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Associations of the *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1* cytokine receptor genes with AIDS progression in a French AIDS cohort

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Abstract We have performed an extensive analysis of Th1/Th2 cytokine receptors *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1* gene polymorphisms to evaluate their impact on AIDS progression. The coding regions and promoters of these genes were sequenced in the genetics of resistance to immunodeficiency virus cohort, composed of 327 HIV-1-positive patients with extreme progression phenotypes, slow and rapid progressors, and of 446 healthy control subjects, all of them of Caucasian descent. Overall, 104 single nucleotide polymorphisms and four insertions/deletions with a minor allelic frequency higher than 1% were identified, 21 of them being newly characterized. We observed weak associations for 13 polymorphisms of *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1*, and 11 haplotypes of *IL2Rα*, *IL4Rα*, and *IFNγR1*. However, we could not relate these positive signals to any relevant biological information on the gene function. To affirm these putative associations in AIDS, further confirmation on other AIDS cohorts will be needed. This complete catalog of polymorphisms in *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1* cytokine receptor genes should also be useful for investigating associations in other immune-related diseases.

Keywords Cytokine receptors · AIDS progression · Genotyping · Genetic polymorphism · Haplotype · GRIV cohort

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Introduction

The cytokine network plays a central role in the functioning of the immune system, and it is involved in the proliferation, differentiation, and death of multiple cell types such as lymphocytes, monocytes, or dendritic cells (Curfs et al. 1997; Thomson and Lotze 2003). The cytokine receptors are essential components of this network, and it has been shown for example that the absence of IL2Rα is functionally equivalent to the absence of the corresponding cytokine (Gaffen 2001). Other studies have shown that inactivating mutations in the alpha chains of the cytokine receptors can lead to severe, possibly life-threatening immune disorders (autoimmunity, immunodeficiency and deregulation of the production of T lymphocytes for IL2Rα, severe immunodeficiency, and higher susceptibility to mycobacterial infections for IFNγR1) (Jouanguy et al. 2000; Sharfe et al. 1997; Willerford et al. 1995).

In human immunodeficiency virus type 1 (HIV-1) infection, the role and status of the cytokine network has been extensively investigated by in vivo and in vitro approaches (Alfano and Poli 2002, 2005; Clerici and Shearer 1993). If many studies have pointed out the role of cytokines in acquired immunodeficiency syndrome (AIDS), only a few have addressed the role of the cytokine receptors in HIV-1 infection. It has been reported that the viral protein Tat inhibits the expression of IL2Rα (Puri et al. 1995) and up-regulates the expression of IL4Rα (Husain et al. 1996), which is consistent with the dysregulation of the Th1/Th2 cytokine balance observed in HIV-1 infection (Clerici and Shearer 1993). Another study highlighted a disruption of the IL2R production during

HIV-1 infection, leading to a dysregulation of the IL2/IL2R system (David et al. 1998).

To better understand the involvement of the cytokine network in AIDS development, our group has previously completed an extensive genomic analysis of Th1/Th2 cytokine genes (Vasilescu et al. 2003). We found significant associations between disease progression and some haplotypes of the *IL4* and *IL10* genes, which have also been pointed out by other studies (Breen et al. 2003; Nakayama et al. 2002). Only one genetic study on *IL4Rα* has been performed on cytokine receptor genes regarding their association with AIDS (Wang et al. 2004), and to extend our previous work on Th1/Th2 cytokine genes in AIDS, we have investigated thoroughly the polymorphisms of the cytokine receptor genes *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1*. We have analyzed these genes by extensive nucleotide sequencing to clarify whether their genetic variations could influence disease onset and progression. For that, we used the genetics of resistance to immunodeficiency virus (GRIV) cohort, which consists of two subpopulations of French Caucasian HIV-1 seropositive individuals with extreme progression phenotypes: 82 patients with rapid progression (RP) and 245 patients with slow progression (SP), which are equivalent to the extreme 1% subset of a cohort of 24,500 seroconverter patients (Huber et al. 2003). We also included 446 healthy control (CTR) subjects of similar ethnic origin. The GRIV cohort is, as far as we know, the largest cohort of its kind in the world, and its usefulness has already been validated for several gene associations such as CCR5 (Rappaport et al. 1997; Hendel et al. 1998; Winkler et al. 2004) and human leukocyte antigens (HLA) (Flores-Villanueva et al. 2003; Hendel et al. 1999). Single nucleotide polymorphisms (SNPs) and other genetic variations identified through sequencing were evaluated for their association with disease susceptibility and progression.

Materials and methods

The GRIV cohort

The GRIV cohort was established in 1995 in France to generate a large collection of DNAs for genetic studies on candidate polymorphisms associated with RP and SP to AIDS. Only Caucasians of European descent living in France were recruited to limit the influence of the virogenetic and environmental factors. Patients with SP were defined as seropositive asymptomatic individuals for 8 or more years with a $CD4^+$ cell count above 500 per cubic millimeter in the absence of antiretroviral therapy. Patients with RP were defined as patients with a drop in their $CD4^+$ cell count below 300 per cubic millimeter in less than 3 years after the last seronegative test. The DNA was obtained from fresh peripheral blood mononuclear cells or from Epstein–Barr virus (EBV)-transformed cell lines. The CTR subjects are seronegative Caucasians of European descent living in France.

Genotyping

The primers and conditions used for polymerase chain reaction (PCR) amplification of the different fragments are available in Table S1 (supplementary material online). Sequencing reactions and analysis were performed as previously described (Do et al. 2005). For practical reasons, an initial screening was performed on 150 SP patients, 50 RP patients, and 150 CTR subjects for polymorphism discovery. The screening was extended to more subjects when a p value <0.1 was obtained.

Linkage disequilibrium and haplotyping

Linkage disequilibrium (LD) was computed for each pair of polymorphisms using the r standard method (Hill and Weir 1994). Haplotype estimates were obtained using the EM algorithm (Laird 1993) and the Phase2 algorithm (Stephens and Donnelly 2003; Stephens et al. 2001) either for all polymorphisms or for selected ones. The haplotype-tagging polymorphisms have been computed using the SNP-tagger software (Ke and Cardon 2003).

