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ORIGINAL INVESTIGATION

Programmed cell death-1 single-nucleotide polymorphism rs10204525 is associated with human immunodeficiency virus type 1 RNA viral load in HIV-1-infected Moroccan subjects

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Abstract

Human Immunodeficiency Virus (HIV-1) infections are characterized by dysfunctional cellular and humoral antiviral immune responses. The progressive loss of effector functions in chronic viral infection has been associated with the up-regulation of programmed death-1 (PD-1), a negative regulator of activated T cells and Natural Killer cells. In HIV-1 infection, increased levels of PD-1 expression correlate with CD8 + T-cell exhaustion. In vitro, PD-1 blockade using PD-1 antibodies led to an increase in HIV-1 specific CD8 + T and memory B cell proliferation. We aimed to investigate the impact of *PDCD1* rs10204525 polymorphism on HIV-1 susceptibility, AIDS development, and treatment response outcomes in HIV-1 infection in a Moroccan population. A total of 214 HIV-1 seropositive and 250 seronegative subjects were enrolled to investigate the association between the between the single-nucleotide polymorphism (SNP) rs10204525 of *PDCD1* gene and HIV-1 pathogenesis using a predesigned TaqMan SNP genotyping assay. No significant association was found between rs10204525 and susceptibility to HIV-1 infection and AIDS development ($p > 0.05$). Genotype frequencies were significantly associated with the viral load before ART ($p = 0.0105$). HIV-1 viral load was significantly higher among subjects with the CC compared to TT genotype ($p = 0.0043$). In treated subjects, the median of viral load levels was significantly higher in CC and CT groups than TT subjects ($p < 0.005$). However, analysis of the correlation between CD4 + T-cell levels and *PDCD1* polymorphism before and after ART showed no significant difference ($p > 0.05$). Our results demonstrated that rs10204525 polymorphism does not affect HIV-1 infection. However, this polymorphism may affect the response to treatment as measured by RNA viral load levels.

Keywords HIV-1 · PD-1 · rs10204525 · Polymorphism · ART

Introduction

The Human Immunodeficiency Virus type 1 (HIV-1) pandemic is one of the main causes of death worldwide. Globally, the United Nations Programme on HIV/AIDS (UNAIDS) estimated that 38 million people were living with HIV at the end of 2019 [1]. In Morocco, about 22,000 people were living with HIV-1 [2]. Infection with HIV-1 is characterized by dysfunctional cellular and humoral antiviral immune responses [3–6]. CD8 + T cells are a crucial component of the cellular immune response and they play a major role in controlling viral infection [7–11]. These cytotoxic T lymphocytes (CTL) can also eliminate infected cells by the engagement of death-inducing ligands expressed by

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CD8 + T cells with death receptors on the surface of the infected cells [12].

However, the virus escapes this immunological barrier by altering certain functional mechanisms of the immune system during primary infection [13] and by changing continuously the shape of the envelope proteins through mutation accumulation and immune-evading carbohydrates "glycans" associated with these proteins [14] and by infecting and depleting T helper cells and macrophages [15]. Infection with HIV-1 is accompanied by a loss of the ability of memory T cells to reduce viral replication [4]. The regulation of T responses to HIV-1 infection is governed by a certain balance between the implementation of these effector functions [16] necessary for the elimination of the pathogen and the control of the level of these responses to avoid tissue damage that could result from exacerbating this response [17]. Thus, the molecules that regulate the functioning of the immune system play an important role in maintaining the balance between an effective response and the development of autoimmune pathologies [18]. Among these modulating molecules, the Programmed Death-1 (PD-1) molecule which is encoded by the Programmed Cell Death-1 (*PDCD1*) gene, located on the human chromosome 2q37.3, has attracted wide interest since the acceleration of its use in the immuno-oncology therapeutic arsenal [19]. PD-1 is a member of the immunoglobulin receptor super-family (CD28) that encodes a 55-kd type 1 trans-membrane inhibitory immuno-receptor and is localized on the surface of activated B cells, activated monocytes, $CD8^+$ T cells, CD4 + T cells, natural killer T cells, and macrophages [15, 20–22]. Moreover, the PD-1 is a central regulator of $CD8^+$ T-cell exhaustion, and blocking the PD-1/PD-L1 pathway has a beneficial effect on enhancing T-cell immunity in chronic viral infections [8–10]. Several studies have demonstrated that PD-1 expression was important and highly expressed on HIV-1-specific $CD8^+$ T cells in subjects with high viremia [23–26].

