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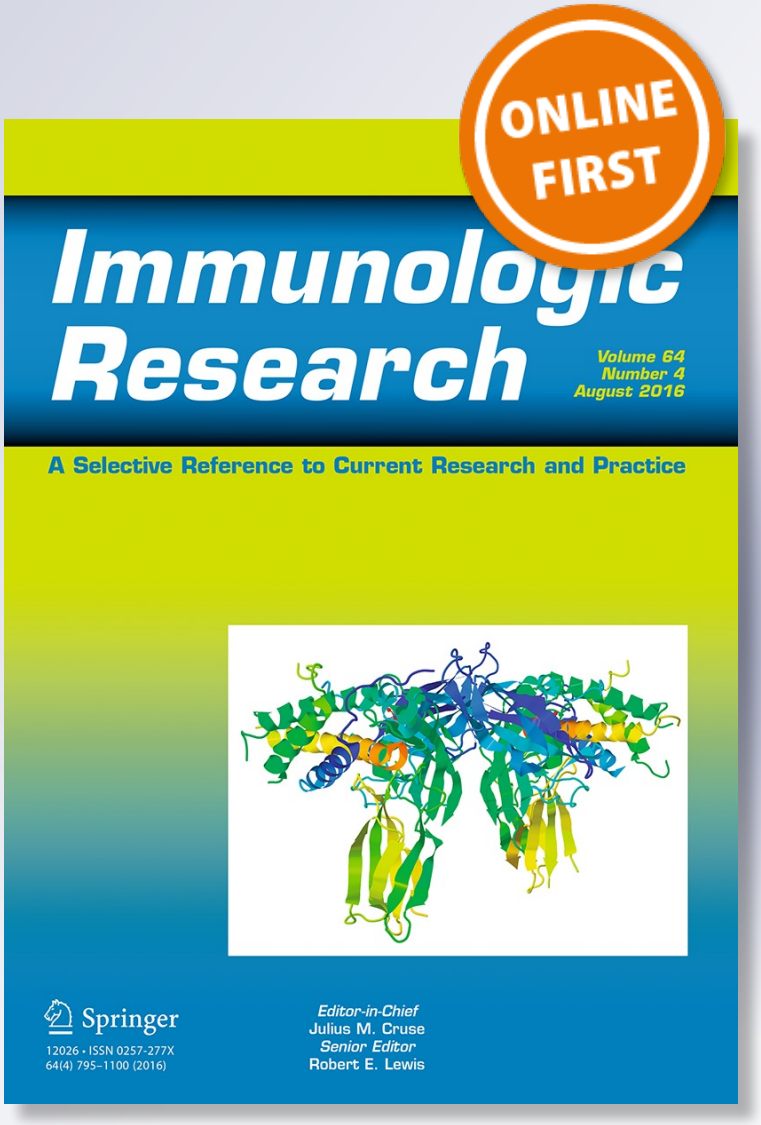
Immunologic Research

ISSN 0257-277X

Immunol Res
DOI 10.1007/s12026-016-8875-x



Springer

Immunol Res
DOI 10.1007/s12026-016-8875-x

ORIGINAL ARTICLE

Genome-wide scan in two groups of HIV-infected patients treated with dendritic cell-based immunotherapy

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Abstract We performed a retrospective genome-wide association study in HIV-infected individuals who were treated with dendritic cell-based immunotherapy in clinical trials performed by two research groups (Spain and Brazil). We aimed to identify host genetic variants influencing treatment response. The Illumina Human Core Exome 12 v 1.0 Bead Chip with over 250,000 markers was used to analyze genetic factors affecting treatment response. Additionally, we performed a meta-analysis of the results obtained from Spanish and Brazilian patients. We identified a genetic variation (rs7935564 G allele) in *TRIM22* gene, which encodes TRIM22 protein acting like a HIV restriction factor, as being associated with good response to dendritic cell-based immunotherapy. We then verified the impact of *TRIM22* rs7935564 SNP in susceptibility to HIV infection and disease progression by assessing the influence of biogeographic ancestry in the distribution of allelic and genotype frequencies in three populations from Italy, Brazil and Zambia. *TRIM22* rs7935564 genotyping indicated association of G rs7935564 allele with long-term non-progression of HIV disease in Italian patients, thus corroborating our hypothesis that it is involved as a restriction factor in dendritic cell-based immunotherapy response. *TRIM22* rs7935564 polymorphism was associated with good response to dendritic cell-based immunotherapy. We hypothesize that in selecting patients for treatment, there is a possible bias related to the natural presence of restriction factors that are genetically determined and could influence final outcome of therapy.