Statistical analysis

Statistical analyses were performed only on the polymorphisms with a minor allele frequency greater than 1% in our whole population, termed "frequent" polymorphisms. The differences in the allelic distributions (SNPs or haplotypes) between the three groups were examined as follows: for each allele (with a frequency greater than 1% in the whole population), the expected numbers of individuals in each group with and without that allele were compared using Fisher's exact test. Four modes of calculation were used for the genetic analysis: allelic frequency, dominant mode, recessive mode, and genotypic distribution. For SNPs, there are only two alleles, and the p value for the dominant mode on one allele is identical to the p value for the recessive mode on the other allele. As a consequence, when obtaining a positive signal, the mode of calculation (dominant or recessive) is precised for the A1 allele in Table 2.

Results

SNP discovery

We systematically screened the *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1* genes for polymorphisms by resequencing the exons with their flanking regions and the promoter region (*IL2Rα*, *IL4Rα*, and *IFNγR1*). In the case of *IL10Rα*, the promoter was not known, and we sequenced the 1-kb region upstream of the first exon, assuming that putative regulatory elements are located within that region. We identified 108 frequent (minor allele frequency equal or superior to 1% in our whole population) polymorphisms on

these genes, most of them being SNPs (Fig. 1). Out of these 108 polymorphisms, 27 polymorphisms were on the *IL2Rα* gene, 51 polymorphisms on the *IL4Rα* gene, 19 polymorphisms on the *IL10Rα* gene, and 11 polymorphisms on the *IFNγR1* gene (Fig. 1). Twenty-one out of these 108 polymorphisms are newly characterized by our study. On average, we found one polymorphism with a frequency higher than 1% in every 300 bp (Table 1), which is consistent with previous studies (Cargill et al. 1999; Nelson et al. 2004).

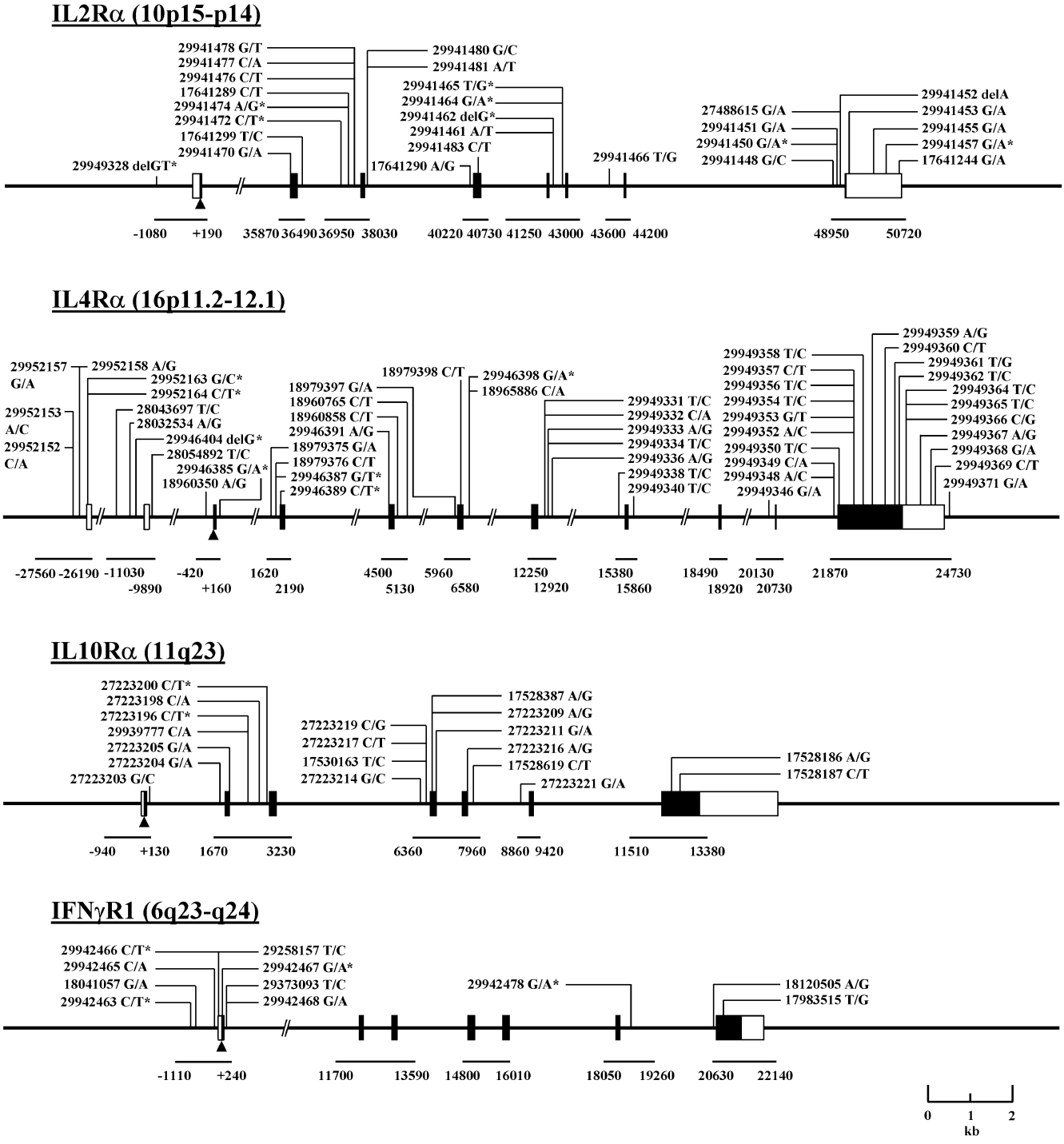

Fig. 1 Genetic organization of the *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1* genes. Coding and untranslated regions are indicated by *solid* and *open rectangles*, respectively. The regions that have been sequenced are indicated by a *horizontal line*, with start and end positions according to the first nucleotide of the initiation codon as +1 (indicated by a *black triangle*). The polymorphism numbers are the attribution numbers from the CNG database (the correspondence with Genbank SNP database is given in Tables 2 and S2). The newly characterized polymorphisms are indicated by an *asterisk*. The genomic sequences used for alignment are NT_077569.2 (IL2Rα), NT_010393.15 (IL4Rα), NT_033899.7 (IL10Rα), and NT_025741.13 (IFNγR1)