It has also been shown that high viral load and low $CD4^+$ T-cell counts are associated with both the level of PD-1 expression on HIV-specific $CD8^+$ T cells and the percentage of cells expressing PD-1 [27]. As well, the entry of PD-1 with its ligand PD-L1 (also known as CD274) attenuates the T-cell receptor signaling pathway responsible for the specific recognition of viral antigens and the initiation of T-cell response, so it induces the inhibition of proliferation and the IL-2 production [28]. Otherwise, PD-1 expression is associated with HIV-1 disease progression [27].

Some studies have concluded that chronic immunodeficiency virus infections are associated with B cell dysfunction [29, 30]. Furthermore, the colligation PD-1 and B cell receptors lead to the attenuation of B cells receptors signaling by recruiting the protein tyrosine phosphatase SHP-2 to the phosphorylated tyrosine residue of PD-1. All that above led to the conclusion that PD-1 regulates the immune responses negatively by competing for stimulation [31]. It has been also approved that PD-1 and PD-L1 are up-regulated in HIV-1 infection [27] which suggests that the PD-1/ PD-L1 pathway could, in fact, be operating during the chronic HIV infection [32].

In vitro, blocking the PD-1/PD-L1 interaction with an anti-PD-L1 antibody enhanced the effector function of HIV-specific T cells [18, 27, 33, 34], restore $CD8^+$ T-cell function, and reduces viral load [35]. In vitro evenly, it was demonstrated that the PD-1 regulates the survival of HIV-specific $CD8^+$ T cells [27]. Therefore, the induction of apoptosis could be a key mechanism used by the PD-1/PD-L1 system to affect the effect of an HIV-specific T-cell response [13]. In vivo as well, it has been proved that the PD-1 inhibits immune responses [31].

Recently, a single-nucleotide polymorphism (SNP) rs10204525 has been shown to be associated with the susceptibility and disease course of chronic HBV infection [36–38] and HCV infection [34, 39]. This SNP is located at + 8669 base pairs in the 3′-untranslated region (3′ UTR) at the position 241,850,169 in the PDCD1 gene and was previously called PD-1.6 and 8738 G/A. The mechanisms underlying this correlation are likely due to the rs10204525 A allele, disrupting the binding sequence for miR-4717 inhibitor within the 3′ UTR of PD-1 mRNA, which leads to increased PD-1 expression [40]. Actually, the miRNA-4717 was demonstrated to affect the luciferase activity in a dose-dependent manner in cells transfected with a recombinant vector expressing the luciferase reporter gene under the transcription control of the PD-1 promoter containing the rs10204525 G polymorphic variant [40].

To our knowledge, this study is the first to analyze the association between rs10204525 SNP and the HIV-1 infection. It focuses on the study of the potential association between the *PDCD1* polymorphism rs10204525 and HIV-1 susceptibility, AIDS development, and treatment response outcomes in HIV-1 infection among a Moroccan population.

Materials and methods

HIV-1subjects and healthy HIV-uninfected controls

A total of 464 consented Moroccan subjects including 214 subjects (120 women (56.07%); 94 men (43.92%)) living with HIV-1 (PLWH) and 250 healthy HIV-uninfected controls (176 women (70.04%); 74 men (29.6%)) were recruited. For subjects living with HIV-1, they were divided into two groups: the AIDS group ($n = 148$), for those who attained the AIDS stage according to the CDC 93 classification; and the Non-AIDS group ($n = 66$), those who did not reach the AIDS stage yet (Table 1). The PLWH subjects were medically monitored at the Infectious Disease Center, University

Table 1 Clinico-immunological classification of the HIV-1 subjects (CDC 1993)

CD4 + T-Cell categories	Clinical categories		
	(A) Asymptomatic, Acute (Primary) HIV ($N=75$)	(B) symptomatic, not (A) or (C) conditions (25)	(C) AIDS-Indicator conditions (114)
(1) > 500/mm3	10	9	18
(2) 200–499/mm3	42	5	24
(3) < 200/mm3	23	11	72

Hospital Center, Ibn Rochd in Casablanca, and the healthy HIV-uninfected controls were collected in the Pasteur Institute of Casablanca (Morocco). All PLWH subjects are now receiving a first-line antiretroviral treatment regimen with tenofovir/emtricitabine plus either nevirapine or lopinavir/ritonavir or efavirenz. The demographic and clinical features of the study subjects are summarized in Table 2.

