Original article

Relationship between CCR5$^{(WT/\Delta32)}$ heterozygosity and HIV-1 reservoir size in adolescents and young adults with perinatally acquired HIV-1 infection

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ABSTRACT

Background: Several host factors contribute to human immunodeficiency virus (HIV) disease progression in the absence of combination antiretroviral therapy (cART). Among them, the CC-chemokine receptor 5 (CCR5) is known to be the main co-receptor used by HIV-1 to enter target cells during the early stages of an HIV-1 infection.
Objective: We evaluated the association of CCR5$^{(WT/\Delta32)}$ heterozygosity with HIV-1 reservoir size, lymphocyte differentiation, activation and immunosenescence in adolescents and young adults with perinatally acquired HIV infection receiving cART.
Methods: CCR5 genotype was analysed in 242 patients with vertically transmitted HIV-1 infection from Paediatric Spanish AIDS Research Network Cohort (coRISpe). Proviral HIV-1 DNA was quantified by digital-droplet PCR, and T-cell phenotype was evaluated by flow cytometry in a subset of 24 patients (ten with CCR5$^{(\Delta32/WT)}$ genotype and 14 with CCR5$^{(WT/WT)}$ genotype).
Results: Twenty-three patients were heterozygous for the $\Delta32$ genotype but none was homozygous for the mutated CCR5 allele. We observed no difference in the HIV-1 reservoir size (455 and 578 copies of HIV-1 DNA per million CD4$^+$ T cells in individuals with CCR5$^{(WT/WT)}$ and CCR5$^{(\Delta32/WT)}$ genotypes, respectively; p 0.75) or in the immune activation markers between both genotype groups. However, we found that total HIV-1 DNA in CD4$^+$ T cells correlated with the percentage of memory CD4$^+$ T cells: a direct correlation in CCR5$^{(WT/\Delta32)}$ patients but an inverse correlation in those with the CCR5$^{(WT/WT)}$ genotype.
Conclusions: This finding suggests a differential distribution of the viral reservoir compartment in CCR5$^{(WT/\Delta32)}$ patients with perinatal HIV infection, which is a characteristic that may affect the design of strategies for reservoir elimination. **M. Martínez-Bonet, Clin Microbiol Infect 2017;23:318**

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Introduction

AIDS-related mortality has decreased significantly with the wide availability of combination antiretroviral therapy (cART). Some people infected with human immunodeficiency virus type 1 who

initiate cART soon after infection do not show HIV-1-specific antibodies or cellular responses, indicating the early control of viral replication [1]. An HIV-1 infection quickly establishes a latent reservoir primarily in the resting memory CD4+ T-cells, and several factors could affect the size of this viral reservoir. With respect to these factors, it has been demonstrated that when cART is initiated earlier, the size of the proviral reservoir in the peripheral CD4+ T cells in HIV-infected children [2–5] or adults [6] is smaller. In terms of host factors, a 32-base-pair deletion in one allele of the CC-chemokine receptor (CCR5) gene decreases the surface expression of viral co-receptor. The CCR5(WT/Δ32) genotype in HIV-infected individuals has been associated with lower pre-cART HIV-1 RNA levels and slower progression of HIV-1 disease in the absence of cART [7–9]; however, the impact of the CCR5(WT/Δ32) genotype on the size of the latent HIV-1 reservoir is still controversial. Previous studies in adults recently infected with HIV-1 [10] or in children infected perinatally [11], suggested that cell-associated HIV-1 DNA levels are lower in CCR5(WT/Δ32) HIV-1-infected individuals compared with CCR5(WT/WT) individuals. In contrast, a recently published study that involved a large cohort of HIV-1-infected CCR5(WT/Δ32) adults on long-term suppressive cART did not show significant differences in cell-associated HIV-1 DNA and RNA levels [12].

Because perinatally HIV-infected individuals have been infected during the course of the maturation of their immune system, they present unique HIV-1 infection features. For instance, the memory T-cell population in the peripheral blood mononuclear cells (PBMC) is small in newborns [13] and develops later in childhood [14]. Memory CD4+ T cells present in the newborn blood or gut are susceptible to HIV-1 infection [15]. Of note, the differential co-receptor surface expression on CD4+ T-cell subsets [16] makes the memory (CD45RO+) CD4+ T cells a significant viral reservoir for R5-tropic strains [17], whereas X4-tropic strains are mainly present in naïve (CD45RA+) CD4+ T cells [18]. Moreover, perinatally HIV-1-infected patients have suffered a persistent chronic activation of their immune system throughout their lifetime, causing specific patterns of immunosenescence of CD4+ T-cell subsets [19].

