# Toll-Like Receptor Polymorphism Associations With *Trichomonas vaginalis* infection among African couples: A Hypothesis-generating Study

Yu-Ting Lin

A thesis
submitted in partial fulfillment of the
requirements for the degree of
Master of Science

University of Washington
2019

Committee:
Jairam Lingappa
Kathleen Kerr
Romel Mackelprang

Program Authorized to Offer Degree:
Public Health Genetics

University of Washington

## Abstract

Toll-Like Receptor Polymorphism Associations with *Trichomonas vaginalis* infection among African couples: A Hypothesis-generating Study

Yu-Ting Lin

Chair of the Supervisory Committee:
Jairam Lingappa
Department of Global Health, Medicine, and Pediatrics

*Trichomonas vaginalis* (Tv) is the most common non-viral, sexually transmitted infection. There is evidence that Tv infection increases risk of HIV-1 acquisition and transmission. The detailed mechanism underlying Tv infection is not clear, although toll-like receptors (TLRs) may be involved in innate immune responses when stimulated by Tv. This study evaluated the association between single-nucleotide polymorphisms (SNPs) that represent common variation across TLR genes and Tv acquisition/transmission. Logistic regression analyses were performed among 70 African heterosexual men to evaluate the association between TLR SNPs and Tv acquisition, and among 61 African heterosexual women for association between TLR SNPs and Tv transmission. Our results showed that SNPs in TLR2, 3, 4, 7, 8, TIRAP, and MyD88 may be associated with Tv acquisition/transmission. *TIRAP* rs1893352 was significantly associated with Tv acquisition among men ($p = 0.002$) , and *TLR7* rs1634319 was significantly associated with Tv transmission from women to study partners ($p = 0.001$). Our finding also indicated that HIV-1 serostatus may modify some associations between TLR SNPs and Tv acquisition/transmission.

**Acknowledgements**

The completion of this project was possible thanks to the guidance, support and valuable feedback of my thesis committee: Jairam Lingappa, Katie Kerr, and Romel Mackelprang. My special thanks to the committee chair Dr. Lingappa who guided me with profound wisdom, patience, and kindness. I also want to thank Aaron Bochner for providing me with access to the epidemiologic dataset compiled in his prior work. And lastly, thanks to my father and mother for their best supports.

## Table of Contents

## List of Tables

## 1. Introduction

### 1.1 *Trichomonas vaginalis* and trichomoniasis

*Trichomonas vaginalis* (Tv) is a protozoan parasite and the pathogen that causes trichomoniasis, the most common non-viral sexually transmitted infection (STI). There are about 100 million prevalent cases of trichomoniasis are reported globally in men and women aged 15-49 years. However, high rates of asymptomatic infection and reluctance to seek treatment may lead to under-reporting [1]. Tv infection may be more prevalent in low/middle income countries.

Tv is spread primarily through sexual intercourse. During infection, the pathogen can be identified in the female lower genital tract and the male urethra and prostate [2]. Infection may last for months or even years in women, often without symptoms. In men, infection is also commonly asymptomatic, but associated with spontaneous resolution within 10 days [3][4] [5]. Among women, symptoms of trichomoniasis includes urethral discharge, vaginal discharge, dysuria, itching, irritation and abdominal pain. In men, Tv infection may cause itching or irritation inside the penis, epididymitis, prostatitis, and decreased sperm cell motility[6].

The majority of infected cases (~70%) are asymptomatic. Infected and asymptomatic carriers are at greatest risk of unknowingly spreading the infection in their community. Asymptomatic carriers might also contribute to high Tv repeat infection rates (5-31%)[7]. Tv may be infected with Trichomonas vaginalis virus, a double stranded RNA totivirus, and two mycoplasma species, which may have important implications for Tv pathogenesis [8].

Identified epidemiologic risk factors for Tv infection include a Tv infected sexual partner, low level of education, a history of STI, unprotected sex and multiple sexual partnerships [9]. There

is evidence that Tv infection is a risk factor HIV-1 acquisition and transmission: Tv infected individuals have 1.5-3 times higher risk of seroconversion when exposed to HIV-1 [10, 11]; and concurrent Tv and HIV infection may increase cervical shedding of HIV, which may increase HIV transmission risk [11, 12]. Among HIV-1 serodiscordant couples (one partner HIV-1 infected and the other HIV-uninfected at enrollment), Bochner et al[13], found that the risk of Tv infection is higher among men with concurrent HIV-1 infection compared to men without concurrent HIV-1 infection .

**1.2 Toll-like receptors**

TLR are a class of transmembrane proteins that play an important role in the innate immune response against microorganisms. Currently, 10 TLRs have been identified in humans and combinations of TLRs are found in different cell types (TLR molecular biology is reviewed in reference [14]). Human TLRs 1, 2, 4, 5, 6 and 10 localize to the cell surface, while TLR 3, 7, 8, 9 localize in endosomes. The extracellular domain of TLRs is involved in the recognition of microbial ligands, which activates the intracellular domain to dimerize or associate with other receptors. The TLR intracellular domain (TIR) is involved in signal transduction by interacting with other TIR containing intracellular proteins, such as MyD88 and TIRAP. All TLRs, except TLR3 transduce signals through MyD88; however, TLR2 and TLR4 need to dimerize with TIRAP to associate with MyD88.

TLR2 associates with TLR1 and TLR6 thereby expanding the range of microbial factors it can recognize; these include Gram-positive bacteria and Mycobacteria through bacterial lipoproteins, lipomannans and lipoteichoic acids; as well as, ligands from fungi, protozoa, and viruses. TLR3 recognizes double-stranded RNA from viruses; TLR4 recognizes bacteria,

protozoa, and viruses; while, TLR7 and TLR8 primarily respond to single-stranded viral RNAs.

Studies have suggested that Tv infection may induce inflammation in both the male and female genital tract through stimulation of TLRs. Cervico-vaginal lavage specimens from Tv-infected women were found to stimulate cytokine production by TLR4-responsive mouse splenic cells, but not TLR4-unresponsitive cells [15]. In a study of *T. vaginalis*-induced macrophage apoptosis, upregulated expression of TLR2, TLR4, and TLR9 was also observed in HeLa cells treated with Tv [16].

### 1.3 The purpose of this study

SNPs are DNA variations that occur at a single nucleotide in the genome sequence. Applications of SNPs analyses allow researchers to explore the influence of genetic variation on disease outcomes. *TLR* gene polymorphisms have been associated with infectious disease outcomes including clinical HIV-1 outcomes [17] and risk of bacterial vaginosis [18]. Given the experimental data that suggests TLRs may play a role in host response to Tv infection, one may hypothesize that variations in one or more TLRs will alter risk of Tv infection. However, there are no studies reporting associations between *TLR* gene polymorphisms and Tv infection. We hypothesized that common variation in selected *TLR* genes is associated with Tv acquisition and transmission. We also hypothesized that HIV-1 serostatus modifies the association between TLR SNPs and Tv transmission/acquisition. The main goal of this study was to evaluate the association between *TLR* SNPs and *T. vaginalis* acquisition or transmission, with the following specific aims:

Aim 1A: Quantify the associations between *TLR* SNPs and risk of Tv acquisition by heterosexual African men with female Tv-infected regular sexual partners;

Aim 1B: Quantify the associations between *TLR* SNPs and risk of Tv transmission from heterosexual African women to their male sexual partners;

Aim 2: Quantify the associations between *TLR* SNPs and risk of Tv acquisition/transmission without and with adjustment of number of unprotected sex acts among individuals who reported <100% condom use;

Aim 3: Determine if HIV serostatus modifies the association between *TLR* SNPs and Tv acquisition/transmission.

