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Exhaustive genotyping of the *interferon alpha receptor 1 (IFNAR1)* gene and association of an IFNAR1 protein variant with AIDS progression or susceptibility to HIV-1 infection in a French AIDS cohort

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Abstract

We have undertaken a systematic genomic approach in order to explore the role of the interferon alpha (IFN-α) pathway in AIDS disease development. As it is very difficult to genotype the *IFN-α* gene itself since it has many pseudo-genes, we have focused our interest on the genetic polymorphisms of the IFN-α receptor 1 (IFNAR1). We genotyped the Genetics of Resistance to Immunodeficiency Virus (GRIV) cohort composed of patients with extreme profiles of progression to AIDS, slow progressors (SP) and rapid progressors (RP), as well as seronegative controls (CTR). We identified 19 single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) greater than 1% among which two were newly characterized by our study. We found putative associations with AIDS disease development for four SNP alleles and for three haplotypes. The most interesting signals were found for two SNPs in linkage disequilibrium, the SNP IFNAR1_18339 corresponding to a Val168Leu mutation in the extracellular domain of the protein and the intronic SNP, IFNAR1_30127. The intronic SNP IFNAR1_30127 yielded a strong signal both when comparing SP with CTR ($P = 0.002$) and RP with CTR ($P = 0.005$) while IFNAR1_18339 yielded a smaller signal because less patients were analyzed; these SNPs could thus be involved in AIDS progression or in susceptibility to human immunodeficiency virus 1 (HIV-1) infection. Interestingly, two independent studies have previously pointed out the SNP IFNAR1_18339 in susceptibility to multiple sclerosis and to malaria. This is the first work investigating the polymorphisms of the *IFNAR1* gene in AIDS. Our results which point out a possible role for the IFN-α pathway in susceptibility to HIV-1 infection or progression to AIDS need a necessary confirmation by genomic studies in other AIDS cohorts.

Keywords: AIDS progression; IFN alpha receptor 1; Gene; Genotyping; Single nucleotide polymorphism; Haplotype; GRIV cohort

1. Introduction

Cytokines constitute a complex network that regulates the proliferation, differentiation, and death of immune cells. Their involvement is critical in the development of an immune response against various pathogens. In the case of human immunodeficiency virus 1 (HIV-1) infection where the primary targets of the virus are immune cells such as $CD4^+$ lymphocytes or macrophages, the role of cytokines becomes twisted about since cell activation can lead also to viral production [1]. Globally, studies have demonstrated that cytokines can be inhibitory, stimulatory or bifunctional on HIV-1 replication in immune cells [2–4].

Role of type I interferons (IFN) namely IFN-α, IFN-β, IFN-ω, IFN-π as anti-viral agents has been well documented for a long time [5,6]. Type I IFNs are produced by monocytes and dendritic cells, stimulating both antigen presentation and cellu-

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lar cytotoxicity. They play a central role in the regulation of immune response by stimulating both pro- and anti-inflammatory cytokines (IL2, IL10, IL12, IL18) [7]. They elicit a pleiotropic antiviral, antiproliferative, immunomodulatory response and constitute a first line of defense against viral infections including HIV-1 infection [8]. IFN-α, in particular, is the best-known type I IFN and its anti-retroviral effects have been demonstrated in vitro [9,10] and also in vivo [11]. But some works suggest that IFN-α overproduction in AIDS could in fact have a deleterious effect on disease evolution, notably due to the immunosuppressive effect of this cytokine [12,13].

One way to explore the role of a cytokine in disease development is the genomic approach. Indeed, genetic association studies have previously been undertaken in AIDS for the cytokines genes such as IL1, IL4, IL2, IL6, IL10, IL12, IFN-γ and their receptors [4,14–18]. A problem for the genomic study of the *IFN-α* gene is that there exist many pseudo-genes (13 different IFN-α subtypes) [19]. However all IFN-α molecules exert their action through one receptor, IFN-α receptor (IFNAR), for which they compete. As a consequence, it is interesting to study the genetic polymorphisms of this receptor. IFNAR is composed of two subunits IFNAR1 and IFNAR2 [20]. There is a single form of the IFNAR1 subunit. However, an alternative processing of the *IFNAR2* gene transcript produces long (IFNAR2c), short (IFNAR2b), and soluble (IFNAR2a) forms of the encoded subunit [21,22]. *IFNAR1* and *IFNAR2* genes lie on chromosome 21q22.1 next to each other, in a cluster of other genes (Fig. 1) encoding important proteins for the immune response, namely interleukin-10 receptor B (IL10RB) and interferon-gamma receptor-2 (IFNGR2) [23,21]. The IFNAR1 subunit is considered the 'signaling' subunit, since it does not bind type I IFNs with detectable affinity but it is absolutely required for the signal transduced by the heterodimeric IFNAR complex and for type I IFN biological activity [24].

