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A single-nucleotide polymorphism in *TLR4* is linked with the risk of HIV-1 infection

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ABSTRACT

Introduction: Toll-like receptors (TLRs) are pattern recognition receptors that play a role in innate immunity. Mounting evidence shows that single-nucleotide polymorphisms (SNPs) in TLRs link to various infectious diseases, including human immunodeficiency virus (HIV). We hypothesized links between two TLR4 SNPs (rs4986790 leading to Asp299Gly and rs4986791 leading to Thr399Ile) and HIV, to investigate the frequency of TLR4 polymorphism and its role in patients infected with HIV.

Materials and methods: We recruited 160 HIV-1 seropositive patients, who were further divided on disease severity based on CD4 count (stages I, II and III), and 270 age- and sex matched healthy HIV-1 seronegative individuals. Subjects were genotyped for TLR4 gene polymorphism by polymerase chain reaction restriction fragment length polymorphism.

Results: The TLR4 Asp299Gly heterozygous genotype (OR=2.160; p=0.004) and the mutant allele G (OR=2.051; p=0.002) was higher in HIV-1 infection than healthy controls and also in stage I (OR=2.559; p=0.034) compared to different clinical stages of infection. There was no link between the Thr399Ile polymorphism and HIV infection.

Conclusion: The TLR4 (Asp299Gly) SNP is a risk factor in HIV-1 disease susceptibility.

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KEYWORDS
TLR 4; polymorphism; HIV; disease susceptibility

Introduction

Acquired immunodeficiency syndrome (AIDS) is a complex and chronic disease, caused by HIV-1 and characterized by a gradual dysfunction of cell-mediated and humoral immunity, which leads to depletion of the CD4 T-lymphocyte cell population and to high levels of HIV RNA and development of opportunistic disease [1]. The course of HIV-1 infection varies widely even among individuals with similar risk exposure levels [2–4] and there is extensive heterogeneity in susceptibility to infection, the time required to deplete the CD4 T-lymphocyte population and to develop AIDS defining diseases [5,6]. Persistent chronic immune activation plays a role in the pathogenesis of HIV infection and the progression to AIDS [7]. Hence, an intact innate immune system has a significant role in controlling chronic inflammation, thereby influencing the occurrence of opportunistic diseases [8,9].

One of the first events in the interaction with an invading microorganism is the engagement of cellular pattern recognition receptors, the best characterized class being Toll-like receptors (TLRs) [10,11]. TLR genes encode a family of type I transmembrane receptors with extracellular leucine-rich repeats and an intracellular signalling domain and are expressed on most immune cells. They play a role in the innate immune response inducing a signal that regulates the activation of several pro-inflammatory genes leading to induction of suppression of genes involved in the inflammatory process [12]. TLR4 is a pathogen-associated molecular pattern receptor of bacterial lipopolysaccharide (LPS) and viral-envelope proteins. The interaction of LPS with TLR4 initiates a downstream intracellular signalling transduction that induces production of inflammatory cytokines such as TNFα, ILβ, IL6 and IL8. *TLR4* consists of three exons mapped to 9q32-33. Several single-nucleotide polymorphisms (SNPs) have been reported in *TLR4*, of which two non-synonymous SNPs in exon 3 are more prevalent, an A/G transition at SNP rs4986790 that causes the substitution of aspartic acid by glycine at amino acid position 299 (Asp299Gly) and a C/T transition at SNP rs4986791 that causes the substitution of threonine by isoleucine at position 399 (Thr399Ile). These SNPs are usually found in co-segregation and have been shown to change the ligand-binding site of the receptor [13,14].

In HIV-infection studies to evaluate the contribution of Asp299Gly and Thr399Ile SNPs in the pathogenesis of HIV infection are limited. Innate immune response such as TLR4 are triggered by HIV infection which is responsible for clinical outcomes of individuals with the disease. Thus, polymorphisms occurring in innate immune genes may serve as possible susceptibility factors for the development of HIV

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infection. Here, we conducted a case–control study to test the hypothesis of a link between TLR4 (Asp299Gly) and (Thr399Ile) and risk of HIV infection.

