J Biosci Vol. 42, No. 3, September 2017, pp. 481–490
DOI: 10.1007/s12038-017-9694-5 © Indian Academy of Sciences

Promoter polymorphism MMP-1 (-1607 2G/1G) and MMP-3 (-1612 5A/6A) in development of HAND and modulation of pathogenesis of HAND

HARI OM SINGH[1,*], SHRUTI D MARATHE[1], SUMITRA NAIN[1], DHARMESH SAMANI[1], VIJAY NEMA[1], MANISHA V GHATE[2] and R R GANGAKHEDKAR[2]

[1]*Department of Molecular Biology, National AIDS Research Institute, Pune 411 026, India*

[2]*Department of Clinical Sciences, National AIDS Research Institute, Pune 411 026, India*

**Corresponding author (Email, hsingh@nariindia.org, hariomsgpgims@gmail.com)*

MS received 11 April 2016; accepted 16 May 2017; published online 4 July 2017

The pathogenesis of HIV-associated neurocognitive disorder (HAND) is modulated by host genetic susceptibility factors such as Matrix metalloproteinases (MMPs). Promoter polymorphism of *MMP-1* and *MMP-3* may modify the expression of the gene. Hence, we evaluated the association of *MMP-1*-16072G/1G and *MMP-3*-1612 5A/6A polymorphisms with development of HAND and the modulation of pathogenesis of HAND. We enrolled a total of 180 individuals, 50 HIV-infected individuals with HAND, 130 without HAND, and 150 healthy controls. Polymorphism of *MMP-1* and *MMP-3* were genotyped by PCR-RFLP. *MMP-1*-1607 2G1G, -16071G/2G-1G/1G genotypes and -1607 1G allele were associated with the development of HAND (OR = 1.64, P = 0.05; OR = 1.45, P = 0.04; OR = 1.69, P = 0.05). *MMP-1*-16071G1G, *MMP-3*-16125A5A genotypes increased the risk for the development of HAND (OR = 1.78, P = 0.25; OR = 2.39, P = 0.13). *MMP-3*-1612 5A5A, -1612 6A/5A-5A/5A genotypes and -1612 5A allele were associated with the reduced risk of HAND (OR = 0.40, P = 0.05; OR = 0.53, P = 0.04; OR = 0.40, P = 0.01). Haplotype 5A1G increased the risk of development of HAND (OR = 1.93, P = 0.05). As observed in advanced HIV disease stage, *MMP-1*-1607 1G1G genotype enhance the risk for advancement of HIV disease (OR = 1.69, P = 0.89). *MMP-3*-1612 6A5A genotype showed higher risk for development of HAND in alcohol users (0R = 1.65, P = 0.44). *MMP-1* genotype may have an influence on development of HAND whereas *MMP3*-1612 5A5A genotype may reduce risk for pathogenesis of HAND.

Keywords. Development of HAND; genetic susceptibility; HAND; MMP-1; MMP-3

1. Introduction

HIV-associated neurocognitive disorders involve impairment of cognition and its associated function with varying degrees in the HIV-infected individuals (Antinori *et al.* 2007; Grant 2008). After wider use of antiretroviral therapy (ART), the prevalence of the severest form of HIV-associated neurocognitive disorder (HAND), i.e. HIV-associated dementia (HAD), has dramatically decreased. However, the overall prevalence of HAND and associated morbidity remained high (∼50%) (Sacktor *et al.* 2005; Nath *et al.* 2008; Heaton *et al.* 2010; McArthur *et al.* 2010). In India, the prevalence of HAD was estimated at less than 6% until 2005 (Satishchandra *et al.* 2000; Deshpande and Patnaik 2005). However, a study with very high prevalence (32.50%) of HAND patients was reported in India (Saini and Barar 2014). The pathogenesis of HAND is modulated by host genetic susceptibility such as MMPs, subtype (clade), drugs of abuse, hepatitis C and viral gene adaptations (Letendre *et al.* 2010; McArthur *et al.* 2010; Valcour *et al.* 2011). Also, the systemic activation of the immune system modulated by HIV infection may affect the inflammatory state of the central nervous system (Kraft-Terry *et al.* 2010; Roberts *et al.* 2010). The pathogenesis of HAD is arbitrated mainly by mononuclear phagocyte (MP) secretory products and their interactions with neural cells (Ghorpade *et al.* 2001). Mononuclear phagocyte differentiation efficiently increases the expression of Matrix metalloproteinases (MMP-1, -2, -3, and -9) and MMPs are one of the influencing factors for monocyte migration into the central nervous system (CNS) (Ghorpade *et al.* 2001).

Electronic supplementary material: The online version of this article (doi:10.1007/s12038-017-9694-5) contains supplementary material, which is available to authorized users.

Matrix metalloproteinases (MMPs) plays an important role in the degradation of extracellular matrix (EM) and adipocyte differentiation, but its activity is inhibited by the antiretroviral drugs.