The genomic study of the *IFNAR1* gene should thus certainly contribute to the understanding of the mechanisms that

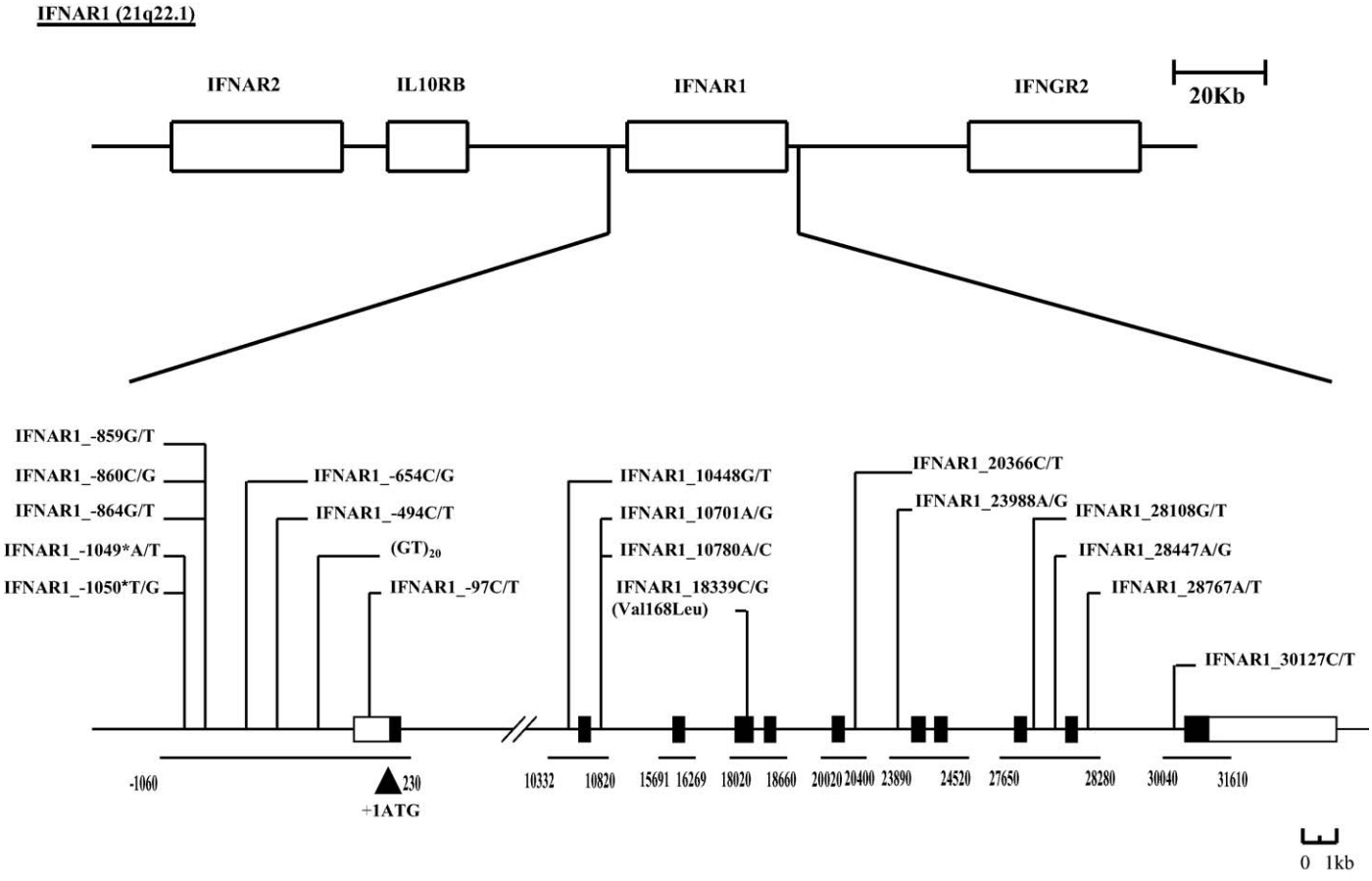

Fig. 1. Chromosome 21q22.1 genes and localization of *IFNAR1* gene markers.
Organization of the chromosome 21q22.1 cytokine receptor cluster. IFNAR2 = interferon-alpha receptor-2, IL10RB = interleukin-10 receptor B, IFNAR1 = interferon-alpha receptor-1 and IFNGR2 = interferon-gamma receptor-2.
Genomic organization of *IFNAR1* gene. 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 (+1ATG). The polymorphism numbers are the attribution number according to first codon site (Met start site). The correspondance with Genebank SNP database is given in Table 2. The newly characterized variants are indicated by an *asterisk*. The genomic sequences used for alignment are *NT_011512.10.(IFNAR1)*.

control the IFN-α pathway in HIV-1 infection. With this in view, we have undertaken the extensive genotyping of the *IFNAR1* gene in the 'Genetics of Resistance to Immunodeficiency Virus' (GRIV) cohort in order to determine if genetic variants could influence disease progression. The GRIV cohort consists of two sub-populations of Caucasian HIV-1 seropositive individuals living in France, with extreme progression phenotypes: 100 patients with rapid progression (RP) and 300 patients with slow progression (SP), which are equivalent to the extreme 1% subset of a cohort of 30.000 seroconverter patients [25]. We also used a group of 400 seronegative CTR subjects of similar ethnic origin. The GRIV cohort is the largest of its kind in the world, and its quality and power have already been validated by several works [26–30].

