

Original Article

Insights into the Role of *IL-12B* and *IFN- γ* Cytokine Gene Polymorphisms in HIV-1/AIDS Infection

(*IL-12B* / *IFN- γ* / pro-inflammatory / cytokine / polymorphism / RFLP / ARMS / HIV-1/AIDS)

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Abstract. One of the main characteristics of HIV-1 infection is persistent systemic immune activation. This immune activation and dysregulation is characterized by a specific pattern of cytokine production, expression of membrane activation molecules on the cells of the immune system, and changes in the levels of several immune parameters in blood. Therefore, the aim of the present work was to evaluate the effect of a *TaqI* polymorphism in the 3'UTR of the *IL-12B* gene at position -1188 (A/C) and the biallelic polymorphism in the first intron of *IFN- γ* at position +874 (T/A) on HIV-1/AIDS among north Indian population. *IL-12B* and *IFN- γ* gene polymorphisms were studied in 300 patients with HIV-1/AIDS and an equal number of negatively diagnosed controls of the matched age, using DNA-based polymerase chain reaction with sequence-specific primers and restriction digestion. The allelic as well as genotypic frequencies of interleukin-12B gene polymorphisms did not significantly differ between HIV-1/AIDS patients and negative healthy controls. A statistically significant correlation was found between *IFN- γ* polymorphism and the risk of the disease. The present study suggested that individuals with mutant homozygous

IFN- γ AA genotype were at risk of HIV-1/AIDS (OR = 1.88, 95% CI 1.14–3.10, P = 0.008).

Introduction

HIV-1 infection is characterized by an insidious deterioration of the cellular immune system. Both the quantity and proportion of plasma CD4⁺ T cells decrease steadily over a period of years to decades, and this progressive loss of CD4⁺ T cells is associated with the development of acquired immunodeficiency syndrome (AIDS) in the infected individuals. The degree of immunodeficiency associated with HIV-1 infection, as defined by the onset of opportunistic diseases, correlates closely with plasma CD4⁺ T-cell counts (Vergis et al., 2002; Smith, 2006). Earlier studies demonstrated that individuals with a higher HIV load tend to progress to AIDS and death at a more rapid rate than those with lower viral loads, and that different prognostic information can be derived from the CD4⁺ T-cell count and the viral load (Coffin, 1996; Mellors et al., 1996).

The biological correlates of an effective immune response that could contain or prevent HIV infection remain elusive despite substantial scientific accomplishments in understanding the interactions among the virus, the individual and the community. Since the reporting of the first case of AIDS in 1981, there has been substantial scientific progress in the development of both effective antiretroviral therapy and the understanding of virus-host cellular interactions (Marmor et al., 2006). Two phenomena have indicated that natural resistance to HIV-1 infection exists:

First – there are individuals who have been exposed to HIV, in some cases repeatedly and over long periods of time, who have remained HIV uninfected (Fowke et al., 1996; McNicholl et al., 2004; Farquhar et al., 2005).

Second – there are individuals who have become infected with HIV, but whose disease has not progressed or has progressed very slowly as compared to the average experience. Criteria defining these long-term non-progressors have varied among reports, but usually in-

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Abbreviations: 3'UTR – untranslated region, AIDS – acquired immunodeficiency syndrome, ARMS-PCR – amplification refractory mutation system-polymerase chain reaction, CI – confidence interval, CTL – cytotoxic T lymphocytes, EDTA – ethylene-diamine-tetraacetic acid, ELISA – enzyme-linked immunosorbent assay, HLA – human leukocyte antigen, *IFN- γ* – interferon γ , IL-12 – interleukin 12, OR – odds ratio, PBMC – peripheral blood mononuclear cells, PCR – polymerase chain reaction, PGIMER – Postgraduate Institute for Medical Education and Research, SNPs – single-nucleotide polymorphisms, TGF- β – transforming growth factor β , Th1 – T-helper type 1, Th2 – T-helper type 2, WHO – World Health Organization.