Therefore, it is worthwhile to elucidate how CCR5(WT/Δ32) heterozygosity can affect the persistence of HIV-1 in perinatally HIV-infected patients with suppressed viraemia. We retrospectively studied a cohort of adolescents and young adults with perinatally acquired HIV-1 infections on suppressive cART. We evaluated the frequency of the CCR5 genotypes and studied the association of the CCR5(WT/Δ32) genotype with the HIV-1 reservoir size, lymphocyte differentiation, activation and immunosenescence.

Methods

Study participants

The study included 242 participants with a vertically transmitted HIV-1 infection from the Paediatric Spanish AIDS Research Network Cohort (coRISpe). PBMC samples from each individual were subjected to CCR5 genotyping. To quantify proviral HIV-1 DNA, we sampled participants who had maintained viral suppression (≤200 HIV-1 RNA copies/mL) for at least 1 year with no treatment discontinuation and with available cryopreserved PBMC. Twenty-three out of 242 participants were heterozygous for the Δ32 genotype (CCR5(WT/Δ32)), and of these, ten fulfilled the selection criteria. Among the 219 participants homozygous for the wild-type CCR5 genotype (CCR5(WT/WT)), and after assessing the inability of having a perfect 1:1 matched study based on potential confounding effects, including hepatitis C virus co-infection, we finally selected 14 CCR5(WT/WT) participants matched with the CCR5(WT/Δ32) group by sex, age at

virological control (± 1.5 years), and age at cART initiation (± 2.5 years).

Cryopreserved plasma, PBMC and clinical data were provided by the Spanish HIV HGM BioBank [20] and by coRISpe.

The clinical classification of AIDS-defining events and immunological categories were based on international guidelines [21]. The Ethics Committee and Clinical Research of Hospital Gregorio Marañón in Madrid approved this Delta 32-15-01 study.

CCR5 genotyping

DNA samples were obtained from the PBMC using the QIAamp Blood kit (Qiagen, Madrid, Spain). A portion of the CCR5 gene was amplified by PCR with primers that flanked the 32-bp deletion [22]. Wild-type and deleted fragments of 185 bp and 153 bp, respectively, were generated and visualized in 2% agarose gels. Heterozygosity (i.e. CCR5(WT/Δ32)) was indicated by the presence of both fragments.

Quantification of the proviral HIV-1 DNA

To evaluate the size of the proviral reservoir, CD4+ peripheral T cells were purified from the cryopreserved PBMC by negative immunomagnetic separation (CD4+ T-cell Isolation Kit; Miltenyi Biotech, Barcelona, Spain) (purity >90%). Lysed CD4+ T-cell extracts were used to measure cell-associated total HIV-1 DNA through a droplet digital polymerase chain reaction, as previously described [5].

Cellular immunophenotype

Isolated CD4+ T cells were stained for 1 h at 4°C using the conjugated monoclonal antibodies CD45RO-phycoerythrin-Cy7 (Becton Dickinson, Madrid, Spain), (Phycoerythrincyanin 5.1) CD25-PC5 and (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) CD45RA-ECD, CD57-FITC, CD28-phycoerythrin (Beckman Coulter, Madrid, Spain), CD38-allophycocyanin-Cy5.5 (Invitrogen, Madrid, Spain) and HLA-DR-allophycocyanin (Immunostep, Madrid, Spain). Naïve (CD45RA+) and memory (CD45RO+) CD4+ T-cell subsets were defined [23]. Cellular activation in CD4+ T-cell subsets was characterized by CD25, CD38 and HLA-DR expression [24]. Senescent CD4+ T cells were characterized as CD28− CD57+. At least 50 000 events were collected for each sample in a Gallios flow cytometer and analysed with the Kaluza software (Beckman-Coulter).

Determination of viral tropism

In samples taken near birth, viral tropism was determined by syncytium formation in MT-2 cells measured by a standard assay [25], respectively, considering X4-tropic or R5-tropic isolates able or not able to form syncytia.

