MAJOR ARTICLE

Exhaustive Genotyping of the Interleukin-1 Family Genes and Associations with AIDS Progression in a French Cohort

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(See the editorial commentary by Orr et al., on pages 1475–7.)

Interleukin (IL)–1 family members are key players in inflammatory processes but have been the subject of few studies of acquired immunodeficiency syndrome (AIDS). To better evaluate the impact of the IL-1 family on AIDS development, we genotyped the *IL1α*, *IL1β*, *IL1Ra*, and *IL1R1* genes in 245 slow progressor (SP) and 82 rapid progressor (RP) human immunodeficiency virus type 1–seropositive patients as well as in 446 control subjects, all of whom were of white ethnicity. One hundred sixteen frequent polymorphisms were identified, of which 23 were newly characterized by our study. Many putative associations were found between single-nucleotide polymorphism (SNP) or haplotype alleles and the extreme profiles of progression. Most of them corresponded to weak associations ($.01 < P < .05$); however, the SNP IL1Ra_2134 exhibited a consistent association, found at the level of the SNP, haplotypes, and haploblocks, when the SP and control populations were compared ($P = .0002$). The IL-1–dependent inflammatory response is, thus, likely to play a role in AIDS progression via the regulation of IL-1Ra expression. This association will need to be confirmed in other AIDS cohorts, and experiments will also have to be performed to unravel the biological mechanisms at work. The data presented here will be useful for future genomic studies of the IL-1 family members in other infectious and chronic inflammatory diseases.

Interleukin (IL)–1 is a key player in inflammatory responses and can induce various effects that range from fever induction to the increase of the lymphocyte response [1–3]. The term "IL-1" usually designates a group of 3 molecules; 2 of them, IL-1α and IL-1β, are biologically active, and the third member, IL-1Ra, is a receptor antagonist whose function is to moderate the effects of IL-1α and IL-1β [4, 5].

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The role played by cytokines in the development of HIV-1 infection has been extensively investigated [6, 7], but relatively few studies have dealt with the members of the IL-1 family. This is surprising, in light of the importance of IL-1 in inflammation and in the response to infectious diseases, but it is understandable, given that AIDS is mainly known as a disease of adaptive immunity [8]. The balance between IL-1α/β and IL-1Ra has been reported to modulate HIV-1 expression in monocytes [9, 10]—IL-1Ra has been shown to reduce HIV-1 levels in monocytes, whereas IL-1α/β have been shown to induce HIV-1 expression [11, 12]. Similarly, antiretroviral therapy (ART) caused a reduction of IL-1α/β levels and an increase of IL-1Ra levels in lipopolysaccharide-stimulated HIV-1–infected monocytes [13]. Other studies have pointed out interactions such as an activation of IL-1β production in neuroblastoma cells in vitro by the gp120 protein of HIV-1

envelope [14] and an inhibition of IL-1α expression in vitro by the viral protein Tat [15]. Furthermore, it has been showed that caspase-mediated IL-1β processing can play a major role in $CD4^+$ T cell survival and HIV-1 proliferation [16].

Only few genetic association studies regarding the impact of IL-1 family members on AIDS have been conducted to date—the first study showed that a tandem-repeat polymorphism in the *IL1Ra* gene was associated with reduced HIV-1 levels [17], another study linked single-nucleotide polymorphisms (SNPs) in *IL1α* with the control of HIV viremia in patients receiving highly active ART (HAART) [18], and a third study investigating a few polymorphisms in *IL1α, IL1β, IL1Ra,* and *IL1R1* found no association with AIDS progression [19].

Our group previously completed the exhaustive genotyping of Th1/Th2 cytokines and their receptor genes [20, 21] and looked for associations with AIDS progression in the Genetics of Resistance to Immunodeficiency Virus (GRIV) cohort. As a continuation of this previous work and to clarify the role played by IL-1 family members in AIDS, we present here the extensive genotyping of the *IL1α, IL1β, IL1Ra,* and *IL1R1* genes and their associations with AIDS progression in the GRIV cohort, which is composed of 2 subpopulations of HIV-1–seropositive white individuals with extreme progression phenotypes and living in France: slow-progressor (SP) patients and rapid-progressor (RP) patients. We also genotyped healthy control subjects of similar ethnic origin. The GRIV patients correspond to the extreme 1% subset of a cohort of 30,000 seroconverter patients [22] and is, as far as we know, the largest cohort of its kind in the world. Its quality has already been confirmed for several gene polymorphisms, such as CCR5 [23–25] and HLA [26, 27].

