

Combinatorial Effects of *SDF-1* and *CCL3L1* Gene Variants and Susceptibility to HIV-1/AIDS in Indian Population

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Abstract: HIV-1 infection requires the expression of CD4+ molecules in colligation with C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) as the major coreceptors. The role of SNP in 3' untranslated region of *SDF-1* (*SDF1-3'A*) and low copy number (CN) of the *CCL3L1* gene is reported to confer increased resistance to HIV-1 infection. The aim of the present study was to analyze the combinatorial effect of both the variations in protection towards HIV-1 infection in Indian population. The combinatorial effect of genetic variation in terms of SNP in *SDF-1* gene and *CCL3L1* CN was investigated in 105 healthy individuals and 78 HIV-1 patients. Genotyping of *SDF-1* was performed by RFLP-PCR and *CCL3L1* by real-time PCR using TaqMan chemistry. The genotype frequency distribution of *SDF-1* was found to be (*SDF-1/SDF-1*: 65.4%, *SDF-1/SDF1-3'A*: 29.5% and *SDF1-3'A/SDF1-3'A*: 5.1%) in HIV patients as compared to (*SDF-1/SDF-1*: 64.8%, *SDF-1/SDF1-3'A*: 30.5% and *SDF1-3'A/SDF1-3'A*: 4.7%) in healthy individuals, whereas a range of 1 to 6 copies per diploid genome was observed for *CCL3L1* gene.

Key words: *CCL3L1*, coreceptor, gene copy number, HIV-1, *SDF-1*, SNP.

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) enters into the target cells through the interactions of the viral envelope protein gp120 with CD4+ and chemokine coreceptor (CCR5 or CXCR4) [1]. Macrophage-tropic (M-tropic) or nonsyncytium-inducing (NSI) HIV-1 strains use CCR5 as major coreceptor whereas T lymphocyte-tropic (T-tropic) or syncytium-inducing (SI) HIV-1 strains use CXCR4 as coreceptors [2]. Chemokines are low molecular weight chemoattractants [3] which play a key role in leukocyte activation regulation and recruitment to the sites of inflammation via chemokine receptors interaction [4]. *CCL3L1* [Chemokine (C-C motif) ligand 3-like 1, also known as MIP-1 α P and LD78 β] is a suppressive chemokine and a natural ligand for CCR5 coreceptor

[3]. On the other hand, the only known ligand for CXCR4 is *SDF-1* (Stromal derived factor, also known as CXCL12) which is constitutively expressed by stromal, endothelial, dendritic and other cells [2, 5]. The rate of progression to acquired immunodeficiency syndrome (AIDS) exhibits inter-individual variation owing to the host genetic factors, which play a significant role in susceptibility to HIV-1 infection and rate of progression to the disease [6]. In addition, there are several reported association studies of SNPs [7] and CNVs [8] with disease progression or resistance.

SDF-1 is a potent chemokine that inhibits T tropic HIV-1 entry by competing for the binding to the CXCR4 coreceptor. It has a highly conserved sequence. Human and murine *SDF-1* can be discriminated by a single conservative amino acid substitution of isoleucine to valine at position 18. The *SDF-1* gene has two alternatively spliced variants, *SDF-1 α* and *SDF-1 β* [9]. The two transcripts vary from each other by the

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presence of 4 additional amino acids at the carboxy terminus of SDF-1 β . The expression of the SDF-1 mutation, a single nucleotide polymorphism (designated *SDF1-3'UTR-801G-A*) at position 801 in the 3'-untranslated region (UTR) of the *SDF-1* gene may be entailed in resistance to HIV-1 infection or delayed progression to AIDS [10]. The polymorphism *SDF1-3'A* is located in the 3' UTR region and thus does not affect the SDF1 β protein. However, *SDF1-3'A/3'A* action may involve up-regulation of the quantity of SDF-1 protein available to bind CXCR4 which competes with late stage T-tropic HIV-1 strains [5]. This mechanism would be consistent with the gradation in survival outcome whereby *SDF-1* protection is more pronounced in late stage AIDS outcomes than for earlier stages. According to another hypothesis, SDF-1 α down-regulates CXCR4 coreceptor on cells by induction of endocytosis, effectively blocking infection by T-tropic strains [6].

Human chemokine (C-C motif) ligand 3-like 1 (*CCL3L1*) maps on chromosome 17q11.2 and is a duplicated isoform of the gene encoding *CCL3*. *CCL3L1* is a suppressive chemokine involved in the susceptibility to HIV-1/AIDS susceptibility [11]. The binding of the *CCL3L1* to the CCR5 coreceptor on the CD4+ cells blocks the entry of the HIV-1 virus and thus protect the individuals from infection. *CCL3L1* gene has several SNPs and has hotspots for gene duplication, resulting in distinct haplotypes in population. Thus, it was hypothesized that the individuals with low copy number as compared to the median copies in their respective population confers a risk of acquiring HIV-1. Some ethnic groups were studied for *CCL3L1* gene copy number and it was found that Africans have the highest copy number followed by Asians, Amerindians, Central/South Asians, Middle East individuals and Europeans [11].