Table 1 Incidence of polymorphisms identified in the *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1* cytokine receptor genes

Gene	IL2Rα	IL4Rα	IL10Rα	IFNγR1	Total
Frequent polymorphisms	27	51	19	11	108
Newly identified	8	7	2	4	21
Located in exons	6	21	7	2	36
Synonymous	2	5	3	2	12
Nonsynonymous	0	9	4	0	13
UTR	4	7	0	0	11
Base pair sequenced	7,600	9,950	6,650	7,170	31,370
Frequency (polymorphism/base pair sequenced)	1/281	1/195	1/350	1/652	1/290

Associations with AIDS progression

This case-control study is based on the comparison of the allelic distributions of the polymorphisms between the patient populations (SP and RP) and the CTR population. Table 2 provides information regarding the polymorphisms exhibiting positive (p value from the Fisher's exact test lower than 0.05) or borderline (p value lower than 0.1) association signals, the allelic frequencies, and the localization (intron, promoter, or exons with the amino acid changes when applicable). The SNP allele frequencies shown in Table 2 and Table S2 are consistent with those given in SNP database (dbSNP) except for the SNPs IL10Rα_27223204, IL10Rα_27223209, IL4Rα_29949368, and IL4Rα_29949371, for which a difference in frequencies higher than 10% is observed. Most of the identified polymorphisms respect the Hardy–Weinberg equilibrium (HWE) in cases and in CTRs. Only three polymorphisms did not fully respect the HWE, all in the SP patients group: IFNγR1_18041057 (p=0.19), IL4Rα_28043697 (p=0.12), and IL4Rα_28054892 (p=0.25) (we used a cut-off of 0.3 since p values lower than 0.3 for HWE tests are very rare in our cohort).

In the *IL2Rα* gene, two SNPs (29941451 and 27488615) and one deletion (29941452) exhibited significant associations ($p \leq 0.05$) between SP and CTR populations. In the *IL4Rα* gene, seven SNPs (29952157, 28043697, 28054892, 18979375, 18979376, 29949352, and 29949353) exhibited positive signals. In the *IL10Rα* gene, two SNPs (27223205 and 29939777), which are in LD, exhibit significant associations between the SP and CTR populations. In the *IFNγR1* gene, the SNP IFNγR1_29942463 exhibits a significant association between the RP and CTR populations (p=0.032). Out of the 13 positive signals ($p \leq 0.05$) found in the analysis of the populations two by two, only four remained positive when comparing simultaneously the three groups SP, RP, and CTR with a χ^2 test (Table 2); thus, the comparison between the populations two by two appears more sensitive.

Overall, positive or borderline signals were obtained for only four SNPs introducing an amino acid change (Table 2). These amino acid changes are not located in known active domains (binding or signal transduction) of these proteins. Two SNPs located in promoter regions exhibited significant associations: IL4Rα_29952157 and IFNγR1_29942463.

Given the high number of polymorphisms, the haplotype estimation programs could not take all of them into account for the genes *IL2Rα* and *IL4Rα*. We had to split the polymorphisms into smaller groups. Thus, it appeared more informative to compute the haplotypes based on subgroups of polymorphisms linked to protein variations or located in promoter regions rather than using all of them. Table 3 presents the significant and borderline p values obtained for the estimated haplotypes, and Table S3 of supplementary material online presents the composition of all haplotypes. To perform the haplotype calculation in the *IL2Rα* gene, we had to exclude the relatively rare polymorphisms (with a global minor allele frequency lower than 3%). We were thus able to compute haplotypes and found some positive signals. The lowest p value (p=0.041) was obtained for the haplotype IL2Rα_Over3%_8 for the comparison between the RP and CTR populations (Table 3). We also used a smaller group of polymorphisms, with a global allelic frequency greater than 10%. Using 10% as a cut-off is interesting because it yields the most frequent haplotypes in the population. The lowest p value (p=0.006) was obtained for the haplotype IL2Rα_Over10%_7 between RP and CTR populations (Table 3). This haplotype is actually a subhaplotype of IL2Rα_Over3%_8 (Table S3 of supplementary material online). The number of polymorphisms in *IL4Rα* was also too high to perform a complete calculation. By selecting the SNPs with a minor allele frequency higher than 3%, a signal was identified for haplotype IL4Rα_Over3%_1 (Table 3). By using a 10% threshold, the signal remained with the corresponding subhaplotype IL4Rα_Over10%_1 (Table 3). We also computed haplotypes with polymorphisms located in the promoter region, nonsynonymous SNPs (NS), and nonsynonymous SNPs that introduce changes in the intracellular domain of the receptors (NSintra) (Table 3). The haplotype IL4Rα_NS_3 is based on nine SNPs, only one of them being extracellular: the variation Ile75Val (Table 2). Out of these nine SNPs, five correspond to a serine change in the intracellular domain. The haplotype IL4Rα_NS_3 presents a positive signal (p=0.041) for the comparison between the three populations and for the comparison between the SP and RP populations as well (p=0.02). The haplotype IL4Rα_NSintra_3, which is a subhaplotype of IL4Rα_NS_3, also presented a significant association (p=0.016) between the SP and RP populations. However, it was not possible to correlate this haplotype

Table 2 List of polymorphisms with a positive ($p \leq 0.05$) or borderline ($p < 0.1$) signal for the *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1* genes