Matrix metalloproteinase acts on pro-inflammatory cytokines, chemokines and other proteins, which regulate varied aspects of inflammation and immunity (Parks *et al.* 2004). The mRNA and protein levels of MMP-1, -2, -3, and -9 were increased in infected brain tissue of HAD patients (Johnston *et al.* 2000; Ghorpade *et al.* 2001). MMP-1 can degrade HIV-1 Tat and this effect is blocked by MMP-1 inhibitors (Rumbaugh *et al.* 2006). *MMP-1*-1607 1G/2G and *MMP-3*-1612 5A/6A polymorphism have functional effects on promoter activity (Ye *et al.* 1996; Rutter *et al.* 1998). Insertion of a guanine (G) at position -1607 creates an erythromatosis twenty-six (ETS) binding site adjacent to the activating protein-1 (AP-1) site at position -1602, and this genotype has been shown to correlate with declines in pulmonary function in smokers (Nishioka *et al.* 2000). Montes *et al.* (2010) reported that *MMP-1*-1607 2G2G genotype was more frequent among lipodystrophic syndrome patients compared with those without lipodystrophic syndrome of HIV. Microarray analysis indicates HIV-1 Tat-induced upregulation of matrix metallopeptidase 3 in primary human brain microvascular endothelial cells (Woollard *et al.* 2014). *In vitro* assays showed that the 5A allele has a higher promoter activity than 6A allele, with differential binding of a nuclear protein to the two allelic promoters (Ye *et al.* 1996). Mellanen *et al.* (1996) reported that MMP-1, -3 and -8 associated with saliva from HIV-infected individuals, and these MMPs might play a role in the development of HIV-associated periodontitis (Mellanen *et al.* 1996).

Until now, association of *MMP-1*-1602 2G/1G and *MMP-3*-16125A/6A polymorphism with the modulation of pathogenesis of HAND and its prevalence in HIV-infected individuals has not been demonstrated. Hence, we examined the association of *MMP-1*-16072G/1G and *MMP-3*-1612 5A/6A polymorphisms with development of HAND and modulation of pathogenesis of HAND in western India.

2. Materials and methods

2.1 *Subjects*

From November 2013 to September 2014, 50 HIV-infected individuals with HAND confirmed by International HIV-associated Dementia Score (IHDS) <9.5 and documented evidence of HIV infection, 130 without HAND confirmed by IHDS >9.5 and age- and gender-matched were consecutively taken from the outdoor patient clinics of National AIDS Research Institute, Pune. HIV-infected individuals with HAND and without HAND having concurrent untreated opportunistic infections and febrile illness currently or in past 15 days were excluded from both groups. At the same time, 150 unrelated healthy controls, who were HIV-, hepatitis B, C- and tuberculosis-free, and were age- and ethnicity-matched and serum-negative, from SD Bioline ELISA test, were recruited from clinic belonging to the same institution. Clinical data were obtained by questionnaire, personal interviews and review of case records. Scaling of dementia was done by a trained clinician using guidelines of IHDS 2005 (Grant 2008). Estimation of CD4 count was done by Fluorescently Activated Cell Sorter (FACS). CD4 counts <200, 200–350, and >350 cells/mm^3 were considered as advanced, intermediate and early disease stages, respectively. Tobacco and alcohol usage were recorded in a pre-designed questionnaire. The study was approved by institutional ethics committees, and all participants gave a written and signed informed consent. Ethical approval was obtained: ICF version 1.0 dated 18 April 2011.

2.2 *DNA extraction*

Two milliliters peripheral blood sample was collected and stored at −70°C prior to DNA extraction. Genomic DNA extraction was done from peripheral blood leukocytes pellet using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA) according to the protocol given by the manufacturer.

2.3 *Genotyping*

The *MMP-1* and *MMP-3* polymorphism (-1607 2G/1G) and (-1612 5A/6A) were genotyped in subjects using PCR-restriction fragment length polymorphism (PCR-RFLP). Primers for amplification of *MMP-1* (-1607 2G/1G) and *MMP-3* (-1612 5A/6A) were taken as described (Gnasso *et al.* 2000; Zhu *et al.* 2001). PCR was performed in a total volume of 25 μL with 20 pmol of each primer, genomic DNA (100–150 ng), 10 mM deoxynucleotide triphosphates, PCR buffer containing 100 mMTris-HCl, pH 8.6, 50 mM KCl, 1.5 mM $MgCl_2$ and 1.5 units of Taq polymerase (Bangalore Genei, India). The reaction conditions for *MMP-1* were: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 50 s, extension at 72°C for 1 min and a final extension at 72°C for 7 min. The conditions for PCR amplification of *MMP-3* were: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 45 s and a final extension at 72°C for 7 min. Amplified product of *MMP-1* and *MMP-3* were digested using restriction enzyme *Alu1* and *TthIII* (MBI Fermentas Inc, Hanover, MD, USA). Genotyping of *MMP-1* and *MMP-3* was done in 15 %

polyacrylamide gel using molecular weight markers and visualized after staining with ethidium bromide. Based on sequences and location of SNP, genotypes of *MMP-1* and *MMP-3* were as assigned as follows: 270 bp for -16072G2G; 270 bp, 248 bp, and 28 bp for -1697 2G1G and 248 bp, and 28 bp for -16071G1G genotype and for *MMP-3*: 129 bp for -1612 6A6A; 129 bp, 97 bp, and 32 bp for -1612 6A5A and 97bp, and 32bp for -16125A5A (supplementary figures 1 and 2). All reactions were carried out in a Veriti 96-well Thermal cycler (Applied Biosystems, USA). PCR products and molecular weight markers were visualized in 2% agarose gel. Twenty percent of the samples from both patients and controls were re-genotyped by other laboratory personnel and discrepancy in genotyping was not noticed. Sequencing was done in 10% of samples to assess the genotyping error.

2.4 *Data analysis*

The age variable was expressed as mean ± standard deviation (SD). The χ^2 goodness-of-fit test was used for any deviation from Hardy–Weinberg equilibrium in controls. We used the χ^2 statistic (Fisher's exact test for cell size <5) to compare genotype frequency between HIV-infected individuals with HAND vs. without HAND, HIV-infected individuals vs. healthy controls. SNPStats software was used to compare haplotype frequency between HIV-infected individuals with HAND and without HAND, HIV-infected individuals and healthy controls. Odds ratios (ORs) and 95% confidence interval (CI) were calculated by unconditional binary logistic regression. SPSS software version 17.0 (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0 (Chicago: SPSS Inc.) was used for statistical analysis and tests of statistical significance were two-sided and taken as significant when P-value is less than 0.05. LD was estimated between both the loci by calculating the relative LD value (D') as $D' = Dij/Dmax$ (Cox *et al.* 1998). The Dij values were compared between HIV-infected individuals with HAND and without HAND, HIV-infected individuals and healthy controls by comparison of confidence intervals.

