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The -137G/C polymorphism of interleukin 18 promoter and risk of HIV-1 infection and its progression to AIDS

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Summary – A growing body of evidence suggests that host genetic factors play an important role both in susceptibility to human immunodeficiency virus 1 (HIV-1) infection and in progression to AIDS. Interleukin 18 (IL-18) is a pleiotropic proinflammatory cytokine that serves as an important regulator of immune responses. It plays a key role in induction of both Th1 and Th2 cytokines and, thereby, modulates their immune responses. Single nucleotide polymorphisms in the *IL-18* gene promoter region may lead to an altered transcriptional activity and IL-18 production, and so this may account for individuals' variation to the risk of HIV-1 infection. With this perspective, the *-137G/C* polymorphism in the promoter region of the *IL-18* gene was studied in 500 patients with HIV-1/AIDS and an equal number of sex and age matched healthy controls using sequence specific polymerase chain reaction analysis. We did not observe any significant association of the heterozygous *G/C* genotype with the risk of HIV-1-infection/AIDS. However, statistically significant associations of the *G* allele and homozygous *G/G* genotype of *-137 G/C* polymorphism of *IL-18* promoter with increased risk of HIV-1/AIDS were identified. The data of the present study suggest that *IL-18 -137 G* allele and *G/G* genotype seem to be involved in the pathogenesis of HIV-1 infection among North Indians.

Keywords: HIV-1/AIDS; IL-18 gene; PCR; SNP

Introduction

The susceptibility and clinical manifestations of infectious diseases in human populations are influenced by a variety of factors. Among these, host genetic variability plays a major role in determining individuals' susceptibility to potentially pathogenic infections and subsequent disease severity (Segal and Hill, 2003). In the context of HIV-1 infection, genetic epidemiologic cohort studies have shown polymorphisms in the genes encoding chemokine receptors to be associated with the resistance against acquiring HIV-1 infection and an altered rate of disease progression in infected individuals (O'Brien and Moore, 2000). Moreover, several human leukocyte antigen (HLA) alleles have also been associated with different rates of progression and varying susceptibility to HIV infection (Smith *et al*., 1997; Klein *et al*., 1998).

Single nucleotide polymorphism (SNP) in genes encoding cytokines and their receptors have been shown to influence not only cytokine gene expression, but also susceptibility to or progress of the disease (Bidwell *et al*., 2001; Kaur *et al*., 2007). *IL-18* gene encoding human interleukin 18 (IL-18) cytokine is mapped to chromosome 11q22.2–22.3 (Nolan *et al*., 1998) and within this gene three SNPs in the promoter region have been identified. Of these, polymorphisms at promoter positions *-137* and *-607* influence the activity and expression of *IL-18* gene. The G to C substitution at the position -137 abolishes the human histone 4 transcription factor 1 (H4TF-1) nuclear factor-binding site and the C to A substi-

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Abbreviations: CREB = cAMP responsive element binding protein; HIV-1 = human immunodeficiency virus; HLA = human leukocyte antigen; H4TF-1 = human histone 4 transcription factor 1; IL-18 = interleukin 18; SNP = single nucleotide polymorphism

tution at position-607 disrupts the binding sites of a potential cAMP responsive element binding protein (CREB) thereby, reducing its transcription. Therefore, mutations at these two *IL-18* promoter polymorphisms (*-607C/A* and *-137G/C*) and their haplotypes seem to account for differential IL-18 expression and changes in the production of this cytokine (Giedraitis *et al.,* 2001).

IL-18 is a pleiotropic proinflammatory cytokine that is recognized as an important regulator of innate and acquired immune responses (Dinarello and Fantuzzi, 2003). It is produced mainly by monocytes/macrophages, but also other immune and non-immune cells (Gracie *et al*., 2003; Skurk *et al*., 2005). By virtue of its nature, IL-18 plays a key role in induction of both Th1 and Th2 cytokines and, thereby, modulates their immune responses. IL-18 has been shown to play a protective role in the host defense towards different sources of infection or to have a pathological role in various inflammatory and autoimmune diseases (Nakanishi *et al*., 2001). In the context of HIV-1 infection, IL-18 has also been shown to play a pathogenic role by enhancing viral replication in both monocytes and T-cells (Shapiro *et al*., 1998; Klein *et al*., 2000). Moreover, clinical studies have demonstrated the involvement of IL-18 in the immunopathogenesis of HIV-1 infection (Torre *et al.,* 2000; Wiercinska-Drapalo *et al*., 2004); however, most of the reports of such studies so far have focused primarily on associating the serum level of IL-18 and its clinical outcome with the pathogenesis of HIV-1 infection. Thus, it is believed that SNP studies may provide valuable information about the correlation of this noble cytokine gene with HIV-1 /AIDS. For this reason, the present study was aimed to see the impact of polymorphisms of the *IL-18* gene promoter *-137 (G/C)* position on the risk and progression of HIV-1 infection.