Gene	CNG ID	A1	A2	Allelic frequency (A1) (%)			Localization and amino acid change	Nominal p value for test statistics[a]										References to previous studies of the variant and new IDs
								SP vs CTR					RP vs CTR					SP vs RP vs CTR	
				CTR	RP	SP		AF	OR	D/R	OR	G	AF	OR	D/R	OR	G	AF	
IL2Rα	29941472	C	T	99.28	96.67	99.30	Intron 2	1.000					0.098	0.212	0.096 (R)	0.206		0.081	ss46566602 (New)
	29941476	C	T	77.89	84.44	75.36	Intron 2	0.491				0.092	0.233		0.070 (D)	1.286		0.196	rs12722574
	29941481	A	T	60.14	56.67	63.31	Intron 3	0.484					0.621		0.072 (D)	0.466		0.492	rs12358961
	29941448	G	C	93.53	93.48	90.88	Intron 7	0.140		0.087 (D)	4.993		1.000					0.318	rs10508300
	29941451	G	A	87.02	88.04	92.34	Intron 7	0.018	0.557	0.024 (R)	0.550	0.065	0.871					0.058	rs12243993
	27488615	G	A	82.06	85.87	87.13	Intron 7	0.050	0.676	0.026 (R)	0.601	0.065	0.471					0.116	rs7076103
	29941452	A	–	88.77	88.04	92.96	Intron 7	0.050	0.599	0.038 (R)	0.566	0.035	0.862					0.125	rs12722599
	17641244	G	A	49.31	46.67	42.81	3' UTR	0.130		0.067 (D)	1.658		0.718					0.297	rs1570538
IL4Rα	29952157	G	A	64.24	72.92	57.72	Promoter	0.119		0.047 (R)	1.655		0.135					0.024	rs12927172
	28043697	T	C	72.30	72.64	64.48	Intron 1	0.050	0.696	0.010 (R)	1.878	0.023	1.000					0.085	rs6498013
	28054892	T	C	71.28	73.58	64.60	Intron 2	0.089	1.360	0.017 (R)	1.788	0.036	0.707					0.120	rs7190472
	18979375	G	A	82.68	81.73	89.24	Intron 3	0.025	0.576	0.026 (R)	0.542	0.031	0.882					0.044	rs3024543
	18979376	C	T	82.68	82.69	89.93	Intron 3	0.012	0.534	0.012 (R)	0.497	0.014	1.000					0.027	rs3024544
	29949349	C	A	83.77	81.63	82.37	Intron 10	0.659					0.642		0.096 (D)	0.206		0.849	rs3024676
	29949352	A	C	89.77	84.78	90.37	Exon 11 (E400A)	0.885					0.254		0.050 (D)	0.109		0.309	rs1805011 (Deichmann et al. 1997)
	29949353	G	T	89.77	85.56	90.00	Exon 11 (L414L)	1.000					0.334		0.050 (D)	0.107		0.469	rs2234898 (Deichmann et al. 1997)
	29949354	T	C	89.08	86.67	90.67	Exon 11 (C431R)	0.575					0.570		0.091 (D)	0.200		0.550	rs1805012 (Deichmann et al. 1997)
	29949356	T	C	88.24	86.05	89.84	Exon 11 (L433L)	0.580					0.576		0.091 (D)	0.199		0.612	rs2234900 (Deichmann et al. 1997)
	29949371	G	A	64.54	64.44	56.25	3′ Ter	0.056	1.416	0.063 (R)	1.602		1.000					0.105	rs1029489

Table 2 (continued)

Gene	CNG ID	A1	A2	Allelic frequency (A1) (%)			Localization and amino acid change	Nominal p value for test statistics[a]										References to previous studies of the variant and new IDs
								SP vs CTR					RP vs CTR				SP vs RP vs CTR	
				CTR	RP	SP		AF	OR	D/R	OR	G	AF	OR	D/R	OR	G	AF	
IL10Rα	27223205	G	A	86.82	90.00	92.22	Exon 2 (A60A)	0.041	0.556	0.076 (R)	0.573	0.076	0.497					0.110	rs4252249, (Tanaka et al. 1997)
	29939777	C	A	85.66	90.00	94.08	Intron 2	0.007	0.376	0.015 (R)	0.371	0.022	0.359					0.027	rs4252251
	27223217	C	T	85.53	89.39	91.35	Intron 3	0.054	0.560	0.096 (R)	0.567	0.096	0.554					0.128	rs4252270
	17528387	A	G	51.00	60.94	54.40	Exon 4 (A153A)	0.261					0.098	0.616				0.329	rs2256111, (Gasche et al. 2003)
	17528186	A	G	27.74	30.00	33.82	Exon 7 (R351G)	0.212		0.080 (D)	0.590	0.080	0.677					0.439	rs2229113, (Gasche et al. 2003)
	17528187	C	T	95.29	89.39	96.77	Exon 7 (S420L)	0.600					0.080	2.401	0.072 (R)	0.386	0.058	0.079	rs2229114
IFNγR1	29942463	C	T	98.50	97.86	98.52	Promoter	1.000					0.479		0.032 (R)	0.700		0.841	ss46566618 (New)

For each polymorphism, we indicated the p values from the Fisher's exact tests and the odds ratios (ORs) when applicable, the localization, with the amino acid change when applicable, and the relevant information known to date. A1 represents the nucleotide found in the reference sequences, which are NT_077569.2 (*IL2Rα*), NT_010393.15 (*IL4Rα*), NT_033899.7 (*IL10Rα*), and NT_025741.13 (*IFNγR1*). The amino acid positions are taken from the reference sequences NP_000408.1 (IL2Rα), NP_000409.1 (IL4Rα), NP_001549.1 (IL10Rα), and NP_000407.1 (IFNγR1). The polymorphism numbers attributed by the CNG database (CNG ID) are shown in the second column, and the corresponding dbSNP numbers are also given in the last column. The calculation modes for the Fisher's exact tests are indicated (AF, allelic frequency; D/R, dominant/recessive; G, genotypic distribution). For the dominant/recessive mode, we indicate in parentheses the mode corresponding to the allele A1