The *IL1α, IL1β, IL1Ra,* and *IL1R1* genes are located in the 2q12–2q14 region of chromosome 2. *IL1α* and *IL1β* are spaced by 40 kb, *IL1Ra* is 280 kb telomeric from *IL1β,* and *IL1R1* is 11 Mb centromeric from *IL1α* (figure 1*A*). *IL1α, IL1β,* and *IL1Ra* likely derive from the same ancestral gene [2, 3]. In the present study, SNPs and other genetic variations of the IL-1 family members were identified through sequencing. The data gathered were used to compute the genetic structure of the IL-1 family locus (linkage disequilibrium and haploblocks) and to evaluate associations with AIDS susceptibility and development.

SUBJECTS, MATERIALS, AND METHODS

The GRIV cohort. The GRIV cohort was established in 1995 in France to generate a large collection of DNA for genetic studies of candidate polymorphisms associated with rapid and slow progression to AIDS. Only white individuals of European descent living in France were recruited. These criteria limit the influence of the virogenetic and environmental factors (all subjects are infected by B strains and live in a similar environment) and puts emphasis on the genetic makeup of each individual to determine the various patterns of progression. SP patients were defined as individuals who had been seropositive but asymptomatic for 8 or more years with a $CD4^+$ cell count >500 cells/mm^3 in the absence of ART. RP patients were defined as those who had a decrease in their $CD4^+$ cell count to <300 cells/mm^3 in <3 years after the last seronegative test. DNA was obtained from fresh peripheral-blood mononuclear cells or from Epstein-Barr virus–transformed cell lines. The control subjects were seronegative white individuals of European descent living in France. In the present study, we genotyped up to 245 SP patients, 82 RP patients, and 446 control subjects.

Genotyping. The primers and conditions used for polymerase chain reaction amplification of the different fragments are shown in table 2. Sequencing reactions were performed by the Dye Terminator method, using an ABI PRISM 3700 DNA analyzer (Applied Biosystems). Alignment, SNP discovery, and genotyping were done with the software Genalys (version 3.3.24a), developed by the Centre National de Génotypage [34].

For practical reasons, an initial screening was performed on 150 SP patients, 50 RP patients, and 150 control subjects for polymorphism discovery. The screening was then extended to include more subjects when a positive ($P \leqslant .05$) or borderline ($P < .1$) association was detected.

Hardy-Weinberg equilibrium (HWE). HWE analysis was performed for each SNP in each group according to the standard method: the experimental genotypic distribution was compared with the theoretical distribution estimated on the basis of the SNP allelic frequencies, and a *P* value could thus be derived. It is important to assess the deviations from HWE ($P < .05$), because it suggests an effect of the SNP if a deviation is observed in a case group, and it suggests a mistake in the genotyping otherwise [35, 36].

Haplotypes. Haplotype estimates were obtained using the expectation-maximization (EM) algorithm [37] and the phase 2 algorithm [38, 39] either for all polymorphisms or for selected ones.

Linkage disequilibrium and haploblocks. Linkage disequilibrium was computed for each pair of polymorphisms by the standard r^2 method [40] or the standard D' method [41]. A haploblock is a genetic region for which no evidence of a historical recombination event can be found—in other words, the SNPs located in that region exhibit a significant level of linkage disequilibrium (D' close to 1). The haploblocks in the genes were computed using the method developed by Gabriel et al. [42], which is used by the software Haploview (version 3.2; available at: http://www.broad.mit.edu/mpg/haploview/) [43]. The haplotype tagging (HT) polymorphisms are the polymorphisms that are sufficient to describe the genetic variation inside a haploblock. These HT polymorphisms were also computed using Haploview and served as a basis for the estimation of subhaplotypes in the computed haploblocks.

