Published in final edited form as:

AIDS. 2010 July 31; 24(12): 1813–1821. doi:10.1097/QAD.0b013e32833b5256.

A Trim5alpha Exon 2 Polymorphism is Associated with Protection from HIV-1 Infection in Pumwani Sexworker Cohort

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Abstract

Objective—The innate immune component TRIM5 α has the ability to restrict retrovirus infection in a species-specific manner. TRIM5 α of some primate species restricts infection by HIV-1, while huTRIM5 α lacks this specificity. Previous studies have suggested that certain polymorphisms in *huTRIM5* may enhance or impair the proteins affinity for HIV-1. This study investigates the role of *TRIM5* polymorphisms in resistance/susceptibility to HIV-1 within the Pumwani sex worker cohort in Nairobi, Kenya. A group of women within this cohort remain HIV-1 seronegative and PCR negative despite repeated exposure to HIV-1 through active sex work.

Design—A 1 kb fragment of Trim5alpha gene, including exon 2, from 1032 women enrolled in the Pumwani sex worker cohort was amplified and sequenced. SNPs and haplotypes were compared between HIV-1 positive and resistant women.

Methods—The *TRIM5* exon 2 genomic fragment was amplified, sequenced and genotyped. Pypop32-0.6.0 was used to determine SNP and haplotype frequencies and statistical analysis was carried out using SPSS-13.0 for windows.

Results—A *TRIM5* SNP (rs10838525) resulting in the amino acid change from Arginine to Glutamine at codon 136, was enriched in HIV-1 resistant individuals ($p=1.104E-05$; OR:2.991; CI95%:1.806–4.953) and women with 136Q were less likely to seroconvert ($p=0.002$; Log Rank: 12.799). Wild type TRIM5 α exon 2 was associated with susceptibility to HIV-1 ($p=0.006$; OR: 0.279; 95%CI:0.105–0.740) and rapid seroconversion ($p=0.001$; Log Rank: 14.475).

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Contributors

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Philip Lacap: Assisted with data generation, analysis and interpretation, as well as editing of the manuscript.

Jeff Tuff: Assisted with data generation, analysis and interpretation.

Charles Wachihi: Maintained the Pumwani sex worker cohort and was involved in the acquisition of data.

Joshua Kimani: Maintained the Pumwani sex worker cohort and was involved in the acquisition of data.

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Francis A. Plummer: The overall principal investigator. Secured funding for the study. Established and maintained the Pumwani sex worker cohort and was involved in the acquisition of data.

Conclusions—Our findings suggest that a shift from arginine to glutamine at codon 136 in the coiled-coil region of TRIM5 α confers protection against HIV-1 in the Pumwani sex worker cohort.

Keywords

TRIM5 α ; Single nucleotide polymorphism; HIV-1; Sex Workers; Taxonomy-based Sequence Analysis; Disease Association; Disease Resistance

Introduction

According to the World Health Organization, there were 2.1 million deaths due to HIV and AIDS last year alone and close to 35 million people still live with HIV worldwide [1]. There remains no cure for HIV-1 and the development of an effective vaccine continues to elude researchers. While current antiretroviral therapies can greatly extend the life expectancy of those living with HIV/AIDS, such treatments are expensive and remain largely inaccessible in developing countries. With the knowledge of natural immunity to HIV-1 constantly growing, it is becoming clear that intrinsic immune responses play an important role and may hold the key to an effective and accessible prophylactic [2].

