

**TNF block gene variants associated with pain intensity in black Southern Africans with HIV-associated sensory neuropathy**

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## Abstract

*Objectives:* HIV-associated sensory neuropathy (HIV-SN) is a common neurological complication of HIV infection, and often is painful. Tumour necrosis factor (TNF)- $\alpha$  is implicated in neuropathic pain, but associations between neuropathic pain and polymorphisms in the *TNFA* gene have not been identified. The “TNF block” is a region of high linkage disequilibrium within the central major histocompatibility complex that contains several genes involved in the regulation of inflammation, including *TNFA*. Polymorphisms in the block have been associated with altered risk of HIV-SN, but no investigations into whether this region is associated with the painful symptoms of neuropathy have been undertaken. Therefore, we investigated whether polymorphisms in the TNF block are associated with pain intensity in black Southern Africans with HIV-SN.

*Methods:* Single nucleotide polymorphisms (SNPs) defining TNF block haplotypes and African-specific tagSNPs were genotyped in samples from 150 black Southern Africans with HIV-SN.

*Results:* One SNP allele, rs28445017\*A, was significantly associated with increased pain intensity after correction for age, sex and CD4 T-cell count. A common 3-SNP haplotype containing rs28445017\*G remained associated with reduced pain intensity after correction for covariates and multiple comparisons.

*Discussion:* We identified a novel genetic associations between polymorphisms in the TNF block and pain intensity in black Southern Africans with HIV-SN. Our study implicates rs28445017 in painful HIV-SN, although its precise role and whether it may be causative is unclear. rs28445017 was not associated with risk for HIV-SN as such, highlighting potential differences between the pathophysiology of the neuropathy and the painful features of the neuropathy.

## Keywords:

HIV, neuropathy, neuropathic, pain, TNF- $\alpha$ , African

## Introduction

HIV-associated sensory neuropathy (HIV-SN) is a common neurological complication of HIV infection and its treatment, affecting between 30 and 60% of ambulatory HIV-infected individuals [1]. Most affected individuals experience painful symptoms, which decrease quality of life, with increasing pain intensity associated with greater disability [2-5]. Despite the high prevalence of painful HIV-SN, there are no proven treatments for the pain [6, 7].

Multiple lines of evidence implicate tumour necrosis factor (TNF)- $\alpha$  in the pathogenesis of neuropathic pain, including that associated with HIV-SN [for review 1, 8]. For example, in rodent models of HIV-SN, direct application of HIV viral coat protein gp120 to the sciatic nerve or systemic administration of the antiretroviral drug zalcitabine induced mechanical allodynia and increased expression of TNF $\alpha$  mRNA in the spinal cord [9, 10]. In both models, knockdown of TNF $\alpha$  reduced the allodynia. TNF $\alpha$  was also increased at the site of application of gp120 to rat sciatic nerves [11].

The *TNFA* gene resides on chromosome six in the central major histocompatibility complex (MHC). To date, no studies have identified a link between polymorphisms in *TNFA* and neuropathic pain, but these studies generally were limited to *TNFA* SNPs, such as TNF-308, with known associations with other immune diseases [for review 12]. Complex linkage disequilibrium within the central MHC, which contains many immune-related genes with polymorphisms associated with inflammatory disorders [13-15], gives cause to study polymorphisms in the broader central MHC rather than *TNFA* in isolation [16]. Indeed, we have shown that novel SNPs and haplotypes spanning an ~ 60kb region of the central MHC termed the “TNF block” associated with altered risk of HIV-SN in different population groups [17, 18]. While these studies identified risk factors for developing neuropathy, they did not investigate whether polymorphisms in the TNF block were associated with pain in individuals who had HIV-SN. Painful HIV-SN significantly impairs quality of life [2, 5] and there are no proven treatments for the pain [6, 7], so it is important to identify risk factors associated with pain in this neuropathy, and potential therapeutic targets. Therefore we assessed the broader TNF block in this exploratory analysis of genetic associations with pain intensity in black Southern Africans with HIV-SN.

## Materials and Methods

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa (protocols M080220 and M110754), and written, informed consent was obtained from all participants. An interpreter fluent in English and local African languages facilitated the consent process.

*Population* (see Figure, Supplemental Digital Content 1)

In this study we report on a subset of 150 individuals from a larger cohort of 342 black Southern African HIV-positive out-patients who met the inclusion criteria and genetic quality control criteria for a study on HIV-SN susceptibility at the Virology Clinic of the Charlotte

Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa between July 2008 and April 2009. All 342 participants in the original cohort were  $\geq 18$  years old, and had been on combination antiretroviral therapy (ART) for at least six months. Most (98%) had received stavudine (d4T). The clinical, demographic and genetic associations with neuropathy (but not pain intensity) in the full cohort of 342 individuals have been published, with increasing age and height associated with increased risk of HIV-SN, and carriage of rs11796\*A, rs3130059\*G, rs2071594\*C, rs2071592\*A, rs2071591\*A, rs909253\*G, rs1041981\*C in the TNF block associated with reduced risk of having the neuropathy [4, 18].