Keywords HIV · Dendritic cells · Immune-therapy · Genome-wide · *TRIM22* · SNPs

Electronic supplementary material The online version of this article (doi:10.1007/s12026-016-8875-x) contains supplementary material, which is available to authorized users.

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Introduction

Presently, there are more than 30 million HIV-infected people worldwide, the majority of whom live in countries with limited financial resources. The availability of combined antiretroviral therapy (cART) continues to increase and has become more accessible for those in developing countries. However, although cART effectively controls disease progression, it is not a cure for HIV and it is a lifelong treatment. Lifelong use of cART presents various

Published online: 04 October 2016

problems, including lack of adherence, viral resistance, and adverse effects.

Alternative strategies have been proposed to control HIV infection. The goal of these strategies is to attempt to reduce costs and pharmacological adverse effects and to make treatment available in developing countries [1].

Therapeutic vaccines based on whole inactivated virus (REMUNE) [2] or recombinant gp120 protein [3] have been developed. These aim to restore immune response against HIV, but results thus far have been suboptimal.

Long-term non-progressors (LTNP), a subgroup of genetically predisposed individuals who do not require cART treatment for long-term viral control, inspired research into HIV-specific T cell responses as a means to control viral replication. In this regard, researchers have investigated dendritic cell-based immunotherapy (DC-IT) as an alternative strategy to fight HIV infection.

The first clinical trial using DC-IT was published in 2004 [4] and described the use of autologous monocytes, which were differentiated in vitro in dendritic cells (DC) and pulsed with aldrithiol-2 inactivated virus. Of 18 HIV-infected patients not currently receiving cART, 8 patients demonstrated controlled viral load for 1 year.

Presently, 15 clinical trials investigating DC-IT treatment methods have been completed. These trials demonstrate that DC-IT treatment is safe and well tolerated [5–11]. However, few of these trials demonstrated efficacy in control of viral replication. Lu et al. [4] and Garcia et al. [7] found that viral load (VL) was partially controlled for up to 12 and 6 months, respectively. Interestingly, only a subset of patients in both studies responded positively to DC-IT: 8 of 18 in Lu et al. [4] and 7 of 15 in Garcia et al. [7].

In order to develop a consensus protocol, Garcia and Routy [6] organized a workshop for all current DC-IT clinical trials. The variables analyzed include immune-vaccine preparation, clinical trial design, and immunological monitoring for vaccinated patients. However, the host genome, which may influence response to therapeutic vaccine, was not considered. We therefore speculate that patient selection for undergoing DC-IT therapy could face bias related to natural genetic capacity to control viral replication, as in the LTNP.

The objective of this study was to understand the potential influence of host genetic background on patient response to DC-IT. We performed a retrospective genome scan analysis using individuals from the Garcia et al. [7] clinical trial and compared the results with previous genomic analysis scans conducted on DC-IT patients from the study by Lu et al. [4].

Methods

Patients

This study consists of a retrospective analysis of genomic DNA from 15 HIV-infected patients treated with DC-IT from a DCV2 Phase II clinical trial performed at the Infectious Diseases Service Hospital Clínic, Barcelona (Spain) [7]. HIV-infected patients were classified according to response to DC-IT at 24 weeks following vaccination: poor responders (PR)—patients whose viral load (VL) dropped <1 log compared to baseline value or good responders (GR)—patients whose viral load dropped to >1 log compared to baseline value [7].

All study experiments and procedures were performed in accordance with ethical standards of 1975 Declaration of Helsinki (6th revision, 2008). All patients were informed of the objectives of the study and provided consent to participate. This study was approved by the local ethical committee (CI 14/10/03).

Exome analysis

We used the Illumina Human Core Exome 12 v 1.0 Bead Chip, which contains over 250,000 markers representing European, African, Chinese, and Hispanic populations (coding single nucleotide polymorphism—cSNPs and Tag SNPs with MAF ≥5 %) (ILLUMINA®, San Diego, CA, USA). We conducted a meta-analysis pooling the genomic data from Spanish [7] and Brazilian clinical trials [4] using the fixed-effects model. To correct results for population stratification, we made a principal components analysis (PCA), as suggested by Price et al. [12]. The first ten principal components were used as covariates for meta-analysis.