[a]Bonferroni corrections were not performed on p values

Table 3 Positive ($p \leq 0.05$) and borderline ($p < 0.1$) signals found with the estimated haplotypes of *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1*

IL2R α_Over3%	*Polymorphisms*																		*Global*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	29949328	29941470	17641299	29941474	17641289	29941476	29941477	29941478	29941480	29941481	29941483	29941461	29941462	29941464	29941465	29941466	29941448	29941451	27488615	29941452	29941453	29941455	29941457	17641244	*Global Allelic Frequency (%)*	CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
8	GT	G	T	A	C	T	G	G	G	A	C	G	G	G	T	T	G	G	G	A	G	G	G	G	3.21	1.63	7.69	3.60	0.358		**0.044**	**0.041**	0.250			

IL2R α_Over10%	*Polymorphisms*								*Global Allelic Frequency (%)*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	29949328	17641299	17641289	29941476	29941480	29941481	29941451	27488615	29941452	17641244		CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
1	GT	T	C	T	G	A	G	G	A	A	16.24	15.00	8.06	20.00	0.201		0.202		**0.036**	**0.048**		**0.064**
3	GT	T	C	C	G	A	A	T	G	G	8.22	11.50	9.68	5.91	**0.054**		0.819		0.387			0.123
7	GT	T	C	T	G	A	G	G	A	G	3.91	1.50	9.68	4.55	**0.092**		**0.007**	**0.006**	0.129			**0.013**

IL4R_Over3%	*Polymorphisms*																														*Global*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	29952152	29952153	29952157	29952158	29952163	28043697	28032534	29946404	28054892	18960350	18979375	18979376	29946391	18960658	18960765	18979397	18979398	29949331	29949332	29949334	29949338	29949340	29949349	29949350	29949353	29949354	29949356	29949357	29949358	29949359	29949361	29949367	29949368	29949369	29949371	*Global Allelic Frequency (%)*	CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
1	A	C	A	A	G	C	G	G	C	G	18960350	G	C	G	18960658	T	G	C	C	C	C	T	T	C	A	G	T	T	C	A	T	G	A	C	A	19.77	18.00	10.71	25.25	**0.090**	**0.030**	0.226		**0.019**	**0.018**		**0.032**

IL4Rα_Over10%	*Polymorphisms*																														*Global*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	29952152	29952153	29952157	28043697	28032534	28054892	18960350	18960765	18979375	18979376	29946391	18960658	29949331	29949332	29949334	29949340	29949349	29949350	29949358	29949359	29949367	29949368	29949371	*Global Allelic Frequency (%)*	CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
1	A	C	A	C	G	C	G	G	C	G	T	T	C	C	C	T	C	T	T	A	G	A	A	25.33	22.28	16.67	31.63	**0.042**	**0.016**	0.368		**0.032**	**0.037**		**0.024**

IL4R α_Promoter	*Polymorphisms*				*Global Allelic Frequency (%)*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	29952152	29952153	29952157	29952158		CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
1	A	C	A	A	29.10	27.97	19.79	33.70	0.168	**0.093**	0.139		**0.013**	**0.007**		**0.031**
2	A	C	G	A	14.70	18.18	17.71	10.00	**0.007**	**0.007**	1.000		**0.066**		**0.056**	**0.017**

IL4Rα_NS	*Polymorphisms*									*Global Allelic Frequency (%)*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	29946391	29949352	29949354	29949357	29949358	29949359	29949360	29949361	29949362		CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
3	A	A	T	C	T	G	C	T	T	4.27	5.61	8.62	1.67	**0.056**	**0.051**	0.373		**0.022**	**0.020**		**0.041**
5	G	C	C	C	C	G	C	T	T	2.25	2.04	5.17	1.11	0.687		0.198		**0.095**	**0.092**		0.168

IL4Rα_NSintra	*Polymorphisms*								*Global Allelic Frequency (%)*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	29949352	29949354	29949357	29949358	29949359	29949360	29949361	29949362		CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
3	A	T	C	T	G	C	T	T	3.84	5.00	7.81	1.40	**0.037**		0.470		**0.018**	**0.016**		**0.030**

IL10R α	*Polymorphisms*																*Global*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	27223203	27223204	27223205	29939777	27223198	27223214	17530163	27223217	27223219	17528387	27223209	27223211	27223216	17528619	17528186	17528187	*Global Allelic Frequency (%)*	CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
0	G	A	G	C	C	G	C	C	C	G	A	G	A	C	G	C	50.88	46.35	57.14	54.71	0.097	**0.075**	0.146		0.760			0.130
1	G	G	G	C	C	G	T	C	C	A	A	G	A	C	A	C	16.37	16.79	8.93	18.82	0.609		0.159		**0.097**			0.224

IL10R α_NS	*Polymorphisms*				*Global Allelic Frequency (%)*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	29952152	29952153	29952157	29952158		CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
1	A	A	G	T	4.56	4.48	8.11	2.90	0.598		0.240		0.101	**0.094**		0.223

IFN γR1	*Polymorphisms*									*Global Allelic Frequency (%)*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	29942463	18041057	29942465	29942466	29258157	29942467	29373093	29942468	29942478	18120505	17983515		CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
10	T	A	C	C	T	G	T	G	G	A	T	1.32	0.35	3.49	1.33	0.326		**0.040**	**0.039**	0.352			**0.058**

IFN γR1_promoter	*Polymorphisms*					*Global Allelic Frequency (%)*	*Allelic Frequency (%)*			*SPvsCTR*		*RPvsCTR*		*SPvsRP*			*SPvsRPvsCTR*
Haplotypes	29942463	18041057	29942465	29942466	29258157		CTR	RP	SP	AF	D	AF	D	AF	D	R	AF
4	T	A	C	C	T	1.34	0.34	3.57	1.89	0.166		**0.035**	**0.034**	0.409			**0.053**