In the present study we have resequenced the *IFNAR1* exonic regions as well as the promoter region in the GRIV cohort subjects and in CTR, and the genetic polymorphisms identified were then evaluated for their association with susceptibility and progression to AIDS.

2. Materials and methods

2.1. The GRIV cohort and CTR subjects

The GRIV cohort was established in 1995 in France to generate a large collection of DNA for genetic studies on candidate polymorphisms associated with rapid and slow progression to AIDS. Only Caucasians of European descent living in France were recruited. These criteria limit the influence of the environmental and virogenetic factors (all subjects live in a similar environment, and are infected by B strains) and put emphasis on the genetic make-up of each individual to determine the various patterns of progression. Patients with SP were defined as seropositive asymptomatic individuals for 8 or more years with a $CD4^+$ cell count above 500 per mm^3 in the absence of antiretroviral therapy. Patients with RP were defined as patients with a drop in their $CD4^+$ cell count below 300 per mm^3 in less than 3 years after the last seronegative test. The DNA was obtained from fresh peripheral blood mononuclear cells or from EBV-transformed cell lines. The CTR subjects were seronegative Caucasians of European descent living in France.

2.2. Genotyping

The primers and conditions used for PCR amplification of the different fragments are presented in Table 1. Sequencing reactions were performed according to the Dye Terminator method using an ABI PRISM® 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Alignment, single nucleotide polymorphism (SNP) discovery, and genotyping were performed with the software Genalys®, developed by the Centre National de Genotypage (CNG) [31]. For practical reasons, an initial screening was performed on 150 SP, 50 RP, and 150 CTR subjects for polymorphism discovery. The screening could then be extended on the whole cohort when a positive ($P \leq 0.05$) or borderline ($P \leq 0.1$) association was detected. In this study, we have genotyped up to 253 SP, 84 RP, and 443 CTR subjects.

2.3. Hardy–Weinberg equilibrium (HWE)

HWE analysis was performed for each SNP using a Fisher's exact test. It is important to assess the deviations from HWE ($P < 0.05$), 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 [32,33].

2.4. Haplotypes

Haplotype estimates were obtained using the Phase 2 algorithm [34,35] either for all polymorphisms or for selected ones.

2.5. Linkage disequilibrium and haploblocks

Linkage disequilibrium was computed for each pair of polymorphisms, using the r^2 standard method [36] and the D' standard method [37]. A haploblock is a genetic region for which no evidence of a historical recombination event can be found: in other words, the SNPs located within that region exhibit a significant level of linkage disequilibrium (D' close to 1). The haploblocks in the genes have been computed using the method developed by Gabriel et al. [38], which is utilized by the software Haploview [39]. The Tagging-SNPs (tSNPs) were also computed from the genotype data according to the de Bakker's algorithm [40]. These SNPs are useful to retain the

Table 1
Primers used to amplify the exons of *IFNAR1* by PCR. The reaction mixture was: 5 μl DNA (5 ng/μl), 2.4 μl dNTP mix (2.5 mM each), 1.5 μl 10 × ExTaq™ buffer, 0.3 μl of each primer (10 μM), 0.15 μl ExTaq™ (Takara, Otsu, Shiga, Japan) (5 U/μl), and 5.35 μl water. Thermal cycling conditions for PCR were as follows: 94 °C, 10 min for 1 cycle; denaturation, 94 °C, 30 s; annealing, 55–60 °C, 30 s; extension 72 °C, 2 min for 40 cycles; and 72 °C, 10 min for 1 cycle

Exons	Forward primer	Reverse primer
1	5′-AGTGATGGGATATAGAGATGG-3′	5′-AGAGAGGACCCAGAACACCA-3′
2	5′-TGTGCTGGGAGCAATCATTA-3′	5′-TGGCTATGGGTTAGAGACGC-3′
3	5′-GAAGCAACCACCCACAAAAT-3′	5′-ACAAATGGAGCCATAGCAGG-3′
4–5	5′-TGCTCATTGATCGCTTCATC -3′	5′-GGCTGTTCTCGAACTTCTGG-3′
6	5′-CGTCTCGAACTCCTGACCTC-3′	5′-TGGGGAAATAACACATGCAA-3′
7–8	5′-GCAGAGCACAACATGACCAC-3′	5′-GCATCCAGCCCACATAAAGT-3′
9–10	5′-GGCCAATGTTAGACTGAACA-3′	5′-CTCCCAAAGTGCTGGGATTA-3′
11	5′-TGGCACAGTGTACCTTTCTT-3′	5′-CTATCTTCTGGCATGGCATG-3′

major genotype information and limit the computational power necessary to estimate haplotypes in populations.