This study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee and has been approved by the Ethics Committee of the Faculty of Medicine of Casablanca.

Genomic DNA isolation and SNP genotyping

For all participants and controls, 5 mL of peripheral blood was collected by venipuncture in Vacutainer EDTA Tubes. The genomic DNA was obtained from the whole blood by standard phenol–chloroform method [40, 41]. The evaluation of DNA concentration was assessed using a NanoVue plus spectrophotometer (GE Healthcare, USA). The extracted DNA samples were kept at -20 °C until use.

Genotyping of *PDCD1* rs10204525 polymorphism of each subject was performed by a predesigned TaqMan® allelic discrimination assay (Applied Biosystems; Foster City, USA, assay ID C_172862_10). Real-time PCR was performed with mixes consisting of 20 ng of genomic DNA, 5 µl of 1 × SensiFAST Genotyping Lo-Rox Mix (Bioline, London, UK), 0.125 µl of 20 × assay mix, and Nuclease-Free water up to 10 µl of the final volume. Thermal cycling conditions were as follows: pre-incubation at 60 °C for 1 min, 95 °C for 10 min, 45 cycles of denaturation: 95 °C for 15 s and annealing/ extension at 60 °C for 1 min, then a post-incubation at 60 °C for 1 min and a final cooling at 40 °C for the 30 s. The allelic discrimination design consisted of oligo-nucleotides and two labeled probes, which included the wild-type probe labeled with VIC dye (*C* allele) and the variant probe labeled with FAM dye (T allele). The Thermo Fisher context Sequence [VIC/FAM]: CCAAGGAGAGCTCCCAGGGTGGGCA[C/T]ATGGGGGGCCCTAGGTGCCTGCACT.

After PCR, the genotype of each sample was assigned automatically on the Roche LightCycler® 480 real-time PCR software (LightCycler 480 Instrument; Roche Diagnostics), based on competition of the hybridization probes.

Statistical analysis

The allele and genotype frequencies were evaluated by direct gene counting and compared with the controls using the Chi-square test. Hardy–Weinberg equilibrium (HWE) was calculated using expected and calculated frequencies. HIV-1 viral loads (copies/mL) were $\log_{10}$ transformed before statistical analysis. The odds ratio (OR) and 95% confidence interval (CI) were examined for each allele and genotype. Differences between continuous variables (CD4 + T-cell counts and HIV-1 viral load) were analyzed using the Mann–Whitney *U* test. The demographic data between HIV-1 infection and the control group were compared using the χ^2 test. The differences in survival time were evaluated using the log-rank test. IBM SPSS software package ver. 21.0 (IBM Co., Armonk, NY, USA) and GraphPad PRISM version 7.00 (GraphPad Software, San Diego, CA, USA) were used in the statistical analyses. Differences were considered to be significant if the *p* value was less than 0.05. All tests were two sided.

Table 2 Demographic and clinical features of the study subjects

Parameter (variable)	People living with HIV-1 (PLWH) ($N=214$)	Healthy HIV-uninfected controls ($N=250$)	*p* value
Median age [Range]. years	35 [18–62]	55 [18–93]	0.0153
Biological sex (Male/Female)	94/120	74/176	0.001
CD4 + T cell count [Range] (cells/mm3)	229.45 [2.5–2520]		
HIV-1 viral load [Range]. ($\log_{10}$ copies/mL)	4.82 [1.30–5.67]		

Results

Characteristics of the population's study

The demographic characteristics and clinical features of the 214 recruited people living with HIV-1 (PLWH) and 250 healthy HIV-uninfected controls (HIV^-) are shown in Table 2. The median age of the HIV^+ subjects composed of 94 males (43.92%) and 120 females (56.07%) was 35 (range, 18–62 years). Significant differences were observed in the distributions of age and biological sex between the PLWH group and controls ($p=0.0153$ and $p=0.001$, respectively) as shown in Table 2.

The *PDCD1* rs10204525 polymorphism was genotyped successfully and did not deviate from the Hardy–Weinberg equilibrium in controls.

