# Association of *CD209L* tandem repeats polymorphism with susceptibility to human immunodeficiency virus-1 infection, disease progression, and treatment outcomes: a Moroccan cohort study

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## Abstract

In order to investigate the association between length variation of the *CD209L* neck region and human immunodeficiency virus (HIV)-1 susceptibility, disease progression, and treatment response outcomes, we genotyped 139 HIV-1-seropositive and 109 seronegative individuals. The heterozygous genotype 6/5 showed a significant increased risk of HIV-1 infection (OR 3.03, 95% CI 0.99–9.33, p 0.046). Moreover, after highly active antiretroviral therapy (HAART), HIV-1-seropositive individuals carrying the 6/5, 7/5 and 7/7 genotypes and alleles 5, 6 and 7 showed good $CD4^+$ T-cell recovery. In addition, individuals with the 7/5, 6/6 and 7/7 genotypes showed a significant decrease in viral load during the treatment period as compared with baseline ($p < 0.05$). Interestingly, we found that alleles 4 and 6 were associated with protection against AIDS progression. *D209L* variation may influence susceptibility to HIV-1, response to treatment, and disease progression.



**Keywords:** AIDS, $CD4^+$, L-SIGN, tandem repeats, viral load
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## Introduction

According to the recent UNAIDS report, 35.3 (32.2–38.8) million people were living with human immunodeficiency virus (HIV) and 2.3 (1.9–2.7) million became newly infected with HIV in 2013 [1]. In Morocco, the scale of the local HIV epidemics and populations affected showed a highly heterogeneous geographical distribution [2]. Patients infected with HIV-1 show variable clinical outcomes. This natural variation can be attributed to host genetics, pathogen factors, and environmental factors, and is substantial among infected individuals [3,4]. Early studies showed that liver–lymph node-specific intercellular adhesion molecule-3 (ICAM-3)-grabbing integrin (L-SIGN), also known as dendritic cell-specific ICAM-3-grabbing non-integrin-related (DC-SIGNR) or CD209L, might promote the spread of HIV infection in lymph nodes [5,6]. The *CD209L* gene is highly polymorphic in the neck region; this is responsible for the homo-oligomerization that brings carbohydrate recognition domains (CRDs) into proximity for high-affinity ligand binding, based on the number of repeats in exon 4, with three to ten repeats (alleles 3 to 10) of a 69-bp segment [5,7–9]. Previous studies have shown that CD209L captures HIV-1 by binding to glycoprotein 120, and promotes the enhancement of T-cell infection by HIV-1 in trans [5,6] and also virus degradation in a proteasome-dependent manner [10]. Previous studies on the association between variation in the neck region of *CD209L* and HIV-1 susceptibility have been conducted, with controversial results [11–13]. In addition, the association between *CD209L* neck-repeat length and the response to highly active antiretroviral therapy (HAART) has not been investigated so far. Therefore, the aim of our study was to investigate the associations between length variation of the *CD209L* neck region and HIV-1 susceptibility, disease progression and treatment response outcomes in Moroccan subjects, a North African population.

The cohort was established in September 2012, with 139 antiretroviral-naive HIV-1-seropositive adult subjects (80 women (57.6%); 59 men (42.4%)). All patients are now



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receiving treatment with tenofovir/emtricitabine plus either nevirapine or lopinavir/ritonavir or efavirenz. One hundred and nine unrelated HIV-negative healthy subjects were recruited as described previously [14]. The *CD209L* repeat region was genotyped as reported previously [14].

The characteristics and allele and genotype frequencies of the study subjects are summarized in Table 1.The analysis of the frequency distribution of the *CD209*L neck-region genotypes showed that the frequency of the 6/5 genotype was significantly different between the seronegative and HIV-1-seropositive subjects (4.6% vs. 9.3%, p 0.046). Logistic regression analysis of the association of genotype with susceptibility to HIV-1 demonstrated that the 6/5 genotype was the only genotype significantly associated with the risk of infection with HIV-1 (OR 3.03, 95% CI 0.99–9.33, p 0.046) (Table 1).