## 2.Methods

### 2.1 Data Source

Data were selected from two prospective cohorts of African heterosexual HIV-1 serodiscordant couples, in which one partner is HIV-1 infected and one is HIV-1 uninfected at enrollment:

1. The Partners in Prevention HSV/HIV Transmission Study was a randomized controlled trial that examined the efficacy of twice-daily acyclovir, a herpes simplex virus type-2 (HSV-2) suppressive therapy, in reducing HIV-1 transmission from HIV-1 infected individuals to their heterosexual HIV-1 uninfected partners. The study enrolled 3408 HIV-1 serodiscordant couples from 14 sites in seven East and Southern African countries. All enrolled HIV-1 infected partners were HSV-2 seropositive and had a CD4 count $\geq$250 cells/mm$^3$ at enrollment. [19]
2. The Couples Observational Study (COS) also assessed HIV transmission and used similar recruitment method as the Partners in Prevention HSV/HIV Transmission Study. The study enrolled 485 HIV-1 heterosexual serodiscordant couples from Soweto, South Arica and Kampala, Uganda. There was no restriction on CD4 count or HSV-2 serostatus of the HIV-1 infected partner.[20]

All participants in the two studies were 18 years or older and reported regular sexual intercourse with their study partner in the three months prior to enrollment. At the time of enrollment, data on demographics and participants' sexual behaviors were collected by interviewer-administered standardized questionnaires. The total number of sex acts with the study partner and the number of sex acts with use of a condom in the three months prior to enrollment were also collected from participants. At enrollment endocervical swabs and urine samples (from men) were collected to test for trichomoniasis, chlamydia, and gonorrhea by nucleic acid amplification; in addition, blood samples were tested for HIV-1 serology.

*TLR* genotypes for 863 individuals were generated for a prior analysis that investigated associations between *TLR* variants and HIV-1 acquisition [17]. For that study, 129 seroconverting couples were matched to 246 non-seroconverting couples based on HIV-1 exposure scores (calculated using sex, age, male circumcision, HIV-1 infected partner plasma RNA level, and unprotected sex). Additional seronegative participants with the highest HIV-1 exposure scores were also included. All genotyped participants provided written informed consent for genetic studies.

**2.2 Sample Selection**

683 participants (341 men, 342 women) had both Tv status determined and genotypic data available. Each individual was classified into one of four categories based on results from Tv testing of the individual and his or her study partner at enrollment: 1) Tv negative men partnered with Tv negative women, 2) Tv negative men partnered with Tv positive women, 3) Tv positive men partnered with Tv negative women, and 4) Tv positive men partnered with Tv positive women.

In order to address my three Aims, I divided participants into four groups:

**Group 1 (men in Category 2, 3, and 4 as described above):** All Tv exposed men with *TLR* genotype data were included (for Aim 1A): a total of 70/341 (21%) of men had Tv infected study partners (NOTE: although all these female partners had Tv infection data, some did not have *TLR* genotype data). These n=70 men included n=28 Tv infected men and n=42 Tv uninfected men. Men in Category 2 were considered being exposed to due to their Tv infection, even though their study partners were not infected.

**Group 2 (women in Categories 2 and 4 described above):** All Tv infected women with *TLR* genotype data were included (for Aim 1B): a total of 61/342 (18%) women were identified as

Tv infected and had *TLR* genotype data (Note: although all of these male partners had Tv infection data, not all of them had *TLR* genotype data).

**Group 3:** Subset of Tv exposed men reporting less than 100% condom use (to address Aim 2): n=37/70 men reported condom use at every sex act. For the purpose of Aim 2, these individuals were considered not exposed to Tv leaving 33 men exposed to Tv infected female partners through unprotected sex.

**Group 4:** Subset of Tv infected women reporting less than 100% condom use for (to address Aim 2): n = 35/61 women reported condom use at every sex act. For the purpose of Aim 2, these individuals were considered not being able to transmit Tv, leaving 26 women who have the potential to transmitting Tv to their study partners through unprotected sex.

All of the 4 groups were used to fit a logistic regression model that includes an HIV serostatus-SNP interaction term to investigate HIV-1 infection status as possibly modifying the association between *TLR* SNPs and Tv acquisition/transmission (to address Aim 3).

### 2.3 Genotyping and *TLR* variants selection

*TLR* genotyping was performed using genomic DNA from archived whole blood [16]. Samples were genotyped using an Illumina Custom Oligo Pooled Assay for 123 SNPs across six *TLR* genes (8 in *TLR2*, 13 *TLR3*, 22 *TLR4*, 40 *TLR7*, 25 *TLR8*, 3 *TLR9*), and 2 genes encoding TLR-associated proteins (4 SNPs in *MYD88*, and 8 SNPs in *TIRAP*). Among the 123 SNPs, 7 SNPs were selected as candidate SNPs previously associated with risk of HIV infection, and 116 SNPs were haplotype-tagging SNPs (tagSNPs) with minor allele frequency > 5%. Haplotypes were inferred from the Yoruba HapMap population.

Power analysis was conducted by PS software [21], and Hardy-Weinberg Equilibrium (HWE) tests were performed using the 'HardyWeinberg' R package. In order to eliminate SNPs with low power, I excluded SNPs for which there were fewer than five individuals who were either heterozygous or homozygous for the minor allele. For analyses performed using Group 1 men, 6 SNPs were excluded for having >10% missing results, 1 SNP for failing HWE at alpha level = 0.05, and 17 SNPs for having a sum of heterozygous and homozygous minor allele individuals < 5. For analyses performed using Group 2 women, 2 SNPs were excluded for >10% missing results, 1 SNP for failing HWE, and 17 SNPs for having sum of heterozygous and homozygous minor allele individuals < 5. Due to the smaller sample size for Aim 2, 48 SNPs were excluded from the analyses performed using Group 3 for having less than 5 heterozygous and/or homozygous minor allele individuals; similarly, 46 SNPs were excluded from analyses using Group 4 for low power considerations.

**2.4 Statistical analyses**

Logistic regression analyses were performed using Firth's penalized likelihood tests to assess the association between *TLR* polymorphisms and Tv acquisition among Group 1 men, and the association between *TLR* polymorphisms and Tv transmission from Group 2 women to their study partners. These analyses were performed both without (Aim 1A and 1B) and with (Aim 2) adjustment for the number of unprotected sex acts. These analyses included both a dominant model in which the exposure was binary (either having or not having two major alleles), and an additive model in which the exposure was the dose of the minor allele (0, 1, or 2). Analyses were performed using R V3.5.2 and the 'logistf' package.

A similar approach was used in Aim 3 to assess the evidence that HIV-1 infection status at enrollment modifies the association between *TLR* gene variation and Tv infection. Analogous

to Aims 1 and 2, we performed these analyses both with and without adjustment for the number of unprotected sex acts. For SNPs showing significant evidence of effect modification by HIV-1 infection status, additional logistic regression analyses were performed to assess SNPs association with Tv acquisition/transmission for subgroups stratified by HIV-1 infection. As a hypothesis-generating study examining candidate genes, we did not correct for multiple comparisons. Results with $P<0.05$ were considered significant for further study.

## 3.Results

### 3.1 The association of *TLR* variants with Tv acquisition among men

#### 3.1.1 Without and with adjustment for number of unprotected sex acts

In the analysis of Tv acquisition among men (Group 1), the *TIRAP* rs1893352 G allele was significantly associated with a reduced risk of Tv acquisition (OR = 0.11; 95% CI: 0.01-0.48; p = 0.002) (**Table 1)**. No individual in the sample had the GG genotype, so the dominant and additive models produced identical results. *TLR8* rs5741883 T allele was also significantly associated with a reduced risk of Tv acquisition (OR = 0.26; 95% CI: 0.08-0.74; p = 0.01; dominant model). The *TLR*8 gene is on chromosome X, so men have only a single allele that is either the major allele or minor allele, and only the dominant model was of interest.

