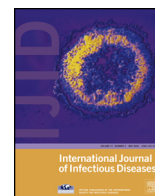


Contents lists available at [SciVerse ScienceDirect](http://SciVerse.Sciencedirect.com)

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Novel genetic association of TNF- α -238 and PDCD1-7209 polymorphisms with long-term non-progressive HIV-1 infection

Milena Nasi^{a,*}, Agostino Riva^b, Vanni Borghi^c, Roberto D'Amico^d, Cinzia Del Giovane^d, Claudio Casoli^b, Massimo Galli^b, Elisa Vicenzi^e, Lara Gibellini^a, Sara De Biasi^a, Mario Clerici^{f,g}, Cristina Mussini^{a,c}, Andrea Cossarizza^a, Marcello Pinti^h

^a Department of Surgery, Medicine, Dentistry and Morphological Sciences, University of Modena and Reggio Emilia, Via Campi 287, 41125 Modena, Italy

^b Infectious Diseases Clinics, University of Milan, Milan, Italy

^c Infectious Diseases Clinics, Azienda Ospedaliero-Universitaria, Modena, Italy

^d Department of Diagnostic and Clinical Medicine and Public Health, University of Modena and Reggio Emilia, Modena, Italy

^e Viral Pathogens and Biosafety Unit, San Raffaele Scientific Institute, Milan, Italy

^f University Medical School, DISP LITA Vialba, Milan, Italy

^g Don C. Gnocchi Foundation, IRCCS, Milan, Italy

^h Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

ARTICLE INFO

Article history:

Received 13 June 2012

Received in revised form 27 December 2012

Accepted 6 January 2013

Corresponding Editor: Ziad Memish, Riyadh, Saudi Arabia

Keywords:

HIV

Long-term non-progressors

Host genetics

Polymorphism

Disease progression

SUMMARY

Objectives: About 2–5% of HIV-1-infected subjects, defined as long-term non-progressors (LTNPs), remain immunologically stable for a long time without treatment. The factors governing this condition are known only in part, and include genetic factors. Thus, we studied 20 polymorphisms of 15 genes encoding proinflammatory and immunoregulatory cytokines, chemokines and their receptors, genes involved in apoptosis, and the gene HCP5.

Methods: We analyzed 47 Caucasian LTNPs infected for >9 years, compared with 131 HIV-1-infected Caucasian patients defined as 'usual progressors'. The genotypes were determined by methods based upon PCR, and the statistical analysis was performed by univariate logistic regression.

Results: The well-known CCR5 Δ 32 del32 allele, the cell death-related TNF- α -238 A and PDCD1-7209 T alleles, and HCP5 rs2395029 G, a non-coding protein associated with the HLA-B*5701, were found positively associated with the LTNP condition. No association was observed for other single nucleotide polymorphisms (SDF-1-801, IL-10-592, MCP-1-2518, CX3CR1 V249I, CCR2V64I, RANTES-403, IL-2-330, IL-1 β -511, IL-4-590, FASL IVS3nt-169, FAS-670, FAS-1377, FASL IVS2nt-124, PDCD1-7146, MMP-7-181, and MMP7-153).

Conclusions: The novel genetic associations between allelic variants of genes TNF- α -238 and PDCD1-7209 with the LTNP condition underline the importance of host genetic factors in the progression of HIV-1 infection and in immunological preservation.

© 2013 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The complexity of HIV-1 infection is influenced by individual variability, especially as far as susceptibility to infection and disease progression are concerned. About 2–5% of HIV-1-infected patients, defined as long-term non-progressors (LTNPs), can remain asymptomatic in the absence of therapy, from 7 up to 20 years, and with a CD4⁺ cell count >500 cells/ μ l.¹ This phenomenon is influenced by virus–host interactions and can be

influenced by the host genetic polymorphisms.² In this regard, polymorphisms in chemokine receptors that mediate virus entry significantly influence the pathogenesis and progression of HIV-1 disease.³ However, genetic variants of cytokines that modulate the infection and replication of HIV-1 can potentially influence disease progression.⁴ Indeed, it has been shown that interleukin (IL)-10 can inhibit viral replication,^{5,6} whereas proinflammatory cytokines, such as tumor necrosis factor alpha (TNF- α) and IL-1 stimulate HIV-1 replication.^{7,8} Conversely, HIV-1 causes impairment in the cytokine network, determining a decrease in T helper type 1 cytokines and an increase of both T-helper type 2 cytokines (IL-4 and IL-10) and proinflammatory cytokines such as TNF- α and IL-1.⁷

* Corresponding author. Tel.: +39 059 2055428; fax: +39 059 2055426.
E-mail address: milena.nasi@unimore.it (M. Nasi).