Statistical analyses. Statistical analyses were performed only

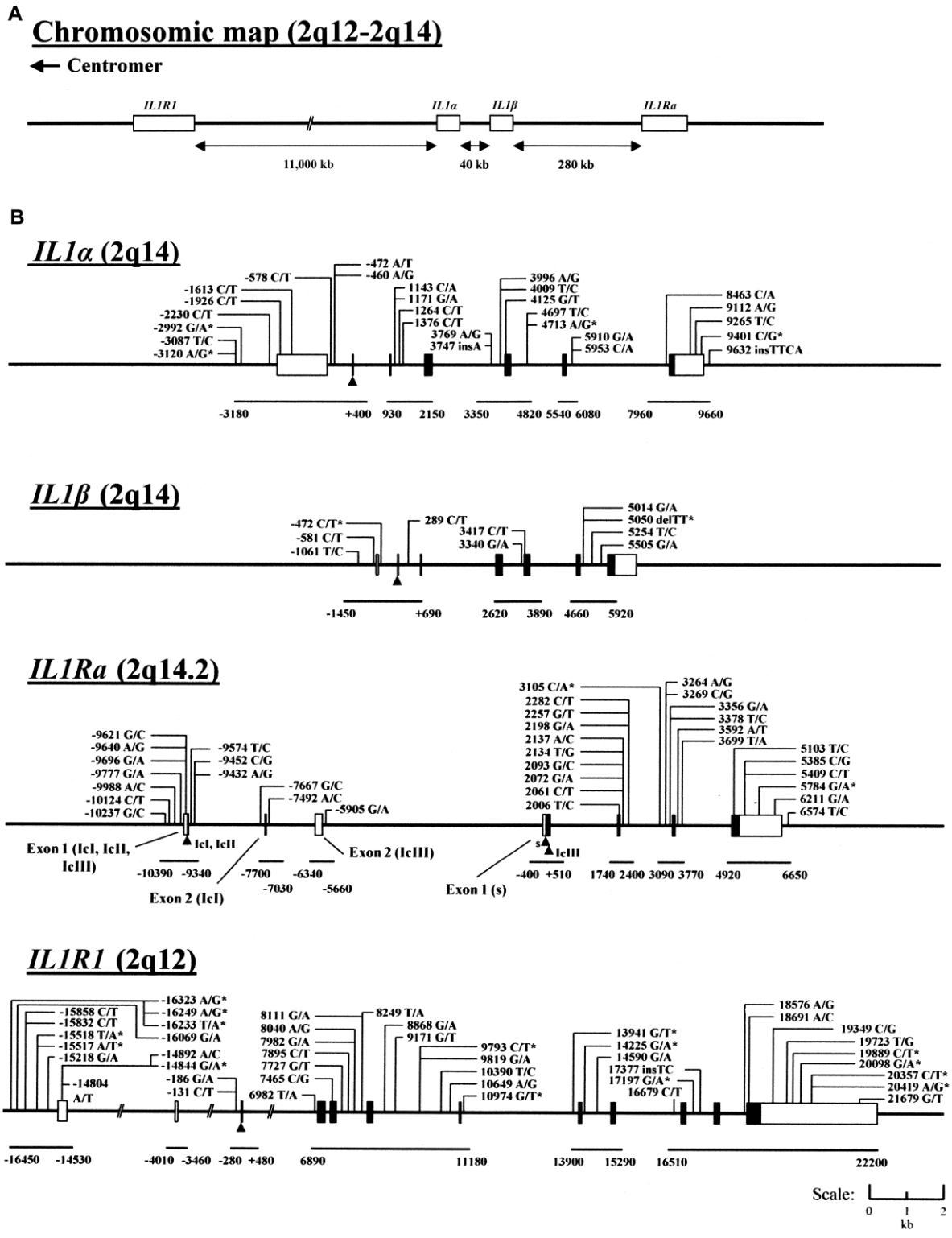

Figure 1. Genetic mapping. *A,* Positions of the *IL1α*, *IL1β*, *IL1Ra,* and *IL1R1* genes on the 2q12–2q14 locus. *B,* Map of the *IL1α*, *IL1β*, *IL1Ra,* and *IL1R1* genes. Coding and untranslated regions are respectively indicated by black and white rectangles. The regions that have been sequenced are indicated by a horizontal line, with start and end positions enumerated in reference to the first nucleotide of the initiation codon as +1. The initiation codon is indicated by a black triangle, and a name is provided for each isoform of interleukin (IL)–1Ra (soluble [s] and intracellular [Ic] isoforms). The polymorphism nos. are their positions relative to the initiation codon as +1 (correspondence with the dbSNP database is given in table 1). The newly characterized polymorphisms are indicated by an asterisk. The genomic sequence used for alignment of the 4 genes is NT_022135.14.

Table 1. *IL1α, IL1β, IL1Ra,* and *IL1R1* polymorphisms.