Of the 342 patients, 190 (56%) had HIV-SN (*see Phenotyping procedures*). Of these 190 patients, 144 (76%) reported having pain as a current symptom, whilst 15 individuals were pain-free. Thirty-one patients (16%) had a history of previous pain in the lower limbs, but were pain free at recruitment, and were excluded from this analysis. One-hundred and fifty (out of the possible 159) individuals were included in the final analyses following quality control of the data (one individual without pain and 8 with pain were removed, *see Quality control*).

#### *Phenotyping procedures*

The study cohort included patients screened for neuropathy using the AIDS Clinical Trials Group (ACTG) Brief Peripheral Neuropathy Screen, a validated tool for identifying symptomatic HIV-SN [19]. Individuals recorded as having symptomatic HIV-SN had bilateral presence of at least one symptom (pain, aching or burning; numbness; pins-and-needles) and at least one clinical sign (reduced vibration sense or absent ankle reflexes). Vibration sense was assessed using a 128Hz tuning fork placed on the interphalangeal joint of each great toe;  $<10$  seconds was considered abnormal. Individuals with other risks for neuropathy (e.g. alcoholism, vitamin B12 deficiency, chemotherapy) were excluded. The intensity of pain was recorded in those individuals with HIV-SN. Pain intensity was rated on an 11-point numerical scale where '0' represented no pain and '10' represented the worst pain they could imagine.

#### *SNP selection* (see Figure, Supplemental Digital Content 2)

Thirty-one SNPs that define the *FV* haplotypes spanning the TNF block [16] were genotyped. These SNPs were supplemented with African-specific tagSNPs, using the Yoruba in Ibadan, Nigeria (YRI) dataset from the International HapMap project (HapMap Data Release 27, Phase II+III, February 2009, on the National Center for Biotechnology Information (NCBI) B36 assembly, dbSNP b126) [20], which was the best proxy African genome dataset publicly available at the time.

A list of tagSNPs was generated using Haploview (version 4.2) [21] using a pairwise approach at  $r^2 = 1.0$  and with minor allele frequency (MAF)  $> 0.01$ . The list was refined using the Illumina® Assay Design Tool (Illumina, Technical Note: DNA Analysis), to eliminate SNPs that could not be genotyped. The tagSNP selection procedure produced 46 SNPs, 12 of

which were amongst the 31 SNPs that define the original *FV* haplotypes. Three additional SNPs (rs1128640, rs3093661 and rs909253) were included based on a review of the literature. Two SNPs were monomorphic in our Southern African samples (rs2239707 and rs3093982) and two failed genotyping (rs2516478 and rs2523502) and were excluded, leaving 64 SNPs to be considered. Alleles 1 and 2 of the 31 *FV* SNPs were assigned based on whites, with allele 1 being the major allele and allele 2 the minor allele. Alleles of the new SNPs were assigned from the forward (+) strand of the genome assembly obtained using BioMart (Ensembl release 67, May 2012) [22, 23]. Details of all SNPs are tabulated in Supplemental Digital Content 3.

### *Genotyping*

DNA was extracted from saliva samples using the QIAamp DNA mini kit (QIAGEN; Valencia, CA, USA) or from blood using a salting-out method [24]. SNPs were genotyped using the GoldenGate® assay on the Illumina BeadXpress™ genotyping platform (Illumina, San Diego, CA, USA). Initial genotyping of three SNPs (rs3179003, rs2516478 and rs2523502) failed. rs3179003 was re-genotyped using a Taqman® SNP genotyping assay (Applied Biosystems, Foster City, CA, USA). DNA extraction and genotyping were carried out in the Division of Human Genetics, National Health Laboratory Services & University of the Witwatersrand (Johannesburg, South Africa).

### *Quality control*

Raw genotype data were examined using the genotyping module of BeadStudio (Framework version 3.1.3.0; module version 3.2.32). Data quality was assessed using Illumina-designed built-in assay controls and samples failing more than two such controls (out of five) were excluded. Data not meeting the following criteria were also excluded: MAF<0.01, SNP missingness rate>0.04, individual missingness rate>0.05, and Hardy–Weinberg equilibrium (HWE)<1x10<sup>-4</sup>. Seventeen SNPs (eight with low MAF, seven with excess SNP missingness and two failing HWE) and nine individuals were excluded leaving 150 individuals (see Figure, Supplemental Digital Content 1) and 47 SNPs (see Figure, Supplemental Digital Content 2).

### *Statistical analyses*

Statistical analyses were carried out using PLINK [25, 26]. As pain intensity is a continuous variable, only an allelic test was carried out to assess the association between the alleles present and reported pain intensity. Multivariate analyses correcting for age, sex and CD4 T-cell count (factors previously associated with risk of pain or pain intensity) [27, 28] were performed using linear regression. These analyses were chosen having ascertained that the data were normal and linear.

Univariate and multivariate analyses, correcting for age, sex and CD4 T-cell count, were also conducted for haplotype association analysis. Because this was an exploratory analysis, haplotypes were constructed using the sliding window approach in PLINK to assess

consecutive 3-SNP combinations for association with pain intensity, moving one SNP along each time. 61-SNP *FV* haplotypes used to describe the region in relation to HIV-SN risk were also visualised.