A recently discovered gene, *TRIM5* (chromosome 11), has the ability to provide innate protection against retroviruses in the form of TRIM5 α , a splice variant of TRIM5 [3,4,5,6,7]. Studies have shown that TRIM5 α of old world monkeys, such as the Rhesus Macaque, is able to effectively inhibit HIV-1 infection [4,5]. While human TRIM5 α (huTRIM5 α) does not completely inhibit HIV-1 infection, it has shown slight anti-HIV activity and is known to restrict other retroviruses, such as the N-tropic murine-leukemia virus [3,5,6]. TRIM5 α has highly variable regions and restricts retroviral infection in a species-specific manner [3,4,5,6,7,8]. The B30.2 SPRY domain at the C-terminus of TRIM5 α is the most variable region and has been shown to be responsible for capsid recognition and binding [9,10,11,12,13]. The coiled-coil is thought to be involved in TRIM5 α multimerization and is essential for effective retroviral restriction [9,10,12,13,14]. It has been suggested that the RING and B-box domains are non-essential for capsid binding and viral restriction. The term “effector” has been applied to the RING and B-box regions as they enhance TRIM5 α antiretroviral activity through possible interactions with other proteins [9,12,15]. The RING domain has been shown to affect cytoplasmic levels and distribution of TRIM5 α [16] and act as an E3 ubiquitin ligase, targeting the virus for proteasomal degradation [12,15].

Previous studies have shown, with conflicting results, that certain polymorphisms may alter the potency of huTRIM5 α against HIV-1 [3,17,18,19]. Two SNPs in particular have been at the centre of many investigations: a G to A change in rs10838525 (R136Q in exon 2) and a C to T change in rs3740996 (H43Y in exon 2). A study by Javanbakht et al. (2006) found the amino acid changes H43Y and R136Q associated with resistance to HIV-1 infection [18]. Another study showed 136Q associated with increased HIV-1 infection in a European population [3]. Neither of these studies found any association between *TRIM5* SNPs and disease progression, however Van Manen et al. (2008) found 136Q correlated with slower disease progression and 43Y correlated with accelerated progression [17]. These discrepancies suggest a need for further investigation into the effect human *TRIM5* SNPs have on HIV-1 infection. This study set out to characterize genetic variations of TRIM5 α within the Pumwani cohort at exon 2. Exon 2 contains the majority of previously identified nonsynonymous coding SNPs and makes up a large part of the functional protein (Figure 1) [3,18]. This locus was also chosen due to the conflicting results obtained by previous studies concerning the effect of two specific SNPs on HIV-1 infection: H43Y and R136Q [3,17].

For over 20 years the HIV-1 infections of women enrolled in the Pumwani sex worker cohort in Nairobi, Kenya have been closely monitored. Within the cohort, a group of women remain HIV-1 seronegative despite heavy exposure to HIV-1 through active sex work [20]. To study the role of TRIM5 α polymorphisms in this observed natural resistance to HIV-1 infection, 1032 women of the Pumwani cohort were genotyped at exon 2. The SNP and haplotype frequencies in the cohort were investigated and correlations with HIV-1 resistance or susceptibility and disease progression are reported. Our findings suggest that TRIM5 α polymorphisms play an important role in HIV-1 infection and the SNP causing the amino acid change to 136Q might play a key role in determining natural resistance to HIV-1 in the Pumwani sex worker cohort.

Methods

Study Population

The study population consisted of 1032 women enrolled in the Pumwani sex worker cohort, established in 1985 in Nairobi, Kenya. Cohort design and follow-up have been discussed elsewhere [20]. The overall HIV-1 infection rate in the Pumwani Sex Worker Cohort is greater than 73.7%. Women were classified as HIV-1 resistant if they remained HIV seronegative and PCR negative for at least 3 years while continuing active sex work and were negative at the time of this study. The 88 women who were classified as resistant in this study were all enrolled in the cohort before 1999 with an average follow-up time of 9.6+/-4.3 years. The HIV negative women who enrolled later and therefore had a shorter follow-up time were not included in the comparison between resistant women and positive women. HIV-1 negative women enrolled after 2001 were not included in the analysis for resistance or susceptibility to HIV-1 infection and all the HIV-1 infected women were included in the analysis for CD4+ T cell decline to below 200/mm³. Informed consent was obtained from all women enrolled in the cohort. The ethics committees of the University of Manitoba and the University of Nairobi have approved this study.