At baseline, we found no association between any *CD209L* genotype and $CD4^+$ T-cell counts (all $p > 0.05$) (Fig. 1(a)). However, during follow-up and after HAART, the $CD4^+$ T-cell count was associated with the *CD209L* genotypes (Fig. 1(a)). Overall, HIV-1-seropositive subjects carrying genotypes 6/5 (449 cells/$mm^3$ at baseline vs. 608 cells/$mm^3$ after HAART, p 0.015), 7/5 (280 cells/$mm^3$ at baseline vs. 358 cells/$mm^3$after HAART, p 0.001) and 7/7 (346 cells/$mm^3$ at baseline vs. 463 cells/$mm^3$ after HAART, p 0.001) had an attenuated loss of $CD4^+$ T-cells, with a significant increase in the numbers of cells as compared with baseline $CD4^+$ T-cell levels (Fig. 1(a)). Regarding allele frequencies, we found that alleles 5 (329 cells/$mm^3$ at baseline vs. 409 cells/$mm^3$ after HAART, $p < 0.0001$), 6 (402 cells/$mm^3$ at baseline vs. 489 cells/$mm^3$ after HAART, p 0.005) and 7 (330 cells/$mm^3$ at baseline vs. 429 cells/$mm^3$ after HAART, $p < 0.0001$) were associated with restoration of $CD4^+$ T-cells as compared with allele 4, which was not associated with a significant increase in the number of $CD4^+$ T-cells after HAART (392 cells/$mm^3$ at baseline vs. 463 cells/$mm^3$ after HAART, p 0.5277) (Fig. 1(b)).

On the other hand, at baseline, no association was observed between any *CD209L* genotype and HIV-1 RNA viral load (all $p > 0.05$) (Fig. 1(c)). However, after HAART, subjects with the 7/5 genotype reduced their HIV-1 RNA viral load (4.75 $log_{10}$ vs. 3.1 $log_{10}$ copies/mL, p 0.0003) more than those with genotype 7/6 (4.8 $log_{10}$ vs. 3.6 $log_{10}$ copies/mL, p 0.0366) and genotype 7/7 (4 $log_{10}$ vs 3 $log_{10}$ copies/mL, p 0.0050) (7/5 > 7/7 > 7/6) (Fig. 1(c)). Moreover, after HAART, a significant correlation was found between *CD209L* alleles and HIV-1 viral load. Subjects with alleles 5, 6 and 7 showed a significant reduction in viral load as compared with subjects with allele 4 (Fig. 1(d)).

We checked whether the frequency distributions of *CD209L* neck-region genotypes and alleles were significantly different between patients with and without AIDS progression (Table S1). Progression to AIDS in adults was defined according to the CDC classification when patients showed opportunistic infections and the CD4 count was <200 cells/$mm^3$. Analysis of the association of genotype with progression outcomes demonstrated that only the 5/5 genotype increased the risk of progression to AIDS, without reaching the significance threshold (p 0.093, OR 3.3, 95% CI 0.78-13.93) (Table S1). In addition, comparison between the non-progressor and progressor groups carrying or not carrying allele 4 showed that this

**TABLE 1. Demographic, clinical characteristics and liver–lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN) neck-region polymorphism of the study subjects**

| | HIV-1-seropositive subjects ($n$ = 139) | Seronegative subjects ($n$ = 109) | OR (95% CI) | p |
|---|---|---|---|---|
| Mean age ± SD (years) | 37.62 ± 9.63 | 56.45 ± 10.86 | — | — |
| Gender (male/female) | 60/79 | 53/56 | — | — |
| Baseline median $CD4^+$ T-cell count ± SD (cells/$mm^3$) | 348 ± 317 | — | — | — |
| Baseline median viral load ± SD ($log_{10}$ copies/mL) | 4.59 ± 1.30 | — | — | — |
| Median antiretroviral treatment in months (range) | 9 (1–14) | — | — | — |
| Fraction of patients who progress to AIDS | 0.52 | — | — | — |
| Genotypes | | | | |
| 4/4 | 1 (0.7) | 2 (1.8) | 0.42 (0.04–4.92) | 0.484 |
| 5/4 | 0 (0) | 1 (0.9) | 0.29 (0.01–7.24) | 0.284 |
| 6/4 | 3 (2.1) | 0 (0) | 0.12 (0.01–2.45) | 0.067 |
| 7/4 | 4 (2.9) | 6 (5.5) | 1.28 (0.34–4.92) | 0.713 |
| 5/5 | 12 (8.6) | 7 (6.4) | 1.51 (0.38–6.09) | 0.556 |
| 6/5 | 13 (9.3) | 5 (4.6) | 3.03 (0.99–9.33) | 0.046 |
| 7/5 | 32 (23) | 20 (18.3) | 0.71 (0.22–2.27) | 0.568 |
| 6/6 | 8 (5.7) | 7 (6.9) | 0.75 (0.25–2.27) | 0.610 |
| 7/6 | 24 (17.3) | 25 (22.9) | 1.18 (0.57–2.37) | 0.671 |
| 7/7 | 42 (30.2) | 36 (33) | 1.00 | — |
| Homozygotes | 63 (45.3) | 52 (47.7) | — | — |
| Heterozygotes | 76 (54.7) | 57 (52.3) | 0.91 (0.53–1.55) | 0.798 |
| L-SIGN alleles | $n$ = 278 | $n$ = 218 | | |
| 4 | 9 (3.2) | 11 (5) | 0.63 (0.23–1.67) | 0.361 |
| 5 | 69 (24.8) | 40 (18.3) | 1.47 (0.93–2.33) | 0.080 |
| 6 | 56 (20.1) | 44 (20.2) | 1.00 (0.63–1.59) | 0.991 |
| 7 | 144 (51.8) | 123 (56.4) | 0.83 (0.57–1.20) | 0.319 |