3. Results

In the present study, 50 HIV-infected individuals with HAND, 130 without HAND and 150 healthy individuals were enrolled. The mean age (years ± SD) of HIV-infected individuals with, without HAND and healthy controls were 39.04 years ± 6.62,, 38.01 years ± 8.03, and 37.01 years ± 7.9 respectively. Characteristics of HIV-infected individuals with, without HAND and healthy controls are shown in table 1.

Table 1. Characteristics of HIV-infected individuals with HAND, without HAND and healthy controls

Subjects	HIV-infected individuals with HAND (%)	HIV-infected individuals without HAND (%)	Healthy controls (%)
Number of individuals	50	130	150
Mean age and standard deviation (Years ± SD)	39.04 ± 6.62	38.01 ± 8.03	37.01 ± 7.9
Females	19 (38%)	51 (39.23%)	44 (29.33%)
Males	31 (62%)	79 (60.76%)	106 (70.66%)
Alcohol habit	N = 50	N = 85	
Alcohol users	11 (28.20%)	27 (37.76%)	-
Alcohol non-users	39 (71.80%)	58 (68.24%)	-
Tobacco habits	N = 50	N = 85	
Tobacco users	13 (26.0%)	17 (20.0%)	-
Tobacco non-users	37 (74.0%)	68 (80.0%)	-
CD4 Status (N = 150)	N = 50	N = 100	
Advanced HIV disease stage (<200)	30 (60%)	13 (13%)	Not applicable (NA)
Intermediate HIV disease stage (201-350)	11 (22%)	27 (27%)	NA
Early HIV disease stage (>351)	9 (18%)	60 (60%)	NA
Ethnicity	Western India	Western India	Western India

Status of CD4 count of 30 HIV-infected individuals without HAND was not available. Tobacco and alcohol status of 45 HIV-infected individuals without HAND was not available. Hence, both data is not included in the analysis.

3.1 *MMP-1(-1607 2G/1G) and MMP-3(-1612 5A/6A) polymorphisms and HIV-infected individuals with HAND*

The frequency of *MMP-1* and *MMP-3* genotypes/alleles in HIV-infected individuals with HAND and without HAND is shown in table 2. *MMP-1*-1607 1G1G genotype was underrepresented in HIV-infected individuals with HAND compared with without HAND (2.0% vs. 10.8%, OR = 0.15, 95% CI: 0.01–1.27, $P = 0.08$). *MMP-3*-1612 6A/5A and -1612 6A/5A-5A/5A genotypes and -1612 5A allele were associated with decreased risk of pathogenesis of HAND (16.0% vs. 30.8%, OR = 0.40, 95% CI: 0.16–1.00, $P = 0.05$; 16%, vs. 26.16%, OR = 0.53, 95% CI: 0.29–0.98, $P = 0.04$ and 24% vs. 41.5%, OR = 0.40, 95% CI: 0.18–0.85, $P = 0.01$ respectively).

Table 2. Frequency distribution of *MMP-1*(-1607 2G/1G) and *MMP-3*(-1612 5A/6A) genotypes/alleles in HIV-infected individuals with and without HAND

Genotypes *MMP-1*-16072G/1G	HIV-infected individuals with HAND N = 50 (%)	HIV-infected individuals without HAND N = 130 (%)	*P*-Value	OR (95% CI)
2G2G	21 (42.0%)	53 (40.8%)	1	Reference
2G1G	28 (56.0%)	63 (48.5%)	0.87	1.12 (0.54-2.32)
1G1G	1 (2.0%)	14 (10.8%)	**0.08**	**0.15 (0.01-1.27)**
MMP-1-16072G/1G Allele	HIV-infected individuals with HAND N = 100 (%)	HIV-infected individuals without HAND N = 260 (%)	*P*-Value	OR (95%CI)
2G	70 (70%)	169 (65.0%)	1	Reference
1G	30 (30%)	91 (35%)	0.36	0.79 (0.48-1.30)
Dominant model				
2G/2G	21 (42%)	53 (44.8%)	1	Reference
1G/2G-1G/1G	29 (58%)	77 (59.2%)	0.83	0.93 (0.47-1.84)
Genotypes *MMP-3*-1612 5A/6A	HIV-infected individuals with HAND N = 50 (%)	HIV-infected individuals without HAND N = 130 (%)	*P*-Value	OR(95%CI)
6A6A	38 (76%)	76 (58.5%)	1	Reference
6A5A	8 (16.0%)	40 (30.8%)	**0.05**	**0.40 (0.16-1.00)**
5A5A	4 (8.0%)	14 (10.8%)	0.50	0.57 (0.15-2.04)
MMP-3-1612 5A/6A Allele	HIV-infected individuals with HAND N = 100 (%)	HIV-infected individuals without HAND N = 260 (%)	*P*-Value	OR (95% CI)
6A	84 (84%)	192 (73.84%)	1	Reference
5A	16 (16%)	68 (26.16%)	**0.04**	**0.53 (0.29-0.98)**
Dominant model				
6A/6A	38 (76%)	76 (58.5%)	1	Reference
6A/5A-5A/5A	12 (24%)	54 (41.5%)	**0.01**	**0.40 (0.18-0.85)**

N, total number of HIV-infected individuals with HAND (50) and without HAND (130), (%) = frequency of genotypes/alleles. Age-adjusted OR (odds ratios) and 95% CI (confidence intervals) were derived from logistic regression models comparing the homozygous wild-type genotype/allele (2G/2G for *MMP-1* and 6A/6A for *MMP-3* were taken as reference) with other genotypes/alleles. Significant P values (<0.05) and respective OR (95% CI) have been shown in bold.