TRIM5 genotyping

DNA samples were isolated from whole blood and PBMCs using QIAamp DNA Mini Kit (QIAGEN Inc., Mississauga, ON). A sequence based genotyping method was used for TRIM5 exon2 genotyping. TRIM5 exon2 was amplified using a primer pair known to have successfully amplified exon2 of *huTRIM5* in previous studies [3]. PCR products were sequenced and analyzed for nucleotide variations using computer software CodonExpressTM. These nucleotide polymorphisms were recorded and grouped into haplotypes for analysis.

Statistical analysis

TRIM5 single nucleotide polymorphism and haplotype frequencies were determined using PyPop-32-0.6.0. Associations with HIV-1 positive or resistant women in the cohort were analyzed using SPSS 13.0 for Windows. Standard univariate methods such as the Fisher's exact test (*p* value) as well as Pearson chi-square analysis (odds ratio as well as Peto odds ratio, confidence interval 95%) were utilized to determine the relationship between binary outcomes and explanatory variables. Kaplan-Meier analysis with log rank test was used to examine the time to seroconversion for enrollees who were HIV-1 negative at enrolment. Only women enrolled in the cohort prior to 2002 were included in the survival analysis for HIV-1 infection. Since we have not retained blood samples for HLA typing from all women enrolled during the first few years of the project, the probability of being HLA genotyped may depend on an individual's HIV status and duration of follow up, therefore this selection mechanism had to be adjusted. According to the Hurvitz-Thompson theory, observations must be weighted inversely to the probability of being included in a sample to reach an unbiased estimate of what is being investigated [21]. We estimated the probability of being

typed using logistic regression with follow-up and HIV status as covariables. The inverse of this probability for each woman, after standardization, was then used as a sample weight. We generated a weighted parameter using logistic regression taking into account patient enrolment and samples being typed. We used this parameter to adjust for crosstab analyses and to compare the results with/without using this parameter. Associations were confirmed using Binary Logistic Regression (backward wald). SNPs or haplotypes found to be significant at the $P < 0.05$ level were tested for independent significance using Binary Logistic Regression and Cox regression. Kaplan-Meier survival analysis (log rank test) was used to examine time to seroconversion and significant correlations were verified by Cox regression analysis. P-values were adjusted for multiple comparisons by means of the Bonferroni method using an SPSS syntax created by David Nichols of SPSS.

Results

***TRIM5* SNPs identified in the Pumwani Sex Worker Cohort**

Thirteen SNPs within the 1 kb genomic fragment containing exon 2 of *TRIM5* were identified in the Pumwani Sex Worker Cohort (Table 1). Among them, 9 SNPs are within exon 2 (7 non-synonymous and 2 synonymous), 3 SNPs are in intron 2, and 1 SNP is in the 5' UTR. Of the coding SNPs identified, 3 are located in the RING, 3 in the B-box and 2 in the coiled-coil domains (Figure 1). Four of the 13 SNPs observed in this study had not been previously identified; these polymorphisms appeared at very low frequencies ($< 1\%$). All 4 new SNPs were identified within exon 2 of *TRIM5α* at bases 404 (codon 49), 505 (codon 83), 616 (codon 120) and 713 (codon 152) of the mRNA (Figure 2). All sequences identified with novel SNPs can be accessed through Genbank (accession numbers FJ561487 to FJ561496).

***TRIM5* SNP and exon 2 haplotype frequencies**

The only *TRIM5α* polymorphism found to be present in more than 5% of the population corresponded to the R136Q amino acid change. The three most common *TRIM5α* SNPs in the Pumwani sex worker cohort are those resulting in the amino acid changes R136Q (10.8%), H43Y (4.84%) and V112F (1.21%).