clude survival with HIV infection for more than seven years with consistently low levels of HIV-1 RNA and little or no loss of the primary target of HIV, CD4⁺ T cells. Long-term non-progressors have been identified among various groups, including homosexual men, women, injection drug users and children. Some common genetic mutations have been found in both exposed uninfected populations and in long-term non-progressor populations, suggesting a unifying theory for both conditions, namely that host traits that prevent or hinder HIV-1 entry into cells will reduce the likelihood of infection and, should infection occur, slow or entirely eliminate the development of serious disease (Easterbrook, 1999; Barretina et al., 2000). Other authors reported that during HIV infection a variety of disturbances in the regulation of cytokine expression were observed. These disturbances included a general decrease in the expression of type 1 T-helper (Th1) cytokines, an increase in the expression of pro-inflammatory cytokines, a possible increase in type 2 T-helper (Th2) cytokines, and increased expression of antiviral interferons and transforming growth factor β (TGF- β) (Clerici et al., 1996; Valdez and Lederman, 1997). These perturbations may contribute to HIV disease pathogenesis by contributing to the impaired cellular immune responses and cell loss that characterize HIV infection and AIDS and by accelerating replication of HIV-1. Moreover, in HIV infection, peripheral blood mononuclear cells (PBMC) from HIV-1-infected patients produced significantly less IL-12B than those from uninfected controls (Chehimi et al., 1994; Marshall et al., 1999).

In humans the *IL-12 B* gene is located at an independent locus on chromosome 5 at 5q31-33 (Huang et al., 2000). Recently, *Taq1* (A/C) single-nucleotide polymorphism in the 3' untranslated region (3'UTR) of the *IL-12B* at position -1188 was identified, which was also found to be functional, and any polymorphism in this region of the gene might affect gene expression (Huang et al., 2000; Seegers et al., 2002). With regard to *IFN- γ* , a biallelic polymorphism, T to A, in the first intron of *IFN- γ* has been determined at position +874. The effect of such polymorphism leads to homozygous T/T, A/A and heterozygous T/A alleles being associated with high, low and intermediate production of IFN- γ , respectively (Pravica et al., 2000).

Therefore, the aim of the current study was to assess whether the -1188 (A to C) 3'UTR in *IL-12B* and *IFN- γ* +874 (T to A) gene polymorphisms correlate with the risk of HIV-1/AIDS infection in north Indian population.

Material and Methods

Subjects

EDTA-anticoagulated 5-ml blood samples were obtained from 300 HIV-positive patients during their visits to Postgraduate Institute of Medical and Research (PGIMER) Chandigarh, India. An equal number of con-

trol blood samples were also collected after they were confirmed to be seronegative for HIV-1/AIDS by the enzyme-linked immunosorbent assay (ELISA) test. Data on age, sex, mode of transmission, medical history and occupation were gathered from subjects in a structured form designed for this purpose. The stages of the disease were classified according to the World Health Organization (WHO) staging guideline for HIV-1/AIDS and to the physician's observations. Also, the mean CD4 count was used for disease stage classification. Genomic DNA was extracted from peripheral blood lymphocytes by proteinase K digestion and by the phenol-chloroform method. DNA samples were amplified to detect a biallelic polymorphism, T to A, in the first intron of *IFN- γ* at position +874, and -1188 (A to C) 3'UTR in the *IL-12B* gene to explore their effect on the HIV-1/AIDS infection among north Indians.

Genotyping

Polymerase chain reaction (PCR) primers for *IL-12B* at -1188 position 5'- GG CA TT CT CT TC CA GG TT CT G and 3'-CC AT GG CA AC TT GA GA GC TG were used to amplify a 243-bp fragment of *IL-12B* from genomic DNA. One hundred ng genomic DNA was used for PCR in a total volume of 25 μ l of reaction mixture in a thermal cycler. In addition, the PCR mixture contained 0.75 unit Taq polymerase 10 mM Tris-HCl (PH 8.3), 50 mM KCl, 1 mmol/l of each primer (Sigma-Aldrich, St. Louis, MO), 0.25 mmol/l dNTPs, and 2 mmol/l MgCl₂. PCR conditions were as follows: denaturation at 95 °C for 1 min followed by 36 cycles of 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C and then for 10 min at 72 °C. The products were digested with Taq 1 at 65 °C for 3 h, which resulted in either two fragments of 173 and 70 bp (allele C) or a single fragment of 243 bp (allele A), and analysed by gel electrophoresis in 3% agarose gel (Fig. 1).