Considering the importance of apoptosis in the maintenance of T cell homeostasis and the ability of HIV-1 to alter the delicate balance in the expression of apoptosis-related genes in the immune system, the study of FAS ligand (FASL/CD178) and FAS (CD95) variants seem particularly interesting. Despite their pivotal role in the pathogenesis of HIV/AIDS,^{9–14} there are very few data on the polymorphisms of these genes and disease progression.¹⁵

Furthermore, variants of genes involved in T cell exhaustion,^{16,17} or in their regulation,^{18,19} could contribute to explain the inter-individual variability of the immune system impairment. Accordingly, the aim of our study was to investigate 20 well-known polymorphisms of 15 key genes involved in different aspects of innate and adaptive immunity in HIV-1 pathogenesis. Some of these genetic variants have been investigated previously in different inflammatory diseases or at different stages of HIV infection, but they have never been evaluated in LTNPs.

2. Methods

2.1. Patients

Forty-seven Caucasian LTNPs were enrolled in our study. LTNPs were defined by CD4 criteria as HIV-positive patients with prolonged, AIDS-free survival in the absence of antiretroviral therapy (ART) for at least 9 years, with an elevated CD4 cell count always higher than 500 cells/ μ l. None of these patients could be defined as elite controllers, as they were heterogeneous in their degree of virologic control and were not characterized by a serial viral load test below the limit of detection.²⁰ LTNPs were enrolled in the framework of the multicenter project ELVIS (Evaluation of Long Term Non Progressors Viro-Immunological Study), which comprises the Infectious Diseases Clinic of Modena University Hospital, the Viral Pathogens and Biosafety Unit of San Raffaele Scientific Institute in Milan, and the University Medical School, DISP LITA Vialba in Milan.

As controls, we studied a population of 131 HIV-1-positive subjects defined as 'usual progressors' (UPs). These were patients who showed a symptomatic infection or initiation of ART and at least one CD4+ T cell count below 250 cells/ μ l from the first documented HIV-1 infection. Each control was enrolled from the cohort followed at the Infectious Diseases Clinic of Modena University Hospital. Demographic and clinical characteristics of the patients at inclusion are summarized in Table 1. Written informed consent was obtained from the patients before study entry, and the human experimentation guidelines of the authors' institutions were followed.

2.2. Analysis of polymorphisms

DNA was extracted from 2 ml of peripheral blood using a QIAamp Blood Midi Kit from Qiagen (Alameda, CA, USA), following the manufacturer's instructions. All the genotypic analyses were based on PCR reactions, performed in a PE 9700 Thermal Cycler (PerkinElmer, Boston, MA, USA). Fourteen polymorphisms were detected by restriction fragment length polymorphism (RFLP) as

previously described: TNF- α -238,²¹ IL-10-592,²² MMP-7-181,²³ SDF-1-801,²⁴ RANTES-403,²⁵ MCP-1,²⁶ CX3CR1 V249I,²⁴ PDCD1-7209,²⁷ CCR2V64I,²⁴ IL-2-330,²⁸ IL-1 β -511,²⁹ IL-4-590,²⁸ FAS-670,³⁰ and PDCD1-7146.²⁷ The 32-bp deletion of CCR5 gene was detected by PCR.³¹ The FAS-1377 single nucleotide polymorphism (SNP)^{30,32} was detected by allele-specific amplification (ASA)-PCR, while MMP-7-153,²³ FASL IVS2nt -124, and FASL IVS3nt -169³⁰ were evaluated by amplification created restriction site (ACRS) assay, as previously described. Finally, HCP5 rs2395029 is a G/T substitution³³ that we have detected by RFLP method using the primers HCPC5dir (TCATTGTGTGACAGCAGCC) and HCP5rev (TCCCATTCCTCAACTCACC). The annealing temperature of the PCR reaction was 61 °C and digestion of the PCR product (268 bp) was performed by the restriction enzyme XcmI, generating two fragments of 151 and 117 bp in the presence of T allele.

2.3. Statistical analysis

Fisher's exact test was used to test if the genotype distribution in both LTNPs and UPs is different from those predicted by the Hardy–Weinberg equilibrium.³⁴ The non-parametric Mann–Whitney test was used to evaluate if groups were matched for age, as the distribution of normality for age was not satisfied, and Fisher's exact was used to test if groups were sex-matched. The above-mentioned analyses were performed using Prism 4.0 software.

The association between the LTNP condition and a genotype or an allele was assessed by univariate logistic regression. The estimate odds ratios (OR) and their 95% confidence intervals (CI) were calculated. For genotype, the homozygous common allele group was used as reference, while for allele the common allele was considered as reference. The OR measures the odds of being in the LTNP condition for the non-common genotype versus the common genotype and for the non-common allele versus the common allele. The Bonferroni correction for multiple testing was applied when necessary;³⁵ the Bonferroni corrected α value was set at 0.0025. All the analyses were performed using STATA 10.