Viral tropism at sampling was based on the ability of the cell-free virus to infect U87-CD4+ CCR5+ and U87-CD4+ CXCR4+ and was assayed with the previously described phenotypic HIV-1 tropism co-receptor assay information [26].

Statistical analysis

We compared numerical and categorical variables between the two CCR5 genotype groups using the Mann–Whitney U test and Fisher's exact test, respectively. Wilcoxon's signed-rank test was used for intragroup comparisons. Spearman's rank correlation coefficient was used to identify associations between the percentage of memory CD4+ T-cells and cell-associated HIV-1 DNA levels.

Results

Cohort characteristics

The initial study population comprised 242 perinatally HIV-1-infected participants who were analysed for CCR5 genotypes, nadir CD4$^+$ T-cell counts, immunological and clinical categories (Table 1). Of 242 HIV-1-infected participants, 219 (90.5%) were homozygous for the wild-type genotype, CCR5$^{(WT/WT)}$ and 23 (9.5%) were heterozygous for the $\varDelta$32 genotype, CCR5$^{(WT/\Delta32)}$. We found no homozygosity for the mutated allele CCR5$^{(\Delta32/\Delta32)}$ (Table 1). We observed no differences in the clinical and demographic characteristics of the two genotype groups, except for the ethnicity of the HIV-1-infected participants, which reflected the previously described higher frequency of the heterozygous CCR5$^{(WT/\Delta32)}$ allele genotype in participants of European ancestry [9,27].

Further analyses were performed on a subset of 24 HIV-1-infected participants of European ancestry (ten CCR5$^{(WT/\Delta32)}$ and 14 CCR5$^{(WT/WT)}$). The selection criteria permitted the similarity of sex, age at virological control and age at cART initiation between the ten CCR5$^{(WT/\Delta32)}$ and 14 CCR5$^{(WT/WT)}$ HIV-1 participants (Table 2). None of the clinical and demographic characteristics was different between the groups.

CCR5$^{(WT/\Delta32)}$ heterozygosity association with HIV-1 reservoir size

Because previous evidence suggested that the total cell-associated HIV-1 DNA levels are lower in CCR5$^{(WT/\Delta32)}$ HIV-1-infected participants compared with CCR5$^{(WT/WT)}$ participants

[10,11], we investigated whether the CCR5 genotype could affect the proviral reservoir size. We found no significant differences in the total HIV-1 DNA/10^6 CD4$^+$ T cells between groups (median (interquartile range) of 455 (308–540) and 578 (259–667) copies of HIV-1 DNA per million CD4$^+$ T cells in individuals with CCR5$^{(WT/WT)}$ and CCR5$^{(WT/\Delta32)}$ genotypes, respectively; Fig. 1), indicating that CCR5$^{(WT/\Delta32)}$ heterozygosity may not affect reservoir establishment in HIV-1 perinatally infected adolescents and young adults.

The association of CCR5$^{(WT/\Delta32)}$ heterozygosity with T-cell subsets and cell-associated HIV-1 DNA levels

As previously described for vertically HIV-infected adolescents [19], the HIV-1-infected participants included in this study presented a highly preserved naive T-cell subset (42.5% and 35.8% of CD4$^+$ CD45RA$^+$ T cells in individuals with CCR5$^{(WT/WT)}$ and CCR5$^{(WT/\Delta32)}$ genotypes, respectively; Fig. 2a). Although no significant differences in the proportion of CD4$^+$ T-cell subsets were evident between CCR5$^{(WT/\Delta32)}$ and CCR5$^{(WT/WT)}$ HIV-1-infected participants, we found that the higher frequency of naive versus memory CD4$^+$ T cells in participants with the CCR5$^{(WT/WT)}$ genotype (p 0.04) was absent in those with CCR5$^{(WT/\Delta32)}$ genotype (Fig. 2a).