Table 3. Frequency distribution of *MMP-1*(-16072G/1G) and *MMP-3*(-1612 5A/6A) genotypes/alleles and HIV-infected individuals and healthy controls

Genotypes *MMP-1*-16072G/1G	HIV-infected individuals N = 130 (%)	Healthy controls N = 150 (%)	*P*-Value	OR (95% CI)
2G2G	53 (40.8%)	81 (54.0%)	1	Reference
2G1G	63 (48.5%)	57 (38.0%)	**0.05**	**1.69 (1.00-2.87)**
1G1G	14 (10.8%)	12 (8.0%)	0.25	1.78 (0.71-4.50)
MMP-1-16072G/1G Allele	HIV-infected individuals N = 260 (%)	Healthy controls N = 300 (%)	*P*-Value	OR (95% CI)
2G	169 (65.0%)	219 (73.0%)	1	Reference
1G	91 (35.0%)	81 (27.0%)	**0.04**	**1.45 (1.01-2.08)**
Dominant model				
2G/2G	53 (40.8%)	81 (54%)		
1G/2G-1G/1G	77 (59.2%)	69 (46%)	**0.05**	**1.64 (0.99-2.72)**
Genotypes *MMP-3*-1612 6A/5A	HIV-infected individuals N = 130 (%)	Healthy controls N = 150 (%)	*P*-Value	OR (95% CI)
6A6A	76 (58.4%)	78 (52.0%)	1	Reference
6A5A	40 (30.8%)	66 (44.0%)	**0.08**	**0.62 (0.36-1.06)**
5A5A	14 (10.8%)	6 (4.0%)	**0.13**	**2.39 (0.80-7.42)**
MMP-3-1612 6A/5A Allele	HIV-infected individuals N = 260	Healthy controls N = 300	*P*-Value	OR (95% CI)
6A	192 (73.84%)	222 (74.0%)	1	Reference
5A	68 (26.16%)	78 (26.0%)	0.95	0.98 (0.67-1.44)
Dominant model				
6A/6A	76 (58.5%)	78 (52%)	1	Reference
6A/5A-5A/5A	54 (41.5%)	72 (48%)	0.73	0.91 (0.55-1.52)

N, total number of HIV-infected individuals (130) and healthy controls (150). (%) = frequency of genotypes/alleles. Age-adjusted OR (odds ratios) and 95% CI (confidence intervals) were derived from logistic regression models comparing the homozygous wild-type genotype/allele (*2G/2G* for *MMP-1* and 6A/6A for *MMP-3* were taken as reference*)* with other genotypes/alleles. Significant P values (<0.05) and respective OR (95% CI) have been shown in bold.

3.2 *MMP-1(-1607 2G/1G) and MMP-3(-1612 5A/6A) polymorphisms and HIV-infected individuals*

The genotype and allele frequency of *MMP-1* and *MMP-3* polymorphism in HIV-infected individuals and healthy controls is shown in table 3. The genotype distributions of *MMP-1* and *MMP-3* polymorphisms in healthy controls were in the Hardy-Weinberg equilibrium ($P = 0.65$, $P = 0.08$). *MMP-1*-16072G1G, -16071G/2G-1G/1G genotypes and 1G allele were overrepresented in HIV-infected individuals compared with healthy controls (48.5% vs. 38.0%, OR = 1.69, 95% CI: 1.00–2.87, P = 0.05, 59.2% vs. 46%, OR = 1.64, 95% CI: 0.99–2.72, P = 0.05, 35.0% vs. 27.0%, OR = 1.45, 95% CI: 1.01–2.08, P = 0.04 respectively). The frequency distribution of *MMP-1*-16072G1G and *MMP-3*-1612 5A5A genotypes was found to be increased in HIV-infected individuals compared with healthy controls (10.8%% vs. 8.0%, OR = 1.78, 95% CI: 0.71–4.50, P = 0.25, 10.8% vs. 4.0%, OR = 2.39, 95% CI: 0.80–7.42, P = 0.13 respectively). The prevalence of *MMP-3*-16126A5A genotype was found to be lower in HIV-infected individuals compared with healthy controls (30.8% vs. 44.0%, OR = 0.62, 95% CI: 0.36–1.06, P = 0.08)

3.3 *Gene–gene interaction*

Haplotype frequency of *MMP-1* (-1607 2G/1G) and *MMP-3* (-1612 5A/6A) polymorphisms in HIV-infected individuals with, without HAND and healthy controls is shown in table 4. In the HIV-infected individuals with HAND vs. without HAND and HIV-infected individuals vs. healthy controls populations, there were no significant linkage disequilibrium (LD) D′ between both the genes (0.1302 and 0.0907**)**. Comparing the LD values (Dij) between HAND and without HAND subjects, no significant difference was found between both the genes. It was expected that there might be the additive or synergistic effect of these variations with the development of HAND and modulation in the pathogenesis of HAND. Haplotype 6A2G (*MMP-1**2G/ *MMP-3* *6A) was taken as reference. The frequency of haplotypes 6A1G, 5A2G (*MMP-1**1G, *2G/*MMP-3**6A,

Table 4. Frequency distribution of haplotype *MMP-1*(-16072G/1G) and *MMP-3*(-1612 5A/6A) genotypes among HIV-infected individuals with HAND, without HAND and healthy controls