Patients and controls for TRIM22 genotyping

We utilized a retrospective cohort of 82 HIV-infected children from the Pediatric Division of the Institute for Maternal and Child Health Burlo Garofolo (Trieste, Italy), classifying disease progression according to 1994 CDC AIDS surveillance case definition [13]. There were 17 rapid progressors (RP), who developed severe clinical manifestations within the first 2 years of infection, (defined as "Category C"), 58 slow progressors (SP), who neither progressed to Category C nor developed severe immune-suppression beyond 8 years of age, and 7 long-term non-progressors (LTNP), who were defined by asymptomatic HIV infection for more than 10 years in the absence of any antiretroviral treatment, a stable CD4+ cell count

persistently greater than 500 cells/ml, and a viral load repeatedly less than 5000 copies/ml.

Nineteen HIV-exposed uninfected children (EU) were also enrolled. Consent was obtained from parents or legal representatives. The local ethical committee (Prot. N. L1106, 11/05/2010) approved the study.

We included 319 infants from the "Zambia Exclusive Breastfeeding Study" (ZEBS), which was performed in two primary health clinics (George Clinic and Chawama Clinic) in Lusaka (Zambia) (ClinicalTrials.gov Identifier: NCT00310726) [14]. The study included 82 HIV-exposed and infected (HIV+) and 237 EU children. Mothers gave their informed consent to join the study.

Finally, we included 76 HIV+ and 31 EU children, all of whom were born to HIV-infected mothers not treated with antiretroviral drugs. These subjects were enrolled from a historic cohort at the "Instituto de Medicine Integral Prof. Fernando Figueira" (IMIP) of Recife (Brazil), which began before the introduction of prevention of mother-to-child transmission (PMTCT) protocols for pregnant women and newborns. We also analyzed 116 unexposed and uninfected children (UUC) from the same geographical region as the controls (metropolitan area of Recife). The IMIP Ethical Committee approved the project (CONEP protocol 3127, process 25000.127120/2001-73), and all participants (parents or legal representatives) gave their informed consent for DNA use for research purposes.

TRIM22 rs7935564 polymorphism was genotyped using TaqMan® SNP genotyping assay (C___1452153_10) and TaqMan GTXpress™ Master Mix on ABI7900HT Real Time PCR platform (Thermo Fisher Scientific, Carlsbad, CA, USA.) following the manufacturer's instructions.

Data quality control and statistical analysis

Illumina exome array has many rare variants, but the default Illumina algorithm for genotype calling, GenCall, could exclude some of them. We used the zCall algorithm [15] specifically tailored for rare variants.

Quality control (QC) for the recalling process was performed through the GenABEL [16] package, with a call-rate cutoff set at 99 % for SNP call rate for individual, along a 95 % threshold for identity by state (IBS) and a cutoff of 0.2 for the false discovery rate applied to Hardy–Weinberg equilibrium (HWE) tests. After the QC procedures, 51,261 SNPs (relating to 4959 genes) passed all criteria and no individuals were excluded due to low call rate.

We performed SNP-based analysis using "GenABEL" R Package [16], and for gene-based analysis we used an in-house implementation of the method proposed by Wang and Abbott [17].

Fixed-effects meta-analyses were conducted using GenABEL and MetABEL packages running in R software v. 3.1.1 [18]. In order to adjust for genetic stratification, we calculated the results by including the first 10 principal components in the model of the statistical tests.

For studies on different populations, Fisher's exact test was used to verify association of the candidate marker exploring the allelic and general (co-dominant) genetic models. Odds ratio (OR) and 95 % confidence intervals were also calculated.

Results

To investigate genetic markers involved in the response to DC-IT, we compared genetic data from the 15 patients included in DCV2 Phase II study, which consisted of GR ($n = 8$) and PR ($n = 7$) groups. We observed trends of different SNP frequency distributions, including SNPs in genes found to be involved with HIV infection, between GR and PR; however, these trends were not statistically significant for genome-wide studies ($p > 9.75 \times 10^{-7}$). Table 1 shows the results for the most significant genes; the complete list of genotyping results is available upon request.