In the analysis of Tv acquisition among men who reported less than 100% condom use (Group 3), 5 *TLR4*, 6 *TLR7*, and 4 *TLR8* SNPs showed a significant association with Tv acquisition (these included *TLR8* rs5741883, but not *TIRAP* rs1893352) (**Table 2)**. Among 5 *TLR4* SNPs, there were 2 intronic (rs5030729, rs5030717), 1 synonymous (rs5030710), 1 3'UTR (rs7869402), and 1 5' promoter region variant (rs11536865). *TLR4* rs11536865/C, rs7869402/T, and rs5030717/G were associated with increased risk of Tv acquisition, while *TLR4* rs5030710/C, rs5030729/G were associated with reduced risk of Tv acquisition. All 6 significant *TLR7* SNPs were intronic variants; for four of them (rs1634323/G, rs1634319/C, rs1634322/A, rs1634321/T) the minor alleles were associated with increased risk, while two (rs1620233/C, and rs179009/G) associated with decreased risk of Tv acquisition. *TLR8* rs1548731 C allele and rs4830805 A allele were associated with increased risk of Tv acquisition and were intronic. *TLR8* rs5741883 T allele, an upstream variant, showed a significant association with a reduced risk of Tv acquisition (OR = 0.07; 95% CI: 0-0.7; p = 0.02; unadjusted dominant model), which was consistent with Group 1 analysis result. *TLR8*

rs5744088, a 3' UTR variant, was associated with reduced risk of Tv acquisition. The odds ratios for all significant SNPs, except for rs5741883, were similar before and after controlling for number of unprotected sex acts. The effect of *TLR8* rs5741883 became stronger after adjustment for number of unprotected sex acts (OR = 0.17; 95% CI: 0.02-0.83; p = 0.03).

### 3.1.2 Assessing effect of HIV-1 serostatus in modifying associations between *TLR* gene polymorphism and Tv acquisition

We found evidence that the association between *TLR7* rs1634319 and Tv acquisition among men (Group 1) was modified by HIV-1 serostatus (p = 0.001). Rs1634319 C allele was significantly associated with an increased risk of Tv acquisition among HIV-1 uninfected men (OR = 14; 95% CI: 2.6-140; p = 0.0014; dominant model) (**Table3**). In the analysis of Tv acquisition among men who reported <100% condom use (Group 3), HIV-1 infection status significantly modified the *TLR3* rs6552950 and Tv association (interaction p-value = 0.001, additive model), and *TLR3* rs13126816 and Tv association (interaction p-value = 0.001, additive model).

## 3.2 The association of *TLR* variants with Tv transmission from Tv infected women to men

### 3.2.1 Without and with adjustment for number of unprotected sex acts

In the analysis of association between Tv transmission from women (Group 2) to their study partners, 2 *TLR2*, 1 *TLR3*, 3 *TLR4*, 4 *TLR7*, and 1 *MYD88* SNPs showed significant association at $\alpha = 0.05$. Among these 11 SNPs, 7 SNPs were significantly associated with Tv transmission in both a dominant model and an additive model (**Table 4**).

We found that rs4696483/T, rs7656411/T in TLR2 and rs11536865/C in aTLR4 were significantly associated with Tv transmission from women who reported less than 100%

condom use (**Table 5**). It is worth noting that while *TLR2* rs4696483 T allele was associated with increased Tv transmission (OR = 2.5; 95% CI: 1.2-5.8; p = 0.014; additive model) from women to their study partners (Group 2)(**Table 4**), this variant was associated with an decreased risk of Tv transmission (OR = 0.08; 95% CI: 0-0.97; p = 0.047; unadjusted dominant model) from the women who reported <100% condom use (Group 4). Analysis of Tv transmission from women who reported <100% condom use (Group 4) showed that the odds ratio of Tv transmission risk were similar with and without adjustment for the number of unprotected sex acts for SNPs *TLR2* rs4696483, *TLR2* rs7656411, and *TLR4* rs11536865).

### 3.2.2 Assessing effect of HIV-1 serostatus in modifying associations between *TLR* gene polymorphism and Tv transmission

In the analysis of Tv transmission from women to study partners, HIV-1 infection significantly modified the association between *TLR8* rs17256081 and Tv transmission (interaction p-value = 0.001, additive model) and between *TLR8* rs5744069 and Tv transmission (interaction p-value = 0.008, additive model) in the additive model. The interaction p-values for the dominant model were also significant. (**Table 6**).

*TLR8* rs17256081 C allele was significantly associated with increased risk of Tv transmission from HIV-1 uninfected women to their study partners in both models (OR = 6; 95% CI: 1.4-41; p = 0.012; unadjusted additive model). In contrary, it was significantly associated with reduced risk of Tv transmission from HIV-1 infected women in an additive model (OR = 0.29; 95% CI: 0.07-0.97; p = 0.044; unadjusted additive model). *TLR8* rs5744069 G allele and *TLR2* rs4696483 T allele were associated with increased Tv transmission among HIV-1 uninfected women in additive model.

In the analysis of Tv transmission from women who reported <100% condom use (**Table 7**), *TLR*8 rs1548731 showed significant association with increased risk of Tv transmission from HIV-1 infected women to their study partners (OR = 48; 95% CI: 2.9-7800; P= 0.0041; dominant model) in both models. Six total *TLR7* and *TLR8* SNPs showed significant interaction p values, indicating evidence that HIV-1 serostatus modifies association between TLR SNPs and Tv transmission.

## 4.Discussion

### 4.1 Implications of association between TLR Genes, TIRAP and MyD88 and Tv

In our analyses, we found evidence that 1) polymorphisms in *TIRAP*, *TLR4*, *TLR7*, and *TLR8* may be associated with Tv acquisition; 2) polymorphisms in *TLR2*, *TLR3*, *TLR4*, *TLR8*, and *MyD88* may be associated with Tv transmission; and 3) some of these associations may be modified by the HIV-1 serostatus of the individuals who acquire or transmit the Tv pathogen. Among these SNPs, *TIRAP* rs1893352, *TLR4* rs1927906, and *TLR7* rs1634319 has smallest p-values (p = 0.002-0.001).

Among the SNPs significantly associated with Tv acquisition or transmission, many were intronic, and a few were in regulatory or coding regions. It is possible that these intronic SNPs are 1) influencing gene splicing or regulation [22] or 2) in strong linkage disequilibrium with causal SNPs. For instance, the *TIRAP* intronic SNP from our study, rs1893352G, was reported to be in strong linkage disequilibrium with a rs817737T, which codes for Ser180Leu and has been linked to resistance to bacterial infections and malaria [23].

A few associations were found between SNPs in *TLR2*, and *TLR4*. These two receptors have been thought to recognize protozoan components. Also, a few SNPs found significantly associated with Tv in this study are located in *TIRAP*, *TLR3*, *TLR7*, *TLR8*, and *MyD88* genes. As TIRAP and MyD88 play an important role in the signaling pathways that activate immune responses by associating with several TLRs [24], TLR3, TLR7 and TLR8 may have a immunological response to Tv, or a immunological response to Tv symbiont.

### 4.1.1 TIRAP rs1893352

TIRAP and MyD88 are adaptor proteins that mediate downstream signaling of TLR2 and TLR4 [24, 25, 26]. In this study, *TIRAP* rs1893352 G, strongly linked to rs8177374T which codes Ser180Leu, was found to be associated with reduced risk of Tv acquisition among men. This is somewhat similar to results from previous studies of malaria that suggested TIRAP Ser180Leu heterozygosity had a protective effect on susceptibility to and severity of malaria [27, 28]. There is also evidence suggesting that *TIRAP* Ser180Leu regulates a more potent cytokine response among heterozygous individuals with Behçet's disease [29]. Based on these findings, it is possible that rs1893352 G, or rs8177374T, could eliminate pathogens more effectively thereby reducing the susceptibility to Tv. among Sub-Saharan African men.

### 4.1.2 Tv in symbiosis with virus and bacteria

This study found evidence suggesting that SNPs in *TLR2* and *TLR3* are associated Tv infection. It is possible that these SNPs act indirectly by recognizing Tv endosymbionts. The symbiosis of Tv, Trichomonasvirus, and eubacterial *Mycoplasma* species has been described in previous studies [8, 30, 31]. Macrophage-stimulating factors elicited by *Mycoplama hominis* have been shown to interact with TLR2, which in turn signals tumor necrosis factor (TNF)-α, IL-1, IL-1β, IL-6 and IL-8 production [32, 33]. We speculate that these innate responses may cause host damage from leukocyte infiltration and high local cytokines concentrations, which are often observed in trichomoniasis [34]. Similar to *M. hominis*, endosymbiotic Trichomonasvirus has recently been shown to activate innate responses via TLR3 [30].