We found a correlation between the total HIV-1 DNA in CD4$^+$ T cells and the percentage of memory CD4$^+$ T cells. This correlation was inverse in CCR5$^{(WT/WT)}$ participants (p 0.03; Fig. 2b) but direct in CCR5$^{(WT/\Delta32)}$ participants (p 0.04; Fig. 2c), and indicated a difference in the viral tropism that could target different T-cell

Table 1
Demographic and clinical characteristics of the study cohort

Characteristic	Total	CCR5 genotype		
		WT/WT	WT/Δ32	p value
	242 (100)	219 (90.5)	23 (9.5)	
Sex, n (%)				
Female	144 (59.5)	129 (58.9)	15 (65.2)	0.658
Race/ethnicity, n (%)				
Caucasian	172 (71.1)	152 (69.4)	20 (87)	0.029
Gypsy	14 (5.8)	11 (5)	3 (13)	
Mulatto	7 (2.9)	7 (3.2)	0	
African	31 (12.8)	31 (14.2)	0	
Other	18 (7.4)	18 (8.2)	0	
HIV-1 subtype, n (%)				
B	137 (87.8)	119 (86.2)	18 (100)	0.602
Non–B	19 (12.2)	19 (13.8)	0	
No. with missing data	86	81	5	
Zenith pVL				
Median (IQR), log$_{10}$ copies/mL	5.5 (5.1–5.9)	5.5 (5.1–6)	5.4 (5.2–5.7)	0.221
No. with missing data	5	5	0	
Nadir CD4$^+$ T-cell count				
Median (IQR), cells/mm^3	322 (175.5–474.8)	321 (182–486.5)	336 (118–447)	0.640
Median (IQR), %	15 (10–22)	15 (10–20.8)	15 (6.5–22.5)	0.990
No. with missing data	6	6	0	
CDC category,[a] n (%)				
N or A	98 (40.5)	90 (41.1)	8 (34.8)	0.535
B	80 (33.1)	70 (32)	10 (43.5)	
C	64 (26.4)	59 (26.9)	5 (21.7)	
Immune category,[a] n (%)				
1	13 (5.5)	12 (5.6)	1 (4.3)	0.809
2	79 (33.2)	70 (32.5)	9 (39.1)	
3	146 (61.3)	133 (61.9)	13 (56.5)	
No. with missing data	4	4	0	

Values are shown as median (interquartile range) or number (%). The clinical classification of AIDS-defining events and immunological categories was based on international guidelines. Data were compared between adolescents/young adults homozygous for the wild-type CCR5$^{(WT/WT)}$ genotype, and adolescents/young adults heterozygous for the CCR5 CCR5$^{(WT/\Delta32)}$ genotype using the Mann–Whitney *U* test and Fisher exact test for numerical or categorical variables, respectively.
Abbreviations: CDC, Centers for Disease Control and Prevention; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; pVL, plasma viral load.
[a] Most severe category until data collection.

Table 2
Study population characteristics

Characteristics	Total	CCR5 genotype	
		WT/WT	WT/Δ32
	24 (100)	14 (58.3)	10 (41.7)
Subject characteristics			
Sex, n (%)			
Female	14 (58.3)	8 (57.1)	6 (60)
Race/ethnicity, n (%)			
Caucasian	22 (91.7)	13 (92.9)	9 (90)
Gypsy	2 (8.3)	1 (7.1)	1 (10)
Gestational age			
Median (IQR), weeks	38.5 (36.5−39.3)	37 (34−39)	39 (37.5−39.5)
No. with missing data	8	3	5
HIV-1 subtype, n (%)			
B	19 (79.2)	11 (78.6)	8 (80)
Non-B	1 (4.2)	1 (7.1)	0
No. with missing data	4 (16.7)	2 (14.3)	2 (20)
HCV co-infection, n (%)			
Yes	5 (20.8)	1 (7.1)	4 (40)
Zenith pVL			
Median (IQR), $\log_{10}$ copies/mL	5.5 (5.2−5.9)	5.3 (5−5.9)	5.5 (5.3−5.8)
No. with missing data	1	1	0
Nadir $CD4^+$ T-cell count			
Median (IQR), cells/mm^3	296 (176−475)	308.5 (249.3−450.8)	189 (116−631)
Median (IQR), %	13.5 (5.8−22)	14.5 (8.5−17.6)	11 (4.3−22.8)
Nadir $CD8^+$ T-cell count			
Median (IQR), %	24 (19.3−28)	23.5 (20−26.8)	25.5 (17.8−34.8)
CDC category[a]			
N or A	17 (70.8)	11 (78.6)	6 (60)
B	4 (16.7)	3 (21.4)	1 (10)
C	3 (12.5)	0	3 (30)
Received previous cART			
Yes, No. (%)	19 (79.2)	11 (78.6)	8 (80)
Median duration (IQR), years	2.9 (1.1−4.1)	3.6 (1.9−4.5)	1.9 (0.5−3)
Median age at cART initiation (IQR), yr	5.5 (2.9−9.5)	6.1 (3.1−9.7)	4.7 (3.1−8.5)
Initial cART regimen, n (%) with protease inhibitors	18 (75)	10 (71.4)	8 (80)
Median age at virological control (IQR), years	11.6 (9.2−13.4)	11.5 (10.2−12.9)	12.1 (6.6−15.1)
Parameters at sampling			
Median age (IQR), years	18.7 (16.5−20.1)	19.8 (17.7−20.6)	18 (14.9−18.7)
cART regimen, No. (%) with protease inhibitors	15 (62.5)	7 (50)	8 (80)
Time on cART			
Median (IQR), yr	11.9 (9.9−14)	16.5 (13.6−17.3)	10.7 (10.1−13)
Time under virologic control			
Median (IQR), yr	8.4 (4.1−9.5)	8.8 (5.1−9.8)	6.2 (3.3−9.3)
$CD4^+$ T-cell count			
Median (IQR), cells/mm^3	886.5 (720−1106.8)	1015.5 (746.5−1124.3)	825.5 (729−1038)
Median (IQR), %	35.2 (30.8−39.1)	38 (33.5−47)	33 (26.8−34.3)