SNP distributions among different populations were compared using data provided by the Hapmap project. The three most common SNPs in the Pumwani Cohort (2n=2046) were rs10838525 (R136Q), rs3740996 (H43Y) and rs11601507 (V112F). These frequencies were compared to a Yoruban population from Nigeria (YRI; 2n=120), Chinese (CHB; 2n=90) and Japanese (JPN; 2n=88) populations and a Caucasian population (CEU; 2n=120). The SNP distributions of the Pumwani cohort and the Nigerian population are quite similar with the Nigerian population showing the following frequencies: rs10838525 at 8.30%, rs3740996 at 6.70% and rs11601507 at 0%. There are substantial differences between the *TRIM5α* SNP frequencies in the Pumwani cohort and those in the Caucasian and Asian populations. The Caucasian population showed higher percentages of SNPs with 34.20% rs10838525, 13.30% rs3740996 and 10.50% rs11601507. *TRIM5* SNP distributions in the Chinese and Japanese populations also displayed variation with frequencies as follows: rs10838525 at 4.40% (CHB) and 2.30% (JPN), rs3740996 at 12.20% (CHB) and 19.30% (JPN) and rs11601507 at 12.50% (CHB) and 9.30% (JPN).

We analyzed *TRIM5α* exon 2 haplotypes observed in the Pumwani sex worker cohort. A total of 12 different haplotypes were observed based on the variations of exon 2 sequences (Table 2). Only SNPs within exon 2 were included in *TRIM5α* haplotypes. Intron SNPs were excluded since no reliable pattern of association was observed between these SNPs and those within the exon haplotypes. Wild type *TRIM5* was the most common haplotype,

presenting in 82.5% of the population. The three most common haplotypes were new2 (containing 136Q) at 10.4%, new1 (containing 43Y) at 4.4% and new4 (containing 112F) at 1.2%.

Association of *TRIM5α* SNPs and haplotypes with resistance or susceptibility to HIV-1 infection

TRIM5 SNPs were tested to identify associations with susceptibility or resistance to infection by HIV-1 using crosstab analysis. Most SNPs were identified at a frequency well below 3% and did not show any significant correlations. The 2 SNPs identified at frequencies above 3% were SNPs rs3740996/T (43Y) at 4.84% and rs10838525/A (136Q) at 10.8%. The SNP rs3740996/T (43Y), enriched in HIV-1 negative individuals in previous studies [18], was evenly distributed between HIV-1 positive and resistant women in our cohort. While studies have shown a SNP causing the amino acid change H43Y to be associated with protection against HIV-1 [18], no correlation was observed in the Pumwani cohort (Table 1). The SNP induced amino acid change R136Q had been associated with both protection [18] and susceptibility [3] to HIV-1 in previous studies. A significant difference in the allele and phenotype frequency distributions of this SNP between HIV-1 positive and resistant women was observed in the Pumwani cohort. R136Q is strongly correlated with resistance to HIV-1 infection with an adjusted P-value of 1.325E-04 (bonferroni) and odds ratio of 2.991 (Table 1). These results were upheld through analysis with Binary Logistic regression (Table 3a).

TRIM5 haplotypes were also analyzed for any associations with HIV-1 resistance or susceptibility (Table 2). Haplotype new2 was enriched in resistant women (34.1%) when compared to HIV-1 positive women (14.7%) with an adjusted P-value of 1.480E-04 and odds ratio of 2.991. Binary Logistic regression confirmed these results (Table 3). Most observed haplotypes were exceedingly rare which made it difficult to determine if any significant distribution differences existed between the two groups of women. Alternatively, the effect of haplotypes as a whole was investigated by combining all individuals with polymorphic *TRIM5α* as compared to individuals with wild type *TRIM5α*. Indeed, wild type *TRIM5α* was more common in HIV-1 positive individuals (97.6%) than resistant individuals (92.0%), suggesting that any polymorphism may be advantageous. These associations remained significant after correcting for multiple comparisons (Table 2). Sensitivity analysis, using weighted analysis yielded associations that were consistent with the results obtained using non-weighted analyses.