Previous studies highlighted the individual role of *SDF-1* polymorphism and gene copy number (CN) variation of *CCL3L1* towards the protection to HIV-1 infection [10, 11]. There has been no report till date for

the study of the combinatorial effect of both *CCL3L1* CN and *SDF-1* polymorphism in HIV-1 infection. The aim of the present study was to analyze the combinatorial effect of both the variations in protection towards HIV-1 infection in Indian population.

2. Materials and Methods

2.1 Subjects

The study was conducted in 105 unrelated healthy individuals and 78 HIV-1 seropositive patients. All the healthy control individuals were recruited at B.V. Patel PERD Centre, Ahmedabad. The HIV-1 patient group consisted of subjects recruited at national referral institute, All India Institute of Medicine Sciences (AIIMS), New Delhi. All the samples in both the healthy and HIV-1 group consisted of heterogeneous population. The age of control subjects and the patients were in the range of 19-50 and 24-69 years respectively. The study was approved by the Institutional Ethics Committee and written informed consent form was obtained prior to blood collection from individuals.

2.2 Genotyping of *SDF-1* Polymorphism

Genotyping of *SDF-1* was performed by RFLP-PCR using a pair of specific primers framing the region surrounding the polymorphic site. The sequence of the primers is: Forward primer 5'-ATTAGAGTGTCTTTCCACGGAGCC-3' and Reverse primer 5'-ATCCCGAGCACCTCCACATC-3'. PCR amplification was performed in a volume of 50 μ L containing 5 μ L of 10x PCR buffer (Fermentas Life Sciences) provided with the Taq polymerase (Fermentas Life Sciences), 1.5 mM of MgCl₂, 2 μ L of 1U/ μ L Taq polymerase (Fermentas Life Sciences), 0.2 μ M of each primer, 0.2 mM of dNTP mix and 280 ng of genomic DNA. PCR cycle reactions were performed on Eppendorf gradient thermocycler. Cycling conditions of PCR comprised 3 min denaturation at 94 °C, 35 cycles of 30 sec at 94 °C, 20 sec at 66 °C and 30 sec at 72 °C and 4 min extension at 72 °C. The amplified

products were electrophoresed and visualized in ethidium bromide stained 2% agarose gels. The amplified product of 366 bp was subjected to restriction fragment length polymorphism (RFLP) using restriction enzyme *Hpa II* (Fermentas, India). The G to A transition in *SDF1-3'A* allele discriminates a *Hpa II* site allowing the use of a RFLP-PCR assay for rapid detection of *SDF-1* genotypes. PCR products were digested with *Hpa II* at 37 °C for 8 h and genotypes are scored as homozygous wild type (260 bp and 106 bp), heterozygous (366 bp, 260 bp and 106 bp) and homozygous mutant (366 bp, no digestion) (Fig. 1).

Lane 1 & 2: undigested PCR products (band 366 bp), Lane M: O' Generuler 100 bp ladder (Fermentas), Lane 3: homozygous wild type *SDF-1/SDF-1* (bands 260 bp and 106 bp), Lane 5 & 6: heterozygous *SDF-1/SDF1-3'A* (bands 106 bp, 260 bp, 366 bp), Lane 7: homozygous mutant *SDF1-3'A/SDF1-3'A* (band 366 bp).

2.3 Genotyping of *CCL3L1* Gene Copy Number

Genotyping for the copy number of *CCL3L1* was

performed using TaqMan real-time PCR with ABI StepOne instrument. The emitted fluorescence as FAM (6-carboxyfluorescein, 6-FAM) from the probe detecting *CCL3L1* and VIC from the probe detecting *RNase P* gene (2 copies per diploid genome) was detected during the amplification. The primer sequences of *RNase P* are as follows: sense primer 5'-AGATTTGGACCTGCGAGCG-3'; antisense primer 5'-GAGCGGCTGTCTCCACAAGT-3'; probe 5'-VIC-TTCTGACCTGAAGGCTCTGCGCG-MGB-3'. The method of genotyping *CCL3L1* copy number and primer sequences are similar to as described previously [11].