We used empirically calculated p-values ( $P_{EMP}$ ) in all analyses, using 1000 permutations in each case. We report  $P_{EMP1}$  (uncorrected for family-wise type I error) and  $P_{EMP2}$  (corrected for family-wise type I error rate). SNPs and haplotypes achieving a  $P_{EMP1} < 0.05$  on univariate analysis were included in multivariate analyses that included other known risk factors for pain. Cohen's *d* (difference between two means divided by standard deviation) values were calculated to estimate the effect size of SNPs associated with pain intensity on univariate analysis ( $P_{EMP1} < 0.05$ ).

## Results

Of the 150 individuals included in the final analyses, 78% ( $n = 117$ ) were female, the mean (SD) age was 41 (8.2) and median CD4 T-cell count was 400 cells/ $\mu$ l (range 81-1091) at the time of assessment. Ninety-eight percent (147/150) were treated with stavudine-based (d4T) ART regimens. The remaining three patients had tenofovir-based ART. All three individuals not exposed to stavudine had painful SN and were all homozygous for the major alleles at rs28445017 and rs2857605, described below. When these individuals were excluded from the analyses, the observed associations between host genetics and pain intensity remained.

Univariate SNP analysis identified a single SNP (rs28445017) associated with altered pain intensity. Carriage of the minor allele of rs28445017\*A (MAF = 0.037) was associated with increased pain intensity ( $P_{EMP1} = 0.035$ ; Figure 1), but not after the p-value was adjusted to correct for the family-wise type I error rate ( $P_{EMP2} = 0.564$ ). Nevertheless, the association with rs28445017 was such that mean pain intensity of carriers of at least one A allele was approximately 30% greater than that of individuals homozygous for the G allele, and assessment of effect size using Cohen's *d*, yielded a value of 0.65, which indicates a moderate effect size. In multivariate analysis of rs28445017, the association between the SNP and pain intensity was retained after correcting for age, sex and CD4 T-cell count ( $P_{EMP1} = 0.045$ ). Note that for the multivariate analysis, only rs28445017 was included in the analysis (only SNP with  $P_{EMP1} < 0.05$  on univariate analysis), so no correction was made for multiple comparisons ( $P_{EMP2} = P_{EMP1} = 0.045$ ).

Haplotype analysis detected six three-SNP haplotype combinations that were associated with pain intensity on univariate and multivariate analysis (Table 1). All but one of these haplotypes spanned rs28445017. The most common haplotype (rs2857605\*T-rs2230365\*C-rs28445017\*G) remained associated with decreased pain intensity following correction for covariates and multiple comparisons ( $P_{EMP2} = 0.048$ ). Inspection of the haplotypes indicated that the combination of a T allele at rs2857605 and a G allele at rs28445017 was associated with reduced pain. No individuals carried the haplotype combination of a C allele at rs2857605 and an A allele at rs28445017 to allow direct



comparison of pain intensity and measurement of effect size between carriers of TCG (rs2857605-rs2230365-rs28445017) and CCA haplotypes.

Examination of the *FV* haplotypes investigated in our study of HIV-SN risk [18] revealed that only one haplotype (*FV*20,21\_ext1) contained the A allele at SNP rs28445017, with all other haplotypes containing a G allele at this SNP (Figure, Supplemental Digital Content 4). The average pain score across the 150 individuals with at least one copy of the *FV*20,21\_ext1 haplotype is seven compared to an average pain score of five for all individuals without the haplotype. *FV*20,21\_ext1 carries no other unique alleles so rs28445017\*A could increase pain intensity.

## Discussion

This is the first assessment of genetic associations between polymorphisms in the TNF block of the central MHC and intensity of neuropathic pain. One allele (rs28445017\*A) associated with increased pain intensity in black Southern Africans with HIV-SN. Several three-SNP haplotypes spanning rs28445017 also associated with altered pain intensity after correcting for factors that may influence pain sensitivity in this cohort (age, sex and CD4 T-cell count). The most common 3-SNP haplotype uniquely contained rs28445017\*G and rs2857605\*T and was associated with lower pain intensity, even after correction for multiple comparisons. The combination of these alleles may thus offer “protection” against increased pain intensity in HIV-SN. Although rs2857605 has no independent association with pain intensity ( $P_{\text{EMPI}} = 0.10$ ), it has been implicated in genetic risk in whites for Non-Hodgkin Lymphoma and bortezomib-induced peripheral neuropathy in multiple myeloma patients [29, 30]. Importantly, SNPs associated with altered risk of HIV-SN (rs11796\*A, rs3130059\*G, rs2071594\*C, rs2071592\*A, rs2071591\*A, rs909253\*G, rs1041981\*C) [18] did not associate with pain intensity. Indeed, the presence of a peripheral polyneuropathy does not mean that the affected individual will have pain (~50% of people with diabetic polyneuropathy [31] and ~75% of individuals with HIV-SN have pain [32]), so the pathogeneses of SN and pain associated with SN are distinct.