Gene	Position	A1	A2	Allelic frequency (A1), %			Localization[a]	Nominal *P* values and ORs from statistical tests[b]										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	
IL1α	−472	A	T	30.97	28.70	24.48	Intron 1	**.083**	1.384	**.064 (D)**	1.550	.169	.716					.208	rs3783525
IL1Ra	2134	T	G	81.75	74.62	71.17	Intron 2	**.002**	1.814	**.0002 (R)**	2.389	**.001**	.114		**.088 (R)**	0.587		**.008**	rs2232354
	3105	C	A	77.27	82.81	80.34	Intron 2	.517		**.064 (D)**	0.155		.380					.595	ss52090882 (new)
	5103	T	C	76.00	67.97	71.22	Exon 4 (Ser130Ser[c])	**.094**	1.279	**.071 (R)**	1.399		**.063**	0.670	**.080 (R)**	0.606		**.059**	rs315952 [28]
	5385	C	G	76.21	68.75	71.60	3′-UTR	.104					**.079**	0.687				**.081**	rs315951
	5409	C	T	96.32	92.19	95.43	3′-UTR	.505					**.054**	0.451	**.085 (R)**	0.485		**.095**	rs4252041
	5784	G	A	96.38	98.08	99.29	3′-UTR	**.022**	0.192	**.021 (R)**	0.186	**.016**	.530					**.055**	ss52090883 (new)
IL1R1	−16233	T	A	81.49	83.02	82.31	Promoter	.833					.772				**.077**	.930	ss52090886 (new)
	−16069	G	A	63.31	65.09	67.69	Promoter	.266		**.049 (R)**	0.629	**.053**	.815					.528	rs956730
	−15858	C	T	63.31	65.09	67.69	Promoter	.266		**.049 (R)**	0.629	**.053**	.815					.528	rs2234650 [29]
	−186	C	T	80.79	84.26	81.69	Intron 1	.833					.471				**.023**	.727	rs2287048
	−131	G	A	63.64	66.67	70.50	Intron 1	**.080**	0.732	**.010 (R)**	0.531	**.010**	.641				**.057**	.211	rs2287047
	9171	G	T	68.21	72.55	71.58	Intron 6	.421					.457				**.022**	.576	rs2160227
	10974	G	T	68.40	72.64	72.60	Intron 7	.276					.460				**.024**	.488	ss52090890 (new)
	13941	G	T	68.09	72.12	72.54	Intron 7	.242					.464				**.029**	.462	ss52090891 (new)
	19349	C	G	75.17	75.47	74.82	3′-UTR	.923		**.039 (D)**	3.378	**.029**	1.000					.990	rs3917324

NOTE. Indicated for each polymorphism are the allelic frequencies in the different populations (control [CTR], rapid progression [RP], and slow progression [SP]), the localization and the amino acid change (when applicable), the *P* values from Fisher's exact tests (and odd ratios [OR] when applicable), and the relevant information known to date. A1 represents the nucleotide that is identical to the reference sequence, which is NT_022135.14 for the 4 genes. The amino acid positions are taken from the references sequences NP_000566.3 (interleukin [IL]–1α), NP_000567.1 (IL-1β), NP_000868.1 (IL-1R1), NP_776214.1 (soluble [s]IL-1Ra), NP_776213.1 (IL-1RaIcI), NP_000568.1 (IL-1RaIcII), and NP_776215.1 (IL-1RaIcIII). The polymorphism nos. correspond to their position relative to the initiation codon as +1, and the corresponding dbSNP numbers are also given in the far right column. The calculation modes for the Fisher's exact tests are indicated (AF, allelic frequency; D/R, dominant/recessive; G, genotypic distribution). Positive ($P \leqslant .05$) and borderline ($P < .1$) correlations are shown in boldface. For the dominant/recessive mode, the mode corresponding to the allele A1 is indicated in parenthesis. The *P* values for the dominant/recessive and genotypic distribution calculation modes are shown only if <.1. UTR, untranslated region.

[a] Exon and introns are indicated for sIL1Ra, unless otherwise specified.

[b] Bonferroni corrections were not performed on *P* values.

[c] The amino acid change corresponds to all 4 isoforms, but the position corresponds only to the soluble isoform (sIL1Ra). The positions for the other isoforms are 133 (IcI), 112 (IcII), and 96 (IcIII).

Table 2. Primers used to amplify the exons of *IL1α, IL1β, IL1Ra,* and *IL1R1* by polymerase chain reaction (PCR).

The table is available in its entirety in the online edition of the *Journal of Infectious Diseases.*

on the polymorphisms (and haplotypes) with a minor allele frequency >1% in our entire population, termed "frequent polymorphisms." The differences in the allelic distributions (SNPs or haplotypes) between the 3 populations were examined as follows: for each allele (with a frequency >1% in the entire population), the expected numbers of individuals in each population with and without that allele were compared by a 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 2 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, table 1, which concerns individual polymorphisms, combines the dominant and recessive modes. Bonferroni corrections were not performed because, in such candidate-gene association studies, confirmation in other cohorts is more relevant [22].

RESULTS

SNP discovery. We systematically screened the *IL1α*, *IL1β*, *IL1Ra,* and *IL1R1* genes for polymorphisms by sequencing the exons with their flanking regions as well as the promoter region. We identified 116 frequent polymorphisms (minor allele frequency >1% in our entire population) in these genes, most of them being SNPs (figure 1*B*). Of these 116 polymorphisms, 27 were in the *IL1α* gene, 10 were in the *IL1β* gene, 35 were in the *IL1Ra* gene, and 44 were in the *IL1R1* gene (figure 1*B*); 22 of these 116 polymorphisms were newly characterized in our study. On average, we found 1 polymorphism with a frequency >1% in every 294 bp (table 3), which is consistent with the findings of previous studies [44, 45]. The allelic frequencies obtained in our study were compared with the data from the National Center for Biotechnology Information's dbSNP database (available at: http://www.ncbi.nlm.nih.gov/SNP/) when the allelic frequencies were available for the European population. Our results are consistent with dbSNP allelic frequencies except for the SNPs IL1α_3769, IL1β_289, and IL1R1_–16069, whose allelic frequencies exhibit differences >10%, compared with those in dbSNP.