Association of *TRIM5* SNPs and haplotypes with reduced or increased risk of HIV-1 seroconversion

The influence of *TRIM5* SNPs and haplotypes on the risk of seroconversion was assessed by Kaplan Meier survival analysis. Most SNPs and haplotypes appeared at frequencies below 3% and did not reveal any significant associations. The SNP conferring an amino acid change H43Y and exon 2 haplotype new1 were shown to have no effect on the risk of seroconversion (Figure 2A, 2C). 136Q and haplotype new2 significantly decreased the risk of seroconversion (Figure 2B, 2D). Women who are homozygous for 136Q, or haplotype new2, had not yet seroconverted at the time of this study (Figure 2B, 2D). These significant associations were also supported by Cox regression analysis (Table 3).

To analyze the combined effect of *TRIM5α* polymorphisms on seroconversion, a Kaplan Meier survival plot was generated for wild type *TRIM5α*. Women who are homozygous for wild type *TRIM5α* showed an increased risk of seroconversion when compared to women who are heterozygous for wild type *TRIM5α* or have no wild type *TRIM5α* (Figure 2E).

Association of *TRIM5* SNPs and haplotypes with disease progression

A previous study had discovered an association between SNP H43Y and accelerated disease progression [17]. Using Kaplan Meier survival analysis, HIV-1 positive women with various SNPs and haplotypes were compared based on the number of days with CD4 counts above 200/mm³ (data not shown). No significant correlations were found between any individual SNPs or haplotypes and the rate of disease progression.

Discussion

The long term monitoring of women in the Pumwani sex worker cohort, particularly the highly exposed persistently seronegative individuals, provides an excellent opportunity to study the natural resistance to HIV-1. Through analysis of *TRIM5α* exon 2 sequences of 88 HIV-1 resistant and 468 HIV-1 infected sex workers in the Pumwani Sex Worker Cohort, we have identified a strong correlation between the SNP causing the amino acid change 136Q in the coiled-coil domain (Table 1) and HIV-1 resistance. No significant associations were found between other *TRIM5* SNPs in the region examined and HIV-1 resistance/susceptibility or disease progression.

The haplotype new2, with only one nucleotide difference from the wild type (R136Q), also significantly associated with HIV-1 resistance (Table 2). Women who were HIV-1 negative at cohort enrolment and have 136Q and haplotype new2 seroconverted significantly slower than those who do not have 136Q and haplotype new2 (Figure 2B, 2D). Multivariate analysis confirmed that both 136Q and haplotype new2 were associated with resistance and a decreased risk of seroconversion independent from other SNPs and haplotypes in the region (Table 3b). These findings are consistent with the results of Javanbakht et al. (2006) that R136Q is a protective polymorphism in HIV-1 infection [18]. It is important to note that the association between R136Q and haplotype new2 with resistance against infection may be the result of linkage disequilibrium with unidentified SNP(s) in relatively close proximity to codon 136. Additional sequencing and analysis should be conducted to determine whether or not these associations are independent of other sequence variations.

Codon 136 is located within the coiled-coil domain of *TRIM5α*, which is required for effective recognition and binding of HIV-1 [9,12]. It has also been suggested that the coiled-coil is needed for multimerization of *TRIM5α* particles, allowing for effective viral binding [12,14,16,22]. It is possible that variations in the amino acid sequence of the coiled-coil may alter multimerization and, in turn, the affinity of viral binding to the protein surface. Of note, most non-human primate *TRIM5α* (excluding chimpanzees) encodes a glutamine at codon 136 [18,11]. Many of these non-human forms of *TRIM5α* have been proven to effectively restrict HIV-1 [3,4,5,11]. Thus, a switch from arginine to glutamine at codon 136 in hu*TRIM5α* could indicate a shift towards a protein that is more active against HIV-1.