The detailed composition of all haplotypes is given in Table S3 (supplementary material online). SP, RP, and CTR represent the slow progression, rapid progression, and control populations respectively. The calculation modes AF, D, and R are for allelic frequency, dominant, and recessive modes, respectively. Promoter, NS, NSintra, Over3%, and Over10% represent, respectively, the SNPs located in the promoter, the non-synonymous SNPs, the non-synonymous SNPs involving mutations in the intracellular part of the protein, and the SNPs whose minor allelic frequency is over 3% or over 10%. The tagging polymorphisms for the different haplotypes are highlighted

with the presence or absence of specific serine residues. For *IL10Rα*, no association has been identified with the haplotypes, taking all polymorphisms into account, even if some trends emerged ($p<0.1$). No significant association was found when computing haplotypes from the four SNPs associated with a nonsynonymous mutation. For *IFNγR1*, we identified some significant associations for the haplotype IFNγR1_10 ($p=0.039$) and for the haplotype IFNγR1_Promoter_4 ($p=0.034$) using the five SNPs located in the promoter region.

Discussion

This study was undertaken to identify the genetic associations between the polymorphisms of the *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1* cytokine receptor genes with resistance/susceptibility to AIDS in the GRIV cohort. Thanks to our systematic approach, 21 out of the 108 frequent polymorphisms were newly characterized. We found 13 polymorphisms exhibiting nominally significant associations (nominal p values ≤0.05) and 13 other polymorphisms exhibiting nominally borderline signals (nominal p values <0.1) with AIDS progression (Table 2). Except for four polymorphisms, the associations found correspond to polymorphisms located in introns, whose biological effects are difficult to interpret. Among these four polymorphisms, two are located in exons of IL4Rα coding for the intracellular domain of the receptor, but one is synonymous (29949353, Leu414Leu), and the other one (29949352, Glu400Ala) could not be associated with any known functional property of the protein. The two other polymorphisms are located in promoter regions (IL4Rα_29952157 and IFNγR1_29942463), although these polymorphisms are not located in known transcription factor binding sites or enhancing/repressors regions. Our results did not take into account the multiple testing (Bonferroni corrections), but as discussed in a previous work, such corrections in this type of genomic study have less relevance than the necessary confirmation in other cohorts (Huber et al. 2003). We also compared the three groups after removing the known prominent CCR5-Δ32 effect (Winkler et al. 2004); the results that we found were similar (data not shown).

IL2Rα, *IL10Rα*, and *IFNγR1* genes had never been previously investigated for associations in AIDS, and the extensive characterization of the polymorphisms existing in these genes will thus be useful for any future genetic study. Only one genetic study had been performed by Wang et al. (2004) in the *IL4Rα* gene dealing with the nonsynonymous SNP IL4Rα_29949359 (Gln576Arg). They found a significant association (nominal p value 0.009) for a higher risk of HIV-1 infection. Our allelic frequencies were different, and no association was observed in our study. This certainly stems from the fact that they worked on an African-American population, while our study deals with a Caucasian population.

Eleven haplotypes also exhibited positive signals (Table 3). Among them, the haplotypes IL2Rα_Over10%_3 and IFNγR1_10 track the SNP alleles IL2Rα_29941451A and IFNγR1_29942463T, respectively. Three haplotypes involve promoter SNPs for *IL4Rα* and *IFNγR1* (Table 3). Two haplotypes of *IL4Rα* involving nonsynonymous SNPs also presented a weak signal (Table 3). One of them is a subhaplotype of the other: it is composed of eight intracellular SNPs, five of them involving a serine mutation (Table S2 in supplementary material online). Serines are known to be important for signal transduction; however, no pattern of amino acids distribution could be identified in the haplotype giving a positive signal. More refined analyses of the haplotypes structures should certainly be performed, but they are not in the focus of the present work.

Numerous genetic studies have been previously undertaken on the *IL4Rα*, *IL10Rα*, and *IFNγR1* cytokine receptor gene polymorphisms regarding their possible associations with various diseases. In the *IL4Rα* gene, the SNPs IL4Rα_29946391, 29949358, and 29949359 have all been associated with atopy and asthma (Hershey et al. 1997; Kruse et al. 1999; Mitsuyasu et al. 1998). These polymorphisms influence the production of immunoglobulin E (IgE) (Kruse et al. 1999; Mitsuyasu et al. 1998) and further signal transduction by the interleukin-4 receptor (Kruse et al. 1999). However, these polymorphisms do not exhibit any signal for their association with AIDS progression in our study (Table S2, supplementary material online), nor do haplotypes extracted from these three polymorphisms (data not shown). Interestingly, a haplotype composed of SNPs located in exons of IL4Rα has been associated with diabetes in a Filipino population (Mirel et al. 2002), and it also presents a borderline signal in our study (nominal p value=0.07; these data are not shown because we have used different SNPs for haplotyping). In the *IFNγR1* gene, the SNP IFNγR1_18041057 was associated with a strong promoter activity for its allele G (Rosenzweig et al. 2004) but was never associated with any disease. In our study, this SNP did not respect well the HWE (p=0.19), but this SNP did not exhibit a positive signal (Table S2, supplementary material online). The SNP IFNγR1_29258157 has been associated with a lower risk of cerebral malaria (Koch et al. 2002) and a lower expression of IFNγR1 for its allele C (Juliger et al. 2003), but it did not present any signal in our study. In the *IL10Rα* gene, the SNP IL10Rα_17528186 has been associated with cirrhosis in hepatitis-C infection (Hofer et al. 2005). In our study, this SNP presents a borderline signal. No associations with diseases have been described for the polymorphisms of the *IL2Rα* gene.

In this thorough investigation of the cytokine receptor genes *IL2Rα*, *IL4Rα*, *IL10Rα*, and *IFNγR1*, we identified new polymorphisms and found putative associations between some polymorphisms/haplotypes and AIDS progression. The cytokine receptors are the assembly of two or more subunits, which are all required for efficient ligand binding and signal transduction (Thomson and Lotze 2003). IL2Rα has a short intracellular domain, and IL4Rα, IL10Rα, and IFNγR1 have long intracellular domains, although none of them are sufficient for signal transduction (Asadullah et al. 2003; Bach et al. 1997; Moore et al. 2001; Mueller et al. 2002; Shtrichman and Samuel 2001). It will thus be necessary to complete this study with a genetic analysis of the beta subunits of the receptors, which are also essential components of these receptors and of the cytokine network.

