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

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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 CCR5Δ32 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-1β-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.

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/μl.[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]

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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/μl. 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/μl 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-1β-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 −169[30] were evaluated by amplification created restriction site (ACRS) assay, as previously described. Finally, HCP5 rs2395029 is a G/T substitution[33] that we have detected by RFLP method using the primers HCPC5dir (TCATTGTGTGACAGCAGCC) and HCP5rev (TCCCATTCCTTCAACTCACC). 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 (LNTP) 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/μl	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	*p*-Value
1. IL-1β-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		
	T	26 (29.5)	76 (29.0)	1.03	0.58–1.79	0.923
2. TNFα-238 (rs361525)	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)	-	-	-
	G	74 (78.7)	245 (95.0)	Reference		
	A	20 (21.3)	13 (5.0)	5.09	2.27–11.65	<0.0001[a]
3. IL-2-330 (rs2069762)	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
	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
4. IL-4-590 (rs2243250)	C/C	29 (63.0)	76 (58.0)	Reference		
	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
5. IL-10-592 (rs1800872)	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		
	A	31 (33.0)	46 (18.5)	2.16	1.21–3.81	0.004
6. CCR5del32 (rs333)	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)	-	-	-
	wt	82 (89.1)	255 (97.3)	Reference		
	del32	10 (10.9)	7 (2.7)	4.44	1.46–14.15	0.002[a]
7. RANTES-403 (rs2107538)	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
	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
8. CCR2 V64I (rs1799864)	A/A	28 (63.6)	102 (77.9)	Reference		
	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
9. MCP-1-2518 (rs1024611)	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		
	G	22 (25.6)	59 (22.5)	1.18	0.64–2.14	0.560
10. CX3CR1 V249I (rs3732379)	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
	C	64 (74.4)	178 (67.9)	Reference		
	T	22 (25.6)	84 (32.1)	0.73	0.40–1.30	0.257
11. SDF-1-801 (rs1801157)	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
	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
12. FAS-670 (rs1800682)	A/A	11 (25.6)	34 (26.2)	Reference		
	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
13. 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
	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
14. FASL IVS2nt-124 (rs5030772)	A/A	30 (65.2)	98 (74.8)	Reference		
	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
15. FASL IVS3nt-169 (rs11385743)	T/T	32 (68.1)	107 (82.3)	Reference		
	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
16. MMP7-153 (rs11568819)	C/C	39 (84.8)	119 (90.8)	Reference		
	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
17. MMP7-181 (rs11568818)	A/A	16 (36.4)	27 (20.6)	Reference		
	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
18. PDCD1-7146 (rs11568821)	G/G	25 (65.8)	99 (75.6)	Reference		
	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
19. PDCD1-7209 (rs41386349)	C/C	27 (65.9)	110 (84.0)	Reference		
	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]
20. HCP5 (rs2395029)	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 CCR5Δ32 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 CCR5Δ32 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.

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