Materials and methods

Ethical approval was granted by the Ethics Committee, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, India, and the study was conducted in accordance with the Ethical Guidelines for Biomedical Research on human blood samples. Each patient was provided with written information about the study, and consent was taken prior to enrolling him/her. Recruitment was from patients visiting Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. From April 2012 to December 2014, 160 patients (144 males and 16 females, mean/SD age 54.9 years ± 9.7) were selected. Patients either had no history or a less than 6-week prior history of antiretroviral therapy. Depending on their CD4 counts and clinical symptoms, they were divided into stage I (n = 57, 51 males, 6 females), asymptomatic HIV-1 patients in Centers for Disease Control and Prevention (CDC) category A1–A3; stage II (n = 72, 64 males, 8 females) symptomatic HIV-1 patients without AIDS in CDC category B1 to B3; stage III (n = 31, 27 males, 4 females) symptomatic HIV-1 patients with AIDS in CDC category C1 to C3 as per the CDC criteria [15]. Only those patients who had a minimum follow-up time of at least 6 months were included. A group of 270 age- and sex-matched healthy individuals (237 males, 33 females, mean age 57.0 years ± 12.6) with HIV-1 seronegative status were recruited as controls.

Approximately 5 mL blood was collected from each subject in EDTA-coated vials and stored at −80 °C. DNA was isolated using the standard salting out method [16] and checked on 0.8% agarose gel before storage at −20 °C for further use. Patients were tested for HIV-1 by ELISA (HIV-1/2 ELISA 4.0, MP Biomedicals) and subsequently confirmed with Western Blot (HIV Blot 2.2, MP Biomedicals). CD4 cell count was measured by flow cytometer FACScan (Becton Dickinson San Jose, CA, USA) using fresh EDTA-treated whole blood at the time of recruitment.

Two non-synonymous SNPs in the exon 3 of *TLR4* were detected by PCR-RFLP [17]. For the Asp299Gly polymorphism, the 249-bp fragment spanning position 896 in *TLR4* was amplified using sense primer 5′-GATTAGCATACTTAGACTACCTCC ATG-**3**′ and antisense primer 5′-GATCAACTTCTGAAAAAGCATTCC CAC-**3**′. Digestion of the PCR product with NcoI (Fermentas INC, NY, USA) yielded 249-bp when G was present and 233-bp fragment only when A was present. For the Thr399Ile polymorphism, the 410-bp fragment spanning position 1196 was amplified using sense primer 5′-GGTTGCTGTTCTCAAAGTGATTTTGGGAGA A-**3**′ and antisense primer 5′-CCAGATGTTCTAGTTGTTCTAAGCCCA AG-**3**′. Digestion of the PCR product with HinfI (Fermentas INC, NY, USA) yielded 129 bp when G was present and 104-bp fragment only when A was present. PCR was carried out in 25 µl reaction volume containing 100 ng genomic DNA, 0.5 µmol/l of each primer, 200 µmol/l of each dNTP, 10× PCR buffer and 1.5 U of Taq DNA polymerase (Bangalore Genei, India). For TLR4 896 A/G, the reaction mixture was subjected to initial denaturation 95 °C for 5 min, followed by 30 cycles at denaturation 95 °C for 30 s, annealing 55 °C for 30 s, extension TLR 72 °C for 30 s and final extension at 72 °C for 5 min. The TLR4 1196 C/T reaction mixture was subjected to initial denaturation 95 °C for 15 min, followed by 40 cycles at denaturation 94 °C for 30 s min, annealing 66 °C for 30 s, extension 72 °C for 30 s and final extension at 72 °C for 10 min.

Statistical analysis used SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Direct gene counting determined the frequency of genotypes and alleles. Fisher's exact or χ^2 test was used to determine differences in allele/genotype frequencies. Odds ratios and its 95% confidence interval were calculated to assess the risk conferred by a particular allele and genotype. Observed and expected genotype frequencies were compared by χ^2 test to check deviation from Hardy–Weinberg Equilibrium. A P-value <0.05 was considered statistically significant.

Results

Genotype and allelic frequency distribution among 160 HIV+ve cases and 270 HIV−ve controls groups is given in Table 1. The overall prevalence of *TLR4* (Asp299Gly) and *TLR4* (Thr399Ile) (heterozygous and homozygous mutant genotypes) was 17.6% (76/430) and 10% (43/430), respectively. The genotypic frequency distribution of TLR4 (Asp299Gly) showed that the heterozygous AG genotype is more prevalent in HIV+ve and brings a significant >2-fold higher risk for HIV-1 infection. Because the frequency of mutant genotype of TLR4 (Asp299Gly) polymorphisms is low, we adopted a recessive model, and links with HIV infection were calculated. The AG+GG carriers, and presence of the G allele, were significantly more frequent in HIV+ve patients. There were no differences in the frequency distribution of the TLR4 (Thr399Ile) polymorphism in HIV+ve compared to HIV−ve.