We next conducted a meta-analysis, pooling data from the DCV2 Phase II Spanish trial [7] and the Lu et al. Brazilian trial [4], containing 15 GR and 18 PR. We used a fixed-effect model and adjusted for genetic stratification using the first ten principal components. A total of 11,937 genes and 39,108 SNPs, present in common in the two exome chips used to scan Brazilian and DCV2 Phase II

Table 1 Exome chip Gene-centered analysis

Gene	Chromosome	Start position	End position	*p* value
NRP1	10	33177492	33336262	0.0005
IGDCC4	15	65381464	65423072	0.0013
BIRC6	2	32357028	32618899	0.0022
TEKT1	17	6789133	6831761	0.0032
ZNF428	19	43607219	43619874	0.0038
ZNF438	10	30820207	31031937	0.0038
SRRM5	19	43596617	43614497	0.0039
PADI2	1	17066761	17119435	0.0042
CHRM5	15	33968720	34067457	0.0045
LRCOL1	12	132603150	132610543	0.0045

Gene-centered analysis reveals genes (here we report the 10 genes with better statistical association *p* values) with SNPs differently distributed in good responders (GR) and poor responders (PR) to DC-IT. The gene start and end positions are based on Ensembl_75 (GRCh37.p13) database

Spanish patients, have been considered for meta-analysis. We did not find any association between genetic variations and response to DC-IT achieving genome-wide significance ($p > 9.75 \times 10^{-7}$).

In addition to investigating the statistical association, we also sought to consider the biological significance of our genetic findings. We searched Panther (http://pantherdb.org), Genemania (http://genemania.org/), String (http://string-db.org/), gene annotation and interaction databases, and NCBI (http://www.ncbi.nlm.nih.gov/) to see if any association (or trend) existed with regard to genes involved in HIV-related processes. Our search included processes such as HIV Receptors and Natural Ligands, Cellular Cofactors Involved in HIV Infection (i.e. Chemokine Receptors), Apoptosis, Inflammatory Response, Antimicrobial Humoral Response, Viral Genome Replication, Innate Immune Response, Antimicrobial Humoral Response, Defense Response Against Pathogens, and Immune Evasion. After scanning the 39,108 SNPs in common between the Brazilian and Spanish DC-IT exome studies, we did not find a significant association with the response to DC-IT, except for rs7935564 SNP (A/G) located at *TRIM22* gene, encoding for the Tripartite Motif Containing 22 (TRIM22) protein known as a restriction factor for HIV (Table 2).

The minor allele (G) showed a protective effect against a poor response to DC-IT ($p = 0.0012$ OR 0.29; 95 % CI 0.14–0.62).

The intergenic rs13001244, rs195204, rs631230 SNPs as well as rs11082304 *CABLES1* SNP showed better p values than *TRIM22* rs7935564 SNP; however, none of them were in linkage disequilibrium with other genetic variations reported in the literature or in OMICs databases as associated with the control of HIV infection. Moreover, *CABLES1* encodes a protein involved in regulation of the cell cycle through interactions with several cyclin-dependent kinases; this gene and the SNP found as the third best p value in our study, has never been related to HIV infection or disease progression.

Moreover, aimed at discarding the effect of genetic factors known to influencing HIV susceptibility and progression, we revised the GWAS literature on the genetic control of HIV infection and interrogated our data set for the most associated SNPs. The most common genomic regions reported to be associated with HIV infection control are *HLA-B*, represented by the SNP rs2523608, rs2395029, rs130072, *HLA-C* with rs9264942 SNP and *CCR5*, where the SNP rs333 is present. None of those markers are associated with the response to DC-cell therapeutic vaccine.

Since genomic DNA data were available from HIV-infected DC-IT-treated patients involved in two clinical trials in Barcelona (performed by two of the authors, FG and MP) [7, 19], we retrieved the DC activation data and stratified them according to their *TRIM22* rs7935564 genotype. We sought to verify the possible impact of *TRIM22* rs7935564 genotype on the immunologic activation of DC. Of the HIV-infected patients whose DNA was available, we found 6 individuals carrying the rs7935564 AA genotype, 16 carrying the AG genotype, and 3 carrying the GG genotype.

It should be noted with regard to the results of the correlation with *TRIM22* rs7935564 genotype that García et al. prepared the DC immune-therapy using a DC maturation