Haplotypes *MMP-1*-16072G/1G and *MMP-3*(-1612 5A/6A)	HIV-infected individuals with HAND n = 100* (%)	HIV-infected individuals without HAND n = 260* (%)	*P*-Value	OR (95% CI)
6A2G	0.58	0.51	1	Reference
6A1G	0.26	0.23	0.88	0.95 (0.49–1.84)
5A2G	0.12	0.14	0.90	0.94 (0.40–2.23)
5A1G	0.04	0.12	0.08	0.26 (0.06–1.21)
Haplotypes *MMP-1*-16072G/1G and *MMP-3*-1612 5A/6A	**HIV-infected individuals n = 260* (%)**	**Healthy controls n = 300* (%)**	***P*-Value**	**OR (95% CI)**
6A2G	0.51	0.55	1	Reference
6A1G	0.23	0.19	0.48	1.20 (0.72–2.01)
5A2G	0.14	0.18	0.66	0.87 (0.47–1.61)
5A1G	0.12	0.08	**0.05**	**1.93 (0.98–3.81)**

*n, number of chromosomes. Age-adjusted Odds ratios and 95% CIs were derived from logistic regression models comparing the haplotype 6A2G with other haplotypes. Significant P values (<0.05) and respective OR (95% CI) have been shown in bold.

*5A) did not differ significantly between HAND and without HAND (26% vs. 23%, 12% vs. 14%). Haplotype 5A1G (*MMP-1**1G/*MMP-3**5A) was found to be decreased the risk for pathogenesis of HAND (0.04 vs. 0.12, OR = 0.26, 95% CI: 0.06–1.21, P = 0.08). The frequency of haplotype 5A1G (*MMP-1**1G/*MMP-3**5A) was observed to be higher in HIV-infected individuals compared with healthy controls (0.12 vs. 0.08, OR = 1.93, 95% CI: 0.98–3.81, P = 0.05)

3.4 *MMP-1(-1607 2G/1G) and MMP-3(-1612 5A/6A) polymorphism and different HIV disease stages*

MMP-1-16071G1G genotype and advanced HIV disease stage observed as the risk for advancement of HIV disease (15.38% vs. 8.0%, OR = 1.69, 95% CI: 0.22–10.37, P = 0.89). *MMP-1*-16072G1G and -16071G1G genotypes and intermediate HIV disease stage enhanced the risk of advancement of HIV disease (55.55% vs. 38.0%, OR = 2.37, 95% CI: 0.90–6.35, P = 0.08 and 11.11% vs. 8.0%, OR = 2.25, 95% CI: 0.41–11.12, P = 0.49) (table 5).

3.5 *Gene–environment interaction*

Presence of tobacco and alcohol habits in HIV-infected individuals was analyzed with respect to *MMP-1* and *MMP-3* polymorphism (table 6 and 7). *MMP-1* and *MMP-3* polymorphism in presence of tobacco and alcohol usage neither associated with development of HAND nor pathogenesis of HAND. However, the prevalence of *MMP-3*-1612 6A5A genotype was found to be increased in alcohol using HIV-infected individuals as compared to non-users (40.74% vs. 25.87% OR = 1.65, 95% CI: 0.56–4.90, P = 0.44).

4. Discussion

This is the first study that describes the impact of both *MMP-1* and *MMP-3* polymorphism, which are located on same chromosome with respect to development of HAND and the modulation of pathogenesis of HAND. The pathogenesis of HAND is modulated by host genetic susceptibility (McArthur *et al.* 2010). MMPs are involved in the degradation of EM and are inhibited by antiretroviral drugs. Inter-individual genetic differences may varies the susceptibility to develop HAND and its pathogenesis. Natural sequence variations in promoters of the *MMP* genes may result in variable expression of *MMPs*. The promoter region polymorphisms of *MMP-1* and *MMP-3* gene associated with different transcriptional activity *in vitro*. In our study, the frequency of *MMP-3* -1612 6A5A genotype in healthy controls was comparable with the study carried by Shalia *et al.* (2010) and differed from that of Chaudhary *et al.* (2010). The frequency of *MMP-1* -16072G1G genotype in healthy controls differed from the study carried by Srivastava *et al.* (2009), Devulapalli *et al.* (2014), and Dey *et al.* (2014).

In the present study, *MMP-1* -16071G1G and *MMP-3* -16125A5A genotypes predominantly represented higher risk for the development of HAND (OR = 1.78, P = 0.25 and OR = 2.39, P = 0.13). In our study, *MMP-3* -1612 5A allele was associated with modulation of pathogenesis of HAND. *MMP-3* 5A allele is known to have higher transcriptional activity of promoter, leading to increased gene expression (Okamoto *et al.* 2010). Martin *et al.* (2014) reported an association between *MMP-13* and *MMP-1* SNPs and sepsis (Martin *et al.* 2014). Mellanen *et al.* (1996) reported that *MMP*-1, -3 and -8 polymorphism was associated with saliva of HIV-infected individuals, and these

Table 5. Frequency distribution of *MMP-1*(-16072G/1G) and *MMP-3*(-1612 5A/6A) genotypes in different HIV disease stages of HIV-infected individuals

		Early HIV disease stage		Intermediate HIV disease stage		Advanced HIV disease stage	
Genotypes*MMP-1*-16072G/1G	Healthy controls N = 150 (%)	N = 60 (%)	OR (P)	N = 27 (%)	OR (P)	N = 13 (%)	OR (P)
2G2G	81 (54.0)	18 (30.0)	1 (Reference	9 (33.34)	1 (Reference)	8 (61.53)	1 (Reference)
2G1G	57 (38.0)	35 (58.33)	2.76 (0.003) 1.36-5.66	15 (55.55)	2.37 (0.08) 0.90-6.35	3 (23.07)	0.53 (0.55)
1G1G	12 (8.0)	7 (11.67)	2.63 (0.12) 0.80-8.54	3 (11.11)	2.25 (0.49) 0.41-11.12	2 (15.38)	1.69 (0.89) 0.22-10.37