The important amount of data provided in this study will provide useful leads for further investigation of these genes in relation with HIV-1 infection because they will need to be investigated in other cohorts. Given the importance of

the cytokine network in immune reactions, the information provided in this study should also prove useful for the study of other immune-related diseases.

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References

Alfano M, Poli G (2002) The cytokine network in HIV infection. Curr Mol Med 2:677–689

Alfano M, Poli G (2005) Role of cytokines and chemokines in the regulation of innate immunity and HIV infection. Mol Immunol 42:161–182

Asadullah K, Sterry W, Volk HD (2003) Interleukin-10 therapy—review of a new approach. Pharmacol Rev 55:241–269

Bach EA, Aguet M, Schreiber RD (1997) The IFN gamma receptor: a paradigm for cytokine receptor signaling. Annu Rev Immunol 15:563–591

Breen EC, Boscardin WJ, Detels R, Jacobson LP, Smith MW, O'Brien SJ, Chmiel JS, Rinaldo CR, Lai S, Martinez-Maza O (2003) Non-Hodgkin's B cell lymphoma in persons with acquired immunodeficiency syndrome is associated with increased serum levels of IL10, or the IL10 promoter -592 C/C genotype. Clin Immunol 109:119–129

Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, Shaw N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, Daley GQ, Lander ES (1999) Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet 22:231–238

Clerici M, Shearer GM (1993) A TH1→TH2 switch is a critical step in the etiology of HIV infection. Immunol Today 14:107–111

Curfs JH, Meis JF, Hoogkamp-Korstanje JA (1997) A primer on cytokines: sources, receptors, effects, and inducers. Clin Microbiol Rev 10:742–780

David D, Bani L, Moreau JL, Treilhou MP, Nakarai T, Joussemet M, Ritz J, Dupont B, Pialoux G, Theze J (1998) Regulatory dysfunction of the interleukin-2 receptor during HIV infection and the impact of triple combination therapy. Proc Natl Acad Sci U S A 95:11348–11353

Deichmann K, Bardutzky J, Forster J, Heinzmann A, Kuehr J (1997) Common polymorphisms in the coding part of the IL4-receptor gene. Biochem Biophys Res Commun 231:696–697

Do H, Vasilescu A, Diop G, Hirtzig T, Heath SC, Coulonges C, Rappaport J, Therwath A, Lathrop M, Matsuda F, Zagury JF (2005) Exhaustive genotyping of the CEM15 (APOBEC3G) gene and absence of association with AIDS progression in a French cohort. J Infect Dis 191:159–163

Flores-Villanueva PO, Hendel H, Caillat-Zucman S, Rappaport J, Burgos-Tiburcio A, Bertin-Maghit S, Ruiz-Morales JA, Teran ME, Rodriguez-Tafur J, Zagury JF (2003) Associations of MHC ancestral haplotypes with resistance/susceptibility to AIDS disease development. J Immunol 170:1925–1929

Gaffen SL (2001) Signaling domains of the interleukin 2 receptor. Cytokine 14:63–77

Gasche C, Grundtner P, Zwirn P, Reinisch W, Shaw SH, Zdanov A, Sarma U, Williams LM, Foxwell BM, Gangl A (2003) Novel variants of the IL-10 receptor 1 affect inhibition of monocyte TNF-alpha production. J Immunol 170:5578–5582

Hendel H, Henon N, Lebuanec H, Lachgar A, Poncelet H, Caillat-Zucman S, Winkler CA, Smith MW, Kenefic L, O'Brien S, Lu W, Andrieu JM, Zagury D, Schachter F, Rappaport J, Zagury JF (1998) J Acquir Immune Defic Syndr Hum Retrovirol 19: 381–386

Hendel H, Caillat-Zucman S, Lebuanec H, Carrington M, O'Brien S, Andrieu JM, Schachter F, Zagury D, Rappaport J, Winkler C, Nelson GW, Zagury JF (1999) New class I and II HLA alleles strongly associated with opposite patterns of progression to AIDS. J Immunol 162:6942–6946

Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA (1997) The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. N Engl J Med 337:1720–1725

Hill WG, Weir BS (1994) Maximum-likelihood estimation of gene location by linkage disequilibrium. Am J Hum Genet 54:705–714

Hofer H, Neufeld JB, Oesterreicher C, Grundtner P, Wrba F, Gangl A, Ferenci P, Gasche C (2005) Bi-allelic presence of the interleukin-10 receptor 1 G330R allele is associated with cirrhosis in chronic HCV-1 infection. Genes Immun 6:242–247

Huber C, Pons O, Hendel H, Haumont P, Jacquemin L, Tamim S, Zagury JF (2003) Genomic studies in AIDS: problems and answers. Development of a statistical model integrating both longitudinal cohort studies and transversal observations of extreme cases. Biomed Pharmacother 57:25–33

Husain SR, Leland P, Aggarwal BB, Puri RK (1996) Transcriptional up-regulation of interleukin 4 receptors by human immunodeficiency virus type 1 tat gene. AIDS Res Hum Retroviruses 12:1349–1359

Jouanguy E, Dupuis S, Pallier A, Doffinger R, Fondaneche MC, Fieschi C, Lamhamedi-Cherradi S, Altare F, Emile JF, Lutz P, Bordigoni P, Cokugras H, Akcakaya N, Landman-Parker J, Donnadieu J, Camcioglu Y, Casanova JL (2000) In a novel form of IFN-gamma receptor 1 deficiency, cell surface receptors fail to bind IFN-gamma. J Clin Invest 105:1429–1436

