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Dossier : Human and animal retroviruses

Exploration of associations between phospholipase A2 gene family polymorphisms and AIDS progression using the SNPlex™ method

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Abstract

Members of the secreted phospholipase A2 (PLA2) protein family can inhibit HIV-1 virus replication *in vitro*. To evaluate the impact of *PLA2* gene polymorphisms on AIDS disease development, we studied 12 family members using SNPlex™ technology that permitted simultaneous typing of 70 tagging Single Nucleotide Polymorphisms (tagSNPs). The study utilized HIV-1 seropositive donors with slow progressor ($n = 168$) or rapid progressor ($n = 54$) status, plus 355 control subjects. All donors were Caucasian (total 577 individuals).

Genetic associations yielded mainly $0.01 < p < 0.05$, but lower p-values were obtained for four tagSNPs and seven haplotype alleles. These stronger associations corresponded to both secreted (*PLA2G2A*, *PLA2G2D* and *PLA2G3*) and cytosolic (*PLA2G4A* and *PLA2G6*) *PLA2* genes, including three (*PLA2G2A*, *PLA2G2D* and *PLA2G4A*) implicated in the pathogenesis of other diseases. Our results suggest that the *PLA2* gene family may represent genes of interest for a larger study targeting all the known tagSNPs in the *PLA2* genes. The data presented in this study will have to be confirmed in other AIDS cohorts and will also be useful for studies undertaken on the *PLA2* gene family in other disease cohorts.
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1. Introduction

Phospholipase A2 (PLA2) proteins constitute a large family of enzymes, which share similarities of genomic sequences and common functions in the hydrolysis of phospholipids and release of free fatty acids and lysophospholipids. The family comprises two main classes of enzymes, the low molecular weight secreted PLA2s (sPLA2s) and the high molecular weight cytosolic PLA2s (cPLA2s). Human sPLA2 proteins comprise the IB, IIA, IID, IIE, IIF, III, V, VII, X, XIIA and XIIB groups, while cPLA2 proteins comprise the IV and VI groups. PLA2 proteins may exert many physiological and pharmacological effects among which phospholipid digestion and metabolism, production of lipid mediators for inflammatory reactions, inhibition of platelet aggregation, and cell membrane remodeling (for a review see Ref. [1] or [2]).

PLA2 proteins may also participate to host defense mechanisms against bacterial infections [3–8], parasitic infections [9,10], and viral infections [11–14]. Regarding HIV infection, it was first found that a bee venom sPLA2 can block target cell penetration by both HIV-1 and HIV-2 virions [12]. This

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mechanism was independent of its catalytic activity and associated with the capacity of sPLA2 to bind host cells. Additionally, the authors have shown that a peptide derived from bee venom sPLA2 displays an anti-HIV activity through the binding with the HIV co-receptor CXCR4 [11,12]. In another study, lysophosphatidylcholine, a product of PLA2 enzymatic activity, could inhibit fusion of HIV-1 with host cell membranes by affecting interactions between gp120/gp41 and CD4 [15]. Finally, group X sPLA2 (PLA2G10) protein could neutralize several enveloped lentiviruses, including HIV-1, through the phospholipid hydrolysis of the viral membranes [13].

As these *in vitro* studies suggest, PLA2 enzymes may influence HIV infection. We have undertaken a genomic approach to assess the possible associations of genetic variations of the human PLA2 family with susceptibility to HIV infection or AIDS progression. We compared the distribution of the *PLA2* gene polymorphisms between groups of HIV-1 seropositive patients displaying extreme patterns of disease progression, namely the slow and rapid progressors of the Genomic of Resistance to Immunodeficiency Virus (GRIV) cohort [16], and a seronegative group. This cohort is composed of Caucasian HIV-1 seropositive individuals living in France: 100 patients with a rapid progression (RP) phenotype and 300 patients with a slow progression (SP) phenotype who correspond to the extreme 1% subset of a cohort of 30,000 seroconverter patients [17]. We also included in our study 355 healthy seronegative control subjects of similar ethnic origin. The GRIV cohort is probably the largest cohort of its kind in the world, and its quality and power have already been validated by several gene associations dealing with *CCR5* [18–20], *HLA* [21,22], and cytokines [23–26] gene polymorphisms. We used the SNPlex™ methodology to genotype tagging Single Nucleotide Polymorphisms (tagSNPs) in the *PLA2* genes and identify genetic associations suggesting the possible involvement of PLA2 proteins in the pathogenesis of HIV-1 infection.

2. Materials and methods

2.1. The GRIV cohort and control subjects

The GRIV cohort was established in 1995 in France to generate a large collection of DNAs for genetic studies on candidate human polymorphisms associated with rapid and slow progression to AIDS [16]. 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 slow progression (SP) were defined as seropositive asymptomatic individuals for 8 or more years with a CD4 T-cell count above 500/mm^3 in the absence of antiretroviral therapy. Patients with rapid progression (RP) were defined as patients with a drop in their CD4 T-cell count below 300/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 control (CTR) subjects are seronegative Caucasians of European descent living in France. In this study, we have genotyped up to 168 slow progression patients, 54 rapid progression patients, and 355 control subjects.