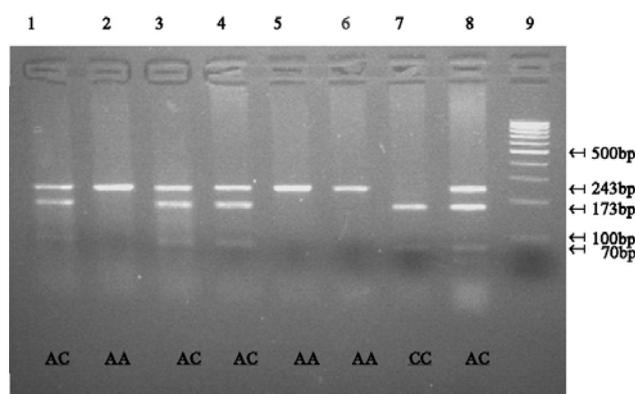


Figure 1: PCR-RFLP (*Taq1*) genotype analysis of *IL-12B* gene of HIV-1/AIDS patients and controls

1. Lane 9	100	bp	DNA Marker	
2. Lane 2, 5, 6	A/A	genotype	243	bp
3. Lane 1, 3, 4, 8	A/C	genotype	243, 173, 70	bp
4. Lane 7	C/C	genotype	173, 70	bp

Fig. 1. PCR-RFLP (*Taq1*) genotype analysis of *IL-12B* gene of HIV-1/AIDS patients and controls

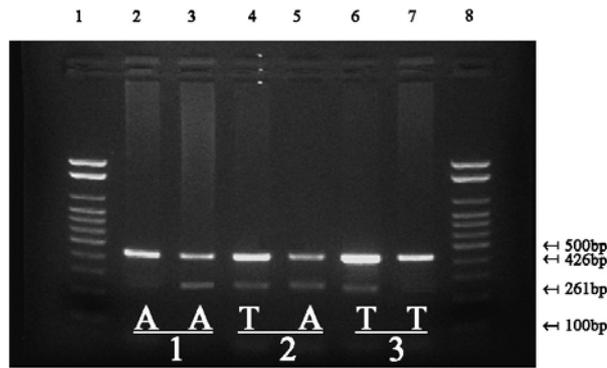


Figure 2: PCR-ARMS genotype analysis of *IFN- γ* gene of HIV-1/AIDS patients and controls.

1. Lane 1, 8 100 bp DNA Marker 5- Upper row internal control 426 bp
 2. Lane 2,3 A/A genotype 6- Lower row A and T Alleles 261 bp
 3. Lane 4,5, T/A genotype
 4. Lane 6,7 T/T genotype

Fig. 2. PCR-ARMS genotype analysis of *IFN- γ* gene of HIV-1/AIDS patients and controls

The polymorphisms in the *IFN- γ* (+874 (T/A) gene were typed by using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) described by Perrey et al. (1999). Primers were 5'-TT TT AC AA CA CA AA AT CA AA TC A-3' for the A allele, 5'-TT C TA CA AC AC AA AA TC AA AT CT-3' for the T allele; 5'-TCA ACA AAG CTG ATA CTC CA-3' was used as a generic primer. For positive control the primers were 5'-GC CT TC CC AA CC AT TC CC T TA-3' and 5'-TC AC GG AT TT CT GTT GT GT TT C-3'. The reaction volume for the amplification reaction was 20 μ l, containing 50 ng/ μ l genomic DNA, 0.1 μ l of 5.0 U/ μ l Taq polymerase, 2 μ l of 10 \times PCR buffer (20 mM Tris-HCl, pH 8.0, 100 mM KCl), 1.2 μ l of 25 mM MgCl₂, 0.4 μ l of 10 mM dNTP and 1 μ l (20 pmol) of each primer, and 0.1 μ l (20 pmol) of internal control primers. Internal control primers were used to check for successful PCR conditions. PCR products are given in Fig. 2.

Results

Subject characteristics

In this study the distribution of mean age, gender, standard deviation, and P values were summarized in Table 1. In terms of mean age and gender distribution,

Table 1. Demographic data of patients and controls used in this study

	Patients	Controls	P value
Number	300	300	
Gender			
Male, N (%)	193 (64.3)	195 (65.0)	0.93
Female, N (%)	107 (35.7)	105 (35.0)	
Age (years)			
Mean \pm SD	35.23 \pm 8.04	36.17 \pm 10.49	0.179

there was no statistically significant difference among patients and controls.

Analysis of the *IL-12B* A/C polymorphism

Tables 2 and 3 summarize the genotypes and allele frequencies for the *IL-12B* gene in patients with HIV-1/AIDS and normal healthy controls. Comparison between the genotype and allelic frequencies in HIV-1/AIDS patients and controls showed no significant association between the patients and controls (P = 0.5 and 0.5 for genotypes AC, CC, respectively). Also, the present data demonstrated no relationship between the allele frequencies and the risk of the disease.