Table 2 shows analysis of HIV+ve patients in stages I and II. A reduced frequency of *TLR4* 896 A/G Asp 299 Gly AG and AG+GG genotypes, and the G allele, were linked to stage progression. There were no links between *TLR4* 1196 C/T Thr 399 Ile and stage. Table 3 shows analysis of HIV+ve patients in stages I and III. There were no links between any *TLR* SNP and stage progression. Thus, overall, there is no link between these SNPs and stage.

Table 1. Allelic and genotype frequency distribution of TLR4 gene polymorphism among HIV-1 seropositive (HIV+ve) and HIV-1 seronegative (HIV−ve) individuals.

	HIV+ve (n = 160)	HIV−ve (n = 270)	OR (95%CI)	P-value
TLR4 896 A/G Asp 299 Gly				
Genotype				
AA	120 (75.0)	234 (86.6)	Reference	–
AG	37 (23.1)	34 (12.5)	2.16 (1.28–3.62)	0.004
GG	3 (1.8)	2 (0.7)	2.56 (0.42–1.54)	0.366
AG+GG	40 (25.0)	36 (13.3)	2.16 (1.31–3.56)	0.003
AG+AA	157 (98.1)	268 (99.2)	0.39 (0.06–2.36)	0.366
Allele				
A	277 (86.5)	502 (92.9)	Reference	–
G	43 (13.4)	38 (7.0)	2.05 (1.29–3.25)	0.002
TLR4 1196 C/T Thr 399 Ile				
Genotype				
CC	141 (88.1)	246 (91.1)	Reference	–
CT	18 (11.2)	23 (8.5)	1.36 (0.71–2.60)	0.397
TT	1 (0.3)	1 (0.3)	1.69 (0.10–2.72)	1.000
CT+TT	19 (11.8)	24 (8.8)	1.38 (0.73–2.61)	0.323
CT+CC	159 (99.3)	269 (99.6)	0.59 (0.03–9.51)	1.000
Allele				
C	300 (93.75)	515 (95.37)	Reference	–
T	20 (6.25)	25 (4.62)	1.37 (0.75–2.51)	0.342

Table 2. Allelic and genotype frequency distribution of TLR4 gene polymorphism among HIV-1 seropositive patients classified into stages I and II.

	Stage I (n = 57) CD4 count >500 cells/μl	Stage II (n = 72) CD4 count 200–499 cells/μl	OR (95%CI)	P-value
TLR4 896 A/G Asp 299 Gly				
Genotype				
AA	37 (64.9)	60 (83.3)	Reference	–
AG	18 (31.5)	11 (15.2)	2.55 (1.09–5.99)	0.034
GG	2 (3.5)	1 (1.3)	2.58 (0.22–2.92)	0.583
AG+GG	20 (35.0)	12 (16.6)	2.70 (1.18–6.16)	0.023
AG+AA	55 (96.4)	71 (98.6)	0.38 (0.03–4.38)	0.583
Allele				
A	92 (80.7)	131 (90.9)	Reference	–
G	22 (19.2)	13 (9.0)	2.41 (1.15–5.02)	0.027
TLR4 1196 C/T Thr 399 Ile				
Genotype				
CC	50 (87.7)	63 (87.5)	Reference	–
CT	6 (10.5)	9 (12.5)	0.82 (0.27–2.46)	0.788
TT	1 (1.7)	0 (0.0)	–	0.422
CT+TT	7 (12.2)	9 (12.5)	0.98 (0.34–2.81)	1.000
CT+CC	56 (98.2)	72 (100)	–	0.442
Allele				
C	106 (92.9)	135 (93.7)	Reference	–
T	8 (7.0)	9 (6.2)	1.13 (0.42–3.03)	0.086

Table 3. Allelic and genotype frequency distribution of TLR4 gene polymorphism among HIV-1 seropositive patients classified into stages I and III.