The role of rs28445017 is unclear. The SNP is intergenic, lying 3' of *NFKBIL1*, a gene with no known function. Being intergenic, it is unlikely that the polymorphism affects protein structure or function directly. Input of the SNP ID into RegulomeDB [33], revealed no evidence to suggest that the SNP disrupts the binding of transcription factors). Hence we postulate that the SNP and its associated haplotypes may mark causative SNPs within the TNF block. Whilst two other *NFKBIL1* SNPs associated with risk of HIV-SN, we could draw no conclusions regarding causality [18]. One SNP tagged by rs28445017 in the YRI population lies in the 3'UTR of *TNFA* (rs3093666) and another is in the 3'UTR of *LTB* (rs3093556). Whether these SNPs (both ungenotyped in this study) have any functional significance is unclear. Interestingly, rs28445017 is monomorphic in several non-African populations (Europeans, Chinese and Japanese), and the minor allele frequency is very low in certain African populations (African Americans: MAF = 0.008, Luhya in Kenya: MAF =

0.005) [34, 35]. Thus the association with rs28445017 and its associated haplotypes presented here may be unique to Southern Africans (MAF = 0.037 in our cohort). Indeed, unique associations in this population are not unusual, as novel associations in *KCNS1* with pain intensity [36] and polymorphisms in the TNF block with risk of HIV-SN [18] were identified in our cohort. Such variations between populations highlight the need for genetic studies of diverse populations, with the genotyping tailored to the population being studied.

Like other genetic association studies investigating *TNFA* and pain [37, 38], and a recent small genome-wide association study related to risk of HIV-SN [39], we found no associations within *TNFA* itself. Due to the small sample and exploratory nature of the analyses, we chose a simple sliding window method for assessing haplotypes to detect a signal, instead of using the extended 61-SNP haplotypes we previously described [18]. Nevertheless, we identified an area of strong signal around the rs28445017 SNP in relation to pain intensity of HIV-SN. A visual assessment of *FV20,21\_ext1* then showed that no alleles of *TNFA* that we genotyped were uniquely marked by the A allele at rs28445017 (Figure, Supplemental Digital Content 4). This suggests that *TNFA* may not influence pain intensity of HIV-SN.

Our screening for symptomatic HIV-SN relied solely on a validated screening tool, the ACTG Brief Neuropathy Screening Tool [19], with no deep phenotyping of pain quality or nerve function (e.g., nerve conduction studies, intra-epidermal nerve fiber density, quantitative sensory testing). Deep phenotyping should be considered in future studies to draw conclusions about, for example, the association between the severity of nerve damage, pain intensity and the genetic region under investigation.

In conclusion, we found a SNP (rs28445017) and an associated haplotype in the TNF block associated with pain intensity in black Southern Africans with HIV-SN. The role of rs28445017 is uncertain, and due to the strong LD in the TNF block, rs28445017 likely tags causative SNPs not tested here. Thus the region requires further investigation. As the SNP and haplotype associated with pain intensity differ from those associated with risk of SN, we demonstrate differences in the pathophysiology of SN and pain in SN.

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## Disclosures

The authors declare no conflicts of interest.

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### Legends to Figures and Tables

Table 1 - Several three-SNP haplotypes associate with pain intensity.

Figure 1 – Mean (SD) pain intensity ratings were significantly different between individuals with the GG and GA genotypes for SNP rs28445017.

Supplemental Digital Content 1 – Patient recruitment and exclusion diagram. Selection of the 150 individuals included in this study from 482 individuals screened for eligibility for a study on risk factors for having HIV-SN [4, 18].

Supplemental Digital Content 2 – SNP selection and exclusion diagram.

Supplemental Digital Content 3 – SNPs included in the analysis. The selection contains previously assessed SNPs and population-appropriate tagSNPs selected from the Yoruba in Nigeria (YRI, HapMap)

Supplemental Digital Content 4 – *FV* haplotype diagram with *FV*20,21\_ext 1 and the A allele of rs28445017 highlighted.

Table 1

**Table 1. Several three-SNP haplotypes associate with pain intensity.**

HAPLOTYPE	HAPLOTYPE FREQUENCIES	UNIVARIATE		MULTIVARIATE			
		PEMP1	BETA *	PEMP1	PEMP2	T	BETA (95% CI)
H1	0.02	0.048	2.59	0.040	0.453	4.68	2.88 (0.27, 5.49)
H2	0.04	0.048	2.05	0.039	0.569	4.06	2.04 (0.06, 4.02)
H3	0.02	0.048	2.59	0.041	0.450	4.69	2.89 (0.27, 5.51)
<b>H4</b>	<b>0.92</b>	<b>0.003</b>	<b>-1.93</b>	<b>0.005</b>	<b>0.048</b>	<b>9.40</b>	<b>-2.02</b> (-3.31, -0.73)
H5	0.03	0.029	2.30	0.028	0.489	4.45	2.28 (0.16, 4.40)
H6	0.03	0.029	2.29	0.029	0.494	4.44	2.28 (0.16, 4.40)

The haplotypes were generated using the sliding window approach.