Values are shown as median (interquartile range) or number (%).
Abbreviations: CDC, Centers for Disease Control and Prevention; HCV: hepatitis C virus; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; pVL, plasma viral load.
[a] Most severe category until data collection.

subpopulations (i.e. memory $CD4^+$ T cells in CCR5$^{(WT/WT)}$ participants and naive $CD4^+$ T cells in CCR5$^{(WT/Δ32)}$ participants) and cause a decrease in the respective subsets as a consequence of a productive infection.

The relationship between the heterozygous CCR5$^{(WT/Δ32)}$ genotype and viral tropism

Considering the relationship differences observed between the reservoir size and the proportion of memory CD4+ T cells in CCR5$^{(WT/Δ32)}$ and CCR5$^{(WT/WT)}$ HIV-1-infected participants, we wanted to determine whether a difference in viral tropism existed between the two genotype groups that could discordantly affect the composition of the $CD4^+$ T-cell population. Contrary to expectations, viral tropism in samples obtained close to birth showed that the isolates from the CCR5$^{(WT/Δ32)}$ participants were R5 tropic (i.e. non-syncytia inducing), whereas four out of ten isolates of the

CCR5$^{(WT/WT)}$ participants were X4-tropic (i.e. syncytia inducing) (Table 3). Moreover, at sample collection, R5-tropic isolates were found in most of the CCR5$^{(WT/Δ32)}$ participants but only in two participants with the CCR5$^{(WT/WT)}$ genotype (Table 3, and Fig. 2b,c).

CCR5Δ32 heterozygosity association with T-cell activation and immunosenescence

Perinatally HIV-1-infected participants presented specific patterns of immunosenescence of $CD4^+$ T-cell subsets because their immune system had been chronically activated throughout their lifetime [19]. Moreover, the higher percentage of memory $CD4^+$ T cells found in CCR5$^{(WT/Δ32)}$ HIV-1-infected participants could indicate a more profound immune exhaustion. Nevertheless, we found no significant differences between CCR5$^{(WT/Δ32)}$ and CCR5$^{(WT/WT)}$ participants with respect to CD25, CD38 or HLA-DR
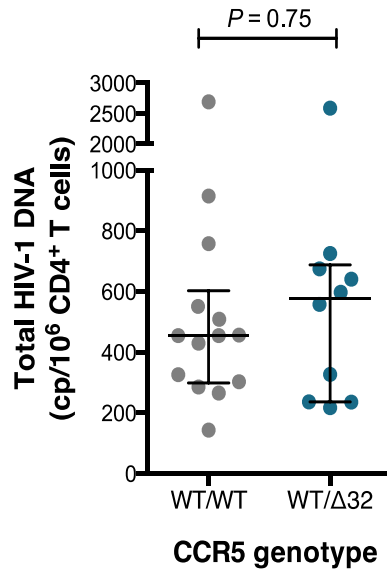
Fig. 1. CCR5(WT/D32) heterozygosity association with HIV-1 reservoir establishment in HIV-1 perinatally infected children.