2.4 Statistical Analysis

The analysis of allelic frequencies and significance of heterogeneity in allele and genotype frequency between control and HIV-1 seropositive subjects was done by chi square test. The Hardy-Weinberg Equilibrium was determined. Measures of central tendency statistics were applied for *CCL3L1* copy number. Odds ratios (ORs), 95% confidence intervals

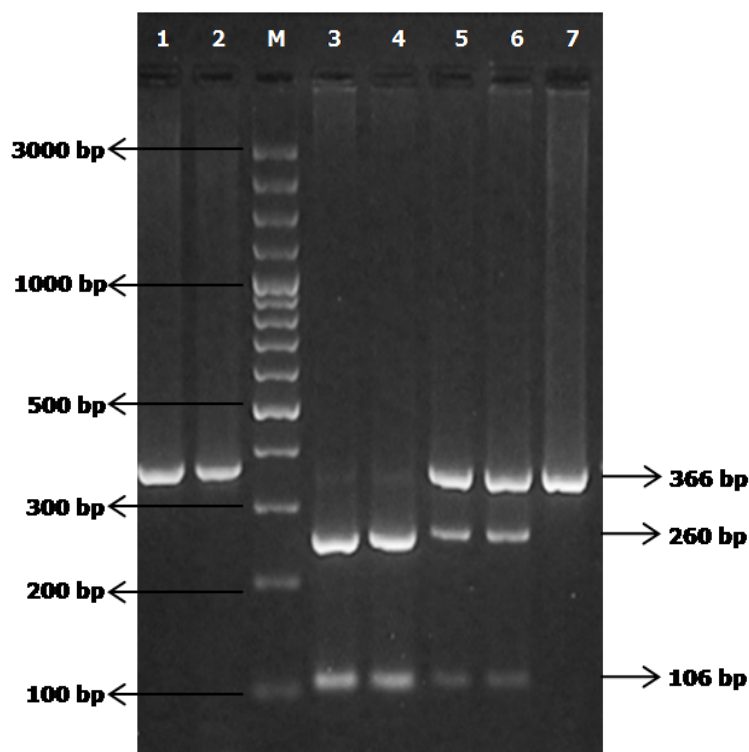


Fig. 1 Restriction fragment length polymorphism (RFLP-PCR) of *SDF-1* gene.

(CI) and *P* values were calculated by unconditional logistic regression and *P* < 0.05 was considered to be significant.

3. Results

3.1 *SDF1-3'A* Polymorphism in HIV-1 Susceptibility

The allelic frequencies of *SDF-1* were determined by the manual counting method. The allelic frequency for the *SDF-1* variants was 80% (*SDF-1*) and 20% (*SDF1-3'A*) in both HIV-1 patients and healthy individuals. The statistical analysis reveals that it follows the Hardy-Weinberg Equilibrium. The genotype frequency of *SDF-1/SDF-1* was 65.4%, *SDF-1/SDF1-3'A* was 29.5% and *SDF1-3'A/SDF1-3'A* were 5.1% in HIV-1 patients and *SDF-1/SDF-1* was 64.8%, *SDF-1/SDF1-3'A* was 30.5% and *SDF1-3'A/SDF1-3'A* were 4.7% in healthy individuals. The homozygous *SDF1-3'A/SDF1-3'A* genotype frequency was found to be similar among HIV-1 patients and healthy individuals (5.1% and 4.7%, respectively). A non-significant association of the *SDF1-3'A* with protection to HIV-1 [odds ratio (OR) = 1.06, 95% CI—0.27 to 4.17, *P* = 0.9244] was found in Indian population (Table 1).

3.2 *CCL3L1* Gene Copy Number in HIV-1 Susceptibility

For *CCL3L1* gene copy number analysis, a range of 1 to 6 copies per diploid genome with an average gene dose of two copies in HIV-1 patients and healthy individuals was observed. The distribution of the copy

number was found to be < 2 copies: 23%, 2 copies: 38.5% and > 2 copies: 38.5% in HIV-1 patients and < 2 copies: 13.3%, 2 copies: 44.8% and > 2 copies: 41.9% in healthy individuals. There was a 2.01 fold increased risk to HIV-1 in individuals with < 2 copies [odds ratio (OR) = 2.01, 95% CI—0.87 to 4.64] when compared to the healthy individuals but this increased risk was not significant (*P* = 0.0979). The *CCL3L1* < 2 copies was more frequent among HIV-1 patients as compared to healthy individuals (23% and 13.3%, respectively) (Table 1).

3.3 *SDF-1* Polymorphism and *CCL3L1* Gene Copy Number Combinatorial Effect in HIV-1 Susceptibility

Given the negative association of *SDF1-3'A* polymorphism, *CCL3L1* gene copy number and HIV-1 susceptibility, the combinatorial effect of *SDF1-3'A* polymorphism and *CCL3L1* copy number in HIV-1 patients and healthy individuals was tested. *SDF1-3'A* allele and *CCL3L1* ≥ 2 copies conferring a protection to HIV-1 were considered as reference combination. The odds ratio (OR) was 0.73 (95% CI—0.27 to 2; *P* = 0.5446) for *SDF1-3'A*, *CCL3L1* ≤ 2 copies, 0.68 (95% CI—0.26 to 1.76; *P* = 0.4208) for *SDF1*, *CCL3L1* > 2 copies and 1 (95% CI—0.42 to 2.41; *P* = 0.9887) for *SDF1-3'A*, *CCL3L1* ≤ 2 copies combinations as compared to *SDF1-3'A*, *CCL3L1* ≥ 2 copies (Table 2). Thus, lack of significant association of the combination of the *SDF1-3'A* polymorphism and *CCL3L1* CN was observed in Indian population.