Association with HIV-1 infection

To investigate a possible relationship between the *PDCD1* rs10204525 polymorphism and HIV-1 susceptibility and AIDS development in the PLWH group, the allele and genotype frequencies of *PDCD1* rs10204525 polymorphism have been determined in 214 Moroccan subjects with HIV-1 and 250 healthy HIV-uninfected controls. The genotype frequencies and distributions of the SNP are shown in Table 3.

In PLWH group, rs10204525 SNP genotype frequencies were 49.53% for CC genotype ($n=106$), 40.65% for CT genotype ($n=87$) and 9.81% for TT genotype ($n=21$). The allele frequencies are 0.70 ± 0.02 for C allele and 0.30 ± 0.02 for T allele. Genotype frequencies in healthy HIV-uninfected controls were 43.6% for CC genotype ($n=109$), 44.4% for CT genotype ($n=111$), 12% for TT genotype ($n=30$). The allele frequencies are 0.66 ± 0.02 for C allele and 0.34 ± 0.02 for T allele.

The distributions of *PDCD1* rs10204525 SNP genotype frequencies in both HIV-1-infected subjects and the control group were in compliance with Hardy–Weinberg equilibrium ($p>0.05$) as assessed using the Chi-square test (χ^2).

Associations of *PDCD1* rs10204525 polymorphism with HIV-1 susceptibility and AIDS development

To examine the impact of the *PDCD1* rs10204525 polymorphism on HIV-1 susceptibility in a Moroccan population, we analyzed the polymorphism in people living with HIV-1 (PLWH) and control groups. Overall, the frequencies of both alleles and genotypes of our investigated *PDCD1* SNP were not significantly associated with HIV-1 susceptibility (Table 3).

No significant association was observed regarding allelic and genotypic distributions among AIDS and non-AIDS groups for this SNP. The SNP genotype and allelic frequencies within these groups are shown in Table 4.

We used the Kaplan–Meier survival statistics to assess the effects of the SNP on the rate of progression to AIDS. Two separate endpoints reflecting advancing AIDS pathogenesis were considered: HIV-1 infection plus a decline of $CD4^+$ T-cell counts <200 cells/mm^3 (CD4 <200) and the 1993 Center for Disease Control definition of AIDS (AIDS-93) [41]. Consistent with the earlier results, we did not find any genotype associations with HIV-1 progression to AIDS among the Moroccan population (Fig. 1).

Table 3 Association between *PDCD1* rs10204525 polymorphism and HIV-1 susceptibility

Parameter (Variable)	People living with HIV-1 (PLWH) ($N=214$)	Healthy HIV-uninfected controls ($N=250$)	OR (95%CI)	*p* value
PDCD1 rs10204525 Genotypes, *n* (%)				
CC	106 (49.53)	109 (43.6)	Reference	–
CT	87 (40.65)	111 (44.4)	0.81 (0.55–1.29)	0.275
TT	21 (9.81)	30 (12)	0.72 (0.39–1.34)	0.296
Recessive model, *n* (%)				
CC + CT	193 (90.19)	220 (88)	Reference	–
TT	21 (9.81)	30 (12)	1.25 (0.69–2.26)	0.453
Dominant model, *n* (%)				
CC	106 (49.53)	109 (43.6)	Reference	–
CT + TT	108 (50.47)	141 (56.4)	0.79 (0.55–1.14)	0.201
PDCD1 rs10204525 Alleles frequencies ± SD				
C allele	0.70 ± 0.02	0.66 ± 0.02	Reference	–
T allele	0.30 ± 0.02	0.34 ± 0.02	0.83 (0.63–1.10)	0.187

HIV human immunodeficiency virus; *OR* odds ration; *PDCD1* programmed cell death-1 gene; *SD* Standard deviation

Table 4 Association between *PDCD1* rs10204525 polymorphism and AIDS development

Parameter (Variable)	AIDS ($N=148$)	Non-AIDS ($N=66$)	OR (95%CI)	p value
PDCD1 rs10204525 Genotypes, *n* (%)				
CC	74 (50)	32 (48.48)	Reference	–
CT	59 (39.86)	28 (42.42)	0.91 (0.494 – 1.680)	0.766
TT	15 (10.14)	6 (9.09)	1.08 (0.385 – 3.039)	0.882
Recessive model, *n* (%)				
CC + CT	133 (89.86)	60 (90.91)	Reference	–
TT	15 (10.14)	6 (9.09)	0.89 (0.328 – 2.397)	0.813
Dominant model, *n* (%)				
CC	74 (50)	32 (48.48)	Reference	–
CT + TT	74 (50)	34 (51.52)	0.94 (0.527 – 1.682)	0.838
PDCD1 rs10204525 Alleles frequencies ± SD				
C	0.70 + / – 0.03	0.70 + / – 0.04	Reference	–
T	0.301 + / – 0.03	0.30 + / – 0.04	0.99 (0.633 – 1.546)	0.961