H1: rs6929796\*G-rs2857605\*C-rs2230365\*C

H2: rs2857605\*T-rs2230365\*C-rs28445017\*A

H3: rs2857605\*C-rs2230365\*C-rs28445017\*G

H4: rs2857605\*T-rs2230365\*C-rs28445017\*G

H5: rs2230365\*C-rs28445017\*A-rs4947324\*C

H6: rs28445017\*A-rs4947324\*C-rs2516390\*T

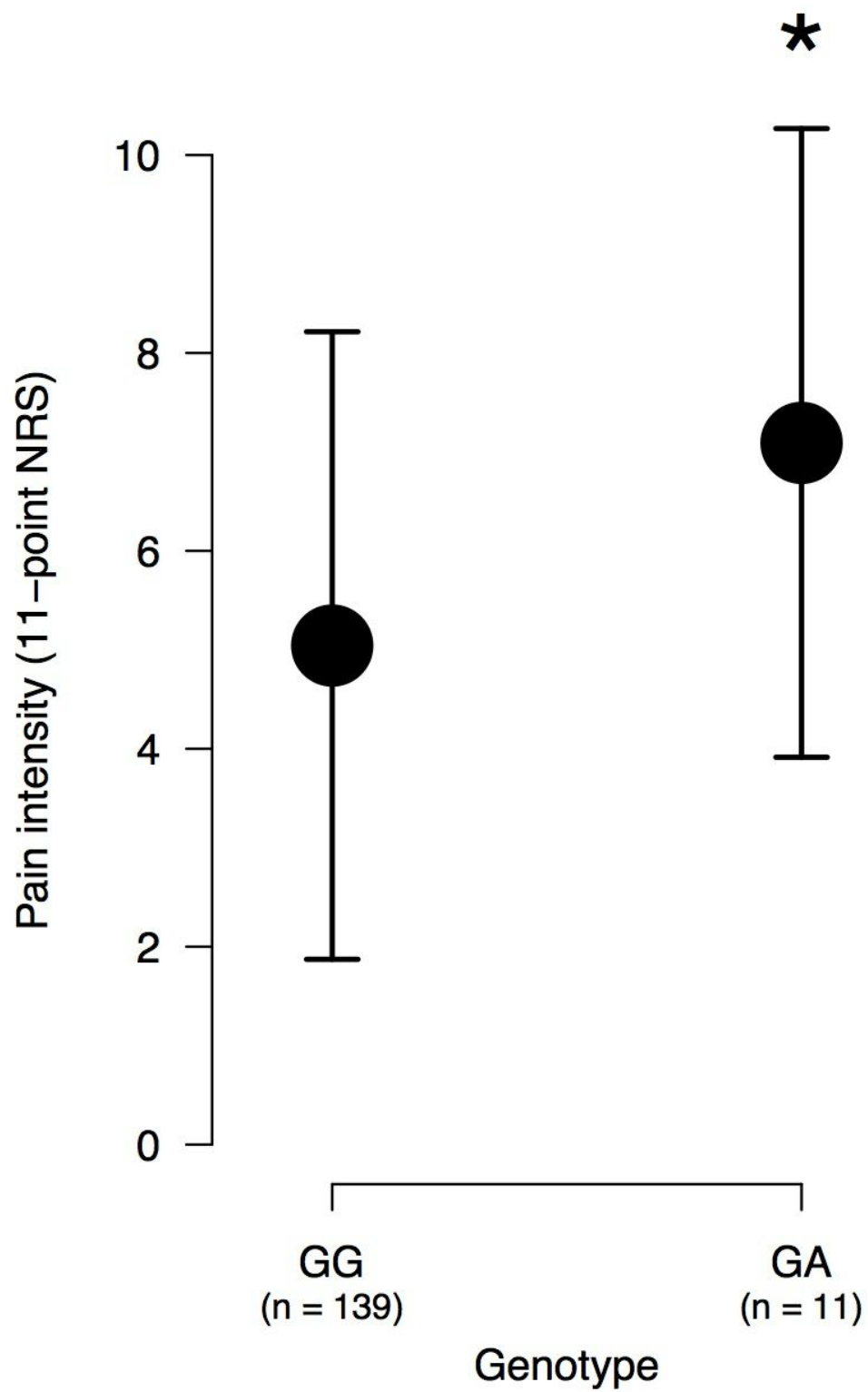
The haplotype in bold is significantly associated with reduced pain intensity after correction for age, sex and CD4 T-cell count and multiple comparisons. All other haplotypes are only significant after correction for age, sex and CD4 T-cell count.

P<sub>EMP1</sub>: pointwise empirical uncorrected p-value.

P<sub>EMP2</sub>: Family-wise correction for multiple comparisons.