IL-1 family gene structure. The 4 genes *IL1α*, *IL1β*, *IL1Ra,* and *IL1R1* are neighbors in the same chromosomal region (figure 1*A*). *IL1α*, *IL1β*, and *IL1Ra* are very close to each other (<300 kb) and, thus, were investigated for linkage disequilibrium between their polymorphisms by use of the software Haploview [43]. As shown in figure 2*A*, *IL1α*, *IL1β*, and *IL1Ra* present strong intragenic linkage disequilibrium (>95%), but there is no intergenic linkage disequilibrium between them even though these genes lie relatively close to each other on the chromosome. Figure 2*B*–2*E* presents the haploblocks for each gene, which correspond to groups of polymorphisms that have never undergone any recombination; we found 1 haploblock for the gene *IL1α*, 2 for *IL1β*, 3 for *IL1Ra,* and 3 for *IL1R1*. As shown in table 4, all the SNPs fitted with Hardy-Weinberg equilibrium in the SP, RP, and control populations.

Associations with AIDS progression: SNPs. The GRIV case-control study is based on the comparison of the allelic distributions of the polymorphisms between the patient populations (SP and RP patients) and the control subjects. Table 1 presents the allelic frequencies in each population, their locations, and *P* values (the abridged version of the table that appears in the print edition of the *Journal* includes only polymorphisms with positive [$P < .05$] or borderline [$P < .1$] associations; the complete results for all polymorphisms with a frequency >1% are given in the version of the table included in the electronic edition of the *Journal*).

Table 3. Frequency of polymorphisms identified in genes of the interleukin (IL)–1 family.

	Gene				
Parameter	*IL1α*	*IL1β*	*IL1Ra*	*IL1R1*	Total
Frequent polymorphisms	27	10	35	44	116
Newly identified	4	2	2	14	22
Located in exons	6	1	8	12	27
Synonymous	0	1	2	1	4
Nonsynonymous	1	0	0	1	2
UTR	5	0	6	10	21
bp sequenced	8510	4670	6380	14,600	34,160
Frequency, polymorphism/bp sequenced	1/315	1/467	1/182	1/332	1/294

NOTE. Data are no. of polymorphisms, unless otherwise specified. UTR, untranslated region.

Table 4. Genotypic frequencies and Hardy-Weinberg equilibrium (HWE) *P* values for all identified polymorphisms in the *IL1α*, *IL1β*, *IL1Ra*, and *IL1R1* genes.

The table is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

In the *IL1α* gene, no polymorphism exhibited any positive association, but the SNP IL1α_–472 exhibited a borderline association (table 1). In the *IL1β* gene, no polymorphism exhibited any positive or borderline association. In the *IL1Ra* gene, 2 SNPs exhibited positive associations (table 1). The lowest *P* value was obtained for the intronic SNP IL1Ra_2134 ($P = .0002$ in the dominant/recessive mode) for the comparison between the SP population and the control population (table 1). In the *IL1R1* gene, 8 polymorphisms exhibited positive associations (table 1). Two were located in the promoter region, 5 were located in introns, and one was located in the 3′ untranslated region. The promoter SNPs did not correspond to known transcription factor binding sites. The lowest *P* value was obtained for IL1R1_–131, located in intron 1 ($P = .010$ in the dominant/recessive and genotypic distribution modes) for the comparison between the SP population and the control population.

Of note, of the 10 polymorphisms exhibiting positive associations, only IL1Ra_2134 remained significant for the simultaneous comparison of the 3 populations by the χ^2 test (table 1). This confirms that comparing the populations 2×2 is more sensitive [21].

Associations with AIDS progression: haplotypes. For each gene, the haplotypes were estimated using the phase 2 and EM algorithms for the polymorphisms with a minor allelic frequency >5% and for the promoter polymorphisms as well. The haplotypes based on the nonsynonymous SNPs (protein variants) were not estimated, because each gene contained no more than 1 nonsynonymous SNP. Table 5 presents the haplotype data; the estimated haplotypes exhibiting a positive ($P \leq .05$) or borderline ($P < .1$) association for comparisons between the populations are shown in the abridged version of the table given in the print edition of the *Journal*, and the detailed composition of all haplotypes is shown in the complete version of the table given in the electronic edition of the *Journal*.