AIDS acquired immune deficiency syndrome; *Non-AIDS* non-acquired immune deficiency syndrome; *OR* odds ration; *PDCD1* programmed cell death-1 gene; *SD* Standard deviation

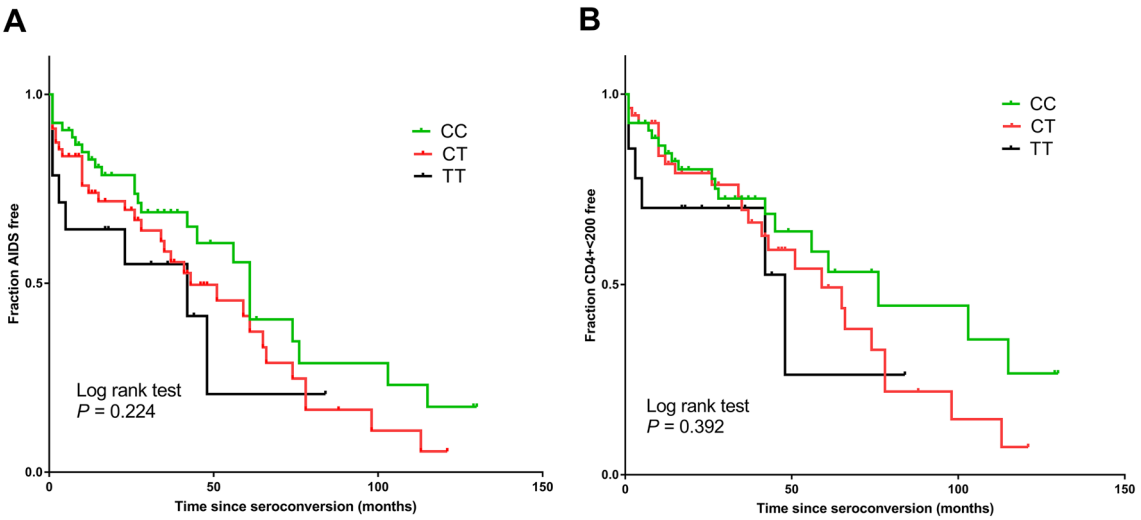

Fig. 1 Kaplan–Meier analysis of progression from HIV-1 sero-conversion to clinical AIDS-1993 (AIDS-defining illness or CD4 + T cell counts < 200 cells/mm^3) by *PDCD1* rs10204525 genotypes Moroccan PLWH. **A** Kaplan–Meier survival curves with AIDS as an endpoint by all *PDCD1* rs10204525 genotype categories. **B** Kaplan–Meier survival curves with CD4 + Cells < 200/mm 3 as end point by all *PDCD1* rs10204525 genotype categories

Associations of *PDCD1* rs10204525 polymorphism with CD4 + T-cell counts and HIV-1 RNA viral loads

To assess the associations of *PDCD1* rs10204525 polymorphism with highly antiretroviral therapy (ART) outcome, we analyzed the CD4$^+$ T-cell counts and HIV-1 RNA viral loads before and after treatment. After treatment, we chose the last viral measurements performed (not less than 1 year), to ensure that the results found will be due to the SNP and not dependent on differences in initial viral load decline.

Our results did not show any significant association between *PDCD1* SNP distribution and CD4 + level before and after ART initiation ($p=0.910$; $p=0.894$, respectively) (Fig. 2A, B).