2.6. Statistical analysis

Statistical analyses were performed only on the polymorphisms (and haplotypes) with a minor allele frequency (MAF) 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 was compared using the 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, Table 2 dealing with individual polymorphisms combines the dominant and recessive modes. Bonferroni corrections were not performed since in such candidate-gene association studies, confirmation in other cohorts is the most relevant [25].

3. Results

3.1. SNP discovery

The *IFNAR1* gene polymorphisms have been systematically screened by sequencing exons with their flanking regions as well as the 1 kb region upstream of the first exon corresponding to the promoter region of the IFNAR1 [41]. We identified 47 SNPs, all SNPs, among which 19 had a MAF greater than 1% in the whole population. Two out of these 19 SNPs were newly characterized by our study (IFNAR1_–1050T/G and IFNAR1_–1049A/T). Fig. 1 shows the positions of the different SNPs with MAF greater than 1%. Only one SNP, IFNAR1_18339G/C, was located in an exon, and it introduces a non-synonymous change in the IFNAR1 subunit (Val168-Leu). The promoter region is more polymorphic than the exonic regions, with eight polymorphisms. The sequencing confirmed the presence of a microsatellite at position -163 as previously described [42] but the number of GT repeat could not be reliably assessed by our sequencing approach. Table 2 summarizes the frequency of each SNP in the three populations SP, RP and CTR, their association with AIDS progression and the relevant information known to date for each SNP.

Table 2
List of all polymorphisms with a minor allele frequency (MAF) greater than 1% in the global population for the *IFNAR1* gene. For each polymorphism we indicate the allelic frequency in the different populations (CTR, RP, SP), the number of genotyped individuals, the *P* values from the Fisher's exact tests, and the NCBI code known to date in the dbSNP database. A1 represents the nucleotide which is identical to the reference sequence *NT_011512.10*. The calculation modes for the Fisher's exact test are indicated (AF: allelic frequency, D: Dominant, R: Recessive). Borderline ($\leq P \leq 0.1$) and significant ($\leq P \leq 0.05$) *P* values are in bold. The *P* values for the dominant/recessive mode was only shown when lower than 0.1. (*: Bonferroni corrections were not performed on *P* values)

SNPs	References to previous studies and NCBI IDs	A1	A2	A1 frequency (%)			Number of individuals			Nominal *P* values for statistical tests*				
				CTR	RP	SP	CTR	RP	SP	SPvsCTR		RPvsCTR		SPvsRPvsCTR
										AF	D/R	AF	D/R	AF
–1050	**NCBI (ss65626678)**	T	G	31.60	36.90	40.31	140	31	102	0.83		0.76		0.87
–1049	**NCBI (ss65626679)**	A	T	31.60	36.90	40.71	140	31	103	0.67		0.75		0.77
–864	NCBI (*rs*17875752)	G	T	26.64	25.00	20.15	118	21	51	0.46		0.73		0.63
–860	NCBI (rs17875753)	C	G	27.08	29.76	21.73	120	25	55	0.90		0.21	*0.08 (R)*	0.67
–859	NCBI (rs2834191)	T	G	27.08	29.76	21.73	120	25	55	0.28		0.48		0.28
–654	NCBI (rs2843710)	C	G	63.88	52.38	57.31	283	44	145	0.50		0.9		0.77
–494	NCBI (rs16997869)	C	T	30.92	36.90	39.52	137	31	100	0.29		1.00		0.51
–163	Muldoon et al., 2001	(GT)20	–	65.01	53.57	66.79	288	45	169	0.73		0.49		0.74
–97	NCBI (rs2850015)	T	C	58.24	55.95	67.19	258	47	170	0.44		1.00		0.72
10448	NCBI (rs2243592, rs17875800)	T	G	29.80	54.761	52.96	132	46	134	0.85		0.71		0.91
10701	NCBI (rs2243594, rs17875801)	A	G	29.12	51.19	52.96	129	43	134	0.92		0.79		0.94
10780	NCBI (rs2253413, rs17875802)	A	C	27.54	51.19	52.56	122	43	133	0.58		0.44		0.67
18339	NCBI (rs2257167, rs17875817)	G	C	32.28	50.00	49.80	143	42	126	0.43	*0.02 (R)*	0.44		0.63
20366	NCBI (rs17875880)	C	T	32.50	55.95	54.54	144	47	138	0.37		0.57		0.49
23988	NCBI (rs2834196, rs17875832)	A	G	33.63	52.38	52.96	149	44	134	0.30		0.28		0.39
28108	NCBI (rs914141, rs17875848)	T	G	34.53	64.28	59.28	153	54	150	0.41		0.13	*0.05 (R)*	0.27
28447	NCBI (rs17875849, rs914142)	G	A	32.95	54.76	55.33	146	46	140	0.51		0.35		0.57
28767	NCBI (rs2856973)	T	A	33.63	57.14	56.12	149	48	142	0.35		0.19		0.35
30127	NCBI (rs2254315, rs17875857)	C	T	93.45	83.33	93.67	414	70	237	0.36	*0.002 (D)*	*0.05*	*0.005 (R)*	0.12

For each SNP, the allelic frequency obtained by our study was similar to that provided in the NCBI dbSNP for European populations. All the SNPs meet the HWE expectations in the three populations.