To the best our knowledge, no report on the association of any SNP of the *IL-18* gene with HIV-1/AIDS is available in the literature. This is therefore the first association study of *IL-18* among North Indian HIV-1/AIDS patients.

Materials and Methods

Materials. A total of 500 seropositive HIV-1 /AIDS patients and an equal number of age and gender matched seronegative healthy controls were recruited from the Immunodeficiency Clinic of the Department of Internal Medicine, Postgraduate Institute of Medical Education and Research (PGIMER) Chandigarh, India. After obtaining informed consent, 2–3 ml of peripheral blood was drawn from the study subjects as well as controls and collected in EDTA-coated tubes.

Methods. Genomic DNA of each subject was extracted from peripheral blood leukocytes by SDS lysis and proteinase K digestion followed by standard phenol-chloroform method described by Sambrook *et al.* (1989). Genotyping of *IL-18-137 G/C* polymorphisms was performed by Sequence Specific Polymerase Chain Reaction analysis using the same oligonucleotide primers and conditions reported previously (Giedraitis *et al.,* 2001).

Statistical analysis. For statistical analysis of the data, SPSS software, version 11.5 (SPSS Inc., Chicago, IL) for Windows was used. The Hardy-Weinberg equilibrium between expected and observed genotype distributions was determined by means of the Chi-square ($\chi 2$) test. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated according to Epi Info Version 3.5.1 2008; CDC, Atlanta, GA, USA.

Results

A total of 500 patients with HIV-1/AIDS infection and an equal number of healthy controls were studied. Although males appeared more frequent in both cases and controls than females, there was no significant difference between cases and controls in gender distribution (65.2% versus 63.4% male and 34.6% versus 36.6% female (P >0.05). The mean age (± standard deviation, years) was 35.4 (±7.9 years) for cases and 36.2 (± 9.8) years for controls. There was no significant mean age difference between the cases and the controls (P >0.05).

In this case-control study, the functional SNP in the promoter region of *IL-18* at the position -137 was genotyped and its association with HIV-1/AIDS infection among North Indian population was analyzed. The genotype distributions and allele frequencies of the two study groups and the statistical comparisons of these data are presented in Table 1. The relative distributions of the *G/G*, *G/C*, and *C/C* genotypes at the position -137 were 176 (35.2%), 301 (60.2%) and 23 (4.6%) in HIV-1 patients, respectively, while in healthy control subjects these were 135 (27.0%), 327 (65.4%) and 38 (7.6%), respectively. Furthermore, the frequency of *G* and *C* alleles were 653 (65.3%) versus 598 (59.8%) and 347 (34.7%) versus 402 (40.2%) in cases and controls respectively. The *-137 G/G* genotype was significantly over-represented in the HIV-1 patients than in healthy controls (P < 0.05) and it was associated with an increased risk of HIV-1 infection.

Table 1. IL-18 promoter -137 G/C polymorphism distribution in the groups

	HIV-1/AIDS patients	Healthy controls
Genotype		
CC	23(4.6%)	38(7.6%)
GC	301(60.2%)	327(65.4%)
GG	176(35.2%)*	135(27.0%)*
Allele		
C allele	347(34.7%)	402(40.2%)
G allele	653(65.3%)*	598(59.8%)*

*Significant differences between groups.

Moreover, the frequency of the *G* allele was significantly higher in cases than in healthy controls ($P < 0.05$). On the other hand, no considerable difference in the frequency of heterozygote *-137 G/C* genotype was observed in the two study groups ($P > 0.05$).