Table 2 Meta-analysis results

SNP	A1	A2	Chr	Pos	Gene	GR A1	GR A2	PR A1	PR A2	OR	95 % CI	*p*
rs13001244	T	C	2	65550494	Intergenic	18 (0.60)	12 (0.40)	16 (0.44)	20 (0.56)	2.67	1.57–4.53	0.0003
rs195204	T	C	1	115191470	Intergenic	21 (0.70)	9 (0.30)	30 (0.83)	6 (0.17)	0.37	0.22–0.63	0.0003
rs11082304	T	G	18	23141009	*CABLES1*	15 (0.50)	15 (0.50)	12 (0.33)	24 (0.67)	2.59	1.54–4.37	0.0004
rs631230	T	C	1	114177396	Intergenic	11 (0.37)	19 (0.63)	23 (0.64)	13 (0.36)	0.41	0.25–0.69	0.0007
rs7935564	A	G	11	5697287	*TRIM22*	13 (0.43)	17 (0.57)	23 (0.64)	13 (0.36)	0.29	0.14–0.62	0.0012
rs14259	T	C	12	121915890	*PSMD9*	3 (0.10)	27 (0.90)	2 (0.06)	34 (0.94)	2.76	1.49–5.12	0.0013
rs17852561	T	C	12	121958489	*WDR66*	3 (0.10)	27 (0.90)	10 (0.28)	26 (0.72)	0.36	0.20–0.67	0.0013
rs6788567	T	G	3	112718200	Intergenic	16 (0.53)	14 (0.47)	20 (0.56)	16 (0.44)	0.48	0.30–0.75	0.0014
rs4704970	T	C	5	156073982	Intergenic	7 (0.23)	23 (0.77)	3 (0.08)	33 (0.92)	2.57	1.43–4.63	0.0016
rs392644	T	C	19	58269137	Intergenic	14 (0.47)	16 (0.53)	20 (0.56)	16 (0.44)	0.52	0.35–0.78	0.0017

Pooled allele distribution, odds ratio (OR), 95 % confidence interval (95 % CI) and *p* value for meta-analysis involving poor (PR) and good responders (GR) to the DC-cell vaccine treatment (DCV2 Phase II and Recife's DC-IT). Genes' positions are based on Ensembl_75 (GRCh37.p13) database

OR = pooled odds ratio, *CI* = confidence intervals; *p* = *p* value, *Chr* = chromosome, *Pos* = position, *A1* = reference allele, *A2* = alternative allele

cocktail, containing TNF-a, IL-6, IL-1beta, and E2 Prostaglandin (PGE2), a powerful activator of cells. This cocktail has been able to drive DC maturation in all patients independently of their *TRIM22* rs7935564 genotype. After 2 days of cell culture, the activation of DC was evaluated and DC activation markers were found to be HLA DR > 90 %, CD80 > 40 %, CD83 > 40 %, CD86 > 90 % and CD40 > 90 % in all DC derived from HIV-1 patients, regardless of their *TRIM22* rs7935564 genotype.

We then genotyped three populations of HIV-infected patients from Italy, Brazil, and Zambia. We sought to verify the impact of *TRIM22* rs7935564 SNP in susceptibility to HIV infection and progression. Moreover, we sought to investigate the influence of biogeographic ancestry in the distribution of allelic and genotype frequencies (Table 3).

TRIM22 rs7935564 polymorphism frequencies resulted in Hardy–Weinberg equilibrium in all groups, except for the Brazilian HIV+. Among Brazilian children, the AA genotype was more frequent in HIV + individuals, while the AG genotype was more represented among EU ($p = 0.03$, OR 0.34, CI 0.10–1.00). No allele or genotype frequency differences were observed when comparing HIV+ with UUC or when comparing EU with UUC in the Brazilian group. In Zambian children, allele and genotype frequencies were similar between EU and HIV+. In the Italian group, *TRIM22* rs7935564 SNP allele and genotype frequencies were not different between HIV + and EU. However, when comparing LTNP with SP, the G allele was more frequent in LTNP with respect to SP (allele: $p = 0.02$, OR 0.26, CI 0.55–0.95). Moreover, when LTNP and RP were compared, G allele and GG genotype were significantly more frequent in LTNP (allele $p = 0.02$, OR 0.20, CI 0.04–0.88, genotype: $p = 0.03$, OR 0.03, CI 0.00–0.89).

Discussion

In our meta-analysis of two genome-wide association studies of HIV-infected DC-IT-treated patients from Spain and Brazil, we identified a genetic variation, (rs7935564) at *TRIM22* gene, associated with better response to DC-IT therapy. *TRIM22* rs7935564 SNP is frequent in the general population, with a minor allele frequency (MAF) of 0.46. Our findings indicate that the minor allele (G) is protective against a poor response to DC-IT.

First, we verified whether the presence of *TRIM22* rs7935564 SNP, particularly the G allele, was correlated with better immunologic activation of DC and therefore also correlated with the response to DC-IT. DC immunologic activation was the same in all patients, independent of their *TRIM22* rs7935564 genotype.