## 4.2 Strengths and Limitations

To our knowledge, this study is the first to examine associations between SNPs in TLR genes and the acquisition/transmission of Tv in Sub-Saharan Africans. Our study design allowed us

to identify men who have likely been exposed to Tv through their sexual partnerships with Tv infected women. Much of the existing Tv concordance data in other Tv studies was collected from transmitted disease clinics. This method may introduce sampling bias because people with symptomatic Tv infection were more likely to be recruited. In contrary, our data may be a better representative of the general population. Our results might be generalized to a greater population. In addition, all individuals in this study were tested for Tv by nucleic acid amplification tests (NAAT), which is likely to reduce misclassification compared to culture tests.

One important concern about using cross-sectional data is that the direction of transmission between partners cannot be definitively determined. However, since the duration of infection in men is usually less than 10 days [3, 4, 5], while infection is much longer lasting in women, we infer that the Tv infected men in this study were more likely to acquire the parasite from their female study partner rather than vice versa. However, this could only be definitively assessed through longitudinal evaluation of Tv.

A few SNPs were significantly associated with Tv acquisition/transmission when we controlled for number of unprotected sex acts. However, we know that self-reported data related to unprotected sex acts can be biased. The fact that we found a few couples had discrepancies in the number of protected sex acts reported by the two study partners and that several Tv infected individuals has reported 100% protected sex acts may suggest the data may be biased.

The samples sizes for association analyses in this investigation were modest, especially for Aims 2 and Aim 3. The Firth's penalized likelihood was selected because it is more appropriate for an analysis with small sample sizes such as these. Firth's method reduces small sample bias

and provides finite and consistent estimates [35]. Nevertheless, these small sample sizes led to large confidence intervals. We consider this investigation to be hypothesis-generating, and our findings need to be replicated.

## 5. Conclusion

In summary, SNPs in TLR genes may be associated with Tv acquisition and transmission, and some associations may be modified by HIV-serostatus of the individuals who acquire or transmit Tv. The most statistically significant associations were *TIRAP* rs1893352 with Tv acquisition and *TLR7* rs1634319 with Tv transmission. SNPs that reached statistical significance ($p = 0.05$) in this hypothesis-generating study should be validated in a larger study.

# Tables

***Table 1*** *TLR SNPs Significantly Associated with Tv Acquisition among Men (Group 1, N = 70). These SNPs had only two genotypes in the data and so only the dominant model is reported*

| GENE | SNP/ Minor Allele | Tv+ | Tv- | OR (lower, upper), P |
|---|---|---|---|---|
| | | +/+*, +/-*,-/-* | | Dominant Model |
| *TIRAP* | rs1893352/G | 27,1,0 | 28,14,0 | 0.11(0.011,0.48), 0.002 |
| *TLR8* | rs5741883/T | 23,0,5 | 22,0,20 | 0.26(0.079,0.74), 0.011 |

+/+: Number of individuals who have a genotype of homozygous major allele
+/-: Number of individuals who have a genotype of heterozygous
-/-: Number of individuals who have a genotype of homozygous minor allele

***Table 2*** *TLR SNPs Significantly Associated with Tv Acquisition among Men reporting less than 100% condom use (Group 3, N = 33)*

| Gene | SNP/Minor allele | Tv+ +/+*, +/-*,-/-* | Tv- | OR (lower, upper), p | | | |
|---|---|---|---|---|---|---|---|
| | | | | Dominant Model | | Additive Model | |
| | | | | Adjusted | Unadjusted | Adjusted | Unadjusted |
| *TLR4* | rs11536865 C | 9,7,0 | 16,1,0 | 8(1.4,85), 0.016 | 8.7(1.5,92), 0.012 | 8(1.4,85), 0.016 | 8.7(1.5,92), 0.012 |
| | rs7869402 T | 6,7,3 | 12,5,0 | 4(1,19), 0.05 | 3.7(0.94,16), 0.062 | 3.7(1.2,16), 0.021 | 3.5(1.2,13), 0.026 |
| | rs5030710 C | 14,2,0 | 9,6,2 | 0.18(0.028,0.86), 0.031 | 0.19(0.031,0.89), 0.03 | 0.22(0.036,0.83), 0.022 | 0.23(0.039,0.86), 0.027 |
| | rs5030729 G | 14,2,0 | 9,6,2 | 0.18(0.028,0.86), 0.031 | 0.19(0.031,0.89), 0.03 | 0.22(0.036,0.83), 0.022 | 0.23(0.039,0.86), 0.027 |
| | rs5030717 G | 9,7,0 | 15,2,0 | 4.6(0.98,29), 0.053 | 4.9(1,31), 0.047 | 4.6(0.98,29), 0.053 | 4.9(1,31), 0.044 |
| **Gene** | **SNP/Minor allele** | **Tv+ +/+, +/-,-/-** | **Tv-** | **Dominant Model** | | | |
| | | | | **Adjusted** | | **Unadjusted** | |
| *TLR7* | rs1620233 C | 11,0,5 | 4,0,13 | 0.14(0.026,0.62),0.008 | | 0.16(0.033,0.65), 0.01 | |
| | rs1634323 G | 9,0,7 | 16,0,1 | 8(1.3,96), 0.023 | | 6.7(1.3,45), 0.02 | |
| | rs1634319 C | 10,0,6 | 16,0,1 | 7(1.2,77), 0.03 | | 6.8(1.2,72), 0.03 | |
| | rs179009 G | 15,0,1 | 11,0,6 | 0.15(0.012,0.92), 0.039 | | 0.19(0.031,0.89), 0.034 | |
| | rs1634322 A | 10,0,6 | 16,0,1 | 6.1(1.1,65), 0.041 | | 6.8(1.2,72), 0.034 | |
| | rs1634321 T | 10,0,6 | 16,0,1 | 6(1.1,64), 0.042 | | 4.9(1,31), 0.044 | |
| TLR8 | rs1548731 C | 6,0,7 | 14,0,2 | 7.9(1.5,59), 0.014 | | 8.7(1.5,92), 0.012 | |
| | rs4830805 A | 9,0,7 | 16,0,1 | 8.4(1.5,90), 0.014 | | 8.7(1.5,92), 0.012 | |
| | rs5741883 T | 14,0,2 | 9,0,8 | 0.17(0.024,0.83), 0.027 | | 0.069(0.00051,0.7), 0.02 | |
| | rs5744088 C | 16,0,0 | 12,0,5 | 0.074(0.00053,0.81), 0.031 | | 0.069(0.00051,0.7), 0.034 | |

+/+: Number of individuals who have a genotype of homozygous major allele
+/-: Number of individuals who have a genotype of heterozygous
-/-: Number of individuals who have a genotype of homozygous minor allele

***Table 3*** *Tv Acquisition Analysis in HIV-1 Positive and Negative Men (Group 1, N = 70 ;Group 3, N = 33)*

| **Group 1** | | | | | | |
|---|---|---|---|---|---|---|
| **Gene** | **SNP/ Minor Allele** | **Stratification** | **Tv+** | **Tv-** | **OR (lower, upper), p** | **Interaction p value** |
| | | | **+/+*, +/-*,-/-*** | | **Dominant Model** | **Dominant Model** |
| *TLR7** | rs1634319 C | HIV+ | 10,0,0 | 7,0,3 | 0.1(0.001,1.3), 0.082 | 0.001 |
| | | HIV- | 11,0,7 | 31,0,1 | 14(2.6,140), 0.001 | |

| **Group 3** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Gene** | **SNP/ Minor Allele** | **Stratification** | **Tv+** | **Tv-** | **OR (lower, upper), p** | | **Interaction p value** | |
| | | | **+/+, +/-,-/-** | | **Dominant Model** | **Additive Model** | **Dominant Model** | **Additive Model** |
| *TLR3* | rs6552950 G | HIV+ | 2,3,0 | 4,0,0, | 13(0.75,2000), 0.082 | 13(0.75,2000), 0.082 | 0.015 | 0.001 |
| | | HIV- | 11,0,0 | 9,3,1 | 0.092(0.001,1), 0.054 | 0.14(0.001,1.1), 0.072 | | |
| | rs1312681 A | HIV+ | 2,3,0, | 4,0,0, | 13(0.75,2000), 0.082 | 13(0.75,2000), 0.082 | 0.041 | 0.001 |
| | | HIV- | 11,0,0 | 11,2,0 | 0.2(0.001,2.8), 0.25 | 0.2(0.001,2.8), 0.25 | | |