		Early HIV disease stage		Intermediate HIV disease stage		Advanced HIV disease stage	
Genotypes *MMP-3*-1612 5A/6A	Healthy controls N = 150 (%)	N = 60 (%)	OR (P)	N = 27 (%)	OR (P)	N = 13 (%)	OR (P)
6A6A	78 (52.0)	32 (53.33)	1 (Reference)	19 (70.37)	1 (Reference)	10 (76.92)	1 (Reference)
6A5A	66 (44.0)	21 (35.0)	0.78 (0.53)	7 (25.93)	0.44 (0.11)	3 (23.08)	0.35 (0.19)
5A5A	6 (4.0)	7 (11/67)	2.84 (0.13) 0.78-10.53	1 (3.70)	0.68 (0.87)	0 (0.0)	NS

Presence of 2G2G for 2G1G for 1G2G genotypes of *MMP-1* and 6A6A for 6A5A, 5A5A genotypes of *MMP-3* were taken as reference group for statistical analysis.

MMPs may play a role in the development of HIV-associated periodontitis (Mellanen *et al.* 1996).

The present study has also attempted to assess the role of two important genes of *MMP* gene cluster individually and in combination, which are closely linked to each other. In our study, haplotype 5A1G indicated a higher risk for the development of HAND (OR = 1.93 P = 0.05). Haplotype studies of *MMP3* and *MMP9* genes in other diseases have been reported (Mellanen *et al.* 1996; Skibola *et al.* 2008; Chen *et al.* 2011).

This is a case-control study where current CD4 count has been considered as a surrogate for HIV -1 infection. Since the time points of acquisition of HIV-1 are not known, the results may be confounded by the duration of HIV infection. In the present study, association of the *MMP-1* and *MMP-* 3 genotypes with different HIV disease stages was carried out to explore the influence of variant genotypes in risk of advancement of HIV disease. In subgroup analysis, *MMP-1* carriage -1607 1G1G genotype increased the risk for the advancement of HIV disease in individuals with advanced HIV disease stage (OR = 1.69, P = 0.89).

The study of gene–environment interaction determines the etiology of disease (Greenland 1980; Deng *et al.* 2004). To evaluate the gene–environment interaction analysis, the case-only method is considered to be better than case-controls since the selection criteria for case-control association studies for environmental influences must have matched controls in the population, else it can lead to false interactions (Chen *et al.* 2011). Therefore, we have employed the case-only study to find out the risk for development of HAND and modulation in the pathogenesis of HAND in alcohol and tobacco users. An individual with HIV infection using heavy alcohol had a negative impact on the CD4 cell count and not on combined antiretroviral therapy (Samet *et al.* 2004). In the present study, *MMP-3*-1612 6A5A genotype among alcohol users was found to be an elevated risk for the development of HAND.

There are certain limitations of this study: being a case-control study, it can only assess association and will not be able to ascertain causality. We have allocated a ratio of 1:3 for case-controls. However, we could not complete matched enrollment in controls. Despite this, our case-control ratio is around 1:2, which may be sufficient for this study.

In summary, *MMP-1* polymorphism showed risk in the development of HAND. *MMP-3* polymorphism independently may decrease the risk of pathogenesis of HAND, but in the presence of alcohol, it increases the risk for the development of HAND. The severity of disease in HAND patients is associated with inflammation. There may be other factors, including additional polymorphisms of the *MMP-1, MMP-3* and other *MMPs* genes, the status of Tissue inhibitory matrix metalloproteinase (*TIMP)*, and the complex cytokine network, which may have a role in modulation of

Table 6. Frequency distribution of *MMP-1*(-16072G/1G) and *MMP-3*(-1612 5A/6A) genotypes in tobacco and alcohol using HIV-infected individuals with HAND

Genotypes *MMP-1*(-6072G/1G)	Tobacco users N = 13 (%)	Tobacco non- users N = 37 (%)	*p*-value	OR 95% CI
HIV-infected individuals with HAND				
2G2G	6 (46.15)	15 (40.54)	1	Reference
2G1G	7 (53.84)	21 (56.75)	0.96	0.83 (0.19-3.57)
1G1G +2G1G	0 (0.0)	1 (2.70)	NS	-
MMP-3(-1612 5A/6A)				
6A6A	10 (76.92)	28 (75.67)	1	Reference
6A5A	2 (15.38)	6 (16.22)	0.71	0.93 (0.11-6.64)
6A5A +5A5A	1 (7.69)	3 (8.10)	0.58	0.93 (0.0-12.50)

Genotypes *MMP-1*(-6072G/1G)	Alcohol users N = 11 (%)	Alcohol non-users N = 39 (%)	*p*-value	OR 95% CI
2G2G	7 (63.64)	14 (35.90)	1	Reference
2G1G	4 (30.77)	24 (61.54)	0.21	0.33 (0.07-1.60)
1G1G +2G1G	0 (0.0)	1 (2.56)	NS	-
MMP-3(-1612 5A/6A)				
6A6A	9 (81.80)	29 (74.36)	1	Reference
6A5A	1 (9.10)	7 (17.95)	0.82	0.46 (0.02-4.81)
6A5A +5A5A	1 (9.10)	3 (7.69)	0.57	1.07 (0.0-14.65)

Presence of 2G1G for 1G1G, 1G1G +2G1G and 6A6A for 6A5A, 6A5A +5A5A genotypes were taken as reference group for statistical analysis.