In fact, the DC maturation cocktail that was used acts as pathogen-associated molecular patterns (PAMPS), nucleotides, inflammatory cytokines or cell damage and activates a complex DC maturation program consisting of the up-regulation of maturation markers such as CD83, co-stimulatory markers (such as CD80, CD86, CD40), and HLA markers, among others, and of the secretion of T cell polarizing cytokines, efficiently activating antigen-specific T cells [20–22].

Based on these findings, we can affirm that *TRIM22* rs7935564 SNP, particularly the G allele, does not affect DC activation status or play a role in the process of preparation of the immune treatment.

TRIM22, previously known as Staf50 [23], is an interferon-induced protein with cytoplasmic and nuclear localization. It is reported to inhibit viral replication, acting as an antiviral against HIV infection. It acts directly on the LTR-HIV promoter or interferes with Tat- and NF-κB-independent LTR-driven transcription [24].

The rs7935564 SNP causes an amino acid substitution at codon 155, localized in the coiled-coil region. This region is known to play a structural role in the protein; it enables networking with other molecules and protein homo-multimerization. Interestingly, this domain is involved in interactions with several other TRIM family proteins [25]. Moreover, heterologous interactions are also promoted by TRIM22 coiled-coil domain, which is crucial in the control of HIV expression [26].

Singh et al. [27] demonstrated that TRIM22 expression correlated negatively with plasma viral load and positively with $CD4^+$ T cell counts in primary HIV infection, suggesting an antiviral role. Also, Rotger et al. described a positive correlation between TRIM22 expression and viremia levels [28], and Singh et al. [29] observed higher TRIM22 expression in HIV-infected patients compared to healthy controls.

Since the rs7935564 SNP is a non-synonymous mutation that changes an asparagine residue (Asn) with an aspartic acid (Asp) residue at codon 155 of the mature protein, its damaging effect has been evaluated using SIFT (http://sift.jcvi.org/) and predicted as deleterious with a 0.01 score. The same variation, when analyzed with Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) has been described as tolerated (score 0.139).

Using SIFT software, Kelly et al. initially reported the rs7935564 genetic variant as having an in silico potential damaging effect [30]. However, in a more recent and complete study, Kelly et al. [31] used six different algorithms, including SIFT, Polyphen, nsSNP (http://snpanalyzer.uthsc.edu/), SNP&GO (http://snps-and-go.biocomp.unibo.it/snps-and-go/), PhD-SNP (http://snps-and-go.biocomp.unibo.it/snps-and-go/) and pMUT (http://mmb.pcb.ub.es/PMut/) and found that the same SNP was

Table 3 *TRIM22* rs7935564 SNP allele and genotype frequencies in the study populations (Brazil, Zambia and Italy)

	HIV+ $N = 76$	EU $N = 31$	UUC $N = 116$	EU versus HIV+	UUC versus HIV+	UUC versus EU
Brazil						
A	87 (0.57)	33 (0.53)	128 (0.55)	Ref	Ref	Ref
G	65 (0.43)	29 (0.47)	104 (0.45)	$p = 0.65$, OR 0.85, CI 0.45–1.61	$p = 0.75$, OR 0.92, CI 0.59–1.42	$p = 0.89$, OR 1.08, CI 0.62–1.9
AA	30 (0.40)	7 (0.23)	36 (0.31)	Ref	Ref	Ref
AG	27 (0.35)	19 (0.62)	56 (0.48)	$p = 0.03$, OR 0.34, CI 0.10–1.00	$p = 0.13$, OR 0.58, CI 0.28–1.19	$p = 0.36$, OR 1.74, CI 0.67–4.5
GG	19 (0.25)	5 (0.16)	24 (0.21)	$p = 1.00$, OR 0.89, CI 0.21–4.10	$p = 1.00$, OR 0.95, CI 0.41–2.20	$p = 0.82$, OR 1.07, CI 0.3–3.77
HWE	$\chi^2 = 5.72$, $p = 0.02$	$\chi^2 = 1.65$, $p = 0.20$	$\chi^2 = 0.07$, $p = 0.80$			

	HIV+ $N = 82$	EU $N = 237$	EU versus HIV+
Zambia			
A	61 (0.37)	198 (0.42)	Ref
G	103 (0.63)	276 (0.58)	$p = 0.31$, OR 1.21, CI 0.83–1.78
AA	11 (0.13)	41 (0.18)	Ref
AG	39 (0.48)	116 (0.49)	$p = 0.71$, OR 1.25, CI 0.56–2.97
GG	32 (0.39)	80 (0.34)	$p = 0.35$, OR 1.49, CI 0.65–3.62
HWE	$\chi^2 = 0.03$, $p = 0.87$	$\chi^2 = 0.01$, $p = 0.92$	

