down	A	0.34	40/65 (62%)	48/85 (56%)	0.53
rs208296	3'down	A	0.17	22/66 (33%)	23/85 (27%)	0.4
rs11065464[c]	*3'down*	*A*	*0.05*	*4/66 (6%)*	*9/86 (10%)*	*0.34*
rs208307	**Intronic**	**G**	**0.44**	**40/65 (62%)**	**61/84 (73%)**	**0.15**
rs12299020	3'down	G	0.17	17/65 (26%)	28/84 (33%)	0.34
rs503720	3'down	G	0.49	50/66 (76%)	62/87 (71%)	0.53
rs504677	3'down	T	0.40	43/64 (67%)	53/86 (62%)	0.48
rs1653609	3'down	A	0.27	31/65 (48%)	43/83 (52%)	0.62
rs2230911	Exonic (non-syn)	G	0.14	17/66 (26%)	25/86 (29%)	0.65
rs1653598	3'down	C	0.47	51/66 (77%)	61/86 (71%)	0.38
rs10160951	**Exonic (non-syn)**	**G**	**0.18**	**17/65 (26%)**	**32/87 (37%)**	**0.17**
rs2230912	**Exonic (non-syn)**	**G**	**0.04**	**2/66 (3%)**	**9/85 (11%)**	**0.08**
rs3751144	Exonic (syn)	T	0.15	16/63 (25%)	27/86 (31%)	0.43
rs3751143	Exonic (non-syn)	C	0.02	1/66 (2%)	4/87 (5%)	0.29
rs12301635	3'down	G	0.31	34/66 (52%)	47/85 (55%)	0.64
			P2X4R			
rs2686387	**5'up**	**C**	**0.30**	**31/66 (47%)**	**51/87 (59%)**	**0.15**

rs2303998	Exonic (syn)	A	0	0/66 (0%)	0/87 (0%)	-
rs7298368	5'up	T	0.08	7/66 (11%)	18/87 (21%)	0.95
rs25643	Exonic (syn)	C	0.41	43/66 (65%)	63/86 (73%)	0.28
rs10849860	**3'down**	**C**	**0.15**	**19/66 (29%)**	**27/86 (31%)**	**0.73**
rs2668252	**3'down**	**C**	**0.32**	**37/65 (57%)**	**58/84 (69%)**	**0.13**
rs11608486	3'down	C	0.04	4/66 (6%)	7/87 (8%)	0.64
rs1169719	**3'down**	**A**	**0.07**	**12/66 (18%)**	**8/86 (9%)**	**0.11**
rs7961979	3'down	A	0.13	18/66 (27%)	20/87 (23%)	0.54
			CAMKK2			
rs1653587	**3'UTR**	**G**	**0.08**	**14/66 (21%)**	**10/85 (12%)**	**0.12**
rs1653588[b]	*3'UTR*	*A*	*0.08*	*12/65 (18%)*	*10/87 (11%)*	*0.23*
rs11065502	3'down	C	0.05	7/66 (11%)	9/87 (10%)	0.96
rs11065503	3'UTR	T	0.11	13/65 (20%)	19/84 (23%)	0.70
rs11065504[b]	*3'down*	*G*	*0.38*	*46/65 (71%)*	*67/87 (77%)*	*0.38*
rs7975295	**3'down**	**C**	**0.34**	**29/65 (45%)**	**56/84 (67%)**	**0.007**
rs2686344	**Intronic**	**T**	**0.19**	**30/65 (46%)**	**24/87 (28%)**	**0.018**
rs1560568	**3'down**	**A**	**0.33**	**30/65 (46%)**	**55/85 (65%)**	**0.023**
rs7314454	5'up	T	0.31	31/66 (47%)	49/86 (57%)	0.22
rs11837114	3'down	G	0.29	29/65 (81%)	39/78 (50%)	0.52
rs1718120	Intronic	T	0.02	1/65 (2%)	4/85 (5%)	0.28
rs3817190	Exonic (non-syn)	T	0.32	35/64 (55%)	43/82 (52%)	0.79
rs2686367	**5'UTR**	**A**	**0.30**	**25/66 (38%)**	**53/86 (62%)**	**0.004**

[a] Sample numbers vary as up to five samples failed genotyping at each SNP.

[b] Twelve SNPs showing a significant or weak association with HIV-SN (p<0.2, bold highlighted) were included in multivariate models (See Table 2).

[c] Four SNPs were excluded from the derivation of haplotypes (See Table 3) as they were not in Hardy-Weinberg Equilibrium.