	Stage I (n = 57) CD4 count >500 cells/μl	Stage III (n = 31) CD4 count <200 cells/μl	OR (95%CI)	P-value
TLR4 896 A/G Asp 299 Gly				
Genotype				
AA	37 (64.9)	23 (74.1)	Reference	–
AG	18 (31.5)	8 (25.8)	1.32 (0.49–3.53)	0.632
GG	2 (3.5)	0 (0.0)	–	0.538
AG+GG	20 (35.0)	8 (25.8)	1.55 (0.58–4.10)	0.474
AG+AA	55 (96.4)	31 (100)	–	0.538
Allele				
A	92 (80.7)	54 (87.0)	Reference	–
G	22 (19.2)	8 (12.9)	1.61 (0.67–3.87)	0.304
TLR4 1196 C/T Thr 399 Ile				
Genotype				
CC	50 (87.7)	28 (90.3)	Reference	–
CT	6 (10.5)	3 (9.6)	1.09 (0.25–4.73)	1.000
TT	1 (1.7)	0 (0.0)	–	1.000
CT+TT	7 (12.2)	3 (9.6)	1.30 (0.31–5.45)	1.000
CT+CC	56 (98.2)	31 (100)	–	1.000
Allele				
C	106 (92.9)	59 (95.1)	Reference	–
T	8 (7.0)	3 (4.8)	1.48 (0.37–5.80)	0.749

Discussion

The TLR-signalling pathway plays a role in susceptibility or resistance to several infectious diseases [18,19]; therefore, we studied the impact of TLR4 (Asp299Gly and Thr399Ile) SNPs on HIV infection. This study of the distribution of these *TLR4* SNPs and their distribution with HIV-1 disease gives a frame of reference that may be useful for other infectious/viral disease studies. To better understand the complex relationship between a genotype and phenotype, a search for functional polymorphism in many candidate genes has emerged as a tool. The overall prevalence of TLR4 Asp299Gly polymorphism in our study population is similar to other reports [17,20,21]. The prevalence of the heterozygous genotype and G allele of the TLR4 Asp299Gly SNP was significantly higher among HIV-infected patients. However, the TLR4 Asp299Gly SNP was not associated with a decrease in CD4 count and is not associated with disease progression. Patients infected with HIV-1 have increased intestinal permeability, which allows the passage of microbial products (PAMPs) into the blood, which leads to chronic immune activation and the decline of CD4 T cells. PAMPs have been found among translocated microbial products which bind to TLR and which trigger a complex signalling pathway which modulates the function of dendritic cells, macrophages and CD4 T cells, the target cells of HIV-1 [22,23]. The TLR4 (Asp299Gly) SNP has been reported to cause LPS hyporesponsiveness, potentially by impairing dimerization of TLR4 and one of its adaptor proteins, MD2, of importance for signal transduction [24,25].

Studies to evaluate the role of TLR4 polymorphisms in the course of HIV-1 infection have been limited and conflicting results have been reported. In Swiss HIV-infected patients, TLR4 SNPs were found to be associated with high peak viral load [26]. However, other studies have shown no association between viral load and these SNPs in Spanish and Omani patients [27,28]. Furthermore, HIV-infected African patients from Tanzania and Caucasian patients from Greece showed a link between TLR4 SNPs and a decreased CD4 count and increased frequency of serious infection in subjects with CD4 count <100 cells/μl [29,30], but a study in Omani individuals reported no association of this SNP with a decrease in CD4 count [28]. Our study is consistent with previous reports showing that TLR4 Asp299Gly gene polymorphism is associated with risk of HIV infection, but we did not observe any association with disease progression when there is decline in CD4 counts [26,28]. We speculate that the HIV-infected patients in our study may have a high viral load and as a consequence are more prone to infection, but we are limited as we do not have the viral load data. Redd et al. [31] reported that the rate of HIV disease progression, as measured by a decline in CD4 T cell, was not found to be related to changing plasma levels of microbial translocation markers. Therefore, it is likely that it is not the degree of microbial translocation but the nature of the host response that determines disease progression.

This study represents an advance in biomedical science as it shows a link between a SNP in a *TLR* SNP and risk of HIV infection.

Summary Table

What is known about this topic:
- SNPs in TLR4 plays a role in modulating the risk and susceptibility to several infectious diseases.
- Studies to evaluate the contribution of *TLR 4* SNPs in the pathogenesis of HIV infection are limited.

What this study adds:
- The *TLR4* Asp299Gly SNP, but not the *TLR4* 1196 C/T Thr399Ile SNP, is linked to HIV-1 infection.
- Neither SNPs are linked to CD4 count stage.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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