Discussion

CCR5 is the main co-receptor used by HIV-1 to enter target cells during the early stages of an HIV-1 infection [28]. Our aim was to describe the relationship between CCR5$^{(WT/\Delta32)}$ heterozygosity, the HIV-1 reservoir size and the immunophenotype of the CD4$^+$ T-cell subpopulations in a Spanish cohort of adolescents and young adults perinatally infected with HIV-1.

Among the 242 participants included in the study, 23 were heterozygous for the $\Delta32$ genotype, but none was homozygous for the mutated CCR5 allele. We observed no differences in the HIV-1 reservoir size nor in the immune activation markers between the CCR5$^{(WT/WT)}$ and CCR5$^{(WT/\Delta32)}$ perinatally HIV-infected genotype groups.

This cohort included Caucasians and gypsies, and the prevalence of the heterozygous allele was 9.5%, which is in agreement with previous studies that describe how the frequency of the CCR5$^{(WT/\Delta32)}$ allele varies between ethnic groups. The prevalence of the heterozygous allele ranges from 0.6% among Asians to 20% among European Caucasians [9,27]. The CCR5$^{(WT/\Delta32)}$ genotype did not affect parameters such as the zenith plasma viral load or nadir CD4$^+$ T cells. Although we did not find significant differences in the pattern of progression to clinical AIDS, we cannot discount a potential role for these genetic characteristics, because the sample size was limited.

If CCR5 is the primary co-receptor used by HIV-1 to enter the target cell during the early stages of an HIV-1 infection, then it can be expected that heterozygous participants (CCR5$^{(WT/\Delta32)}$) may

activation markers. Additionally, we distinguished no specific patterns of immunosenescence (CD28$^-$ and CD57$^+$) on CD4$^+$ T cells. All phenotypic characteristics of the different CD4$^+$ T-cell subsets are shown in the Supplementary material (Table S1).
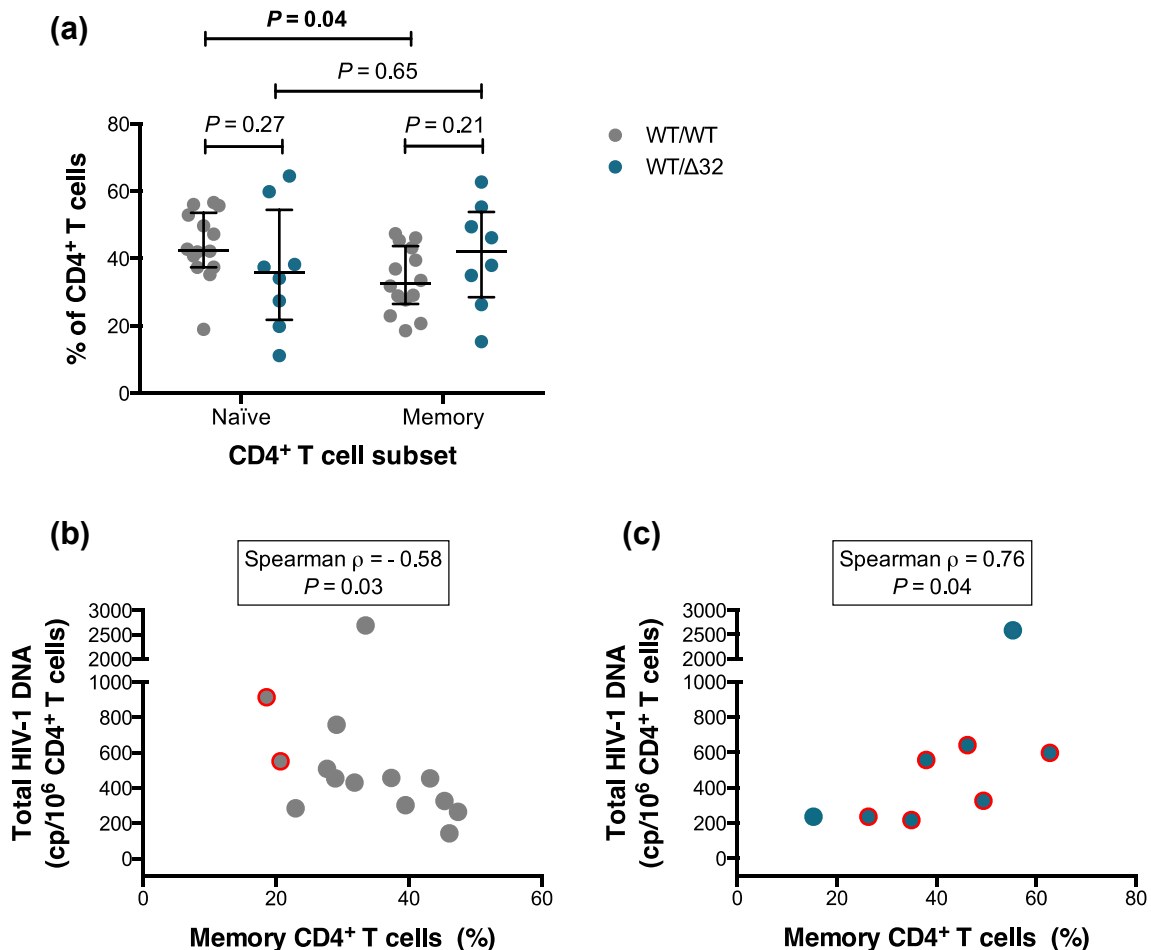