Discussion

The pathogenesis of HIV-1 infection is generally characterized by a long-term, chronic disease that gradually progresses to AIDS. However, the rate of disease progression is substantially different among HIV-1-infected individuals (Shioda and Nakayama, 2006). A small fraction of HIV-1-infected individuals remain clinically and immunologically healthy for more than ten years after seroconversion. On the contrary, the disease of another significant fraction of patients is characterized by extremely rapid progression within a period as short as 1 year. There is still another group of individuals, who do not get infected even after repeated exposures (Fauci and Pantaleo, 1996; Shioda and Nakayama, 2006). Host genetic variability plays a major role in determining individual susceptibility or resistance to the infection with HIV-1, as well as the subsequent rate of disease progression to AIDS following infection. Polymorphisms in chemokine receptors that serve as HIV-1 coreceptors or in their natural ligands have been associated with susceptibility to infection as well as rates of disease progression (Smith *et al*., 1997; O'Brien and Moore, 2000). Furthermore, HLA alleles and closely related genes on MHC have also been suggested to influence HIV-1 disease progression (Klein *et al*., 1998).

IL-18 is a pleiotropic proinflammatory cytokine with effects on both innate and acquired immune responses (Dinarello and Fantuzzi, 2003). It has been shown to play a protective role in host defense towards different sources of infection or to have a pathological role in various inflammatory and autoimmune diseases (Nakanishi *et al*., 2001). As to the effect of IL-18 on HIV-1 infection and disease progression, IL-18 has been shown to enhance the HIV-1 replication in acutely or chronically infected human monocytic and T-cell lines with an intermediate role of HIV-1 inductive TNF-α and IL-6, suggesting a role for IL-18 in HIV-1 pathogenesis (Shapiro *et al*., 1998; Klein *et al*., 2000; Pugliese *et al*., 2002). Moreover, a report by Stylianou *et al.* (2003) has revealed the involvement of IL-18 in enhancing the expression of the HIV-1 co- receptor CXCR4 in peripheral blood mononuclear cells from HIV-1-infected patients. Such upregulation of CXCR4 in turn seems to facilitate viral entry and replication and could possibly contribute to the disease progression (Stylianou *et al*., 2003). They also indicated that IL-18 is not only one of the several inflammation markers that increases during HIV-1 infection, but may also be involved in the pathogenesis of this disease. Increased IL-18 concentrations in the serum of HIV-1-infected patients are likely to play an important role in the development and progression of the infection towards AIDS and the associated clinical conditions (Iannello *et al*., 2009). Apart from these studies, Wiercinska-Drapalo *et al.* (2004) also demonstrated the presence of an association between plasma level of IL-18, the viral load and disease progression in HIV-1-infected patients. In another instance, Song *et al.* (2006) further demonstrated the presence of overall positive correlation between serum IL-18 and HIV-1 viral load accompanied by negative correlation between serum IL-18 and CD4 + T-cell count.

In the current study, five hundred HIV-1 patients and an equal number of healthy control subjects were genotyped for *IL-18 -137 G/C* promoter polymorphism in order to assess possible association with the risk of HIV-1 infection among North Indians. The results of this study demonstrated a statistically significant association between the -137 *G* allele and *G/G* genotype (high IL-18 producer) and the risk of HIV-1/AIDS, however, no significant association between the *-137 G/C* heterozygous genotype and HIV-1/AIDS risk was detected. The findings of the current study are partially consistent with those of Segat *et al.* (2006), who found *-137 G/G* genotype at relatively higher frequency, whereas *-137 C/C* genotype at almost two-fold lower frequency in HIV-1 positive groups than in healthy control subjects.

Elevated protein levels could frequently be the consequence of functional polymorphisms within genes and regulatory regions of genes. These changes in proteins expression levels may influence clinical course of diseases. *IL-18 -137 G/C* promoter polymorphism has been demonstrated to influence the transcriptional activity of the gene and hence may account for observed cytokine level variations. The *G* allele (*G/G* genotype) at the position -137 has been associated with higher transcription activity and higher IL-18 production (Giedraitis *et al*., 2001). An elevated cytokine level in the serum in turn may enhance viral replication and subsequently influence the rate of disease progression. Hence, the disease of HIV-1 patients with the *-137 G* allele and *G/G* genotype may progress to AIDS faster than the disease of those with the -137 *C/C* genotype. Therefore, we believe that this could explain the results of the present study.

In summary, the data of current study demonstrate that the *G* allele and *G/G* genotype at the position -137 of the *IL-18* gene promoter polymorphism could have a role in HIV-1 disease development. This finding may provide further evidence in elucidating the role of host genetic variations in the immunopathogenesis of HIV-1 infection. However, this finding needs to be further confirmed in another study correlating *IL-18* gene promoter polymorphisms and serum levels of IL-18 in HIV-1/AIDS patients for precise determination of the role IL-18 in HIV-1 infection.

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