Analysis of the *IFN- γ* polymorphism

The results of genotypic and allelic frequencies for the patients with HIV-1/AIDS and healthy control subjects are given in Tables 4 and 5. The distribution of TT, TA and AA genotypes in the groups of patients were, respectively, 22, 47 and 31 % versus 24, 58.0 and 18.0 % in the control groups, whereas the frequencies of T and A allele were 45.5 and 54.5 % in the patients and 53.0, 47.0 % in the controls, respectively. The present data demonstrated a significant relationship between the AA genotype and the A allele frequency (OR = 1.88, 95 % CI 1.14–3.10, P = 0.008) and the risk of HIV-1/AIDS (OR = 1.35, 95 % CI 1.07–1.71, P = 0.009). On the other hand, no statistical variation between the T/A genotypes and the disease was observed. The TT genotype was used as a reference. The genotypes of the *IL-12B* and *IFN- γ* gene polymorphisms were in Hardy-Weinberg equilibrium.

Discussion

Control of HIV-1 viraemia and progression to AIDS has been associated with cytokine and human leukocyte antigen (HLA) genes (Carrington et al., 1999; Vasilescu et al., 2003). There are many reports that a number of cytokine genes are polymorphic and their polymorphisms in regulatory regions correlate with their cytokine secretion (Hutchinson et al., 1999). In the current study, we investigated the distribution of a functional polymorphism in the *IL-12B* gene at position -1188 in the 3' UTR region (Huang et al., 2000; Seegers et al., 2002). The presented data showed that the allele and genotype frequencies of the *IL-12B* (A/C) polymorphism did not differ significantly between HIV-1/AIDS patients and healthy control subjects. Prabhu Anand et al. (2007) showed no association between *IL-12B* polymorphism and susceptibility or resistance to pulmonary tuberculosis. Timasheva et al. (2008), however, have reported that carriers of the *IL-12B* 1159 AA genotype had lower risk of stroke. Seegers et al. (2002) reported correlation between *TaqI* polymorphism in 3'UTR of the *IL-12B* gene with increased IL-12 secretion and they also showed that individuals with homozygous AA and heterozygous AC genotypes were higher producers of IL-12 than those with the CC genotype. Several studies

Table 2. Distribution of genotype frequencies of the *IL-12B* A/C polymorphism in HIV-1/AIDS patients and healthy controls

IL-12	Patients (N = 300)	Controls (N = 300)	OR (95% CI)	P value
AA	135 (45.0)	141 (47.0)	Reference	
AC	159 (53.0)	150 (50.0)	1.11 (0.79-1.55)	0.5
CC	6 (2.0)	9 (3.0)	0.82 (0.43-1.54)	0.5

OR was computed by Epi-Info version 3.5.1. (Centre for disease control and prevention).

Table 3. Distribution of allele frequencies of the *IL-12B* A/C polymorphism in HIV-1/AIDS patients and healthy controls

IL-12	Patients (N = 300)	Controls (N = 300)	OR (95% CI)	P value
A	429 (71.5)	432 (72.0)	1.02 (0.79-1.33)	0.8
C	171 (28.5)	168 (28.0)		

OR was computed by Epi-Info version 3.5.1. (Centre for disease control and prevention).

demonstrated increased levels of IL-12 to be playing a critical role in the development of Th1 cells and cell-mediated immune responses (Trinchieri, 1997). In addition, earlier studies reported that IL-12 augments generation of allogeneic HIV-1-specific cytotoxic T lymphocytes (CTL) from peripheral blood mononuclear cells of HIV-1 patients and their increased cytolytic activity (Gately et al., 1992; Gajewski, et al., 1995; McFarland et al., 1998). Our findings demonstrated a lack of association of these single-nucleotide polymorphisms with HIV-1/AIDS infection in north Indian population, even though the *IL-12B* gene dose affects production of the cytokine, but it is believed that the total influence might be weak due to the sample size, or these alleles may not represent a risk factor for HIV-1/AIDS in Indian patients. Further functional studies of *IL-12B* polymorphism with a larger sample size are necessary to give a clearer picture about the role of the interleukin-12 gene expression in HIV-1/AIDS infection and its understanding at the molecular level.