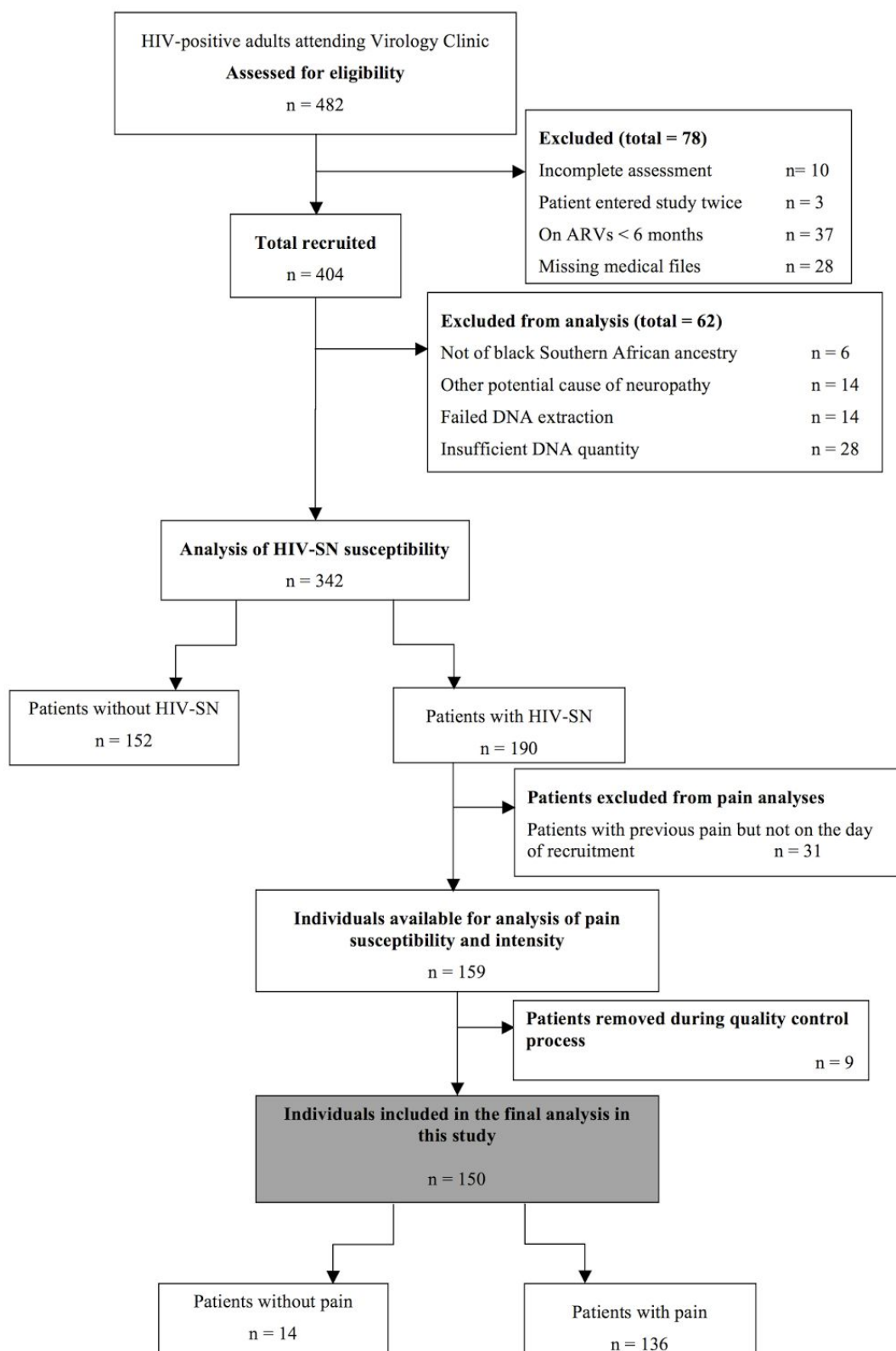
\* A positive beta value indicates increased pain intensity and a negative beta value indicates decreased pain intensity.