Table1 Distribution of *SDF-1* genotype and *CCL3L1* copy number in cases and control in Indian population.

	Case (n)	Control (n)	OR (95% CI)	<i>P</i> value
<i>SDF-1</i> genotype				
<i>SDF-1/SDF-1</i>	51	68	1.00	
<i>SDF-1/SDF1-3'A</i>	23	32	0.96 (0.50-1.83)	0.8975
<i>SDF1-3'A/SDF1-3'A</i>	4	5	1.06 (0.27-4.17)	0.9244
<i>CCL3L1</i> CN				
< 2 copy	18	14	2.01 (0.87-4.64)	0.0979
2 copy	30	47	1.00	
> 2 copy	30	44	1.07 (0.56-2.05)	0.8434

n, number of samples; OR, odds ratio; CI, confidence intervals; *P*, level of significance.

Table 2 Odds Ratio (OR) and 95% CI for assessment of combitorial effect of SNP (*SDF-1* polymorphism) and CNV (*CCL3L1* gene copy number) on susceptibility to HIV-1 in Indian population.

Genotype	OR (95% CI)	P value
<i>SDF1-3'A</i> , <i>CCL3L1</i> > 2 copies	1.00	
<i>SDF1-3'A</i> , <i>CCL3L1</i> ≤ 2 copies	0.73 (0.27-2)	0.5446
<i>SDF-1</i> , <i>CCL3L1</i> > 2 copies	0.68 (0.26-1.76)	0.4208
<i>SDF-1</i> , <i>CCL3L1</i> ≤ 2 copies	1 (0.42-2.41)	0.9887

OR, odds ratio; CI, confidence intervals; P, level of significance.

4. Discussion

Stromal Derived Factor (*SDF-1*) has been reported to block the HIV-1 infection by competing with the T tropic HIV strains for the CXCR4 coreceptor binding and thus influencing the rate of disease progression to AIDS [6]. Similarly the role of *CCL3L1*, a suppressive chemokine and a ligand for the HIV coreceptor CCR5 is well documented and also replicated in various studies that higher copy number of *CCL3L1* confers resistance to HIV-1 infection depending on the population-specific distribution of *CCL3L1* copy number [11].

There are several studies in varied populations where the significant correlation between *SDF1-3'A* SNP [10, 12] and *CCL3L1* CN [11, 13, 14] and susceptibility/protection to HIV was found. However, in the present study significant association of either *SDF1-3'A* polymorphism or *CCL3L1* CN towards susceptibility to HIV infection in Indian population was not found. The allelic frequency of *SDF1-3'A* observed in this study was similar to that reported in North Indian (20.4%) [15] and South Indian (17-35%) [16] populations. The lack of significant association compelled us to think that a single type of genetic variation (like SNP or CNVs) might not show an association towards disease susceptibility or resistance but the combinations of these types of genetic variations could provide a better insight in understanding the mechanism behind the disease development. Therefore, the effect of combination of *SDF1-3'A* SNP and *CCL3L1* CN was evaluated in Indian population. However, the correlation between *SDF1-3'A* or *CCL3L1* CN or even a combined effect of both on progression of HIV-1 towards AIDS in Indian

population could not be established. To the best of our knowledge, a correlation between combination of *SDF1-3'A* SNP and *CCL3L1* CN has not been evaluated till date.

There could be several possible reasons for lack of significant association in this study. Firstly, the sample size of HIV patients was low. Secondly, the protection towards HIV-1 infection is not under the influence of either *SDF1-3'A* SNP or *CCL3L1* CN in Indian population. Lastly, *SDF-1* and *CCL3L1* chemokines bind to different coreceptor, which may cause the lack of combinatorial effect of these variants in populations. Thus, lack of this correlation is not clear and requires further exploration.

The outcome of the complex diseases cannot be explained only by analyzing a single type of variation in a gene. However, combination of several types of genetic variations will facilitate better understanding of their role in health and disease. In conclusion, the frequency data for *SDF-1* SNP and *CCL3L1* CN in Indian population was developed. However, a comparison with HIV-1 patients did not reveal any correlation between genotype of *SDF-1* SNP and *CCL3L1* CN in progression of HIV-1 towards AIDS infection.

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