For the *IL1α* gene, we identified positive associations for the haplotype IL1α_over5%_3 ($P = .013$ in the dominant mode) for the comparison between the SP population and the control population. For the *IL1β* gene, we identified positive associations for IL1β_over5%_0 ($P = .026$ in the recessive mode) and IL1β_over5%_1 ($P = .048$ in the dominant mode) for the comparison between the SP population and the control population. For the *IL1Ra* gene, we identified positive associations for 3 haplotypes. The lowest *P* value was found for IL1Ra_over5%_0 ($P = .0002$ in the dominant mode) for the comparison between the SP population and the control population. The association for this haplotype can be entirely explained by the SNP IL1Ra_2134. No positive association was identified for the haplotypes of *IL1R1*, and no positive association was identified for the haplotypes based on the polymorphisms located in the promoter regions of the genes (data not shown).

We have presented here the results based on SNPs with a minor allelic frequency >5%, to clarify the presentation. We have also performed the haplotype analysis with all the polymorphisms found in each gene, and the results that we obtained were identical (data not shown).

Haploblocks. Haploblocks are presented in figure 2. HT SNPs were computed for the haploblocks IL1α_Block1, IL1β_Block2, IL1Ra_Block1, IL1R1_Block1, and IL1R1_Block3. The other haploblocks were only composed of 2 polymorphisms in linkage disequilibrium and, thus, did not present any interest for this calculation. We computed the haplotypes (which we have termed "HT haplotypes") derived from these HT SNPs and tested them for associations with AIDS progression (table 6). No positive association was found for the HT haplotypes of *IL1α* or *IL1R1*, but some of them presented borderline associations (table 6). For *IL1β*, the HT haplotypes H0 and H2 of the main haploblock presented positive associations for the comparison between the SP populations and the control population table 6: these HT haplotypes were subhaplotypes of the larger haplotypes that were derived from all the SNPs and that exhibited a positive association (table 5). The second haploblock of *IL1β* is minor, because it contains only the 2 polymorphisms IL1β_–1061 and IL1β_–581, which were in linkage disequilibrium. For *IL1Ra*, the HT haplotype IL1Ra_H0 presented a positive association ($P = .0001$ in the allelic frequency mode and $P = .00002$ in the dominant mode) for the comparison between the SP population and the control population (table 6). A positive association was also obtained when the 3 populations were compared for this haplotype ($P = .0005$). IL1Ra_H0 was the only subhaplotype that presented the allele G for the SNP IL1Ra_2134. Furthermore, IL1Ra_H0 is a subhaplotype of IL1Ra_over5%_0, which also exhibited a positive association (table 5) and is linked to the SNP IL1Ra_2134. Thus, the association with AIDS progression for the HT haplotype IL1Ra_H0 is likely due to the effect of the SNP IL1Ra_2134. The HT haplotype IL1Ra_H1 also presented a positive association ($P = .025$) in the recessive mode

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 2. Haploblock analysis. The figure and legend are available in their entirety in the online edition of the *Journal of Infectious Diseases*.

Table 5. Estimated haplotypes of *IL1α*, *IL1β*, *IL1Ra*, and *IL1R1* with an allelic frequency >1%.

Polymorphism	Haplotype			
	IL1α_over5%			
	1	3		
−3120	A	A		
−3087	T	T		
−2992	G	G		
−2230	T	C		
−1926	C	C		
−1613	C	C		
−578	C	C		
−472	A	T		
−460	A	G		
1143	C	A		
1171	G	G		
1264	C	T		
1376	C	T		
3747	–	A		
3769	A	G		
3996	A	A		
4009	T	T		
4125	G	G		
4697	T	C		
5910	G	G		
5953	C	A		
8463	C	A		
9112	A	A		
9265	T	T		
9632	----	TTCA		
Global allelic frequency, %	26.17	11.56		
Allelic frequency, %				
CTR	31.95	8.65		
RP	31.71	7.32		
SP	24.44	15.79		
	Nominal *P* value			
SP vs. CTR				
AF	**.067**	**.017**		
D	**.066**	**.013**		
RP vs. CTR, AF	1.000	.822		
SP vs. RP vs. CTR, AF	.128	**.016**		
	IL1β_over5%			
	0	1	2	5
−1061	C	T	C	T
−581	T	C	T	C
289	T	C	T	C
3340	A	G	G	G

(continued)