3. Results

One hundred seventy-eight Italians of Caucasian origin were included in this study. Of these patients, 131 were UPs and 47 were LTNPs. The groups were matched for sex ($p = 0.320$; Fisher's exact test) and age ($p = 0.127$; Mann–Whitney test; Table 1). Twenty polymorphisms of 15 genes were analyzed, evaluating the differences in genotype and allele frequencies between UPs and LTNPs; 11 polymorphisms are related to cytokine and chemokine genes, eight polymorphisms are related to programmed cell death, and one polymorphism has no direct role in the immune system, but is in linkage disequilibrium with HLA alleles (HCP5 rs2395029). The distribution of the genotypes of each polymorphism in both LTNPs and UPs did not differ significantly from those predicted by the Hardy–Weinberg equilibrium.

In Table 2, we report the number of genotypes and alleles, and the respective frequencies (in percentage), the OR, the 95% CI, and the p -value for the two groups (LTNPs and UPs). As reported in the

Table 1
Characteristics of long-term non-progressors (LNTN) and usual progressors (UP)

	LTNP (n=47)	UP (n=131)	p-Value
Males	29 (61.7)	92 (70.2)	0.356
Age, years	45 (30–58)	46 (29–79)	0.127
Years of infection	20 (9–25)	1 (1–16)	<0.0001
CD4+ count, cells/ μ l	792.0 (511.0–1278.0)	145.0 (27–713.0)	<0.0001
Log ₁₀ plasma VL, copies/ml	3.33 (1.70–5.38)	5.10 (2.20–6.00)	<0.0001
Undetectable viral load	11 (23.4)	0 (0.0)	<0.0001

Results are n (%), or median (range). VL, viral load.

Table 2

Association between genotype/allele frequencies and the condition of long-term non-progressor. For each gene polymorphism, we indicate the number and percentage of long-term non-progressors (LTNPs) and usual progressors (UPs) with a given genotype or allele, the odds ratio (OR), the 95% confidence interval (CI), and the *p*-value. The common allele and the homozygous genotype of common allele are considered as the reference