	HIV+ $N = 82$	LTNP $N = 7$	SP $N = 58$	RP $N = 17$	EU $N = 19$	EU versus HIV+	LTNP versus SP	LTNP versus RP	SP versus RP
Italy									
A	98 (0.60)	4 (0.29)	71 (0.61)	23 (0.67)	21 (0.55)	Ref	Ref	Ref	Ref
G	66 (0.40)	10 (0.71)	45 (0.39)	11 (0.32)	17 (0.45)	$p = 0.71$, OR 0.83, CI 0.38–1.82	$p = 0.02$, OR 0.26, CI 0.05–0.95	$p = 0.02$, OR 0.20, CI 0.04–0.88	$p = 0.55$, OR 0.75, CI 0.30–1.80
AA	28 (0.34)	0 (0.00)	21 (0.36)	7 (0.41)	7 (0.37)	Ref	Ref	Ref	Ref
AG	42 (0.51)	4 (0.57)	29 (0.50)	9 (0.53)	7 (0.37)	$p = 0.56$, OR 1.49, CI 0.39–5.60	$p = 0.15$, OR 0.15, CI 0.00–2.98	$p = 0.25$, OR 0.14, CI 0.00–3.04	$p = 1.00$, OR 0.93, CI 0.26–3.46
GG	12 (0.15)	3 (0.43)	8 (0.14)	1 (0.06)	5 (0.26)	$p = 0.49$, OR 0.61, CI 0.13–2.94	$p = 0.06$, OR 0.05, CI 0.00–1.21	$p = 0.02$, OR 0.03, CI 0.00–0.89	$p = 0.65$, OR 0.38, CI 0.00–3.86
HWE	$\chi^2 = 0.35$, $p = 0.56$	$\chi^2 = 1.12$, $p = 0.29$	$\chi^2 = 0.16$, $p = 0.69$	$\chi^2 = 0.75$, $p = 0.39$	$\chi^2 = 1.23$, $p = 0.27$				

Distribution of *TRIM22* rs7935564 SNP in exposed infected (HIV +), exposed uninfected (EU) and unexposed uninfected children (UUC) from Brazil, Zambia, and Italy

HIV + = HIV-exposed infected children; EU = exposed uninfected children; UUC = unexposed uninfected controls; LTNP = long-term non-progressors; SP = slow progressors; RP = rapid progressors; HWE = Hardy–Weinberg Equilibrium

not predicted as potentially damaging (SNPs predicted to be deleterious by at least four in silico algorithms were defined as damaging SNP).

Kelly et al. [30] also identified a *TRIM22* SNP (rs1063303) with functional impact, able to augment TRIM22 expression and diminish its antiviral activity. We verified whether the rs7935564 SNP was in linkage disequilibrium with the rs1063303 SNP, as well as with other 14 functional SNPs reported by Kelly et al. [31] but no linkage was found ($r^2 < 0.8$).

Next we sought to determine the role of *TRIM22* rs7935564 as a restriction factor acting in the complex interplay between virus replication and the effects of DC-IT. We analyzed the distribution of rs7935564 in the context of HIV vertical transmission (susceptibility to infection) and disease progression (restriction factor limiting virus spread) in three populations from Italy, Brazil, and Zambia, each with different ethnic backgrounds. Despite not having information on HIV genotype in the three study populations, Italian and Brazilian patients were likely infected with clade B HIV, while the Zambian cohort predominantly clade C [32]. We therefore have to be aware that the heterogeneity of virus type could also influence the susceptibility of vertical transmission.

In summary, the rs7935564 AG genotype is protective against vertical transmission in Brazilian children but not in the Italian and Zambia infants. Moreover, carriers of s7935564 G allele and GG genotype, particularly LTNP individuals from Italy, were protected against disease progression. Despite the small number of individuals in our analysis, this finding could corroborate our hypothesis that individuals carrying *TRIM22* rs7935564 G allele are better able to control viral replication, consequently presenting better DC-IT response. However, Ghezzi et al. [33] also studied HIV+ patients and found different results. Their findings show that *TRIM22* rs7935564 AA genotype was associated with long-term non-progression to AIDS. This discrepancy could be due to the low number of individuals included in our study or to age differences, as our study included children and the Ghezzi study included adults.