Fig. 2. Association of CCR5(WT/D32) with T cell subsets, genotype and tropism.

Table 3
Viral phenotype

	Total	CCR5 genotype		
		WT/WT	WT/Δ32	p value
	24 (100)	14 (58.3)	10 (41.7)	
Viral tropism at birth[a]				
R5, *n* (%)	10 (41.7)	6 (42.9)	4 (40)	0.251
X4, *n* (%)	4 (16.7)	4 (28.6)	0	
No. with missing data	10	4	6	
Viral tropism at sample[b]				
R5, *n* (%)	9 (37.5)	2 (14,3)	7 (70)	0.002
X4, *n* (%)	9 (37.5)	9 (64.3)	0	
No. with missing data	6	3	3	

Viral tropism was determined as explained in the Material and methods section and viral isolates were classified as R5- or X4-tropic strains. The number (%) of patients with each viral tropism is depicted. Data were compared between adolescents/young adults homozygous for the wild-type CCR5$^{(WT/WT)}$ genotype, and adolescents/young adults heterozygous for the Δ32 genotype CCR5$^{(WT/Δ32)}$ using the Fisher exact test.

[a] Viral tropism closest to the date of birth.
[b] Viral tropism closest to sampling time.

harbour a smaller proviral reservoir than homozygous participants (CCR5$^{(WT/WT)}$). Previous studies suggested that cell-associated HIV-1 DNA levels are lower in CCR5$^{(WT/Δ32)}$ individuals compared with CCR5$^{(WT/WT)}$-infected adults [10]. In contrast, data from a large cohort of HIV-1-infected CCR5$^{(WT/Δ32)}$ adults on long-term suppressive cART revealed no significant differences in the cell-associated HIV-1 DNA and RNA levels or in the frequency of detectable RNA and 2-LTR circles [12]. Sei *et al.* [11] found lower cell-associated HIV-1 DNA in CCR5$^{(WT/Δ32)}$ perinatally infected children younger than 5 years old compared with CCR5$^{(WT/WT)}$, but no significant differences in proviral burden were present in those examined after 5 years of age. In accordance with that study, our results for viral reservoir size in CD4$^+$ T cells also showed no difference between CCR5$^{(WT/WT)}$ and CCR5$^{(WT/Δ32)}$ (Fig. 1) in perinatally HIV-1-infected adolescents (median age 18.7 years; interquartile range 15.5–19.9). Nevertheless, we cannot exclude differences in the viral reservoir size in other non-blood compartments, such as gut-associated lymphoid tissues.

Additionally, in agreement with the previously mentioned studies [11,12], we found no significant differences between the two genotype groups in terms of CD4$^+$ T-cell activation (i.e. CD25$^+$, CD38$^+$ or HLA-DR$^+$) or in specific patterns of immunosenescence (i.e. CD28$^-$ CD57$^+$) in CD4$^+$ T cells (see Supplementary material, Table S1). Intriguingly, the significant difference in the percentage between naive and memory CD4$^+$ T cells (Fig. 2a) found in CCR5$^{(WT/WT)}$ individuals and also previously observed in perinatally HIV-1-infected children [19] was not present in the CCR5$^{(WT/Δ32)}$ participants.