SNP frequencies in the Pumwani cohort were similar to that of the Yoruban population from Nigeria, but quite different from those in other populations (Chinese, Japanese, Caucasian). This variation in frequency could be an important factor when considering the innate immunity of a population, particularly given the variation of the SNP encoding R136Q: 34.2% in Caucasian populations and only 4.4% or 2.3% in Chinese and Japanese populations respectively. If R136Q exhibits a protective effect in all populations, the frequency of the SNP could have huge implications in the ease of HIV-1 transmission.

While Javanbakht et al. (2006) found a connection between H43Y and protection against HIV-1 infection [18], we found no significant correlations between H43Y and HIV-1 resistance/susceptibility (Table 1) or risk of seroconversion (Figure 2A, 2C) in the Pumwani Sex Worker Cohort.

This study has not identified associations between TRIM5 α SNPs or haplotypes and HIV-1 disease progression based on CD4 T-cell counts (data not shown). These results conflict with a recent study by Van Manen et al. (2008) in which H43Y was found to be associated with accelerated disease progression [17]. The same study also suggested a possible protective effect of 136Q in disease progression after the emergence of CXCR4-using HIV-1 variants, a factor we have not taken into consideration. However, based on what is known of the mechanism of TRIM5 α restriction, an effect on disease progression may be unexpected. TRIM5 α particles able to recognize and restrict HIV-1, do so potently, inhibiting the establishment of an infection. A TRIM5 α variant which is unable to prevent infection may not be efficient at recognizing or binding to HIV-1 and thus would have a limited effect on disease progression.

The ability of huTRIM5 α to restrict HIV-1 infection is likely dependent on the mode of viral transmission whether it is cell-free or cell-associated. There is convincing evidence that rhesus TRIM5 α (rhTRIM5 α) restriction of HIV-1 is sensitive to the mode of viral transmission; cell-free transmission is efficiently blocked by rhTRIM5 α , while the extent of cell-associated restriction is much lower [23]. Cell-associated transmission has been shown to be the principle mode of infection during HIV disease progression [24]. Thus, restriction of the initial cell-free viral infection by the appropriate form of huTRIM5 α may be possible while cell-associated transmission during disease progression may remain largely uncontrolled.

While altering levels of TRIM5 α production during the course of an infection may have an effect on the degree of viral restriction, this has not yet been observed to link directly to viral load [17]. However, there is evidence for interferon induced TRIM5 α transcription [25,26], raising the possibility that an HIV-1 infection could trigger more effective HIV-1 restriction through large quantities of TRIM5 α production.

The specific mechanism of TRIM5 α mediated restriction remains largely unknown and it is likely that many factors are involved. Further investigation is required to enhance our understanding of TRIM5 α mediated resistance to HIV-1 infection. Analysis of the full genomic sequence of TRIM5 α new2 haplotype may further clarify protective TRIM5 α polymorphisms.

Acknowledgments

This work was supported by the National Institute of Health, The Canadian Institutes of Health Research, National Microbiology Laboratory of Canada and The Bill and Melinda Gates Foundation. We thank Tony Kariri for maintaining the databases of both cohorts at the University of Nairobi and Bing-hua Liang for managing the database at the University of Manitoba. We also thank the staff of the Majengo clinic, Jane Njoki, Jane Makene, Elazabeth Bwibo, Edith Amatiwa; and the women of the Pumwani Sex Worker Cohort for their continued participation and support. Dr. Francis A. Plummer is a Canadian Institutes of Health Research Senior Investigator and is currently a Tier I CIHR Canada Research Chair.

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Figure 1. Identified SNP locations in the human TRIM5 gene. Domain structure of the TRIM5α protein is shown below (not drawn to scale).

Figure 2.