Regarding the impact of the rs10204525 polymorphism on the level of viral load, our results showed a significant difference between genotypes CC, CT, and TT frequencies before ART initiation ($p=0.0105$) (Fig. 2C). Moreover, the viral load median was significantly higher in subjects with

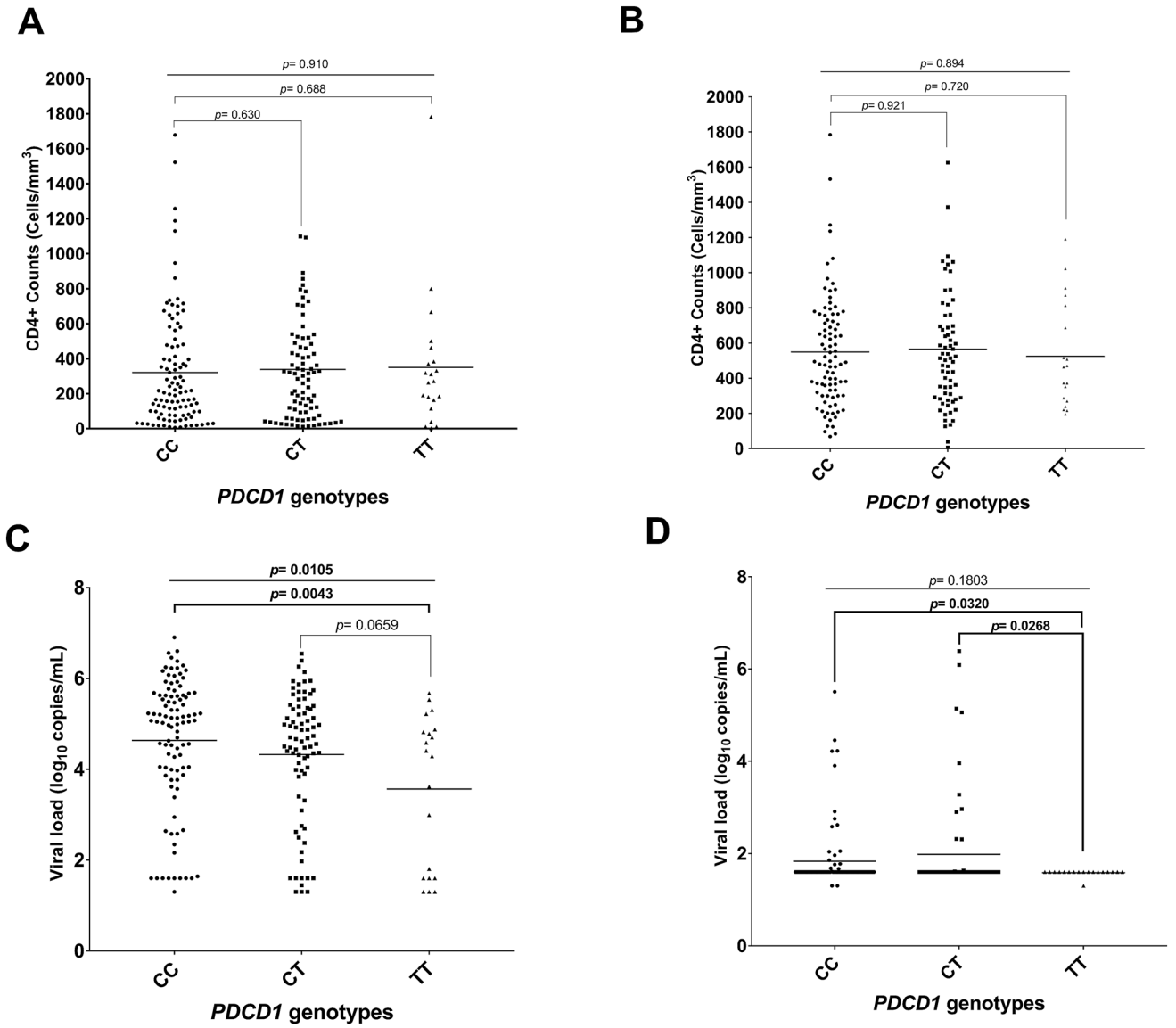

Fig. 2 Immunological and virological variables of HIV-1-infected subjects according to the *PDCD1* rs10204525 at baseline treatment and after antiretroviral treatment. **A** *PDCD1* rs10204525 genotypes and CD4 + T cell counts in subjects before ART. No significant association between different rs10204525 genotypes and CD4 + T cell counts in untreated subjects. **B** *PDCD1* rs10204525 genotypes and CD4 + T cell counts in subjects after ART. No significant association between different rs10204525 genotypes and CD4 + T cell counts in treated subjects. **C** *PDCD1* rs10204525 genotypes and HIV-1 RNA viral load in subjects before ART. A significant association between different rs10204525 genotypes and HIV-1 RNA viral load in untreated subjects. A high significant association between subjects with the CC genotype and those with the TT genotype. Data are presented as a scatter plot with median. **D** *PDCD1* rs10204525 genotypes and HIV-1 RNA viral load in subjects after ART. No significant association between different rs10204525 genotypes and HIV-1 RNA viral load in treated subjects. A significant association in CC and CT group than TT subjects. Data are presented as a scatter plot with median. Statistical tests were performed using Mann–Whitney *U* test and ANOVA test

the CC genotype compared to those with the TT genotype ($p = 0.0043$) (Fig. 2C). After ART regimen initiation, we found that the median of viral load levels was significantly higher in CC and CT group than TT subjects ($p = 0.0320$ and $p = 0.0268$, respectively), as shown in Fig. 2D. Nevertheless, no significant differences were observed between RNA viral load levels and different rs10204525 genotypes distribution.

Discussion

PD-1 is an important co-inhibitory molecule expressed on immune cells, and its regulatory function was exerted in different stages of T-cell immune responses. Several studies have identified the correlations between genetic polymorphisms in the *PDCD1* gene and many immune-related diseases, in which the results demonstrated its potential role in the prediction and evaluation of disease progression [20–22, 42].