Figure 1

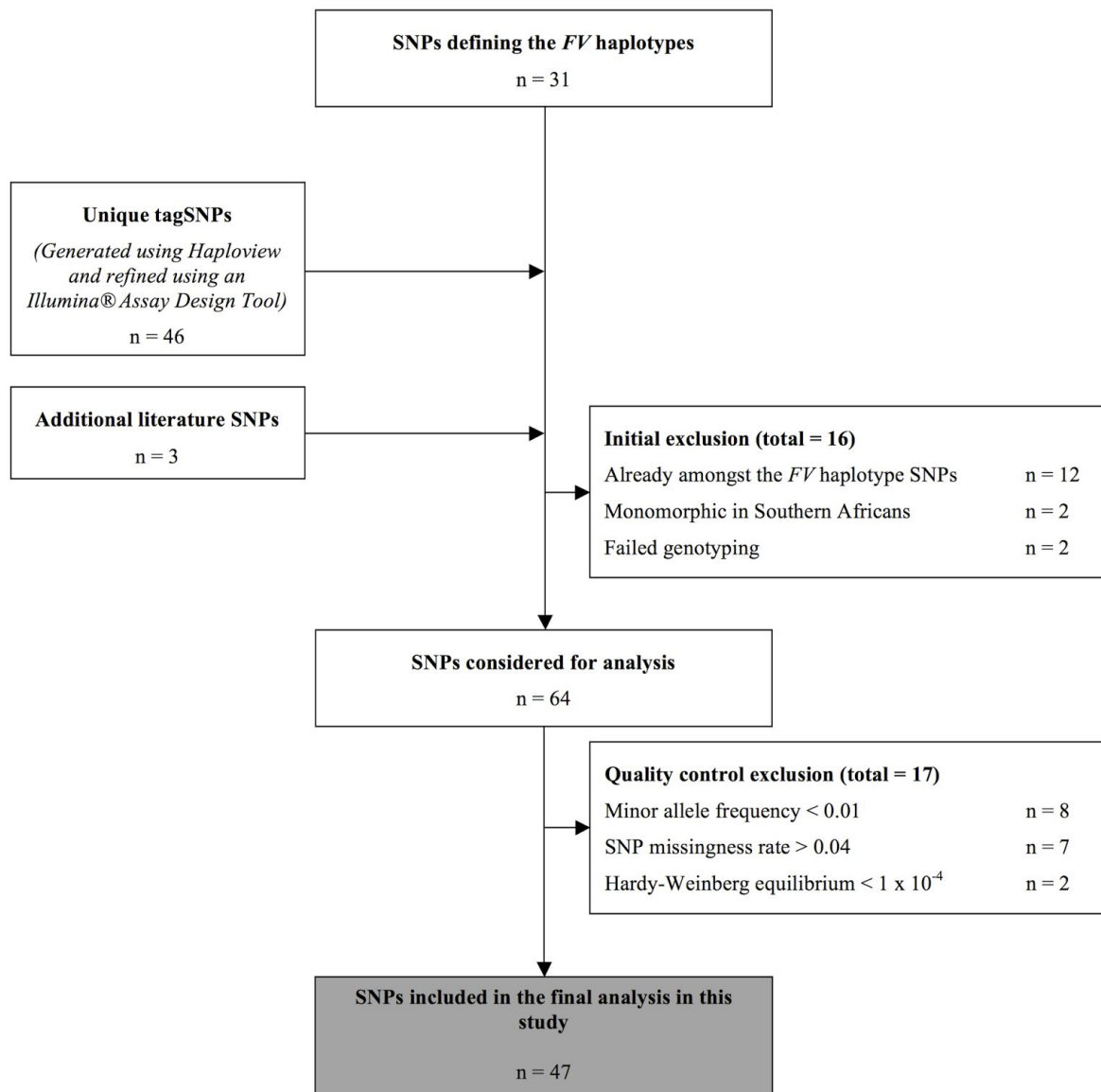




Supplemental Digital Content 1



Supplemental Digital Content 2



## Supplemental Digital Content 3

Table, Supplemental Digital Content 3. SNPs included in the analysis. The selection contains previously assessed SNPs and population-appropriate tagSNPs selected from the Yoruba in Nigeria (YRI, HapMap)