Kaplan-meier survival plots depicting time to HIV-1 seroconversion for women who are homozygous (thick line), heterozygous (thin line) or have no copies (broken line) of (A) rs3740996, (B) rs10838525, (C) Haplotype new1, (D) Haplotype new2 (E) wild type TRIM5α.

TRIM5α single nucleotide polymorphism frequencies in HIV-1 positive and resistant women in the Pumwani Sex Worker Cohort

Table 1

rs ID	Base Change	AA Change	Frequency							P-value	Adjusted P-value ^I	Odds Ratio	95% CI ²
			HIV-1+ (n=468)			Resistant (n=88)							
			Homozygote Mutant	Heterozygote	Mutant Phenotype	Homozygote Mutant	Heterozygote	Mutant Phenotype					
rs28381979	C/T	Intron2	14 (0.030)	127 (0.271)	141 (0.301)	3 (0.034)	16 (0.182)	19 (0.216)	0.105	1.000	0.639	(0.370, 1.101)	
rs10769175	A/G	Intron2	0 (0.000)	1 (0.002)	1 (0.002)	0 (0.000)	0 (0.000)	0 (0.000)	1.000	1.000	0.305	(0.001, 65.488)	
rs61173048	A/G	Intron2	0 (0.000)	3 (0.006)	3 (0.006)	0 (0.000)	0 (0.000)	0 (0.000)	1.000	1.000	0.304	(0.014, 6.777)	
Novel SNP-1	713G/C	I152I	0 (0.000)	2 (0.004)	2 (0.004)	0 (0.000)	0 (0.000)	0 (0.000)	1.000	1.000	0.304	(0.007, 13.604)	
rs10838525	664G/A	R136Q	5 (0.011)	67 (0.143)	72 (0.154)	3 (0.034)	28 (0.318)	31 (0.352)	1.104E-05	1.325E-04	2.991	(1.806, 4.953)	
rs11601507	591G/T	V112F	0 (0.000)	8 (0.017)	8 (0.017)	0 (0.000)	4 (0.045)	4 (0.045)	0.106	1.000	2.738	(0.806, 9.298)	
rs58477024	584C/T	D109D	0 (0.000)	3 (0.006)	3 (0.006)	0 (0.000)	2 (0.023)	2 (0.023)	0.180	1.000	3.605	(0.594, 21.893)	
Novel SNP-3	505T/C	V83A	0 (0.000)	1 (0.002)	1 (0.002)	0 (0.000)	0 (0.000)	0 (0.000)	1.000	1.000	0.305	(0.001, 65.488)	
Novel SNP-4	404C/G	D49E	0 (0.000)	1 (0.002)	1 (0.002)	0 (0.000)	0 (0.000)	0 (0.000)	1.000	1.000	0.305	(0.001, 65.488)	
rs3740996	384C/T	H43Y	1 (0.002)	44 (0.094)	45 (0.096)	0 (0.000)	8 (0.091)	8 (0.091)	1.000	1.000	0.940	(0.427, 2.069)	
rs59896509	348G/A	G31S	0 (0.000)	5 (0.011)	5 (0.011)	0 (0.000)	0 (0.000)	0 (0.000)	1.000	1.000	0.302	(0.027, 3.365)	
rs3824949	C/G	5'UTR	3 (0.006)	99 (0.212)	102 (0.218)	1 (0.011)	15 (0.170)	16 (0.182)	0.447	1.000	0.797	(0.444, 1.431)	

¹ Bonferroni

² Confidence Interval

TRIM5α exon 2 coding haplotypes (nucleotide changes in grey)