Another cohort of patients has been recently identified as able to successfully control viral replication; this cohort is the posttreatment controllers (PTC) [34]. PTC are characterized by long-time viremia control after the disruption of antiretroviral treatment started through primary HIV infection.

Unfortunately PTC were not available to us. However, since their genetic background has been described in terms of HLA-B alleles (the HLAB*3503 and HLAB*3501 found to be more frequent in PTC), we could apply the same analysis strategy used in our study. *TRIM22* rs7935564 SNP could be analyzed to assess whether the genetic component of PTC affects control of HIV infection.

Different *TRIM22* polymorphisms have also been correlated with other viral infection such as rubella virus [35] and HBV [36] in American and Chinese Han patients, respectively. Nevertheless, rs7935564 SNP was not included in the list of genetic variations associated with other viral infections. Recently, publication investigating *TRIM22* variants have reported associations with Very-Early-Onset Inflammatory Bowel Disease. Once again, in this case the rs7935564 SNP was not implicated in the disease [37].

Finally, we interrogated our data set looking for the SNPs reported in the most recent GWAS as associated with the control of HIV infection and diseases progression. All SNPs localized at *TRIM5α*, *Mx2/MxB, SAMHD1, p21/CDKN1, tetherin/BST2/CD137, APOBEC3G* and *APOBEC3F* genes, known to be HIV restriction factors [38], were present in our meta-analysis data set but none of them correlated with the response to DC-IT. We also scanned our meta-analysis findings for the presence of the most common genomic regions reported to be associated with HIV infection control such as are HLA-B, represented by the SNP rs2523608, rs2395029, rs130072, HLA-C with rs9264942 SNP, and CCR5, where the SNP rs333 is present [39, 40]. Also in this case no association between the most known HIV infection markers and response to DC-IT was found.

Conclusions

Being aware of the main limitations of our study, related to the small sample size and consequent low statistical power not allowing to reaching the genome-wide significance of our findings, we suggest that a genetic variation (rs7935564) in *TRIM22* gene, which encodes an HIV restriction factor protein, may be involved in the response to DC-IT.

TRIM22 rs7935564 SNP genotyping in HIV-infected patients from two clinical trials demonstrated a lack of association between the genetic variant and DC immunologic activation status. This reinforces our hypothesis that *TRIM22* rs7935564 SNP may impact HIV-1 replication. Individuals carrying the *TRIM22* rs7935564 G allele could be genetically predisposed to better control HIV-1 replication and present better DC-IT response independently of DC immune treatment. Considering the absence of functional validation of the impact of *TRIM22* SNP on HIV replication control, our hypothesis should be considered as preliminary needing to be validated on higher number of subjects submitted to DC-IT.

Nevertheless our findings suggest that there may be bias in the choice of patients submitted to DC-IT based on their natural capacity to control HIV-1 replication due to genetic

background, an important variable that we suggest be considered in the recruitment of patients for future clinical trials.

Finally, it is important to note that the response to DC-IT in HIV-1 patients is a multifactorial trait. Several genes are involved, each one with low penetrance and different expression, together with other nongenetic variables, widely discussed in the review of Garcia and Routy (reference 6 in our manuscript): *TRIM22* is just one of the genetic actors involved in the complex phenotype DC-IT response.

We hope that our preliminary findings will allow us to join all patients participating to ongoing DC-IT (with availability of DNA, monocytes and DC from patients), thus allowing us to increase sample size and to perform the genome scan at worldwide level, obviously considering the different ethnic contribution of all participants in order to definitively disclose the role of host genome in the response to DC-IT.

Acknowledgments We thank Sheila Pierson for editing the manuscript. Grants from Conselho Nacional Desenvolvimento Cientifico e Tecnologico (CNPq, Brazil) n. 552195/2011-1, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) "Rede Intersys" and the Italian Ministry of Health (RC07/08) supported this study. This work was also supported by HIVACAT (HIV Vaccine Research and Development Programme in Catalonia), Fondo Europeo para el Desarrollo Regional (FEDER), Fondo de Investigación Sanitaria (FIS), FIS PI070291, PI12/00969, Red de Investigación en Sida (RIS), Instituto de Salud Carlos III (ISCIII-RETIC RD06/0006/0000), Grant # 108821-55-RGRL, FP7-HEALTH-2013-INNOVATION-1 Proposal No: 602570-2 and SAF 2012-39075, SAF2015-66193-R.

Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests

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