Gene polymorphism	Genotype/allele	LTNPs, n (%)	UPs, n (%)	OR	95% CI	<i>p</i> -Value
1. IL-1 β -511 (rs16944)	C/C	23 (52.3)	64 (48.9)	Reference		
	C/T	16 (36.4)	58 (44.3)	0.78	0.37–1.62	0.508
	T/T	5 (11.4)	9 (6.9)	1.54	0.47–5.09	0.474
	C	62 (70.5)	186 (71.0)	Reference		
2. TNF α -238 (rs361525)	T	26 (29.5)	76 (29.0)	1.03	0.58–1.79	0.923
	G/G	27 (57.4)	116 (89.9)	Reference		
	G/A	20 (42.6)	13 (10.1)	6.55	2.90–14.79	<0.0001 ^a
	A/A	0 (0.0)	0 (0.0)	-	-	-
3. IL-2-330 (rs2069762)	G	74 (78.7)	245 (95.0)	Reference		
	A	20 (21.3)	13 (5.0)	5.09	2.27–11.65	<0.0001 ^a
	T/T	25 (52.3)	89 (67.9)	Reference		
	T/G	21 (44.7)	40 (30.5)	1.85	0.93–3.68	0.081
4. IL-4-590 (rs2243250)	G/G	1 (2.1)	2 (1.5)	1.76	0.15–20.22	0.650
	T	71 (75.5)	218 (83.2)	Reference		
	G	23 (24.5)	44 (16.8)	1.60	0.86–2.93	0.102
	C/C	29 (63.0)	76 (58.0)	Reference		
5. IL-10-592 (rs1800872)	C/T	13 (28.3)	49 (37.4)	0.71	0.34–1.50	0.369
	T/T	4 (8.7)	6 (4.6)	1.75	0.46–6.64	0.413
	C	71 (77.2)	201 (76.7)	Reference		
	T	21 (22.8)	61 (23.3)	0.97	0.52–1.76	0.929
6. CCR5del32 (rs333)	C/C	21 (44.7)	83 (66.9)	Reference		
	C/A	21 (44.7)	36 (29.0)	2.28	1.11–4.68	0.025
	A/A	5 (10.6)	5 (4.0)	3.90	1.03–14.75	0.045
	C	63 (67.0)	202 (81.5)	Reference		
7. RANTES-403 (rs2107538)	A	31 (33.0)	46 (18.5)	2.16	1.21–3.81	0.004
	wt/wt	36 (78.3)	124 (94.7)	Reference		
	wt/del32	10 (21.7)	7 (5.3)	4.88	1.73–13.74	0.003
	del32/del32	0 (0.0)	0 (0.0)	-	-	-
8. CCR2 V64I (rs1799864)	wt	82 (89.1)	255 (97.3)	Reference		
	del32	10 (10.9)	7 (2.7)	4.44	1.46–14.15	0.002 ^a
	G/G	32 (69.6)	73 (55.7)	Reference		
	G/A	12 (26.1)	57 (43.5)	0.49	0.23–1.03	0.061
9. MCP-1-2518 (rs1024611)	A/A	2 (4.3)	1 (0.8)	4.56	0.40–52.14	0.222
	G	76 (82.6)	203 (77.5)	Reference		
	A	16 (17.4)	59 (22.5)	0.72	0.37–1.37	0.300
	A/A	28 (63.6)	102 (77.9)	Reference		
10. CX3CR1 V249I (rs3732379)	A/G	16 (36.4)	29 (22.1)	2.08	0.99–4.37	0.053
	G/G	0 (0.0)	0 (0.0)	-	-	-
	A	72 (81.8)	233 (88.9)	Reference		
	G	16 (18.2)	29 (11.1)	1.78	0.85–3.62	0.085
11. SDF-1-801 (rs1801157)	A/A	23 (53.5)	81 (61.8)	Reference		
	A/G	18 (41.9)	41 (31.3)	1.53	0.74–3.14	0.251
	G/G	2 (4.7)	9 (6.9)	0.77	0.15–3.83	0.753
	A	64 (74.4)	203 (77.5)	Reference		
12. FAS-670 (rs1800682)	G	22 (25.6)	59 (22.5)	1.18	0.64–2.14	0.560
	C/C	22 (51.2)	58 (44.3)	Reference		
	C/T	20 (46.5)	62 (47.3)	0.83	0.41–1.69	0.618
	T/T	1 (2.3)	11 (8.4)	0.23	0.03–1.93	0.178
13. FAS-1377 (rs2234767)	C	64 (74.4)	178 (67.9)	Reference		
	T	22 (25.6)	84 (32.1)	0.73	0.40–1.30	0.257
	G/G	31 (70.5)	65 (49.6)	Reference		
	G/A	13 (2.5)	54 (41.2)	0.50	0.24–1.04	0.065
14. FAS-1377 (rs2234767)	A/A	0 (0.0)	12 (9.2)	-	-	-
	G	75 (85.2)	184 (70.2)	Reference		
	A	13 (14.8)	78 (29.8)	0.41	0.20–0.80	0.005
	A/A	11 (25.6)	34 (26.2)	Reference		
15. FAS-1377 (rs2234767)	A/G	23 (53.5)	58 (44.6)	1.25	0.54–2.87	0.604
	G/G	9 (20.9)	38 (29.2)	0.73	0.27–1.98	0.539
	A	45 (47.7)	126 (48.5)	Reference		
	G	42 (52.3)	134 (51.5)	0.86	0.51–1.44	0.534
16. FAS-1377 (rs2234767)	G/G	29 (74.4)	102 (79.1)	Reference		

Table 2 (Continued)