rs ID	Gene	Common name	Chromosome position (bp)	Alleles		Minor allele (our sample)	Genotype frequencies (counts)†			MAF (our sample)
				1	2		1 1	1 2	2 2	
rs2734586	<i>MCCD1</i>	-	31497342	C	G	G	0.76 (113)	0.23 (34)	0.01 (2)	0.13
rs3130055 *	<i>MCCD1</i>	-	31497399	T	C	C	0.89 (133)	0.11 (17)	0.00 (0)	0.06
rs2075582	<i>MCCD1</i>	-	31497706	T	C	C	0.82 (123)	0.18 (27)	0.00 (0)	0.09
rs3130056	<i>BATI</i>	-	31499354	T	C	C	0.90 (134)	0.10 (15)	0.00 (0)	0.05
rs11796	<i>BATI</i>	-	31501212	A	T	A	0.11 (16)	0.40 (59)	0.50 (74)	0.31
rs1055388 *	<i>BATI</i>	-	31501737	T	C	C	0.81 (122)	0.19 (28)	0.00 (0)	0.09
rs2734583	<i>BATI</i>	-	31505480	A	G	G	0.71 (107)	0.27 (40)	0.02 (3)	0.15
rs2516393 *	<i>BATI</i>	-	31506744	C	A	A	0.90 (131)	0.10 (15)	0.00 (0)	0.05
rs2239709	<i>BATI</i>	-	31507447	T	C	T	0.00 (0)	0.10 (15)	0.90 (132)	0.05
rs11757236	<i>BATI</i>	-	31508029	T	C	T	0.00 (0)	0.05 (7)	0.95 (143)	0.02
rs3130059 *	<i>BATI</i>	-	31509284	G	C	G	0.10 (15)	0.39 (59)	0.51 (76)	0.30
rs2239528 *	<i>BATI</i>	BAT1-348	31510105	G	A	A	0.82 (123)	0.18 (27)	0.00 (0)	0.09
rs2523504 *	<i>Intergenic</i>	BAT1-1101	31510858	C	T	T	0.76 (114)	0.23 (35)	<0.01 (1)	0.12
rs2844509 *	<i>Intergenic</i>	BAT1-1167	31510924	A	G	G	0.82 (120)	0.17 (25)	0.01 (2)	0.10
rs9267487	<i>Intergenic</i>	-	31511350	T	C	C	0.87 (130)	0.12 (18)	<0.01 (1)	0.07
rs2251824 *	<i>Intergenic</i>	-	31511857	G	A	A	0.79 (119)	0.19 (29)	0.01 (2)	0.11
rs2071594 *	<i>ATP6V1G2</i>	-	31512720	G	C	C	0.50 (75)	0.40 (60)	0.09 (14)	0.30
rs2071593 *	<i>ATP6V1G2</i>	-	31512799	G	A	A	0.91 (137)	0.09 (13)	0.00 (0)	0.04
rs2239705	<i>ATP6V1G2</i>	-	31513402	A	G	A	<0.01 (1)	0.02 (3)	0.97 (145)	0.02
rs2071592 *	<i>NFKBIL1</i>	NFKBIL1-62	31515340	T	A	A	0.49 (74)	0.41 (61)	0.10 (15)	0.30
rs2071591 *	<i>NFKBIL1</i>	-	31515799	G	A	A	0.50 (75)	0.41 (61)	0.09 (14)	0.30
rs6929796	<i>NFKBIL1</i>	-	31522669	A	G	A	0.07 (11)	0.36 (53)	0.57 (84)	0.25
rs2857605 *	<i>NFKBIL1</i>	-	31524851	T	C	C	0.95 (142)	0.05 (7)	<0.01 (1)	0.03
rs2230365 *	<i>NFKBIL1</i>	NFKBIL1+446	31525448	C	T	T	0.95 (142)	0.05 (8)	0.00 (0)	0.03
rs28445017	<i>NFKBIL1</i>	-	31527756	A	G	A	0.00 (0)	0.07 (11)	0.93 (139)	0.04
rs4947324 *	<i>Intergenic</i>	-	31528130	C	T	T	0.82 (123)	0.17 (25)	0.01 (2)	0.10
rs2516390	<i>Intergenic</i>	-	31529883	T	C	C	0.87 (129)	0.13 (19)	<0.01 (1)	0.07
rs2516312 *	<i>Intergenic</i>	LTA-1085	31539435	A	G	G	0.94 (141)	0.06 (9)	0.00 (0)	0.03
rs2844482	<i>Intergenic</i>	-	31539767	T	C	T	0.01 (2)	0.23 (35)	0.75 (113)	0.13
rs2071590 *	<i>Intergenic</i>	LTA-752	31539768	G	A	A	0.89 (132)	0.09 (13)	0.03 (4)	0.07
rs2239704	<i>LTA</i>	-	31540141	C	A	A	0.87 (130)	0.12 (18)	<0.01 (1)	0.07
rs909253	<i>LTA</i>	LTA+252	31540313	A	G	G	0.50 (73)	0.40 (58)	0.10 (15)	0.30
rs2229094	<i>LTA</i>	-	31540556	T	C	C	0.57 (86)	0.38 (57)	0.05 (7)	0.24
rs1041981	<i>LTA</i>	-	31540784	C	A	C	0.10 (15)	0.41 (60)	0.49 (73)	0.30
rs3093544	<i>LTA</i>	-	31541779	A	G	G	0.99 (147)	0.00 (0)	0.01 (2)	0.01
rs1799964 *	<i>Intergenic</i>	TNFA-1031	31542308	T	C	C	0.66 (99)	0.30 (45)	0.04 (6)	0.19
rs1800630	<i>Intergenic</i>	TNFA-863	31542476	A	C	A	<0.01 (1)	0.20 (30)	0.79 (119)	0.11
rs1800750 *	<i>Intergenic</i>	TNFA-376	31542963	G	A	A	0.92 (138)	0.07 (11)	<0.01 (1)	0.04
rs1800629 *	<i>Intergenic</i>	TNFA-308	31543031	G	A	A	0.69 (100)	0.27 (39)	0.04 (6)	0.18
rs2228088	<i>TNFA</i>	-	31543605	T	G	T	0.00 (0)	0.18 (26)	0.82 (122)	0.09
rs1799769 *	<i>TNFA</i>	TNFA-509	31544027	G	(A)	A	0.99 (148)	<0.01 (1)	0.00 (0)	0.00
rs3093662 *	<i>TNFA</i>	TNFA+671	31544189	A	G	G	0.89 (132)	0.11 (16)	<0.01 (1)	0.06
rs3093665 *	<i>TNFA</i>	TNFA+1873	31545391	A	C	C	0.98 (145)	0.02 (3)	0.00 (0)	0.01
rs3093668 *	<i>Intergenic</i>	-	31546495	G	C	C	0.90 (135)	0.09 (14)	<0.01 (1)	0.05
rs3093671	<i>Intergenic</i>	-	31546980	A	G	A	<0.01 (1)	0.15 (22)	0.85 (126)	0.08
rs7769073	<i>LST1</i>	-	31554607	A	T	A	<0.01 (1)	0.16 (24)	0.83 (125)	0.09
rs1052248 *	<i>NCR3</i>	-	31556581	T	A	A	0.55 (83)	0.35 (52)	0.10 (15)	0.27

† = Genotype frequencies are for the 150 individuals included in the study

\* = SNPs from the previous study that defined the original FV haplotypes [16]

(A) this SNP is an insertion deletion and thus when deleted the non-bracketed allele is replaced by an A allele

## Supplemental Digital Content 4

[illegible]

31-SNP haplotypes spanning the TNF block have previously been derived as *FVY* haplotypes [16] and have been extended to 61-SNP haplotypes in Southern Africans [18]. *FVY* mark unknown alleles in less specific haplotypes derived from non-Southern African populations. *FVY20\_21\_ext1* marked in tan. This was the only haplotype to contain an A allele at rs28445017. rs28445017-A associated here with pain intensity of HIV-SN. No variation can be seen in alleles in *TNF-A* in *FVY20\_21\_ext1* compared to haplotypes which did not contain an A allele at rs28445017.