*TLR7* is a X chromosome gene, there only dominant model result is reported for men.
+/+: Number of individuals who have a genotype of homozygous major allele
+/-: Number of individuals who have a genotype of heterozygous
-/-: Number of individuals who have a genotype of homozygous minor allele

***Table 4*** *TLR SNPs Significant Associations with Tv Transmission from Women (Group 2, N = 61)*

| GENE | SNP/Minor Allele | Tv+ | Tv- | OR (lower, upper), p | |
|---|---|---|---|---|---|
| | | +/+*, +/-*,-/-* | | Additive Model | Dominant Model |
| *TLR2* | rs4235232/G | 15,6,1 | 35,4,0 | 3.6(1.1,14.0), 0.031 | 3.8(1.1,15.3), 0.041 |
| | rs4696483/T | 2,9,11 | 12,18,9 | 2.5(1.2,5.8), 0.014 | 3.7(1,20.7), 0.056 |
| *TLR3* | rs7657186/A | 9,12,1 | 27,8,4 | 1.7(0.8,3.8), 0.194 | 3.1(1.1,9.4), 0.033 |
| *TLR4* | rs1927906/C | 13,8,1 | 8,17,13 | 0.3(0.1,0.6), 0.001 | 0.2(0.1,0.6), 0.003 |
| | rs11536887/G | 22,0,0 | 31,7,1 | 0.1(0.0,0.8), 0.023 | 0.1(0,0.7), 0.019 |
| | rs11536888/T | 17,5,0 | 37,2,0 | 4.7(1.0,28.4), 0.047 | |
| *TLR7* | rs1634323/G | 13,9,0 | 34,5,0 | 4.4(1.3,16.0), 0.015 | |
| | rs179018/C | 15,7,0 | 35,2,1 | 2.9(0.9,11.2), 0.073 | 4.9(1.3,22.6), 0.02 |
| | rs5743776/T | 14,8,0 | 33,4,1 | 2.4(0.8,8.0), 0.105 | 3.6(1.1,13), 0.04 |
| | rs179013/A | 16,6,0 | 36,3,0 | 4.1(1.0,19.1), 0.045 | |
| *MYD88* | rs6853/G | 5,14,3 | 22,14,3 | 2.5(1.1,6.1), 0.025 | 4.1(1.4,13.8), 0.011 |

+/+: Number of individuals who have a genotype of homozygous major allele
+/-: Number of individuals who have a genotype of heterozygous
-/-: Number of individuals who have a genotype of homozygous minor allele

***Table 5*** *TLR SNPs Significant Associations with Tv Transmission from Women reporting less than 100% condom use (Group 4, N = 26)*

| Gene | SNP/Minor allele | Tv+ | Tv- | OR (lower, upper), p | | | |
|---|---|---|---|---|---|---|---|
| | | +/+*, +/-*,-/-* | | Dominant Model | | Additive Model | |
| | | | | Unadjusted | Adjusted | Unadjusted | Adjusted |
| *TLR2* | rs4696483/T | 4,2,6 | 0,9,5 | 0.065(0, 0.73) 0.024 | 0.082(0.001,0.97) 0.047 | 0.71(0.24,2), 0.52 | 0.76(0.24,2.3), 0.62 |
| | rs7656411/T | 8,4,0 | 4,9,1 | 0.23(0.042,1.1), 0.059 | 0.32(0.055,1.6), 0.17 | 0.24(0.045,0.99), 0.048 | 0.32(0.059,1.4), 0.14 |
| *TLR4* | rs11536865/C | 7,5,0 | 13,0,1 | 6.6(1,74), 0.045 | 5.6(0.84,64), 0.076 | 2.4(0.61,15), 0.22 | 2.2(0.54,12), 0.28 |

+/+: Number of individuals who have a genotype of homozygous major allele
+/-: Number of individuals who have a genotype of heterozygous
-/-: Number of individuals who have a genotype of homozygous minor allele

***Table 6*** *TLR SNPs and Tv Transmission Analysis in HIV-1 Positive and Negative Women (Group 2, N = 61)*

| Group 2 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Gene** | **SNP/ Minor Allele** | **Stratification** | **Tv+** | **Tv-** | **OR (lower, upper), p** | | **Interaction p value** | |
| | | | **+/+*, +/-*,-/-*** | | **Dominant Model** | **Additive model** | **Dominant Model** | **Additive Model** |
| *TLR8* | rs17256081/C | HIV+ | 9,4,0 | 9,14,2 | 0.27(0.063,1), 0.057 | 0.29(0.069,0.97), 0.044 | 0.003 | 0.001 |
| | | HIV- | 2,5,2 | 10,4,0 | 7(1.3,51), 0.025 | 6(1.4,41), 0.012 | | |
| | rs5744069/G | HIV+ | 9,4,0 | 13,9,2 | 0.51(0.12,1.9), 0.33 | 0.5(0.13,1.5), 0.24 | 0.026 | 0.008 |
| | | HIV- | 1,6,2 | 8,6,0 | 7.4(1.2,83), 0.031 | 6.9(1.5,71), 0.011 | | |
| | rs5741883/T | HIV+ | 2,7,4 | 9,11,4 | 2.6(0.6,16), 0.21 | 2(0.78,5.6), 0.15 | 0.083 | 0.019 |
| | | HIV- | 4,5,0 | 3,6,5 | 0.37(0.061,2.1), 0.26 | 0.32(0.071,1.1), 0.064 | | |
| *TLR2* | rs4696483/T | HIV+ | 2,6,5 | 8,9,8 | 2.2(0.5,13), 0.3 | 1.4(0.62,3.5), 0.4 | 0.428 | 0.029 |
| | | HIV- | 0,3,6 | 4,9,1 | 8.1(0.71,1100), 0.099 | 12(2.2,150), 0.002 | | |

+/+: Number of individuals who have a genotype of homozygous major allele
+/-: Number of individuals who have a genotype of heterozygous
-/-: Number of individuals who have a genotype of homozygous minor allele

***Table 7*** *TLR SNPs and Tv Transmission Analysis in HIV-1 Positive and Negative Women (Group 4, N = 26)*

| GENE | SNP/Minor Allele | Stratification | Tv+ | Tv- | OR (lower, upper), P | | Interaction p value | |
|---|---|---|---|---|---|---|---|---|
| | | | +/+*, +/-*,-/-* | | Dominant Model | Additive model | Dominant Model | Additive model |
| *TLR8* | rs1548731 C | HIV+ | 1,3,2 | 6,0,0 | 48(2.9,7800), 0.004 | 15(1.4,2400), 0.015 | 0.005 | 0.002 |
| | | HIV- | 3,2,0 | 2,2,3 | 0.32(0.03,2.8), 0.31 | 0.31(0.046,1.2), 0.091 | | |
| | rs17256081 C | HIV+ | 3,3,1 | 5,1,0 | 4.7(0.55,64), 0.16 | 3.7(0.65,49), 0.16 | 0.029 | 0.026 |
| | | HIV- | 3,2,0 | 1,6,1 | 0.14(0.001,2), 0.17 | 0.17(0.011,1.3), 0.086 | | |
| | rs5744080 C | HIV+ | 6,1,0 | 2,4,0 | 0.13(0.009,1.2), 0.067 | | 0.01 | |
| | | HIV- | 1,4,0 | 6,2,0 | 7.8(0.87,120), 0.067 | | | |
| | rs4830805 A | HIV+ | 3,4,0 | 6,0,0 | 17(1.2,2500), 0.033 | | 0.013 | |
| | | HIV- | 5,0,0 | 5,3,0 | 0.14(0.001,2), 0.17 | | | |
| *TLR7* | rs5743728 A | HIV+ | 3,4,0 | 6,0,0 | 17(1.2,2500), 0.033 | | 0.013 | |
| | | HIV- | 5,0,0 | 5,3,0 | 0.14(0.001,2), 0.17 | | | |
| | rs864058 A | HIV+ | 5,2,0 | 1,2,3 | 0.12(0.008,1.1), 0.061 | 0.18(0.016,0.82), 0.024 | 0.021 | 0.009 |
| | | HIV- | 1,2,2 | 5,2,1 | 4.7(0.55,64), 0.16 | 2.6(0.71,13), 0.15 | | |
| | rs5741881 G | HIV+ | 1,6,0 | 5,1,0, | 16(1.6,310), 0.017 | | 0.009 | |
| | | HIV- | 5,0,0 | 5,3,0 | 0.14(0.001,2), 0.17 | | | |