With regard to the *IFN-γ* gene, which was also included in the present work, several studies reported that IFN-γ is an important immune regulator and pro-inflammatory cytokine implicated in the pathogenesis of many infectious diseases. A single-nucleotide polymorphism, T to A, at position +874 in the first intron has been shown previously. This polymorphism is associated with the

IFN-γ production level (Izad et al., 2004). We screened genomic DNA samples from clinically defined HIV-1/AIDS patients and healthy control subjects. In previous studies, Izad et al. (2004) reported that patients with multiple sclerosis showed lower TT and higher AA genotypes compared to controls, although there were no statistically significant differences in the *IFN-γ* genotype distribution. Another study performed by Kang et al. (2006) examined the frequency of a variable-length CA repeats in intron 1 of the *IFN-γ* gene. They showed significantly decreased frequency of allele 2 (12 CA repeats) whereas that of allele 2-negative homozygote increased in HIV-1-infected patients as compared with uninfected healthy controls. The present data indicated that individuals with the AA mutant genotype were at significantly elevated risk of HIV-1/AIDS (OR = 1.88, 95 % CI 1.14–3.10, P = 0.008). On the other hand, no association between the AT heterozygous genotype and the risk of disease was shown. The present data suggested that the AA mutant genotype may be playing a role as a risk factor of the disease through the direct effect of this mutant genotype (AA) on the total IFN-γ production. Such hypothesis may be supported by the study of Pravica et al. (2000), who reported that individuals with AA genotype have low levels of IFN-γ production when compared with TT and TA genotype individuals, as it is well known that interferons play an

Table 4. Distribution of genotype frequencies of the *IFN-γ* T/A polymorphism in HIV-1/AIDS patients and healthy controls

IFN	Patients (N = 300)	Controls (N = 300)	OR (95% CI)	P value
TT	66 (22.0)	72 (24.0)	Reference	
TA	141 (47.0)	174 (58.0)	0.88 (0.58-1.35)	0.5
AA	93 (31.0)	54 (18.0)	1.88 (1.14-3.10)	0.008*

OR was computed by Epi-Info version 3.5.1 (Centre for disease control and prevention),* Significant P value.

Table 5. Distribution of allele frequencies of the *IFN-γ* T/A polymorphism in HIV-1/AIDS patients and healthy controls

IFN	Patients (N = 300)	Controls (N = 300)	OR (95% CI)	P value
T	273 (45.5)	318 (53.0)	1.35 (1.07-1.71)	0.009*
A	327 (54.5)	282 (47.0)		

OR was computed by Epi-Info version 3.5.1 (Centre for disease control and prevention),* Significant P value.

important role in the first line of defence against viral infections (Knight, 2008), or indirectly through the effect on activity of the immune system components such as macrophages and other immune cells.

Several studies demonstrated that IFN- γ played an important role in up-regulation of cell-surface class I MHC genes, subsequently increasing the potential for cytotoxic T-cell recognition of foreign peptides and thus promoting induction of cell-mediated immunity (Belich et al., 1994; Groettrup et al., 2001; Decker et al., 2002). Other authors explained another role of IFN- γ through efficient up-regulation of the MHC class II antigen-presenting pathway and thus promotion of peptide-specific activation of CD4 T cells (Mach et al., 1996; Boehm et al., 1997). Our previous and present data that individuals with the AA genotype are at risk of HIV-1/AIDS are in line with the findings of Pravica et al. (2000). Our data suggested that such risk of the disease may occur due to the role of IFN- γ in apoptosis, which is considered as one of the mechanisms of immune system cell depletion in patients infected with HIV-1 (Saksena et al., 2007), since Xaus et al. (2001) indicated that IFN- γ arrests the cell cycle and provides a survival signal to immune system cells, and other authors stressed that it serves as a pro-apoptotic signal (Breen et al., 1991). To the best of our knowledge, there is no published report regarding the association between *IFN- γ* (+874T/A) and *IL-12B* (-1188A/C) gene polymorphisms and HIV-1/AIDS. Therefore, it is not easy to give a thorough and precise explanation of our findings of *IFN- γ* and *IL-12B* gene polymorphism association with increased risk of HIV-1/AIDS disease.

Further functional studies are necessary to give a clearer insight into the role of these polymorphisms. In conclusion, the present study is the first to explore the significance of the *IFN- γ* and *IL-12B* gene polymorphism in HIV-1/AIDS patients and has suggested that *IFN- γ* AA genotype may be associated with an increased risk of HIV-1/AIDS infection in north Indian population. Further functional studies of the polymorphisms with a larger sample size are necessary to give a clearer picture about the role of the interleukin-12 gene expression in HIV-1/AIDS infection and its understanding at the molecular level.

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