3.2. Gene structure

Linkage disequilibrium was observed for many SNPs as shown in Fig. 2. We investigated the presence of haploblock structures in *IFNAR1* gene (see Section 2). We could find a major haploblock composed of 11 'successive' SNPs IFNAR1_–654 C/G to IFNAR1_28767 A/T for which the confidence interval for D' was 0.83–1. Overall, it seems that he *IFNAR1* gene is part of a larger haploblock structure. We identified eight tSNPs from our genotype data: IFNAR1_–1049, IFNAR1_–494, IFNAR1_–163, IFNAR1_10448, IFNAR1_20366, IFNAR1_23988, IFNAR1_30127.

3.3. Associations with AIDS: SNPs

The statistical analysis is based on the comparison of the distribution of the SNP alleles in AIDS patients (SP and RP) with that in the CTR population. A Fisher's exact test was used to look for positive signals for an association between polymorphisms and disease progression. Table 2 presents all the SNPs with their respective *P* values when RP subjects are compared with CTR subjects, SP compared with CTR, and the three groups RP, SP, and CTR compared together.

Weak or borderline signals were found for three SNPs: IFNAR1_–860 located in the promoter region ($P = 0.05$), and IFNAR1_28108 T/G located in Intron 9–10 ($P = 0.080$) and IFNAR1_18339 G/C corresponding to a protein mutation Val168Leu (*P*-value of $P = 0.02$).

For the intronic SNP IFNAR1_30127, significant *P* values were also found both when RP subjects are compared with CTR subjects ($P = 0.005$) and SP with CTR ($P = 0.002$). All the results are given in Table 2. As shown in Fig. 2, there is easy to see that there is a strong LD between the two SNPs IFNAR1_18339 and IFNAR1_30127. The *P* values obtained for each of these two SNPs are different because the number of patients genotyped was different (Table 2).

3.4. Association with AIDS: haplotypes

We estimated the haplotypes using the Phase 2 algorithm as described in Section 2. The computation of the haplotypes derived from the 19 SNPs yielded eight different haplotypes with a global frequency greater than 1% (Table 3A). As shown in Table 4A the haplotype 7 (IFNAR1-H7) exhibited a positive signal for the comparison of the three SP, RP and CTR groups ($P = 0.03$), and a borderline signal for the comparison of the RP with the CTR groups ($P = 0.08$). The association found for this haplotype could not be explained by any individual SNP allele (Table 3A).

We also computed the haplotypes derived from the SNPs located in the promoter region. The computation yielded eight