+/+: Number of individuals who have a genotype of homozygous major allele
+/-: Number of individuals who have a genotype of heterozygous
-/-: Number of individuals who have a genotype of homozygous minor allele

***Table 8*** *Summary of Dominant Model Analyses*

| Acquisition | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gene** | **SNP/Minor Allele** | **Group 1 (N=70)** | | | | **Group 3(N=33)** | | | | **OR (lower, upper), p** | | | **Interaction p-value** | |
| | | **Tv+** | | **Tv-** | | **Tv+** | | **Tv-** | | **Group 1** | **Group3** | | **Group 1** | **Group3** |
| | | **+/+*** | **+/-,-/-*** | **+/+** | **+/-,-/-** | **+/+** | **+/-,-/-** | **+/+** | **+/-,-/-** | **unadjusted** | **unadjusted** | **adjusted** | **unadjusted** | **unadjusted** | **adjusted** |
| *TIRAP* | rs1893352/G | 27 | 1 | 28 | 14 | 15 | 1 | 13 | 4 | 0.11(0.011,0.48), 0.002 | 0.29(0.027,1.83), 0.195 | 0.27(0.024,1.8), 0.18 | 0.657 | 0.729 | 0.838 |
| *TLR3* | rs6552950/G | 23 | 5 | 32 | 10 | 13 | 3 | 13 | 4 | 0.72(0.21,2.3), 0.58 | 0.78(0.15,3.8), 0.76 | 0.81(0.15,4), 0.8 | 0.075 | 0.01 | 0.014 |
| | rs1312681/A | 24 | 4 | 37 | 5 | 13 | 3 | 15 | 2 | 1.3(0.31,4.9), 0.74 | 1.6(0.27,11), 0.6 | 1.7(0.29,12), 0.54 | 0.571 | 0.041 | 0.057 |
| *TLR4* | rs11536865/C | 17 | 11 | 33 | 9 | 9 | 7 | 16 | 1 | 2.3(0.83,6.7), 0.11 | 8.7(1.54,91.9), 0.01 | 8(1.4,85), 0.016 | 0.561 | 0.963 | 0.938 |
| | rs7869402/T | 14 | 14 | 28 | 14 | 6 | 10 | 12 | 5 | 2(0.75,5.2), 0.17 | 3.7(0.94,15.9), 0.06 | 4(1,19), 0.05 | 0.23 | 0.507 | 0.586 |
| | rs5030710/C | 19 | 9 | 29 | 13 | 14 | 2 | 9 | 8 | 1.1(0.38,2.9), 0.9 | 0.2(0.03,0.9), 0.03 | 0.18(0.028,0.86), 0.031 | 0.922 | 0.278 | 0.238 |
| | rs5030729/G | 19 | 9 | 29 | 13 | 14 | 2 | 9 | 8 | 1.1(0.38,2.9), 0.9 | 0.2(0.03,0.9), 0.03 | 0.18(0.028,0.86), 0.031 | 0.922 | 0.278 | 0.238 |
| | rs5030717/G | 15 | 13 | 31 | 11 | 9 | 7 | 15 | 2 | 2.4(0.89,6.6), 0.084 | 4.9(1.04,30.8), 0.044 | 4.6(0.98,29), 0.053 | 0.313 | 0.791 | 0.831 |
| *TLR7* | rs1634319/C | 21 | 7 | 38 | 4 | 10 | 6 | 16 | 1 | 3(0.85,12), 0.088 | 6.8(1.19,72), 0.03 | 7(1.2,77), 0.03 | 0.001 | 1 | 1 |
| | rs1620233/C | 17 | 11 | 17 | 25 | 11 | 5 | 4 | 13 | 0.45(0.17,1.2), 0.1 | 0.16(0.03,0.7), 0.01 | 0.14(0.026,0.62), 0.008 | 0.666 | 0.833 | 0.905 |
| | rs1634323/G | 21 | 7 | 36 | 6 | 9 | 7 | 16 | 1 | 2(0.6,6.6), 0.26 | 8.7(1.54,91.9), 0.01 | 8(1.3,96), 0.023 | 0.418 | 0.963 | 0.985 |
| | rs1634321/T | 20 | 8 | 34 | 8 | 10 | 6 | 16 | 1 | 1.7(0.56,5.1), 0.35 | 6.8(1.19,72.5), 0.03 | 6(1.1,64), 0.042 | 0.968 | 0.686 | 0.677 |
| | rs179009/G | 26 | 2 | 32 | 10 | 15 | 1 | 11 | 6 | 0.29(0.053,1.1), 0.075 | 0.17(0.016,1), 0.047 | 0.15(0.012,0.92), 0.039 | 0.315 | 0.984 | 0.903 |
| | rs1634322/A | 20 | 8 | 31 | 11 | 10 | 6 | 16 | 1 | 1.1(0.39,3.2), 0.81 | 6.8(1.19,72.5), 0.03 | 6.1(1.1,65), 0.041 | 0.833 | 0.686 | 0.693 |
| *TLR8* | rs5741883/T | 23 | 5 | 22 | 20 | 14 | 2 | 9 | 8 | 0.26(0.079,0.74), 0.011 | 0.19(0.031,0.89), 0.034 | 0.17(0.024,0.83), 0.027 | 0.514 | 0.671 | 0.401 |
| | rs1548731/C | 15 | 10 | 24 | 14 | 6 | 7 | 14 | 2 | 1.1(0.41,3.2), 0.79 | 6.7(1.34,44.6), 0.02 | 7.9(1.5,59), 0.014 | 0.779 | 0.735 | 0.572 |
| | rs4830805/A | 19 | 9 | 34 | 8 | 9 | 7 | 16 | 1 | 2(0.67,5.9), 0.21 | 8.7(1.54,91.9), 0.01 | 8.4(1.5,90), 0.014 | 0.157 | 0.579 | 0.543 |
| | rs5744088/C | 26 | 2 | 37 | 5 | 16 | 0 | 12 | 5 | 0.64(0.11,2.9), 0.58 | 0.07(0,0.7), 0.02 | 0.074(0.001,0.81), 0.031 | 0.524 | 0.723 | 0.738 |
| **Transmission** | | | | | | | | | | | | | | | |
| **Gene** | | **Group 2 (N=61)** | | | | **Group 4 (N=26)** | | | | **OR (lower, upper), p** | | | **Interaction p-value** | | |