Table 7. Frequency distribution of *MMP-1*(-16072G/1G) and *MMP-3*(-1612 5A/6A) genotypes in tobacco and alcohol using HIV-infected individuals without HAND

Genotypes *MMP-1*(-6072G/1G)	Tobacco users N = 17 (%)	Tobacco non-users N = 68 (%)	*p*-value	OR 95% CI
HIV-infected individuals without HAND				
2G2G	9 (52.94)	24 (35.30)	1	Reference
2G1G	8 (47.06)	37 (54.41)	0.46	0.58 (0.17-1.93)
1G1G +2G1G	0 (0.0)	7 (10.29)	NS	-
MMP-3(-1612 5A/6A)				
6A6A	11 (64.71)	41 (60.29)	1	Reference
6A5A	5 (29.41)	21 (30.88)	0.92	0.89 (0.23-3.23)
6A5A +5A5A	1 (5.88)	6 (8.82)	0.93	0.62 (0.03-6.38)

Genotypes *MMP-1*(-6072G/1G)	Alcohol users N = 27 (%)	Alcohol non-users N = 58 (%)	*p*-value	OR 95% CI
2G2G	10 (37.03)	23 (39.65)	1	Reference
2G1G	15 (55.56)	30 (51.72)	0.96	1.15 (0.39-3.38)
1G1G +2G1G	2 (7.41)	5 (8.62)	0.71	0.92 (0.10-7.00)
MMP-3(-1612 5A/6A)				
6A6A	16 (59.26)	36 (62.06)	1	Reference
6A5A	11 (40.74)	15 (25.87)	0.44	1.65 (0.56-4.90)
6A5A +5A5A	0 (0.0)	7 (12.07)	NS	-

Presence of 2G1G for 1G1G, 1G1G +2G1G and 6A6A for 6A5A, 6A5A +5A5A genotypes were taken as reference group for statistical analysis.

pathogenesis of HAND. In future, additional studies with inflammation-related candidate gene polymorphisms and possible interactions between gene cluster polymorphism and environmental factors are required for better understanding of modulation of pathogenesis of HAND.

Acknowledgements

We gratefully acknowledge Seema Sahay, in charge of the clinic, for the arrangement of community staff for recruitment of healthy control; Mansa Angadi, Shradha Bapat and

Jyoti Pawar for filling the clinical research proforma; Tumnan Katendra, Jai and Iyesha for counselling of subject participants; and sisters Ujawala Ghule and Sunita for the collection of blood samples. The study was supported by a research grant provided by NARI- ICMR India. The study was supported by research grant from Indian Council of Medical Research (ICMR) India (grant number HIV/50/152/10/2012-ECD-II).

References

Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Clifford DB, Cinque P, *et al.* 2007 Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* **69** 1789–1799

Chaudhary AK, Singh M, Bharti AC, Singh M, Shukla S, Singh AK and Mehrotra R 2010 Synergistic effect of stromelysin-1 (matrix metalloproteinase-3) promoter (-1171 5A->6A) polymorphism in oral submucous fibrosis and head and neck lesions. *BMC Cancer* **10** 369

Chen TY, Li YC, Liu YF, Tsai CM, Hsieh YH, Lin CW, Yang SF and Weng CJ 2011 Role of MMP14 gene polymorphisms in susceptibility and pathological development to hepatocellular carcinoma. *Ann. Surg. Oncol.* **18** 2348–2356

Cox A, Camp NJ, Nicklin MJ, Di Giovine FS and Duff GW 1998 An analysis of linkage disequilibrium in the interleukin-1 gene cluster, using a novel grouping method for multiallelic markers. *Am. J. Hum. Genet.* **62** 1180–8

Deng Y, Newman B, Dunne MP, Silburn PA and Mellick GD 2004 Case-only study of interactions between genetic polymorphisms of GSTM1, P1, T1 and Z1 and smoking in Parkinson's disease. *Neurosci. Lett.* **366** 326–331

Deshpande AK and Patnaik MM 2005 Nonopportunistic neurologic manifestations of the human immunodeficiency virus: an Indian study. *MedGenMed* **7** 2

Devulapalli K, Bhayal AC, Porike SK, Macherla R, Akka J, Nallari P, Ananthapur V 2014 Role of interstitial collagenase gene promoter polymorphism in the etiology of gastric cancer. *Saudi J. Gastroenterol.* **20** 309–314

Dey S, Ghosh N, Saha D, Kesh K, Gupta A, Swarnakar S (2014) Matrix metalloproteinase-1 (MMP-1) Promoter polymorphisms are well linked with lower stomach tumor formation in eastern Indian population. *PLoS One* **9** e88040

Ghorpade A, Persidskaia R, Suryadevara R, Che M, Liu XJ, Persidsky Y and Gendelman HE 2001 Mononuclear phagocyte differentiation, activation, and viral infection regulate matrix metalloproteinase expression: implications for human immunodeficiency virus type 1-associated dementia. *J. Virol.* **75** 6572–6583

Gnasso A, Motti C, Irace C, Carallo C, Liberatoscioli L, Bernardini S, Massoud R, Mattioli PL, *et al.* 2000 Genetic variation in human stromelysin gene promoter and common carotid geometry in healthy male subjects. *Arterioscler. Thromb. Vasc. Biol.* **20** 1600–1605

Grant I 2008 Neurocognitive disturbances in HIV. *Int. Rev. Psychiatry* **20** 33–47

Greenland S 1980 The effect of misclassification in the presence of covariates. *Am. J. Epidemiol.* **112** 564–569

Heaton RK, Clifford DB, Franklin DR, Jr, Woods SP, Ake C, Vaida F, Ellis RJ, Letendre SL, *et al.* 2010 HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology* **75** 2087–2096

Johnston JB, Jiang Y, van Marle G, Mayne MB, Ni W, Holden J, McArthur JC and Power C 2000 Lentivirus infection in the brain induces matrix metalloproteinase expression: role of envelope diversity. *J. Virol.* **74** 7211–7220