Gene polymorphism	Genotype/allele	LTNPs, n (%)	UPs, n (%)	OR	95% CI	p-Value
14. FASL IVS2nt-124 (rs5030772)	G/A	9 (23.1)	21 (16.3)	1.49	0.62–3.61	0.374
	A/A	1 (2.6)	6 (4.7)	0.58	0.07–5.02	0.621
	G	67 (85.9)	225 (87.2)	Reference		
	A	11 (14.1)	33 (12.8)	1.12	0.48–2.42	0.763
	A/A	30 (65.2)	98 (74.8)	Reference		
15. FASL IVS3nt-169 (rs11385743)	A/G	15 (32.6)	32 (24.4)	1.51	0.72–3.17	0.269
	G/G	1 (2.2)	1 (0.8)	3.23	0.20–53.27	0.412
	A	75 (81.5)	228 (87.0)	Reference		
	G	17 (18.5)	34 (13.0)	1.52	0.75–2.98	0.196
	T/T	32 (68.1)	107 (82.3)	Reference		
16. MMP7-153 (rs11568819)	T/delT	15 (31.9)	22 (16.9)	2.26	1.05–4.86	0.037
	delT/delT	0 (0.0)	1 (0.8)	-	-	-
	T	79 (84.0)	236 (90.8)	Reference		
	delT	15 (16.0)	24 (9.2)	1.87	0.86–3.92	0.074
	C/C	39 (84.8)	119 (90.8)	Reference		
17. MMP7-181 (rs11568818)	C/T	7 (15.2)	12 (9.2)	1.94	0.70–5.35	0.200
	T/T	0 (0.0)	0 (0.0)	-	-	-
	C	85 (92.4)	250 (95.4)	Reference		
	T	7 (7.6)	12 (4.6)	1.71	0.55–4.89	0.267
	A/A	16 (36.4)	27 (20.6)	Reference		
18. PDCD1-7146 (rs11568821)	A/G	22 (50.0)	71 (54.0)	0.65	0.30–1.39	0.267
	G/G	6 (13.6)	33 (25.2)	0.46	0.16–1.33	0.152
	A	54 (61.4)	137 (52.3)	Reference		
	G	34 (38.6)	125 (47.7)	0.57	0.34–0.97	0.027
	G/G	25 (65.8)	99 (75.6)	Reference		
19. PDCD1-7209 (rs41386349)	G/A	11 (28.9)	30 (22.9)	1.44	0.63–3.26	0.385
	A/A	2 (5.3)	2 (1.5)	3.92	0.52–29.21	0.183
	G	61 (80.3)	228 (87.0)	Reference		
	A	15 (19.7)	34 (13.0)	1.65	0.78–3.35	0.141
	C/C	27 (65.9)	110 (84.0)	Reference		
20. HCP5 (rs2395029)	C/T	11 (26.8)	20 (15.3)	2.22	0.95–5.18	0.065
	T/T	3 (7.3)	1 (0.8)	12.11	1.21–121.05	0.034
	C	65 (79.3)	240 (91.6)	Reference		
	T	17 (20.7)	22 (8.4)	2.85	1.33–5.99	0.002 ^a
	T/T	30 (69.8)	126 (96.2)	Reference		
	T/G	13 (30.2)	5 (3.8)	10.83	3.58–32.73	<0.0001 ^a
	G/G	0 (0.0)	0 (0.0)	-	-	-
	T	73 (84.9)	257 (98.1)	Reference		
	G	13 (15.1)	5 (1.9)	9.15	2.92–33.61	<0.0001 ^a

^a $p \leq 0.002$.

OR column, we have considered as reference the common allele and the homozygous genotype. As far as polymorphisms of cytokines and chemokines are concerned, in our study the alleles TNF- α -238 A and CCR5 Δ 32 del32 were positively associated with the LTNP condition, whereas no association was observed for the other polymorphisms.

Regarding polymorphisms of genes involved in cell death and in the regulation of the immune response, the allele PDCD1-7209 T was associated with the LTNP condition, whereas the alleles of the other polymorphisms did not show any association with the LTNP condition. Finally, HCP5 rs2395029 G allele was significantly overrepresented in LTNPs as compared to UPs; none of these variants showed any significant co-segregation.

4. Discussion

Allelic variants in the human genome regulate either susceptibility or resistance to HIV-1 infection and disease progression; thus, the analysis of frequencies of polymorphisms in patients able to control viral replication and to maintain a high number of CD4+ T cells is crucial to identify those alleles that could contribute to limit viral replication.²⁰ We have studied a cohort of 47 LTNPs,

found in the framework of a collaborative study that has involved several Italian clinical centers.

As expected, a significant number of these patients presented the CCR5 Δ 32 deletion, crucial in the control of the virus (reviewed in Reiche et al.³⁶). In contrast, we found no correlation between the condition of LTNP and the allelic variants of other genes (RANTES-403, SDF-1-801, CCR2-64I, MCP-1-2518, and CX3CR1 V249I). However, it should be noted that the role of these variants in the progression of HIV-1 infection, including their role in LTNP, is still controversial,^{36–40} and further studies are required to clarify this point.

The most striking result that we found in the analysis of the polymorphisms of proinflammatory cytokines was the favorable role of the allele TNF- α -238 A in delaying the progression of HIV-1 infection. Although the functional role of the TNF- α -238 rare allele A on transcriptional activity is not clear,⁴¹ this variant could be related to a different production of the molecules, at least in some particular subjects, and thus to a diminished viral replication, contributing to the immunological preservation and disease control that characterize LTNPs.

The over-representation of the PDCD1-7209 T allele in LTNPs suggests a possible protective role of the T allele in HIV-1 infection.

Along with functional studies, the analysis of a larger number of patients will be necessary to test this possibility and to identify the mechanisms underlying the single, and eventually additive, effects. As the engagement of PDCD-1 by its ligands inhibits immune responses, and as PDCD1 is highly expressed on exhausted T cells during different infections, it is possible that during HIV-1 infection these polymorphisms modulate the expression of PDCD1/PDCD1L1 and reduce their capability to exhaust virus-specific CD8+ T cells.^{16,17} Moreover, since it has recently been demonstrated that the microbial translocation during chronic HIV-1 infection leads to an up-regulation of PDCD1 with a subsequent increase in IL-10,⁴² it would be interesting to evaluate the impact of PDCD1-7209 T allele on PDCD1 expression. Indeed, even if the frequency of the IL-10-592 A allele does not reach statistical significance after multiple comparison correction, such a variant shows an interesting higher frequency in LTNPs.^{43–45}

In vitro studies on CD4+ T cells from patients carrying or not the two mutations indicated above, after infection with HIV-1, could clarify if the presence of alternative alleles leads to different levels of protein expression, both for TNF- α and PDCD-1.