SD, standard deviation.

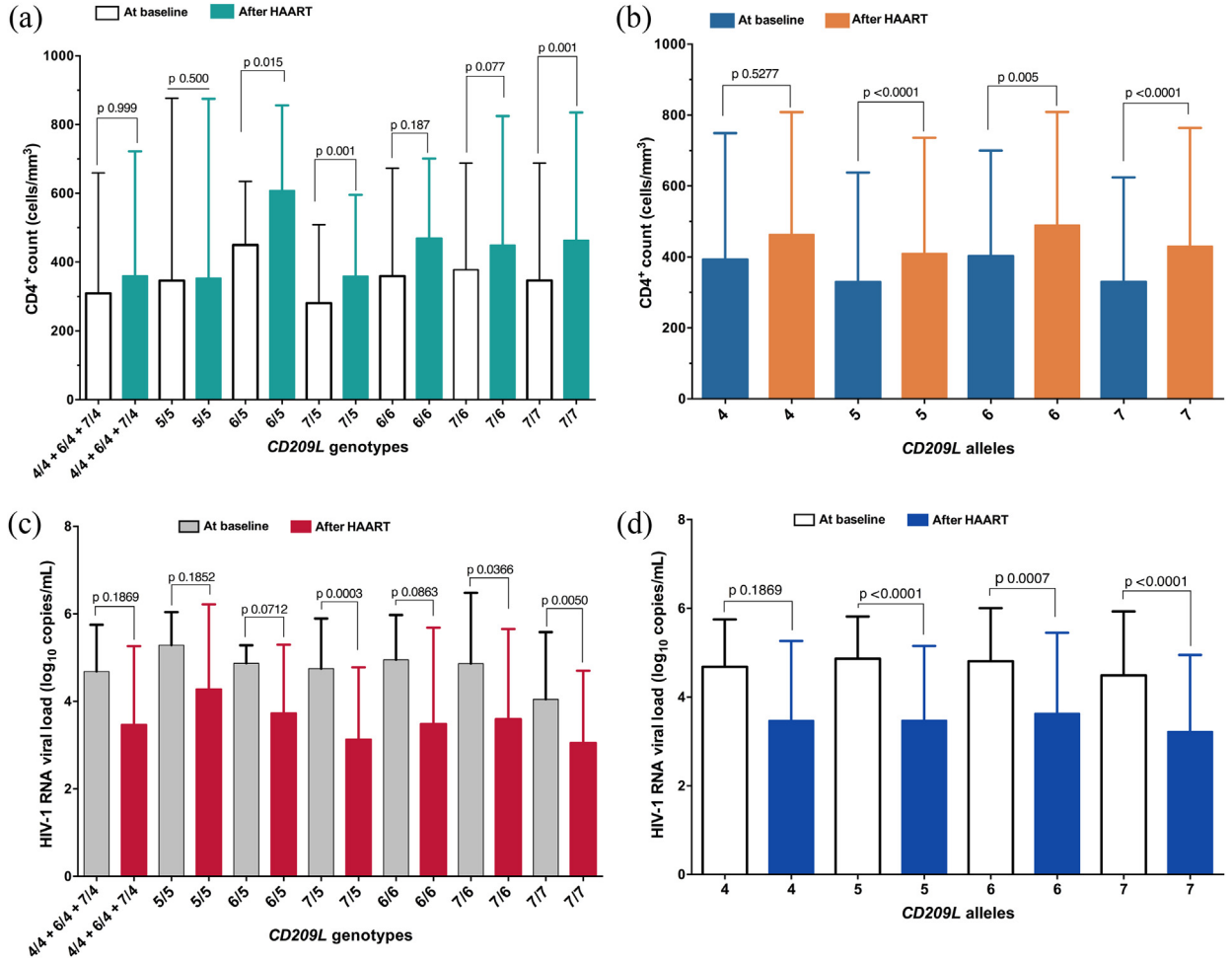


**FIG. 1.** $CD4^+$ T-cell counts and human immunodeficiency virus (HIV)-1 RNA viral load according to *CD209L* neck-repeat variation. (a) Association between genotypes of *CD209L* and $CD4^+$ T-cell counts. (b) The improvement in $CD4^+$ T-cell counts between the initial and final measurements after highly active antiretroviral therapy (HAART) according to alleles of *CD209L*. For all graphs, error bars represent mean ± standard error of the mean (SEM). (c) HIV-1 RNA viral load according to *CD209L* genotype at baseline and after HAART. (d) Impact of *CD209L* alleles on HIV-1 RNA viral load outcomes after HAART. For all graphs, error bars represent mean ± SEM.

allele was more prevalent in the non-progressor group than in the progressor group (5.3% vs. 1.4%, respectively, p 0.02). Moreover, we found that the prevalence of allele 6 was significantly much high in the non-progressor group than in the progressor group (22% vs. 18.5%, respectively, p $8 \times 10^{-3}$) (Table S1).

In the present study, we found that, overall, the heterozygous 6/5 genotype was associated with HIV-1 susceptibility. However, previous studies demonstrated that the homozygous 7/7 genotype was significantly associated with an increased risk of HIV-1 infection, whereas the heterozygous 7/5 genotype was correlated with relative resistance to HIV-1 infection [12,13,15]. Moreover, a previous study showed that the medium length 6-tandem or 7-tandem repeats form a rigid neck region that projects the carbohydrate recognition domains away from the membrane, ensuring strong multivalent adhesion to the target membrane, and thus promoting access to pathogens [16].

We also showed that, at baseline prior to initiation of treatment, there was no association between the number of *CD209L* repeats and viral load. However, Xu et al. found that patients with the DC-SIGNR 9/7 or 7/7 genotype had significantly higher HIV RNA loads than those with the 7/5 genotype [17]. Interestingly, our work suggests that variation in the number of *CD209L* repeats was linked to HIV viraemia after HAART.