The PD-1 has been evenly suggested as a promising therapeutic approach to eradicate HIV [43]. This eradication could be mediated by improving the function of T cells after blocking the interaction between PD-1 and its ligands (PD-L1 and PD-L2) which triggers the inhibitory pathway to induce the exhaustion of lymphocyte T cells. Blocking this interaction could, therefore, contribute to the restoration of functional capacities of anti-HIV-1 T lymphocytes [18, 27, 33, 44]. On the other hand, improving T-cell functions could be a critical factor in implementing a well-spaced intermittent treatment regimen without prolonged and continuous antiretroviral therapies [45].

The discovery of new markers associated with the infection is of great importance in the diagnostic, prophylactic, and therapeutic strategies for anti-HIV-1 treatment. The *PDCD1* rs10204525 polymorphism could provide a potential target for designing immunotherapeutic intervention and for screening and selecting HIV-1 subjects with a specific immuno-genetic background who could benefit from rationally designed and effective personalized immunotherapy regimen against HIV-1-associated diseases.

In this present study, we analyzed the impact of rs10204525 polymorphism in the *PDCD1* gene on the susceptibility to HIV-1 infection, AIDS development, and treatment response outcomes in 214 Moroccan subjects. After analyzing our results regarding the relationship between the distribution of genotypes and susceptibility to HIV-1 infection, we observed no significant difference in SNP rs10204525 polymorphism between infected subjects and healthy HIV-uninfected controls as shown by the similarity of the frequency distribution of the different alleles among these groups. These results are in accordance with the previous findings reported on hepatitis C virus infection in which no significant association of rs10204525 SNP with HCV infection susceptibility was observed [46]. However, previous association studies have described a critical role of the rs10204525 polymorphism on the susceptibility to other viral infections. Yan Li et al. had shown that the frequencies of rs10204525 genotypes TT were significantly lower in enterovirus 71 infected cases than in the negative controls (Yan Li et al., The 13th congress of asian society for pediatric research, Abstract 382). A significant association was also obtained for this same SNP in HBV-infected subjects and healthy controls. It has been shown that allele C of rs10204525 and allele C-comprising genotypes (CT and CC) may have a protective role, while rs10204525 genotype TT and allele T may have a predisposing role in HBV infection [36, 38, 47, 48]. However, in the Moroccan population, a previous HBV infection study showed that rs10204525 TT polymorphism has a protective effect, but only for homozygous carriers [49].