Finally, the association of the HCP5 rs2395029 G allele with the LTNP condition is in agreement with recent reports from genome-wide association studies, which identified this allele as positively associated with the viral set point as predictor of disease progression.³³ It is well known that the HCP5 allele G is in linkage disequilibrium with HLA-B*5701 in populations with European ancestry,^{46,47} and can be used as a screening tool for HLA typing;⁴⁸ our data further confirm the central role of this locus in the ability to control disease progression.

The main limitation of this study is the limited sample size and the relative diversity of the two groups, which is due to the difficulty in recruiting LTNP patients. However, our data will help to understand possible mechanisms that characterize non-progressive infections.

In conclusion, we found two novel genetic associations between allelic variants of TNF- α -238 and PDCD1-7209 genes and the LTNP condition. The significance of these differences is still not completely clear, but our data could indicate that the LTNP condition is likely due to a complex association of several genetic variants that collectively contribute to the observed phenotype, rather than to a single, crucial gene variant. Our data further underline the importance of host genetic factors in the progression of HIV-1 infection, evidencing the possible role of PDCD1 variants. We are well aware of the difficulty in identifying a possible, complex genotype that unequivocally characterizes LTNPs; however, if our data are confirmed by further analyses that consider larger cohorts of patients, they could represent another piece of this complex puzzle.

Acknowledgements

This study has been supported by the “Concerted Action ELVIS: Evaluation of Long term Non Progressors: Viro-Immunological Study”, in the framework of the Progetto Nazionale AIDS 2007 – Istituto Superiore di Sanità, Rome, Italy (grants to MG, MC, EV, and AC).

Conflict of interest: The authors declare no conflict of interest.