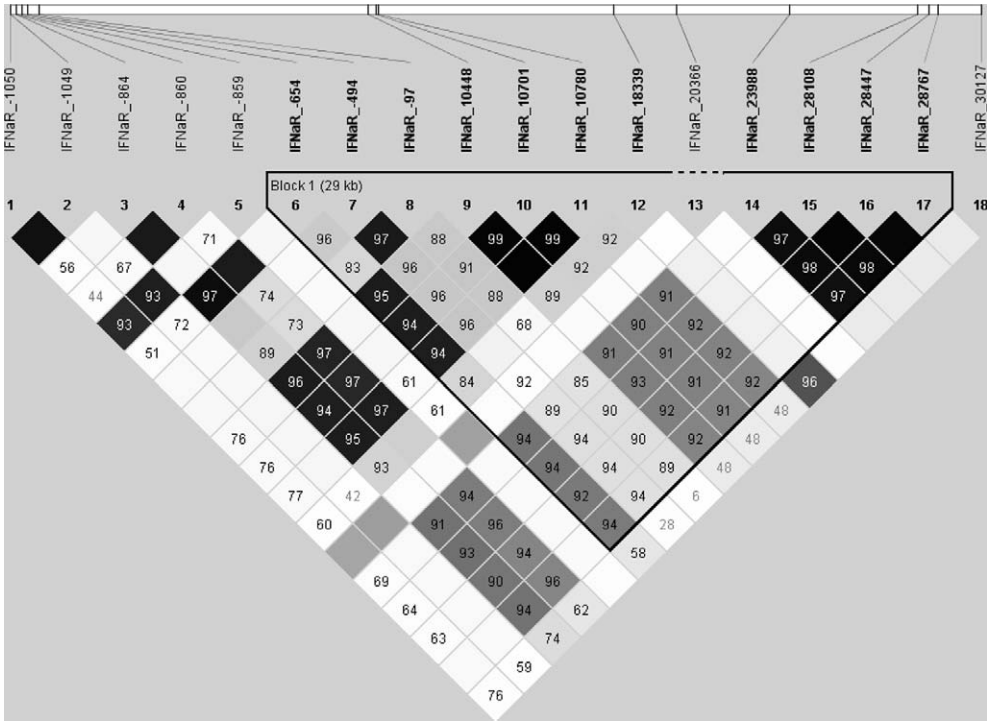

Fig. 2. Linkage disequilibrium map of the *IFNAR1* gene provided by the software Haploview. The different shades of grey correspond to the level of LD according to the r^2 coefficient. The values indicated in the squares correspond to the LD according to the D' coefficient. An empty square indicates that $D' = 1$. The Lewontin's D' coefficient is correlated with the level of recombination: it is useful for the finding of haploblocks. r^2 is a more stringent coefficient that detects more perfect LD. r^2 has a value of 1 if only two haplotypes can be derived from the two SNPs analyzed, whereas D' takes a value of 1 if three haplotypes can be derived.

Table 3A
Global haplotypes. Detailed description for the global haplotypes of *IFNAR1* gene estimated with the Phase 2 algorithm

Haplotypes	Polymorphisms of IFNAR1 gene																	
	-1050	-1049	-864	-860	-859	-654	-494	-97	10448	10701	10780	18339	20366	23988	28108	28447	28767	30127
H1	T	A	G	C	T	C	C	C	T	A	A	G	C	A	G	A	A	C
H2	T	A	T	G	T	G	C	C	G	G	C	G	C	G	T	G	T	C
H3	T	A	G	C	T	C	T	T	T	A	A	G	C	A	G	A	A	C
H4	T	A	T	G	T	G	C	C	G	G	C	C	C	A	G	A	A	T
H5	T	A	G	C	T	C	T	T	T	A	A	G	C	A	G	A	A	T
H6	G	T	G	C	G	C	C	C	T	A	A	G	C	A	G	A	A	C
H7	T	A	G	G	T	C	T	T	T	A	A	G	C	A	G	A	A	C
H8	G	T	G	C	G	C	C	C	T	A	A	G	T	A	G	A	A	C

Table 3B
Promoter haplotypes. Detailed description for the promoter haplotypes of *IFNAR1* gene estimated with the Phase 2 algorithm

Global Haplotypes	Promoter Polymorphisms of IFNAR1 gene							
	-1050	-1049	-864	-860	-859	-654	-464	-97
Prom-H1	T	A	G	C	T	C	C	C
Prom-H2	T	A	T	G	T	G	C	C
Prom-H3	T	A	G	C	T	C	T	T
Prom-H4	T	A	G	G	T	G	C	C
Prom-H5	T	A	T	G	T	G	T	T
Prom-H6	G	T	G	C	G	C	C	C
Prom-H7	T	A	G	G	T	C	C	C
Prom-H8	T	A	G	C	T	C	C	T

Table 4A

Global Haplotypes	Allelic frequency (%)			Nominal p values (P) for statistics test*				
	CTR	RP	SP	SPvsCTR		RPvsCTR		SPvsRPvsCTR
				AF	D/R	AF	D/R	AF
H1	27.82 (74)	31.67 (19)	27.89 (53)	1.00	0.40 (R)	0.53		0.82
H2	25.94 (69)	25.00 (15)	21.58 (41)	0.31	0.22 (D)	1.00		0.55
H3	19.55 (52)	13.33 (8)	24.21 (46)	0.24	0.21 (D)	0.35		0.16
H4	10.53 (28)	11.67 (7)	10.00 (19)	0.87	0.42 (R)	0.81		0.93
H5	2.26 (6)	3.33 (2)	4.21 (8)	0.276	0.26 (D)	0.64		0.49
H6	3.76 (10)	5.00 (3)	1.58 (3)	0.254	0.24 (D)	0.71		0.27
H7	1.13 (3)	5.00 (3)	0.53 (1)	0.644	1.00	0.078	0.076 (D)	0.029
H8	1.50 (4)	0.00 (0)	2.63 (5)	0.500	0.49	1.00		0.67

Comparative distribution of global haplotypes. For each haplotype we indicated the frequency in each population CTR, RP, and SP with the absolute number of haplotypes in parenthesis, the *P*-value (Fisher's exact test) for the comparisons *SPvsCTR, RPvsCTR*, and *P*-value (χ^2 test) for the comparison *SPvsRPvsCTR*. SP, RP and CTR represent respectively the Slow Progression, Rapid Progression and Control Groups. The calculation modes AF, D, and R correspond, to the Allelic Frequency, the Dominant, and the Recessive modes respectively. Borderline ($P \leq 0.1$) and significant ($P \leq 0.05$) P values are in bold. The *P* values for the D/R calculation modes were only shown when lower than 0.1.