| | SNP/Minor Allele | Tv+ | | Tv- | | Tv+ | | Tv- | | Group 2 | Group4 | | Group 2 | Group4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | +/+ | +/-,-/- | +/+ | +/-,-/- | +/+ | +/-,-/- | +/+ | +/-,-/- | unadjusted | unadjusted | adjusted | unadjusted | unadjusted | adjusted |
| *TLR2* | rs4235232/G | 15 | 7 | 35 | 4 | 10 | 2 | 10 | 4 | 3.6(1.1,14), 0.031 | 0.56(0.081,3.1), 0.51 | 0.71(0.1,4.2), 0.71 | 0.813 | 0.263 | 0.172 |
| | rs4696483/T | 2 | 20 | 12 | 27 | 4 | 8 | 0 | 14 | 2.5(1.2,5.8), 0.014 | 0.065(0,0.73), 0.024 | 0.082(0.001,0.97), 0.047 | 0.428 | 0.76 | 0.633 |
| | rs7656411/T | 13 | 9 | 18 | 21 | 8 | 4 | 4 | 10 | 0.54(0.21,1.3), 0.17 | 0.23(0.042,1.1), 0.059 | 0.32(0.055,1.6), 0.17 | 0.133 | 0.473 | 0.406 |
| *TLR3* | rs7657186/A | 9 | 13 | 27 | 12 | 6 | 6 | 8 | 6 | 1.7(0.77,3.8), 0.19 | 1.3(0.29,6), 0.72 | 1.8(0.37,9.9), 0.48 | 0.431 | 0.431 | 0.461 |
| *TLR4* | rs1927906/C | 13 | 9 | 8 | 30 | 4 | 8 | 6 | 8 | 0.26(0.1,0.59), 0.001 | 1.4(0.31,7), 0.64 | 1.3(0.26,6.3), 0.78 | 0.371 | 0.551 | 0.536 |
| | rs11536887/G | 22 | 0 | 31 | 8 | 12 | 0 | 13 | 1 | 0.097(0.001,0.79), 0.023 | NA* | NA | 0.918 | NA | NA |
| | rs11536888/T | 17 | 5 | 37 | 2 | 12 | 0 | 13 | 1 | 4.7(1,28), 0.047 | NA | NA | 0.522 | NA | NA |
| | rs11536865/C | 17 | 5 | 28 | 10 | 7 | 5 | 13 | 1 | 0.75(0.25,2), 0.58 | 6.6(1,74), 0.045 | 5.6(0.84,64), 0.076 | 0.169 | 0.598 | 0.663 |
| *TLR7* | rs1634323/G | 13 | 9 | 34 | 5 | 9 | 3 | 13 | 1 | 4.4(1.3,16), 0.015 | NA | NA | 0.558 | NA | NA |
| | rs179018/C | 15 | 7 | 35 | 3 | 12 | 0 | 11 | 3 | 2.9(0.91,11), 0.073 | NA | NA | 0.643 | NA | NA |
| | rs5743776/T | 14 | 8 | 33 | 5 | 11 | 1 | 10 | 4 | 2.4(0.83,8), 0.11 | 0.3(0.027,2), 0.23 | 0.32(0.027,2.3), 0.26 | 0.755 | 0.312 | 0.224 |
| | rs179013/A | 16 | 6 | 36 | 3 | 11 | 1 | 12 | 2 | 4.1(1,19), 0.045 | NA | NA | 0.088 | NA | NA |
| | rs5743728/A | 19 | 3 | 30 | 9 | 8 | 4 | 11 | 3 | 0.58(0.13,2.1), 0.41 | 1.7(0.33,9.8), 0.51 | 1.1(0.17,6.8), 0.9 | 0.613 | 0.013 | 0.028 |
| | rs864058/A | 8 | 14 | 21 | 17 | 6 | 6 | 6 | 8 | 1.8(0.87,3.7), 0.11 | 0.76(0.17,3.4), 0.72 | 0.92(0.19,4.4), 0.91 | 0.336 | 0.021 | 0.014 |
| | rs5741881/G | 15 | 7 | 26 | 13 | 6 | 6 | 10 | 4 | 0.78(0.3,1.9), 0.59 | 2.3(0.51,12), 0.28 | 1.7(0.32,8.7), 0.53 | 0.705 | 0.009 | 0.012 |
| *TLR8* | rs17256081/C | 11 | 11 | 19 | 20 | 6 | 6 | 6 | 8 | 1.1(0.46,2.5), 0.86 | 0.76(0.17,3.4), 0.72 | 0.79(0.17,3.8), 0.77 | 0.003 | 0.029 | 0.051 |
| | rs5744069/G | 10 | 12 | 21 | 17 | 4 | 8 | 7 | 7 | 1.4(0.62,3.3), 0.41 | 1.9(0.42,9.2), 0.41 | 1.8(0.38,9.5), 0.45 | 0.026 | 0.13 | 0.09 |
| | rs5741883/T | 6 | 16 | 12 | 26 | 4 | 8 | 3 | 11 | 0.98(0.47,2), 0.96 | 0.57(0.1,3), 0.51 | 0.89(0.15,5.9), 0.9 | 0.083 | 0.103 | 0.217 |
| | rs1548731/C | 6 | 13 | 17 | 19 | 4 | 7 | 8 | 5 | 0.98(0.45,2.1), 0.96 | 2.6(0.54,14), 0.24 | 2.5(0.51,14), 0.27 | 0.471 | 0.005 | 0.011 |
| | rs5744080/C | 14 | 8 | 29 | 10 | 7 | 5 | 8 | 6 | 1.6(0.69,4.1), 0.26 | 0.96(0.21,4.4), 0.96 | 1.1(0.24,5.6), 0.86 | 0.641 | 0.01 | 0.014 |
| | rs4830805/A | 15 | 7 | 27 | 12 | 8 | 4 | 11 | 3 | 1.1(0.43,2.8), 0.81 | 1.7(0.33,9.8), 0.51 | 1.5(0.27,9), 0.62 | 0.186 | 0.013 | 0.013 |
| *MyD88* | rs6853/G | 5 | 17 | 22 | 17 | 6 | 6 | 7 | 7 | 2.5(1.1,6.1), 0.025 | 1(0.22,4.5), 1 | 1(0.22,5), 0.96 | 0.975 | 0.594 | 0.588 |

+/+: Number of individuals who have a genotype of homozygous major allele
+/-,-/-: Number of individuals who have a genotype of heterozygous plus Number of individuals who have a genotype of homozygous minor allele
Any SNP that had a combined number of +/- and -/- less than 5 was not analyzed. These are indicated as NA.

**References**


[1] Newman, L., Rowley, J., Unemo, M., Low, N., Stevens, G., Gottlieb, S., . . . Temmerman, M. (2015). Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting. *PLoS One*, *10*(12), E0143304.

[2] Kissinger, P. (2015). Trichomonas vaginalis: A review of epidemiologic, clinical and treatment issues. *Bmc Infectious Diseases*,*15*(1), 307.

[3] Krieger, J. (1995). TRICHOMONIASIS IN MEN - OLD ISSUES AND NEW DATA. *Sexually Transmitted Diseases*, *22*(2), 83-96.

[4] Cudmore, S. L., Delgaty, K. L., Hayward-McClelland, S. F., Petrin, D. P., & Garber, G. E. (2004). Treatment of infections caused by metronidazole-resistant Trichomonas vaginalis. *Clinical microbiology reviews*, *17*(4), 783–793. doi:10.1128/CMR.17.4.783-793.2004

[5] Seña, A., Miller, W., Hobbs, M., & Schwebke, J. (2007). Trichomonas vaginalis Infection in Male Sexual Partners: Implications for Diagnosis, Treatment, and Prevention. *Clinical Infectious Diseases*, *44*(1), 13.

[6] Martinez-Garcia, Regadera, Mayer, Sanchez, & Nistal. (1996). Protozoan Infections in the Male Genital Tract. *The Journal of Urology*, *156*(2), 340-349.

[7] Kissinger P, Secor WE, Leichliter JS, Clark RA, Schmidt N, Curtin E, Martin DH. Early repeated infections with Trichomonas vaginalis among HIV-positive and HIV-negative women. Clin Infect Dis.

[8] Fichorova, Fraga, Rappelli, & Fiori. (2017). Trichomonas vaginalis infection in symbiosis with Trichomonasvirus and Mycoplasma. *Research in Microbiology*, *168*(9), 882-891.