References

- Okulicz JF, Marconi VC, Landrum ML, Wegner S, Weintrob A, Ganesan A, et al. Clinical outcomes of elite controllers, viremic controllers, and long-term non-progressors in the US Department of Defense HIV natural history study. *J Infect Dis* 2009;**200**:1714–23.
- Fellay J, Shianna KV, Telenti A, Goldstein DB. Host genetics and HIV-1: the final phase? *PLoS Pathog* 2010;**6**:e1001033.
- Chatterjee K. Host genetic factors in susceptibility to HIV-1 infection and progression to AIDS. *J Genet* 2010;**89**:109–16.
- van Manen D, Delaneau O, Kootstra NA, Boeser-Nunnink BD, Limou S, Bol SM, et al. Genome-wide association scan in HIV-1-infected individuals identifying variants influencing disease course. *PLoS One* 2011;**6**:e22208.
- Kollmann TR, Pettoello-Mantovani M, Katopodis NF, Hachamovitch M, Rubinstein A, Kim A, et al. Inhibition of acute in vivo human immunodeficiency virus infection by human interleukin 10 treatment of SCID mice implanted with human fetal thymus and liver. *Proc Natl Acad Sci U S A* 1996;**93**:3126–31.
- Weissman D, Poli G, Fauci AS. Interleukin 10 blocks HIV replication in macrophages by inhibiting the autocrine loop of tumor necrosis factor alpha and interleukin 6 induction of virus. *AIDS Res Hum Retroviruses* 1994;**10**:1199–206.
- Kedzierska K, Crowe SM. Cytokines and HIV-1: interactions and clinical implications. *Antivir Chem Chemother* 2001;**12**:133–50.
- Kreuzer KA, Dayer JM, Rockstroh JK, Sauerbruch T, Spengler U. The IL-1 system in HIV infection: peripheral concentrations of IL-1beta, IL-1 receptor antagonist and soluble IL-1 receptor type II. *Clin Exp Immunol* 1997;**109**:54–8.
- Cossarizza A. Apoptosis and HIV infection: about molecules and genes. *Curr Pharm Des* 2008;**14**:237–44.
- Franceschi C, Franceschini MG, Boschini A, Trenti T, Nuzzo C, Castellani G, et al. Phenotypic characteristics and tendency to apoptosis of peripheral blood mononuclear cells from HIV+ long term non progressors. *Cell Death Differ* 1997;**4**:815–23.
- Pinti M, Nasi M, Moretti L, Mussini C, Petrusca D, Esposito R, et al. Quantitation of CD95 and CD95L mRNA expression in chronic and acute HIV-1 infection by competitive RT-PCR. *Ann N Y Acad Sci* 2000;**926**:46–51.
- Cossarizza A, Stent G, Mussini C, Paganelli R, Borghi V, Nuzzo C, et al. Deregulation of the CD95/CD95L system in lymphocytes from patients with primary acute HIV infection. *AIDS* 2000;**14**:345–55.
- Pinti M, Pedrazzi J, Benatti F, Sorrentino V, Nuzzo C, Cavazzuti V, et al. Differential down-regulation of CD95 or CD95L in chronically HIV-infected cells of monocytic or lymphocytic origin: cellular studies and molecular analysis by quantitative competitive RT-PCR. *FEBS Lett* 1999;**458**:209–14.
- Cossarizza A, Mussini C, Borghi V, Mongiardino N, Nuzzo C, Pedrazzi J, et al. Apoptotic features of peripheral blood granulocytes and monocytes during primary, acute HIV infection. *Exp Cell Res* 1999;**247**:304–11.
- Vasilescu A, Heath SC, Diop G, Do H, Hirtzig T, Hendel H, et al. Genomic analysis of Fas and FasL genes and absence of correlation with disease progression in AIDS. *Immunogenetics* 2004;**56**:56–60.
- Freeman GJ, Wherry EJ, Ahmed R, Sharpe AH. Reinvigorating exhausted HIV-specific T cells via PD-1-PD-1 ligand blockade. *J Exp Med* 2006;**203**:2223–7.
- Holm M, Pettersen FO, Kvale D. PD-1 predicts CD4 loss rate in chronic HIV-1 infection better than HIV RNA and CD38 but not in cryopreserved samples. *Curr HIV Res* 2008;**6**:49–58.
- Powell WC, Fingleton B, Wilson CL, Boothby M, Matrisian LM. The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. *Curr Biol* 1999;**9**:1441–7.
- Jormsjo S, Whatling C, Walter DH, Zeiher AM, Hamsten A, Eriksson P. Allelic-specific regulation of matrix metalloproteinase-7 promoter activity is associated with coronary artery luminal dimensions among hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol* 2001;**21**:1834–9.
- Okulicz JF, Lambotte O. Epidemiology and clinical characteristics of elite controllers. *Curr Opin HIV AIDS* 2011;**6**:163–8.
- Fargion S, Valenti L, Dongiovanni P, Scaccabarozzi A, Fracanzani AL, Taioli E, et al. Tumor necrosis factor alpha promoter polymorphisms influence the phenotypic expression of hereditary hemochromatosis. *Blood* 2001;**97**:3707–12.
- Naicker DD, Werner L, Kormuth E, Passmore JA, Misana K, Karim SA, et al. Interleukin-10 promoter polymorphisms influence HIV-1 susceptibility and primary HIV-1 pathogenesis. *J Infect Dis* 2009;**200**:448–52.
- Lugli E, Pinti M, Nasi M, Troiano L, Prada N, Mussini C, et al. MMP-7 promoter polymorphisms do not influence CD4+ recovery and changes in plasma viral load during antiretroviral therapy for HIV-1 infection. *Int J Immunogenet* 2005;**32**:269–71.
- Puissant B, Roubinet F, Massip P, Sandres-Saune K, Apoil PA, Abbal M, et al. Analysis of CCR5, CCR2, CX3CR1, and SDF1 polymorphisms in HIV-positive treated patients: impact on response to HAART and on peripheral T lymphocyte counts. *AIDS Res Hum Retroviruses* 2006;**22**:153–62.
- Moissidis I, Chinoy B, Yanamandra K, Napper D, Thurmon T, Bocchini Jr J, et al. Association of IL-13, RANTES, and leukotriene C4 synthase gene promoter polymorphisms with asthma and/or atopy in African Americans. *Genet Med* 2005;**7**:406–10.
- Szalai C, Duba J, Prohaszka Z, Kalina A, Szabo T, Nagy B, et al. Involvement of polymorphisms in the chemokine system in the susceptibility for coronary artery disease (CAD). Coincidence of elevated Lp(a) and MCP-1-2518 G/G genotype in CAD patients. *Atherosclerosis* 2001;**158**:233–9.
- Wang SC, Chen YJ, Ou TT, Wu CC, Tsai WC, Liu HW, et al. Programmed death-1 gene polymorphisms in patients with systemic lupus erythematosus in Taiwan. *J Clin Immunol* 2006;**26**:506–11.
- Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito RB, Line SR. Frequencies of the -330 (T \rightarrow G) IL-2 and -590 (T \rightarrow C) IL-4 gene polymorphisms in a population from south-eastern Brazil. *Eur J Immunogenet* 2002;**29**:293–6.
- D’Aiuto F, Parkar M, Brett PM, Ready D, Tonetti MS. Gene polymorphisms in pro-inflammatory cytokines are associated with systemic inflammation in patients with severe periodontal infections. *Cytokine* 2004;**28**:29–34.
- Nasi M, Pinti M, Bugarini R, Troiano L, Lugli E, Bellodi C, et al. Genetic polymorphisms of Fas (CD95) and Fas ligand (CD178) influence the rise in