Table 5. ***(Continued.)***

Polymorphism	Haplotype			
3417	C	C	T	T
5014	G	G	A	A
5050	−−	−−	TT	TT
5254	C	C	C	C
5505	A	G	G	G
Global allelic frequency, %	45.33	18.34	16.23	2.12
Allelic frequency, %				
CTR	41.86	21.32	16.67	1.16
RP	40.48	21.43	19.05	1.19
SP	50.95	14.29	14.76	3.81
	Nominal *P* value			
SP vs. CTR				
AF	**.051**	**.054**	.612	**.071**
D		**.048**		**.068**
R	**.026**			
RP vs. CTR				
AF	.899	1.000	.620	1.000
R			**.067**	
SP vs. RP vs. CTR, AF	**.094**	.118	.652	.119
	IL1Ra_over5%			
	0	4	9	
−10237	G	G	C	
−10124	C	C	T	
−9988	A	A	C	
−9777	G	G	A	
−9696	G	G	A	
−9640	A	A	G	
−9621	G	G	C	
−9574	T	T	C	
−9452	C	C	G	
−9432	A	A	G	
−7667	G	G	C	
−7492	A	A	C	
−5905	G	A	G	
2006	T	T	C	
2061	C	C	T	
2072	G	G	A	
2093	G	G	C	
2134	G	T	T	
2137	A	A	C	
2198	G	G	A	
2257	G	G	T	
2282	C	T	C	
3105	C	C	C	
3264	A	A	G	

(continued)

Table 5. ***(Continued.)***

Polymorphism	Haplotype		
3269	C	C	G
3356	G	G	A
3378	T	T	C
3592	A	A	T
3699	A	T	A
5103	T	C	T
5385	C	G	C
6211	G	G	A
6574	T	T	C
Global allelic frequency, %	22.80	12.01	1.13
Allelic frequency, %			
CTR	18.50	11.02	0.00
RP	23.61	19.44	2.78
SP	32.52	11.17	0.97
	Nominal *P* value		
SP vs. CTR			
AF	**.0007**	1.000	.200
D	**.0002**		
RP vs. CTR			
AF	.400	**.073**	**.048**
D		**.049**	**.048**
SP vs. RP vs. CTR, AF	**.0024**	.132	.178
	IL1R1_over5%		
	11		
−16323	A		
−16233	T		
−16069	A		
−15858	T		
−15832	C		
−15517	A		
−15218	G		
−14892	C		
−14804	A		
−186	C		
−131	A		
6982	T		
7465	C		
7727	G		
7895	T		
7982	G		
8249	A		
8868	G		
9171	G		
10390	T		
10649	A		
10974	G		

(continued)

Table 5. ***(Continued.)***

Polymorphism	Haplotype
13941	G
14225	A
14590	A
17197	G
17377	––
18576	A
18691	A
19349	G
20098	A
20357	C
20419	A
21679	G
Global allelic frequency, %	2.36
Allelic frequency, %	
CTR	4.46
RP	2.50
SP	1.43
	Nominal *P* value
SP vs. CTR, AF	**.090**
RP vs. CTR, AF	.738
SP vs. RP vs. CTR, AF	.167

NOTE. Indicated for each haplotype are the global frequency, the frequency in each population (control [CTR], rapid progression [RP], and slow progression [SP]), the *P* value from Fisher's exact tests for the comparisons SP vs. CTR, RP vs. CTR, SP vs. RP, and SP vs. RP vs. CTR, and the calculation mode (allelic frequency [AF], dominant [D], and recessive [R]). Positive ($P \leq .05$) and borderline ($P < .1$) comparisons are shown in boldface. The *P* values for the D and R calculation modes are shown only if <0.1. The haplotypes presented are based on polymorphisms with a minor allelic frequency >5%. Tagging polymorphisms for the different haplotypes are shaded.

for the comparison between the SP population and the control population.

DISCUSSION

We undertook the exhaustive genotyping of the *IL1α*, *IL1β*, *IL1Ra*, and *IL1R1* genes to look for genetic factors involved in AIDS progression. The interest in such a study is to provide clues to the molecular mechanisms of disease development and help for the rational design of new diagnostic or therapeutic targets.

Of 116 frequent polymorphisms detected in the IL-1 family genes, 22 were newly characterized in our study. We identified 10 polymorphisms exhibiting positive associations ($P \leq .05$) and 6 other polymorphisms exhibiting borderline associations ($P < .1$) with AIDS progression (table 1). We identified 6 haplotypes exhibiting positive associations (table 5). These positive associations were found by comparing the SP population with the control population or the RP population with the control population, which was the basis of the present case-control study. It is noteworthy that comparison of the RP, SP, and control populations simultaneously appears to be less sensitive, given that only 1 association of 10 remained positive for the polymorphisms and 2 of 6 remained positive for the haplotypes.

Almost all polymorphisms involved in an association were located in introns, and their biological effect is, thus, difficult to interpret. Only 3 SNPs exhibiting a positive association were located in a promoter region, all in the promoter region of *IL1R1* (table 1). Two of these SNPs were in full linkage disequilibrium, and they belonged to the same haploblock. The

Table 6. Detailed information for the haplotypes derived from the haplotype tagging (HT) single-nucleotide polymorphism of haploblocks of *IL1α, IL1β, IL1Ra,* and *IL1R1*.