Abbreviations: 3'down – 3' downstream region; 5'up - 5 upstream region; UTR - untranslated region; non-syn - non-synonymous change; syn - synonymous change; MA - minor allele in this cohort; MAF - minor allele frequency.

Table 2: Logistic regression models establish *CaMKK2* genotypes as optimal predictors of HIV-SN

Logistic regression model combining demographics and SNPs (n=143[a], p<0.0001; R^2=0.19)

Variable	Odds ratio	P value	95% Conf. Interval
Age	1.08	0.007	1.02 – 1.13
Height	1.05	0.03	1.00 – 1.11
rs208307*G (*P2X7R*)	1.96	0.10	0.86 – 4.88
rs2668252*C *(P2X4R*)	2.10	0.09	0.90 – 4.93
rs1169719*A *(P2X4R*)	0.37	0.11	0.11 – 1.23
rs1560568*A (*CAMKK2*)	2.39	0.03	1.09 – 5.25
rs2686367*A (*CAMKK2*)	2.85	0.01	1.26 – 6.42

Logistic regression model combining demographics and haplotypes (n=137[b], p<0.0001; R^2=0.18).

Age	1.10	0.001	1.04 - 1.166
Height	1.04	0.17	0.99 - 1.09
211121211112121111111 (*P2X7R*)	3.91	0.15	0.61 - 25.23
11112111111 (*CAMKK2*)	0.15	0.10	0.36 - 0.64
11112121111 (*CAMKK2*)	0.27	0.04	0.08 - 0.94
21111111121 (*CAMKK2*)	0.25	0.03	0.07 - 0.87
11111111121 (*CAMKK2)*	Never seen with SN	-	-
11121212111 (*CAMKK2)*	Never seen with SN	-	-
11121221112 (*CAMKK2)*	Always seen with SN	-	-

Haplotypes are defined by SNPs according to their positions in the genes, as specified in Table 1.

[a] excluding samples with genotyping failures

[b] excluding samples with genotyping failures or carrying *CAMKK2* haplotypes perfectly aligned with SN

537 **Table 3:** Haplotypes in *P2X7R, P2X4R* and *CAMKK2* associated with HIV-SN
538

	***P2X7R* haplotypes**		
	SN Free (n=66)	**SN (n=87)**	**P (chi^2)**
111111211111121111111	4 (6%)	6 (7%)	1[b]
111111211112112111212	5 (8%)	8 (9%)	0.7
111111211112112111211	4 (6%)	5 (6%)	1 [a]
111111211112121111111	**6 (9%) [a]**	**3 (3%)**	**0.18 [b]**
112121111221211221112	12 (18%)	15 (17%)	0.9
121111121111111211111	5 (8%)	6 (7%)	0.9
121121111211211211111	4 (6%)	4 (5%)	0.7 [b]
211121211112121111111	**2 (3%)**	**9 (10%)**	**0.12 [b]**
	***P2X4R* haplotypes**		
111121121	12 (18%)	15 (17%)	0.9
111211111	58 (88%)	74(85%)	0.6
211111111	**5 (8%)**	**12 (13%)**	**0.2**
211111121	2 (3%)	4 (5%)	0.7 [b]
211111212	**11 (17%)**	**8 (9%)**	**0.17**
211121111	4 (6%)	9 (10%)	0.4 [b]
212111111	**7 (11%)**	**17 (20%)**	**0.13**
	***CAMKK2* haplotypes**		
11111111111	9 (13%)	13 (15%)	0.8
11111111112	**9 (14%)**	**20 (23%)**	**0.14**
11111111121	**7 (11%)**	**0**	**0.002 [b]**
11111112111	4 (6%)	3 (3%)	0.5[b]
11111112112	**1 (2%)**	**6 (7%)**	**0.14 [b]**
11111121111	4 (6%)	4 (5%)	0.7 [b]
11112111111	**9 (14%)**	**3 (3%)**	**0.03 [b]**

11112112122	3 (5%)	5 (6%)	1 [b]
11112121111	**10 (15%)**	**5 (6%)**	**0.05**
11121211112	3 (5%)	4 (5%)	1 [b]
11121212111	**5 (8%)**	**0**	**0.014 [b]**
11121212121	6 (9%)	8 (9%)	1
11121221111	**15 (23%)**	**33 (38%)**	**0.05**
11121221112	**0**	**4 (5%)**	**0.13 [b]**
11211112111	3 (5%)	5 (6%)	1 [b]
11211112121	7 (11%)	6 (7%)	0.4
21111111121	**11 (17%)**	**5 (6%)**	**0.03**

[a] Haplotypes showing a significant or weak association with HIV-SN (p<0.2, bold highlighted) were included in multivariate models (See Table 2).

[b] Fisher's Exact tests