Kraft-Terry SD, Stothert AR, Buch S and Gendelman HE 2010 HIV-1 neuroimmunity in the era of antiretroviral therapy. *Neurobiol. Dis.* **37** 542–548

Letendre SL, Ellis RJ, Ances BM and McCutchan JA 2010 Neurologic complications of HIV disease and their treatment. *Top. HIV Med.* **18** 45–55

Martin G, Asensi V, Montes AH, Collazos J, Alvarez V, Carton JA, Taboada F and Valle-Garay E 2014 Role of plasma matrix-metalloproteases (MMPs) and their polymorphisms (SNPs) in sepsis development and outcome in ICU patients. *Sci. Rep.* **4** 5002

McArthur JC, Steiner J, Sacktor N and Nath A 2010 Human immunodeficiency virus-associated neurocognitive disorders: mind the gap. *Ann. Neurol.* **67** 699–714

Mellanen L, Ingman T, Lahdevirta J, Lauhio A, Ainamo A, Konttinen YT, Sukura A, Salo T, *et al.* 1996 Matrix metalloproteinases-1, -3 and -8 and myeloperoxidase in saliva of patients with human immunodeficiency virus infection. *Oral. Dis.* **2** 263–271

Montes AH, Valle-Garay E, Alvarez V, Pevida M, Garcia Perez E, Paz J, Meana A and Asensi V 2010 A functional polymorphism in MMP1 could influence osteomyelitis development. *J. Bone. Miner. Res.* **25** 912–919

Nath A, Schiess N, Venkatesan A, Rumbaugh J, Sacktor N and McArthur J 2008 Evolution of HIV dementia with HIV infection. *Int. Rev. Psychiatry* **20** 25–31

Nishioka Y, Kobayashi K, Sagae S, Ishioka S, Nishikawa A, Matsushima M, Kanamori Y, Minaguchi T, *et al.* 2000 A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter in endometrial carcinomas. *Jpn. J. Cancer. Res.* **91** 612–615

Okamoto K, Ishida C, Ikebuchi Y, Mandai M, Mimura K, Murawaki Y and Yuasa I 2010 The genotypes of IL-1 beta and MMP-3 are associated with the prognosis of HCV-related hepatocellular carcinoma. *Intern. Med.* **49** 887–895

Parks WC, Wilson CL and Lopez-Boado YS 2004 Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat. Rev. Immunol.* **4** 617–629

Roberts L, Passmore JA, Williamson C, Little F, Bebell LM, Mlisana K, Burgers WA, van Loggerenberg F, *et al.* 2010 Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression. *AIDS* **24** 819–831

Rumbaugh J, Turchan-Cholewo J, Galey D, St Hillaire C, Anderson C, Conant K and Nath A 2006 Interaction of HIV Tat and matrix metalloproteinase in HIV neuropathogenesis: a new host defense mechanism. *FASEB J.* **20** 1736–1738

Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ and Brinckerhoff CE 1998 A single nucleotide polymorphism in

the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res.* **58** 5321–5325

Sacktor NC, Wong M, Nakasujja N, Skolasky RL, Selnes OA, Musisi S, Robertson K, McArthur JC, *et al.* 2005 The International HIV Dementia Scale: a new rapid screening test for HIV dementia. *AIDS* **19** 1367–1374

Saini S and Barar KV 2014 Assessment of neurocognitive functions in HIV/AIDS patients on HAART using the international HIV dementia scale. *Int. J. Nutr. Pharmacol. Neurol. Dis.* **4** 252–255

Samet JH, Phillips SJ, Horton NJ, Traphagen ET and Freedberg KA 2004 Detecting alcohol problems in HIV-infected patients: use of the CAGE questionnaire. *AIDS Res. Hum. Retroviruses* **20** 151–155

Satishchandra P, Nalini A, Gourie-Devi M, Khanna N, Santosh V, Ravi V, Desai A, Chandramuki A, *et al.* 2000 Profile of neurologic disorders associated with HIV/AIDS from Bangalore, south India (1989–96). *Indian J. Med. Res.* **111** 14–23

Shalia KK, Shah VK, Mashru MR, Soneji SL, Vasvani JB, Payannavar S, Walvalkar A, Mokal R, *et al.* 2010 Matrix metalloproteinase-3 (MMP-3) -1612 5A/6A promoter polymorphism in coronary artery disease in Indian population. *Indian J. Clin. Biochem.* **25** 133–140

Skibola CF, Bracci PM, Halperin E, Nieters A, Hubbard A, Paynter RA, Skibola DR, Agana L, *et al.* 2008 Polymorphisms in the estrogen receptor 1 and vitamin C and matrix metalloproteinase gene families are associated with susceptibility to lymphoma. *PLoS One* **3** e2816

Srivastava P, Kapoor R and Mittal RD 2009 Influence of matrix metalloproteinase gene polymorphisms in healthy North Indians compared to variations in other ethnic groups worldwide. *Asian Pac. J. Cancer Prev.* **10** 1127–1130

Valcour V, Paul R, Chiao S, Wendelken LA and Miller B 2011 Screening for cognitive impairment in human immunodeficiency virus. *Clin. Infect. Dis.* **53** 836–842

Woollard SM, Bhargavan B, Yu F and Kanmogne GD 2014 Differential effects of Tat proteins derived from HIV-1 subtypes B and recombinant CRF02_AG on human brain microvascular endothelial cells: implications for blood-brain barrier dysfunction. *J. Cereb. Blood Flow Metab.* **34** 1047–1059

Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE and Henney AM 1996 Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J. Biol. Chem.* **271** 13055–13060

Zhu Y, Spitz MR, Lei L, Mills GB and Wu X 2001 A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances lung cancer susceptibility. *Cancer Res.* **61** 7825–7829

Corresponding editor: Partha P Majumder