Furthermore, we found that variation in *CD209L* genotypes/alleles was not associated with $CD4^+$ T-cell numbers at baseline. This finding seems to be in accordance with a previous study, in which no significant differences were found between $CD4^+$ T-cells in patients with different *CD209L* genotypes/alleles [17].

However, after HAART, the $CD4^+$ T-cell counts were associated with *CD209L* genotypes/alleles. Thus, our preliminary evidence suggests that the neck-region variation of *CD209L* may explain why the majority of patients have evidence of slow increases in their $CD4^+$ T-cell count over time, but many do not. Moreover, most patients with *CD209L* genotypes that showed reduction in HIV-1 viraemia after HAART are able to increase their $CD4^+$ T-cell counts. Studies that have addressed the predictors of discordant responses (with virological suppression but incomplete $CD4^+$ T-cell recovery) have shown different results. Among patients with HIV-1 infection, a substantial proportion (10–30%) of individuals showed complete or partial $CD4^+$ T-cell recovery while on HAART, despite suppression of HIV-1 viraemia to undetectable levels [18]. Therefore, further investigations are needed to confirm these findings and elucidate the molecular basis of $CD4^+$ T-cell recovery variation. In this study, we found that mainly allele 6 of *CD209L* was associated with protection against disease progression to AIDS. Moreover, it is conceivable that receptors with fewer repeats form smaller oligomers and are less effective in binding to target molecules such as HIV-1 glycoprotein 120 [19].

In conclusion, *CD209L* gene variation seems to be an important factor affecting the host's susceptibility to HIV-1 infection and progression to AIDS. Furthermore, we found, for the first time, that the number of *CD209L* repeats influences $CD4^+$ T-cell counts and HIV-1 RNA viral load after the start of HAART, and could potentially help to guide treatment strategies. However, owing to the small sample size and differences in allele frequencies worldwide, other investigations are warranted to confirm these findings.

## Transparency declaration

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this article.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2014.12.012.

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