Table 4B

Promoter Haplotypes	Allelic frequency and number of individuals			Nominal p values for static test* in allelic frequency mode		
	CTR	RP	SP	SPvsCTR	RPvsCTR	SPvsRPvsCTR
Prom-H1	0,38 (90)	0,41 (19)	0,32 (33)	0.32	0.74	0.48
Prom-H2	0,29 (68)	0,30 (14)	0,32 (32)	0.69	0.85	0.88
Prom-H3	0,24 (56)	0,15 (7)	0,25 (26)	0.78	0.25	0.37
Prom-H4	0,05 (13)	0,02 (1)	0,02 (2)	0.24	0.48	0.25
Prom-H5	0,01 (3)	0,06 (3)	0,010 (1)	1.00	0.05	0.03
Prom-H6	0,02 (4)	0,00 (0)	0,00 (0)	0.31	1.00	0.83
Prom-H7	0,00% (0)	0,00 (0)	0,03 (3)	0.02	NS	0.14
Prom-H8	0,00% (0)	0,04 (2)	0,01 (1)	0.30	0,02	0.05

Comparative distribution of promoter haplotypes. For each promoter haplotype, we indicated the frequency in each population CTR, RP, SP with the absolute number of haplotypes in parenthesis, the *P*-values (Fisher's exact test) for the comparisons SP vs. CTR, RP vs. CTR, SP vs. RP, SP vs. RP vs. CTR. Bonferroni corrections were not performed on nominal P values. SP, RP and CTR represent respectively the Slow Progression, Rapid Progression and Control Groups. The calculation is in Allelic Frequency mode. Borderline ($P \leq 0.1$) and significant ($P \leq 0.05$) *P*-values are in bold.

haplotypes with a frequency greater than 1%. Table 3B presents the haplotypes with their allelic frequency. As shown in Table 4B, the promoter haplotypes 5 and 8 (IFNAR1 prom-H5 and IFNAR1 prom-H8) both yielded positive nominal *P* values in associations tests for the comparison of the RP with the CTR groups, and for the simultaneous comparison of the three groups ($P = 0.03$). The associations found for the IFNAR1 prom-H5 and H8 haplotypes could not be explained by any individual SNP allele, and they were not derived from the IFNAR1-H7 global haplotype.

4. Discussion–conclusion

In our systematic analysis of the *IFNAR1* gene polymorphisms in the French GRIV cohort, we have identified 19 frequent SNPs (MAF greater than 1%) among which two were newly characterized. We found four polymorphisms exhibiting positive or borderline signals (nominal P values < 0.1) with AIDS progression (Table 2). Among these four polymorphisms, two are located in introns (IFNAR1_28108T/G, IFNAR1_30127C/T), one in the promoter region (IFNAR1_–860C/G), and one in the exon 4 region (IFNAR1_18339G/C) which induces a non synonymous Val168Leu change in the protein. We also computed haplotypes and weak positive signals were also observed for the estimated haplotype IFNAR1-H7, and similarly for the promoter haplotypes IFNAR1_prom-H5 and IFNAR1_prom-H8. These haplotype associations could not be explained by any individual SNP allele. The functional significance of polymorphisms located in introns is difficult to interpret. The polymorphism IFNAR1_–860 located in the promoter region presents a positive signal ($P = 0.05$), but using the TRANSFAC site [43], we could not find a similarity to any known transcription factor binding site. The way to explore further the associations found with the SNP IFNAR1_-860 and with the promoter haplotypes IFNAR1_prom-H5 and IFNAR1_prom-H8 would be to perform functional tests such as luciferase assays. Of course, these putative associations will also need to be validated by genomic studies on other AIDS cohorts.

Overall, the most interesting findings were related to the SNPs IFNAR1_18339G/C and IFNAR1_30127C/T and the following discussion will focus on these two SNPs. These two SNPs are in fact in strong LD ($r^2 = 0.86$, see Fig. 2) and if the P values observed for these SNPs are different (Table 2), it is mainly because less patients were genotyped for the SNP IFNAR1_18339. When looking at the HapMap database [44], we found another two intronic *IFNAR1* SNPs, namely rs1041868 and rs2254180, in strong LD with IFNAR1_18339 and IFNAR1_30127 (data not shown). These two SNPs were not sequenced in our study since we put our focus around the exonic and promoter regions (Fig. 1). From the HapMap database, it appears unlikely that the associations observed for the *IFNAR1* SNPs might be caused by LD with SNPs of the neighboring cytokine receptor genes present in the 21q22.1 locus but we could not observe any LD. The biological explanation for the associations is thus likely linked to the *IFNAR1* SNPs.

Table 5 gives the detail of the genotypic distributions of the IFNAR1_18339G/C and IFNAR1_30127C/T alleles in the SP, RP and CTR populations. Since RP and SP groups have a similar distribution regarding the SNPs IFNAR1_18339 and IFNAR1_30127 but they are significantly different from the CTR group ($P < 0.05$, Table 5), it is possible that these SNPs could also be linked to the susceptibility to HIV-1 infection.