The table is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

haplotypes derived from these promoter SNPs did not exhibit any positive or borderline associations (data not shown). The biological effect of the promoter SNPs on IL-1R1 expression is at present unknown, and we did not find any similarity with previously described transcription factor binding sites. Thus, it will be of interest to perform reporter gene experiments or competitive gel shift assays, to determine whether the alleles of these SNPs can have a differential effect on gene expression.

Most of the associations for the individual polymorphisms were rather weak, with *P* values between .01 and .05, except for the intronic SNP IL1Ra_2134 ($P = .0002$, in the dominant/recessive mode) for the comparison between the SP population and the control population. Similarly, most of the associations found for haplotypes and HT haplotypes were weak ($.01 < P < .05$), except for the ones linked with the SNP IL1Ra_2134 (table 5). We also evaluated whether sex, age, or route of infection could influence these results; there was no such influence (data not shown).

The allele IL1Ra_2134G was carried by 32.12%, 44.62%, and 53.06% of the control, RP, and SP populations, respectively. Because the *P* value comparing the RP and control populations was also low for this allele ($P = .08$), there was a possibility that this SNP could be associated with susceptibility to HIV-1 infection rather than with disease progression; we thus analyzed 180 seropositive subjects from the SEROCO cohort [46] and found that the distribution of the alleles for this SNP was identical to that of the control population (data not shown). The effect of IL1Ra_2134 is, thus, mainly associated with slow disease progression. This SNP is located next to a well-known variable-number tandem repeat of *IL1Ra* (located in the same intron) that has been associated with many diseases [4, 47], including AIDS [17]. This repeat is composed most often by two or by four 86-bp units, but it could not be genotyped in the present study because of technical reasons. There have been contradictory reports of the IL1Ra*2 allele (2-U repeat) being associated with higher [48, 49] or lower [50] levels of IL-1Ra. The association found for the IL1Ra_2134 SNP emphasizes the importance of this intronic region, and experiments will have to be performed to determine whether the effect is mediated solely through this SNP or through linkage disequilibrium with this variable-number tandem repeat.

Besides the study of this repeat conducted by Witkin et al. [17], there have been 2 other studies of the association between the IL-1 gene family and AIDS. Wang et al. [19] reported no association between AIDS and the SNPs IL1β_–1061 and IL1β_3417, which is consistent with our results. Price et al. also identified associations between the control of HIV-1 replication and the SNPs IL1α_–1613 and IL1α_4125 (which are in linkage disequilibrium) in patients receiving HAART [18]. Of note, the allele IL1α_−1613T has been associated with increased IL-1α expression [51, 52] but none of these SNPs exhibited any associations in our study. The SNPs IL1β_−1061, IL1β_−581, IL1β_3417, IL1α_−1613, IL1α_4125, and IL1Ra_2006 have been associated with other diseases, such as rheumatoid arthritis [30], osteoarthritis [53–55], periodontal disease [52], asthma [56, 57], allergic rhinitis [58], and lupus [59]. None of these SNPs presented a positive association in our study.

In the present study, we thoroughly investigated the genes of the IL-1 family (*IL1α, IL1β, IL1Ra,* and *IL1R1*), providing an important set of information. We identified new polymorphisms in these genes and also found putative associations between some polymorphisms, haplotypes, and haploblocks of these genes with AIDS progression. Of all the associations, one appears to be strongest, pointing to an intronic SNP in *IL1Ra;* this SNP definitely deserves more extensive analyses, either by genetic association investigations in other AIDS cohorts or by further biological experimentation to assess its impact on IL-1Ra expression. Another interesting methodological observation, which confirms the observation made in our previous work [21], is that use of the 2×2 case-control comparisons is more sensitive for the detection of associations. On the other hand, it appears more robust to compare simultaneously the 3 populations.

Cytokine receptors are an assembly of 2 or more subunits, which are all required for efficient ligand binding and signal transduction. The α subunit IL-1R1 studied here is involved in both ligand binding and intracellular signaling, but the β subunit IL-1RacP (accessory protein) is required for efficient signal transmission [5]. There is another receptor in this family, IL-1R2, which preferentially binds to IL-1β. Its intracellular domain is very short, and it apparently fails to initiate any biological response [60]. It is believed to act as a natural moderator for IL-1. Our work will, thus, need to be completed by the analysis of these last 2 members of the IL-1 family.

The important amount of data provided and the putative associations discovered in this work will be useful for future genetic association studies of the role IL-1 family members play in HIV-1 infection. Given the importance of IL-1 in the inflammatory process, this information should also prove to be useful for the study of other infectious and chronic inflammatory diseases.

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