[9] Cudmore, S., & Garber, G. (2010). Prevention or treatment: The benefits of Trichomonas vaginalis vaccine. *Journal of Infection and Public Health*, *3*(2), 47-53.

[10] McClelland, R. Scott, Sangare, Laura, Hassan, Wisal M., Lavreys, Ludo, Mandaliya, Kishorchandra, Kiarie, James, . . . Baeten, Jared M. (2007). Infection with Trichomonas vaginalis increases the risk of HIV-1 acquisition. *Journal of Infectious Diseases*, *195*(5), 698-702.

[11] Termeulen, Mgaya, Changclaude, Luande, Mtiro, Mhina, . . . Pawlita. (1992). RISK-FACTORS FOR HIV-INFECTION IN GYNECOLOGICAL INPATIENTS IN DAR-ES-SALAAM, TANZANIA, 1988-1990. *East African Medical Journal*, *69*(12), 688-692.

[12] Hobbs, M., Kazembe, P., Reed, A., Miller, W., Nkata, E., Zimba, D., . . . Hoffman, I. (1999). Trichomonas vaginalis as a cause of urethritis in Malawian men. *Sexually Transmitted Diseases*, *26*(7), 381-7.

[13] Bochner, A., Baeten, J., Rustagi, A., Nakku-Joloba, E., Lingappa, J., Mugo, N., . . . Mcintyre, James. (2017). A cross-sectional analysis of Trichomonas vaginalis infection among heterosexual HIV-1 serodiscordant African couples. *Sexually Transmitted Infections*, *93*(7), 520-529.

[14] Jiménez-Dalmaroni, Gerswhin, & Adamopoulos. (2016). The critical role of toll-like receptors — From microbial recognition to autoimmunity: A comprehensive review. *Autoimmunity Reviews*, *15*(1), 1-8.

[15] Zariffard, Harwani, Novak, Graham, Ji, & Spear. (2004). Trichomonas vaginalis infection activates cells through toll-like receptor 4. *Clinical Immunology*, *111*(1), 103-107.

[16] Chang JH, Kim SK, Choi IH, et al. Apoptosis of macrophages induced by *Trichomonas vaginalis* through the phosphorylation of p38 mitogen-activated protein kinase that locates at downstream of mitochondria-dependent caspase activation. Int J Biochem Cell Biol. 2006;38(4):638–47.

[17] Mackelprang, Romel D., Bigham, Abigail W., Celum, Connie, Bruyn, Guy de, Beima-Sofie, Kristin, John-Stewart, Grace, . . . Lingappa, Jairam R. (2014). Toll-like receptor polymorphism associations with HIV-1 outcomes among Sub-Saharan Africans. *Journal of Infectious Diseases*, *209*(10), 1623-1627.

[18] R D Mackelprang, C W Scoville, C R Cohen, R O Ondondo, A W Bigham, C Celum, . . . J R Lingappa. (2015). Toll-like receptor gene variants and bacterial vaginosis among HIV-1 infected and uninfected African women. *Genes and Immunity*, *16*(5), 362-365.

[19] Celum, C., Wald, A., Lingappa, J. R., Magaret, A. S., Wang, R. S., Mugo, N., … Partners in Prevention HSV/HIV Transmission Study Team (2010). Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2. *The New England journal of medicine*, *362*(5), 427–439. doi:10.1056/NEJMoa0904849

[20] Lingappa, J., Petrovski, S., Kahle, E., Fellay, J., Shianna, K., McElrath, M., . . . Speck, R. (2011). Genomewide Association Study for Determinants of HIV-1 Acquisition and Viral Set Point in HIV-1 Serodiscordant Couples with Quantified Virus Exposure (GWAS for HIV-1 Acquisition). *PLoS ONE*, *6*(12), E28632.

[21] Dupont WD, Plummer WD: 'Power and Sample Size Calculations for studies Involving Linear Regression', Controlled Clinical Trials 1998; 19:589-601.

[22] Edwards, Beesley, French, and Dunning. "Beyond GWASs: Illuminating the Dark Road from Association to Function." *The American Journal of Human Genetics* 93.5 (2013): 779-97. Web.

[23] Khor CC, Chapman SJ, Vannberg FO, et al. A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. Nat Genet. 2007;39:523–28.
[24] Tiffany Horng, Gregory M. Barton, Richard A. Flavell, and Ruslan Medzhitov. "The Adaptor Molecule TIRAP Provides Signalling Specificity for Toll-like Receptors." *Nature* 420.6913 (2002): 329-33. Web.
[25] Katherine A. Fitzgerald, Eva M. Palsson-Mcdermott, Andrew G. Bowie, Caroline A. Jefferies, Ashley S. Mansell, Gareth Brady, Elizabeth Brint, Aisling Dunne, Pearl Gray, Mary T. Harte, Diane Mcmurray, Dirk E. Smith, John E. Sims, Timothy A. Bird, and Luke A. J. O'Neill. "Mal (MyD88-adapter-like) Is Required for Toll-like Receptor-4 Signal Transduction." *Nature* 413.6851 (2001): 78-83. Web.
[26] Masahiro Yamamoto, Shintaro Sato, Hiroaki Hemmi, Hideki Sanjo, Satoshi Uematsu, Tsuneyasu Kaisho, Katsuaki Hoshino, Osamu Takeuchi, Masaya Kobayashi, Takashi Fujita, Kiyoshi Takeda, and Shizuo Akira. "Essential Role for TIRAP in Activation of the Signalling Cascade Shared by TLR2 and TLR4." *Nature* 420.6913 (2002): 324-329. Web.

[27] Mehrizi Akram A, Pirahmadi Sakineh, Zakeri Sedigheh, and Djadid Navid D. "Genetic Variation of TLR-4, TLR-9 and TIRAP Genes in Iranian Malaria Patients." *Malaria Journal* 10.1 (2011): 77. Web.
[28] Chiea C Khor, Stephen J Chapman, Fredrik O Vannberg, Aisling Dunne, Caroline Murphy, Edmund Y Ling, . . . Adrian V S Hill. (2007). A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. *Nature Genetics, 39*(4), 523-528.
[29] Durrani, O., Banahan, K., Sheedy, F., Mcbride, L., Ben-Chetrit, E., Greiner, K., . . . Wallace, G. (2011). TIRAP Ser180Leu polymorphism is associated with Behcet's disease. *Rheumatology, 50*(10), 1760-1765.
[30] Fichorova, R., Lee, Y., Yamamoto, H., Takagi, Y., Hayes, G., Goodman, R., . . . Fasel, N. (2012). Endobiont Viruses Sensed by the Human Host – Beyond Conventional Antiparasitic Therapy (Endobiont Protozoan Viruses Impact the Human Host). *7*(11), E48418.
[31] Margarita, Valentina, Rappelli, Paola, Dessi, Daniele, Pintus, Gianfranco, Hirt, Robert P., & Fiori, Pier L. (2016). Symbiotic Association with Mycoplasma hominis Can Influence Growth Rate, ATP Production, Cytolysis and Inflammatory Response of Trichomonas vaginalis. *Frontiers in Microbiology, 7*, 953.
[32] Peltier, M., Freeman, A., Mu, H., & Cole, B. (2005). Characterization and Partial Purification of a Macrophage-Stimulating Factor from Mycoplasma hominis. *American Journal of Reproductive Immunology, 54*(6), 342-351.
[33] Mercer, Frances, Ghedin, Elodie, Diala, Fitz Gerald I., Chen, Yi-Pei, Molgora, Brenda M., Ng, Shek Hang, & Johnson, Patricia J. (2016). Leukocyte Lysis and Cytokine Induction by the Human Sexually Transmitted Parasite Trichomonas vaginalis. *PLOS Neglected Tropical Diseases, 10*(8), E0004913.
[34] Fichorova, R. (2009). Impact of T. vaginalis infection on innate immune responses and reproductive outcome. *Journal of Reproductive Immunology, 83*(1), 185-189.
[35] Xuefeng Ewang. (2014). Firth logistic regression for rare variant association tests. *Frontiers in Genetics, 5*, 187.