The effect would be recessive (Table 5) and it is quite tempting to link it to the SNP allele IFNAR1_18339C which corresponds to a mutation Val168Leu in the extracellular domain of IFNAR1. Indeed, Leyva *et al.* [45] found also an association with this allele in the recessive mode in MS. The G allele is the ancestral allele (it is found in non human primates and in bovines while the C allele is not) and the prevalence observed in our CTR group is similar to that found for Caucasians in the HapMap database [44]. It is important to note that these SNPs also exist in African and in Asian populations with different distributions: hence, the IFNAR1_18339 C allele (Leu variant) has a frequency of 11.2% in Caucasians but increases to 15% in the African population and reaches to 30% in the Asian population.

IFNAR1 is the only member of the helical cytokine receptors superfamily II (hCRII). It possesses an extracellular domain composed of four fibronectin type III domains, denoted for sake of simplicity SD1–SD4 (Fig. 3) and the substitution Val168Leu is located in the SD2 domain. The fine molecular mechanisms of the IFN-α-IFNAR1 interaction remain largely unknown and identification of critical residues for a productive contact could contribute to the understanding of the activation of IFN-α pathway. Cajean-Feroldi et al. [46] performed mutagenesis experiments and found that the specific residues ^{62}FSSLKLNVY^{70} and the tryptophan^{129} of the SD1 and SD2 domains were crucial for IFN-α binding and signaling. But they did not study the valine residue at position 168. Their results suggest however that the SD2 domain may be important for signaling and why not the residue 168? The answer to this question is found by performing functional analyses on the wild type and the mutant receptor.

To our knowledge, this study is the first to analyze the involvement of polymorphisms of the *IFNAR1* gene in AIDS disease development. However many genetic studies have dealt with the involvement of these polymorphisms in other immune and infectious diseases. It is remarkable to observe that the polymorphism IFNAR1_18339 (Val168Leu) was previously associated with severe and cerebral malaria with respective P values $P = 0.002$ and $P = 0.003$ [47] and with susceptibility to

Table 5
Detailed description of the genotypic distribution in the three SP, RP, and CTR groups of the SNPs IFNAR1_18339G/C and IFNAR1_30127C/T which are in LD. The P values observed for the comparison of the SP with CTR groups and of the RP with CTR groups suggest that we likely deal with a recessive effect

SNP	Genotype	GRIV groups			Nominal p-values for Fisher test		
		SP	RP	CTR	SP-CTR	RP-CTR	SP-RP
IFNAR1_18339	C|C	5 (3.97%)	0 (0.00%)	0 (0.00%)	0.02	1	0.33
	G|G	97 (76.98%)	29 (70.73%)	111 (77.62%)	1	0.4	0.53
	C|G	24 (19.05%)	12 (29.27%)	32 (22.38%)	0.54	0.4	0.19
IFNAR1_30127	T|T	15 (6.33%)	6 (8.70%)	7 (1.69%)	0.002	0.005	0.58
	C|C	161 (67.93%)	42 (60.87%)	279 (67.39%)	0.93	0.33	0.25
	C|T	61 (25.74%)	21 (30.43%)	128 (30.92%)	0.18	1	0.36

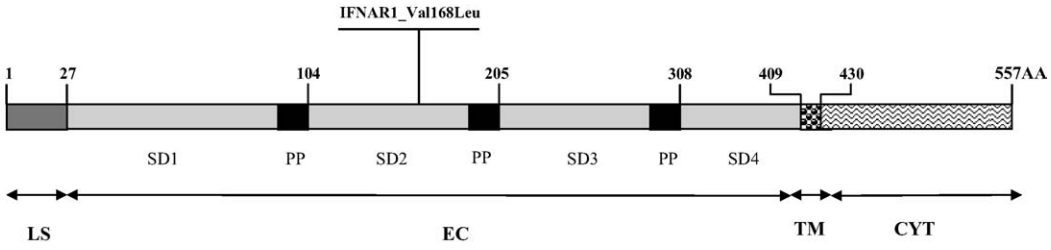

Fig. 3. Representation of the IFNAR1 protein (557AA) and its domains.
SD1, SD2, SD3, and SD4 are the four extracellular subdomains of IFNAR1 delimited by proline motifs (PP). The leader sequence is designated LS; the transmembrane sequence is designated TMS; the extracellular domain is designated EC; the cytoplasmic domain is designated CYT. Protein variant IFNAR1_Val168Leu is mentioned.

multiple sclerosis with a *P*-value $P = 0.001$ [45]. Surprisingly, in these studies the authors apparently did not investigate the other intronic SNPs which are in LD such as IFNAR1_30127 and functional studies have yet to be performed to know which SNP could be the biological cause for the observed associations.

A confirmation of our results by the genomic analysis of other cohorts needs to be done in order to validate these associations. Finally, it appears that the whole chromosomal 21q22.1 locus should be of interest for further genomic studies in AIDS and other immune-related diseases since it contains many cytokine receptor genes (Fig. 1).

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