The relationship between the rs10204525 polymorphism and the development of AIDS has also been investigated. Here again, the results do not reveal any significant differences between the frequency distribution of the rs10204525 alleles and genotypes between the subjects who developed AIDS and those who did not (Non-AIDS subjects), as evidenced in Table 4, Fig. 1. These results were consistent with those of Hong Peng et al. and Valli De Re et al. in HCV and HBV infection, respectively [39, 50]. In both studies, the authors had investigated, respectively, the role of rs10204525 polymorphism in chronic HCV and HBV infections outcome from chronic infection to liver cirrhosis and hepatocellular carcinoma, and showed that this polymorphism had an insignificant role neither in subjects with hepatic disorders related to HCV nor in subjects with cirrhosis and hepatocellular carcinoma in chronic HBV infection. However, our results are inconsistent with other previous reports which have described an association between the rs10204525 polymorphism of the *PDCD1* gene and liver disease progression in chronic hepatitis B virus infection [36–38, 51]. All of these studies showed that allele C and genotypes containing allele C (CT and CC) may have a protective role from chronic HBV infection. These discrepancies between these findings in different studies might be partially attributed to racial and ethnic differences of individuals with a specific immuno-genetic background [48].

Regarding the impact of the *PDCD1* rs10204525 genotype polymorphism on the response to antiretroviral therapy, our results revealed no significant association between the allele frequency distribution of the rs10202545 polymorphism and the number of $CD4^+$ T cells neither before nor after treatment. However, before the initiation of ART, analysis of viral load levels according to the distribution of alleles and genotypes of this polymorphism showed a significant difference between the frequencies of our different genotypes (CC, CT, and TT). Although, we found a significant increase in HIV-1 viremia of the CC genotype compared to the TT genotype. After the initiation of ART, subjects with CC and CT genotypes had significant high viremia levels compared to subjects with TT genotype. Since before ART no significant difference was observed between the CT and TT genotype and after treatment a high significant difference was found, this allows us to conclude that this

polymorphism may affect the response to treatment as measured by RNA viral load levels. Interestingly, it has already been reported that PD-1 rs10204525 CC and CT genotypes were correlated with high levels of PD-1 mRNA expression facilitating viral HBV replication [49]. In contrast, the allele frequency and genotype distribution were found independent of HCV viral load and HCV genotype [39]. Besides, a previous study showed that in untreated HIV-1 individuals, the frequency of productively infected cells strongly correlated with plasma viral load. Those cells preferentially displayed a transitional memory phenotype and are enriched in cells preferentially expressed immune checkpoint molecules as PD-1 [27, 52–54]. Likewise, longitudinal investigation of the relationship between PD-1 expression, viral load, and HBeAg in chronic hepatitis B subjects undergoing oral antiviral treatment revealed a strong correlation between HBV viremia and hyper-expression of PD-1 on all T lymphocyte cells [36]. Further, it has been reported that PD-1 is significantly up-regulated on HIV-specific $CD8^+$ T cells and that the expression level is correlated with predictors of higher viral load and reduced $CD4^+$ T-cell count [27, 53, 55].

Even though, the biologic effect of *PDCD1* rs10204525 remains unknown. It is, therefore, possible that this SNP, either by itself or by other functional SNPs, may affect the expression or function of PD-1 and thereby modify the immune response against the virus infection. Interpretation of our findings may also be impacted by the multiple factors involved in different patho-physiological mechanisms that lead to the development of AIDS, and therefore, further investigations are warranted to clarify the implications.

Conclusion

In conclusion, to the best of our knowledge, this study was the first population-based study carried out worldwide to investigate the associations of *PDCD1* rs10204525 polymorphism and HIV-1 susceptibility infection, AIDS development, and treatment response. Our data revealed that this SNP is not associated with susceptibility to HIV-1 and AIDS development in Moroccan individuals. However, we found that viral load levels were significantly different in subjects based on *PDCD1* rs10204525 genotypes before ART and that the *PDCD1* CC genotype was associated with a higher viral load compared to the TT genotype. Similar results were observed in subjects under treatment with ART in which carriers of the CC and CT genotypes have a higher viral load than carriers of the TT genotype, although this difference is statistically significant.

In the absence of a validating trial, further case–control genetic association studies involving larger sample sizes are needed to further shed light on these relationships between *PDCD1* rs10204525 polymorphism and HIV-1 susceptibility infection, AIDS development, and treatment response to replicate and enrich the genetic data for the role of multiple inhibitory receptors. It is suggested that more attention should be paid to other representative *PDCD1* polymorphisms that affect the susceptibility and progression of HIV-1. Besides, replication studies in different ethnical groups are also needed considering that the findings in this study are obtained only from the Moroccan population.

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Declarations

Conflict of interest The authors have no conflicts of interest to disclose.

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