- CD4+ T cell count after antiretroviral therapy in drug-naïve HIV-positive patients. *Immunogenetics* 2005;**57**:628–35.
31. Nadif R, Mintz M, Rivas-Fuentes S, Jedlicka A, Lavergne E, Rodero M, et al. Polymorphisms in chemokine and chemokine receptor genes and the development of coal workers' pneumoconiosis. *Cytokine* 2006;**33**:171–8.
 32. Huang QR, Morris D, Manolios N. Identification and characterization of polymorphisms in the promoter region of the human Apo-1/Fas (CD95) gene. *Mol Immunol* 1997;**34**:577–82.
 33. Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, et al. A whole-genome association study of major determinants for host control of HIV-1. *Science* 2007;**317**:944–7.
 34. Guo SW, Thompson EA. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* 1992;**48**:361–72.
 35. Rice WR. Analyzing tables of statistical tests. *Evolution* 1989;**43**:223–5.
 36. Reiche EM, Bonametti AM, Voltarelli JC, Morimoto HK, Watanabe MA. Genetic polymorphisms in the chemokine and chemokine receptors: impact on clinical course and therapy of the human immunodeficiency virus type 1 infection (HIV-1). *Curr Med Chem* 2007;**14**:1325–34.
 37. McDermott DH, Beecroft MJ, Kleeberger CA, Al-Sharif FM, Ollier WE, Zimmerman PA, et al. Chemokine RANTES promoter polymorphism affects risk of both HIV infection and disease progression in the Multicenter AIDS Cohort Study. *AIDS* 2000;**14**:2671–8.
 38. Smith MW, Carrington M, Winkler C, Lomb D, Dean M, Huttley G, et al. CCR2 chemokine receptor and AIDS progression. *Nat Med* 1997;**3**:1052–3.
 39. Vidal F, Peraire J, Domingo P, Broch M, Knobel H, Pedrol E, et al. Lack of association of SDF-1 3'A variant allele with long-term nonprogressive HIV-1 infection is extended beyond 16 years. *J Acquir Immune Defic Syndr* 2005;**40**:276–9.
 40. Vidal F, Peraire J, Domingo P, Broch M, Cairo M, Pedrol E, et al. Polymorphism of RANTES chemokine gene promoter is not associated with long-term nonprogressive HIV-1 infection of more than 16 years. *J Acquir Immune Defic Syndr* 2006;**41**:17–22.
 41. Pociot F, D'Alfonso S, Compasso S, Scorza R, Richiardi PM. Functional analysis of a new polymorphism in the human TNF alpha gene promoter. *Scand J Immunol* 1995;**42**:501–4.
 42. Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, et al. Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. *Nat Med* 2010;**16**:452–9.
 43. D'Alfonso S, Rampi M, Rolando V, Giordano M, Momigliano-Richiardi P. New polymorphisms in the IL-10 promoter region. *Genes Immun* 2000;**1**:231–3.
 44. Shin HD, Winkler C, Stephens JC, Bream J, Young H, Goedert JJ, et al. Genetic restriction of HIV-1 pathogenesis to AIDS by promoter alleles of IL10. *Proc Natl Acad Sci U S A* 2000;**97**:14467–72.
 45. Vasilescu A, Heath SC, Ivanova R, Hendel H, Do H, Mazoyer A, et al. Genomic analysis of Th1-Th2 cytokine genes in an AIDS cohort: identification of IL4 and IL10 haplotypes associated with the disease progression. *Genes Immun* 2003;**4**:441–9.
 46. de Bakker PI, McVean G, Sabeti PG, Miretti MM, Green T, Marchini J, et al. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat Genet* 2006;**38**:1166–72.
 47. Catano G, Kulkarni H, He W, Marconi VC, Agan BK, Landrum M, et al. HIV-1 disease-influencing effects associated with ZNRD1, HCP5 and HLA-C alleles are attributable mainly to either HLA-A10 or HLA-B*57 alleles. *PLoS One* 2008;**3**:e3636.
 48. Colombo S, Rauch A, Rotger M, Fellay J, Martinez R, Fux C, et al. The HCP5 single-nucleotide polymorphism: a simple screening tool for prediction of hypersensitivity reaction to abacavir. *J Infect Dis* 2008;**198**:864–7.