The rapid establishment of latent reservoirs as a consequence of HIV-1 infection, primarily in resting memory CD4$^+$ T cells, has been widely described, and it causes a depletion of the resting memory CD4$^+$ T cells because of the intrinsic nature of the HIV-1 infection. This could explain the lower percentage of memory CD4$^+$ T cells as opposed to naive CD4$^+$ T cells and the inverse correlation between the reservoir size and percentage of memory CD4$^+$ T cells in individuals with a CCR5$^{(WT/WT)}$ genotype (Fig. 2b). Nevertheless, HIV-1-infected participants with a CCR5$^{(WT/Δ32)}$ genotype seem to present a direct correlation (Fig. 2c). These data may suggest differential cell targeting depending on the CCR5 genotype: memory CD4$^+$ T cells in CCR5$^{(WT/WT)}$ participants and naive CD4$^+$ T cells in CCR5$^{(WT/Δ32)}$ participants. It is known that different CD4$^+$ T-cell subsets have different co-receptors expressed on the cell surface [16], so naive CD4$^+$ T cells are a significant viral reservoir for X4-tropic strains [18], whereas R5-tropic strains are mainly present

in memory CD4$^+$ T cells [17]. Therefore, to elucidate the reason for this differential cell targeting, it was necessary to determine the viral tropism of the HIV-1 isolates in each individual. At first glance, tropism in participants close to birth failed to confirm this hypothesis, as all isolates from the CCR5$^{(WT/Δ32)}$ HIV-1-infected participants were R5 tropic, whereas four of ten isolates from CCR5$^{(WT/WT)}$ were X4-tropic (Table 3). Moreover, at sample collection, R5-tropic isolates were found in most of the CCR5$^{(WT/Δ32)}$ individuals but in only two with the CCR5$^{(WT/WT)}$ genotype (Table 3 and Fig. 2b,c). This preferential evolution towards X4-tropism in CCR5$^{(WT/WT)}$ participants was in accordance with previous studies that reported a high prevalence of X4- or dual mix-tropic viruses in perinatally HIV-1-infected participants [26] and a correlation between this high prevalence and the switch from R5 to X4-tropic viruses due to the long-term exposure to HIV-1 infection [29]. In contrast, all the isolates from the CCR5$^{(WT/Δ32)}$ individuals remained purely R5-tropic.

It may be possible that during the course of the disease, R5 viruses evolve towards a more efficient usage of CCR5 that favours an increased ability to infect target cells with a low surface density of CCR5 [30], as occurs in CCR5$^{(WT/Δ32)}$ participants, so enhancing the infection of naive CD4$^+$ T cells. This R5-tropism evolution may indicate that all CCR5$^{(WT/Δ32)}$ participants maintain such viral tropism, suggesting that an HIV-1 infection progresses differentially in CCR5$^{(WT/Δ32)}$ than in CCR5$^{(WT/WT)}$ perinatally HIV-1-infected participants and explaining the disparity of results when we correlated the reservoir size with the percentage of memory CD4$^+$ T cells.

The restricted sample amounts limit the quantitative determination of the surface CCR5 density in each cell subpopulation, but CCR5 cell-surface density could be a determining factor in future studies to explain the differential targeting of the cell subsets. Another limitation of this study was the use of two different techniques to determine viral tropism. The reason is partly due to the time interval between birth and sample collection, considering the median age of the individuals from our study is 18.7 years. Nevertheless, both techniques are phenotypic assays with good concordance between them as well as with the widely accepted Trofile assay.

In summary, the CCR5 genotype did not affect the HIV-1 reservoir size in CD4$^+$ T cells in the perinatally HIV-infected adolescent and young adult cohort studied, but a correlation was evident between the total HIV-1 DNA in CD4$^+$ T cells and the percentage of memory CD4$^+$ T cells, which was direct in CCR5$^{(WT/Δ32)}$ participants and inverse in those with the CCR5$^{(WT/WT)}$ genotype. Although the underlying mechanism remains to be elucidated, the differential cell targeting observed in HIV-1-infected youngsters with the CCR5$^{(WT/Δ32)}$ genotype could cause a change in the reservoir cell compartment. Therefore, it might be important to design different strategies for the elimination of the viral reservoir according to the CCR5 genotype.

Appendix A. Supplementary data

Additional Supporting Information may be found in the online version of this article can be found at http://dx.doi.org/10.1016/j.cmi.2016.12.020.

Transparency Declaration

There is no conflict of interest for any of the authors.

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