Juliger S, Bongartz M, Luty AJ, Kremsner PG, Kun JF (2003) Functional analysis of a promoter variant of the gene encoding the interferon-gamma receptor chain I. Immunogenetics 54:675–680

Ke X, Cardon LR (2003) Efficient selective screening of haplotype tag SNPs. Bioinformatics 19:287–288

Koch O, Awomoyi A, Usen S, Jallow M, Richardson A, Hull J, Pinder M, Newport M, Kwiatkowski D (2002) IFNGR1 gene promoter polymorphisms and susceptibility to cerebral malaria. J Infect Dis 185:1684–1687

Kruse S, Japha T, Tedner M, Sparholt SH, Forster J, Kuehr J, Deichmann KA (1999) The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction. Immunology 96:365–371

Laird N (1993) The EM algorithm. In: Rao CR (ed) Handbook of statistics, vol 9: computational statistics. Elsevier, Amsterdam

Mirel DB, Valdes AM, Lazzeroni LC, Reynolds RL, Erlich HA, Noble JA (2002) Association of IL4R haplotypes with type 1 diabetes. Diabetes 51:3336–3341

Mitsuyasu H, Izuhara K, Mao XQ, Gao PS, Arinobu Y, Enomoto T, Kawai M, Sasaki S, Dake Y, Hamasaki N, Shirakawa T, Hopkin JM (1998) Ile50Val variant of IL4R alpha upregulates IgE synthesis and associates with atopic asthma. Nat Genet 19: 119–120

Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683–765

Mueller TD, Zhang JL, Sebald W, Duschl A (2002) Structure, binding, and antagonists in the IL-4/IL-13 receptor system. Biochim Biophys Acta 1592:237–250

Nagarkatti R, Ghosh B (2002) Identification of single-nucleotide and repeat polymorphisms in two candidate genes, interleukin 4 receptor (IL4RA) and signal transducer and activator of transcription protein 6 (STAT6), for Th2-mediated diseases. J Hum Genet 47:684–687

Nakayama EE, Meyer L, Iwamoto A, Persoz A, Nagai Y, Rouzioux C, Delfraissy JF, Debre P, McIlroy D, Theodorou I, Shioda T (2002) Protective effect of interleukin-4 -589T polymorphism on human immunodeficiency virus type 1 disease progression: relationship with virus load. J Infect Dis 185:1183–1186

Nelson MR, Marnellos G, Kammerer S, Hoyal CR, Shi MM, Cantor CR, Braun A (2004) Large-scale validation of single nucleotide polymorphisms in gene regions. Genome Res 14:1664–1668

Ober C, Leavitt SA, Tsalenko A, Howard TD, Hoki DM, Daniel R, Newman DL, Wu X, Parry R, Lester LA, Solway J, Blumenthal M, King RA, Xu J, Meyers DA, Bleecker ER, Cox NJ (2000) Variation in the interleukin 4-receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations. Am J Hum Genet 66:517–526

Puri RK, Leland P, Aggarwal BB (1995) Constitutive expression of human immunodeficiency virus type 1 tat gene inhibits interleukin 2 and interleukin 2 receptor expression in a human CD4+ T lymphoid (H9) cell line. AIDS Res Hum Retroviruses 11:31–40

Rappaport J, Cho YY, Hendel H, Schwartz EJ, Schachter F, Zagury JF (1997) 32 bp CCR-5 gene deletion and resistance to fast progression in HIV-1 infected heterozygotes. Lancet 349: 922–923

Rosenzweig SD, Schaffer AA, Ding L, Sullivan R, Enyedi B, Yim JJ, Cook JL, Musser JM, Holland SM (2004) Interferon-gamma receptor 1 promoter polymorphisms: population distribution and functional implications. Clin Immunol 112:113–119

Sharfe N, Dadi HK, Shahar M, Roifman CM (1997) Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. Proc Natl Acad Sci U S A 94: 3168–3171

Shtrichman R, Samuel CE (2001) The role of gamma interferon in antimicrobial immunity. Curr Opin Microbiol 4:251–259

Stephens M, Donnelly P (2003) A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 73:1162–1169

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68:978–989

Tanaka Y, Nakashima H, Otsuka T, Nemoto Y, Niiro H, Yamaoka K, Ogami E, Arinobu Y, Tachida H, Imamura T, Niho Y (1997) Detection of polymorphisms within the human IL10 receptor cDNA gene sequence by RT-PCR RFLP. Immunogenetics 46:439–441

Thomson A, Lotze M (eds) (2003) The cytokine handbook. Elsevier, Amsterdam

Vasilescu A, Heath SC, Ivanova R, Hendel H, Do H, Mazoyer A, Khadivpour E, Goutalier FX, Khalili K, Rappaport J, Lathrop GM, Matsuda F, Zagury JF (2003) Genomic analysis of Th1-Th2 cytokine genes in an AIDS cohort: identification of IL4 and IL10 haplotypes associated with the disease progression. Genes Immun 4:441–449

Wang C, Song W, Lobashevsky E, Wilson CM, Douglas SD, Mytilineos J, Schoenbaum EE, Tang J, Kaslow RA (2004) Cytokine and chemokine gene polymorphisms among ethnically diverse North Americans with HIV-1 infection. J Acquir Immune Defic Syndr 35:446–454

Willerford DM, Chen J, Ferry JA, Davidson L, Ma A, Alt FW (1995) Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. Immunity 3:521–530

Winkler CA, Hendel H, Carrington M, Smith MW, Nelson GW, O'brien SJ, Phair J, Vlahov D, Jacobson LP, Rappaport J, Vasilescu A, Bertin-Maghit S, An P, Lu W, Andrieu JM, Schachter F, Therwath A, Zagury JF (2004) Dominant effects of CCR2–CCR5 haplotypes in HIV-1 disease progression. J Acquir Immune Defic Syndr 37:1534–1538

Wu X, Di Rienzo A, Ober C (2001) A population genetics study of single nucleotide polymorphisms in the interleukin 4 receptor alpha (IL4RA) gene. Genes Immun 2:128–134