Table 2

										Frequency										
Amino Acid Change	G31S	H43Y	D49E	V83A	S120F	R136Q	713C/G	HIV-1+ (n=468)			Resistant (n=88)			P-value	Adjusted P-value ¹	Odds Ratio	95% CI ²			
								384C/T	404C/G	445T/C	584C/T	591G/T	616C/T					644G/A	Homozygote	Heterozygote
Base Change	348G/A	G	C	C	T	T	C	T	C	C	G	G	340 (0.726)	117 (0.250)	457 (0.976)	48 (0.545)	33 (0.375)	81 (0.920)		
Haplotype	wt	G	C	C	T	T	C	T	C	C	G	G	1 (0.002)	40 (0.085)	41 (0.088)	0 (0.000)	8 (0.091)	8 (0.091)	0.279	
	new1	G	T	C	C	T	C	A	C	C	A	G	5 (0.011)	64 (0.137)	69 (0.147)	3 (0.034)	27 (0.307)	30 (0.341)	1.041	
	new2	G	C	C	C	T	C	C	C	C	G	G	0 (0.000)	8 (0.017)	8 (0.017)	0 (0.000)	4 (0.045)	4 (0.045)	2.991	
	new4	G	C	C	C	T	C	T	C	C	G	G	0 (0.000)	2 (0.004)	2 (0.004)	0 (0.000)	1 (0.011)	1 (0.011)	2.738	
	new5	G	C	C	C	T	T	C	T	C	G	G	0 (0.000)	1 (0.002)	1 (0.002)	0 (0.000)	0 (0.000)	0 (0.000)	2.678	
	new6	G	C	G	T	C	T	C	T	C	G	G	0 (0.000)	5 (0.011)	5 (0.011)	0 (0.000)	0 (0.000)	0 (0.000)	0.305	
	new7	A	C	C	T	C	T	C	C	C	G	G	0 (0.000)	1 (0.002)	1 (0.002)	0 (0.000)	0 (0.000)	0 (0.000)	0.302	
	new8	G	T	C	C	T	T	C	A	C	C	A	C	0 (0.000)	1 (0.002)	1 (0.002)	0 (0.000)	0 (0.000)	0 (0.000)	0.305
	new9	G	T	C	C	C	C	C	G	C	C	G	G	0 (0.000)	1 (0.002)	1 (0.002)	0 (0.000)	0 (0.000)	0 (0.000)	0.305
	new11	G	C	C	C	T	T	T	A	C	C	A	G	0 (0.000)	1 (0.002)	1 (0.002)	0 (0.000)	1 (0.011)	1 (0.011)	5.368
	new12	G	T	C	C	C	G	C	G	C	C	G	C	0 (0.000)	1 (0.002)	1 (0.002)	0 (0.000)	0 (0.000)	0 (0.000)	0.305

¹ Bonferroni

² Confidence Interval

Note. Haplotype new3 (GCTCTGGAG) and new 10 (GCTCTGGAG) were not observed in HIV-1+ or resistant women and thus were not included in Table 2.

Table 3

Binary logistic regression and Cox regression analysis

Logistic Regression Analysis				
	Sig. ¹	Exp(B) ²	95.0% C.I. ³ for Exp(B)	
			Lower	Upper
Haplotypes^a				
New2	2.308E-5	0.331	0.199	0.553
New4	0.095	0.345	0.099	1.204
Constant	0.753	1.231		
SNPs^b				
rs10838525	1.860E-5	0.331	0.199	0.549
rs11601507	0.092	0.341	0.098	1.192
Constant	0.752	1.232		
Cox Regression Analysis				
	Sig.	Exp(B)	95.0% C.I. for Exp(B)	
			Lower	Upper
Haplotypes^a				
New2	1.212E-3	2.959	1.533	5.710
SNPs^b				
rs10838525	2.122E-3	2.839	1.459	5.522
rs28381979	0.080	0.676	0.436	1.048

^aVariables entered on step 1: Haplotypes new1 to new12 inclusive

^bVariables entered on step 1: rs28381979, rs10769175, rs61173048, novel SNP-1, rs10838525, novel SNP-2, rs11601507, rs58477024, novel SNP-3, novel SNP-4, rs3740996, rs59896509, rs3824949

¹ Significance

² Exponent of B, odds ratio

³ Confidence interval