2.2. SNPlex™ Genotyping System

The SNPlex™ Genotyping System (Applied Biosystems, Foster City, CA, USA) is a high-throughput production-scale genotyping system which allows multiplexed genotyping (multiple reactions in one well) of 48 bi-allelic SNPs for a single biological sample [27,28].

2.3. SNP selection

Using HapMap ([29], www.hapmap.org), we listed all bi-allelic SNPs with a minor allele frequency >5% in Caucasians (termed 'frequent SNPs', total = 408), in the *PLA2* genes and 10 kb flanking regions. The latter were included to encompass regulatory sequences. Linkage disequilibrium (LD) was computed for each pair of polymorphisms, using the r^2 standard method [30] or the D' standard method [31]. We determined 183 SNPs sufficient to cover the maximum genotypic information and to limit the computational power necessary to estimate haplotypes in populations, termed tagging SNPs (tagSNPs). For this, we made a pairwise tagging method with the Haploview software [32,33]. We submitted this list of SNPs for customized assay design from the Applied Biosystems Website (www.appliedbiosystems.com). During this screening step, some SNP sequences were eliminated if they were redundant in the human genome, contained non-target polymorphisms near the target SNP or sequence motifs incompatible with the assay, presented cross-reactivity with assay components.

2.4. Experimentation

Fragmented genomic DNA (50 ng) was dried into each well of a 384-well plate (approximately 1 ng DNA per genotype). After phosphorylation of Oligonucleotide Ligation Assay (OLA) probes and universal linkers, allele-specific ligation and enzymatic purification were performed. PCR utilized universal biotinylated primers, so amplicons could be captured on streptavidin-coated plates. Single-strand PCR products were hybridized with a universal set of fluorescently dye-labeled mobility modifiers, the ZipChute™ probes that have a unique sequence corresponding to each SNP. ZipChute™ probes were eluted and separated for detection by capillary electrophoresis on ABI PRISM® 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

2.5. Analysis and quality control

The GeneMapper™ Analysis Software v4.0 collects, formats, processes and analyzes the data and assigns individual genotypes. This tool simplifies quality control (elimination of ambiguous genotypes).

2.6. Bioinformatic analysis

2.6.1. Hardy-Weinberg equilibrium (HWE)

HWE analysis was performed for each SNP in each group using Fisher's exact tests. Deviations from HWE ($p < 0.05$) in one group of patients suggest that the SNP has a biological effect, while deviations in all groups suggest a systematic error in genotyping [34,35].

2.6.2. Haplotypes

Haplotypes inference was obtained using the ISHAPE2 algorithm [36] either for all polymorphisms of each candidate gene or for selected ones.

2.6.3. Statistical analysis

The differences in the allelic distributions between the three groups were examined using Fisher's exact tests for SNP and haplotype alleles. Four modes of calculation were used for the genetic analysis: allelic frequency, dominant mode, recessive mode and genotypic distribution. As SNPs are bi-allelic, the dominant and recessive modes yield identical *p*-values and are combined in Tables 2−4, S1 (Supplementary data online) and S2 (Supplementary data online). Multitest corrections were not performed since confirmation in other cohorts is the most relevant in such candidate gene association study [17].

2.6.4. Biological and disease markers

The progression of HIV disease in SP patients was monitored via the number, percentage and ratio of CD4 and CD8 T-cells and the plasma HIV RNA concentration (viral load) assessed during routine clinical follow-up. The putative year of infection and CDC status was also available for some SP patients. For each marker, we compared the allelic distributions between SP patients in the lower quartile (or tertile) with those in the highest quartile (or tertile) using Fisher's exact tests. This study on biological or disease markers is complementary to the study comparing directly the genotypes of patients between the SP, RP and CTR groups.

3. Results

3.1. SNP selection

The location of the *PLA2* genes is presented in Fig. 1A and information regarding their structure and sequence are provided in Table 1. *PLA2G10* was excluded in our study as no polymorphisms in this gene are provided in HapMap. Table 1 shows that we determined 183 tagSNPs in order to extensively cover the diversity of the 12 remaining genes (*PLA2G1B*, *PLA2G2A*, *PLA2G2D*, *PLA2G2E*, *PLA2G2F*, *PLA2G3*, *PLA2G4A*, *PLA2G5*, *PLA2G6*, *PLA2G7*, *PLA2G12A* and *PLA2G12B*). Due to technical limitations, we decided to do a preliminary screening of 70 tagSNPs with highest r^2 cut-off spread over the 12 genes. The description of the selected SNPs is given in Fig. 1B. Five SNPs (PLA2G2F_159, PLA2G3_208, PLAG3_5191, PLAG3_5208 and PLA2G6_35934) out of 70 did not yield reliable results because of an aspecific reaction during the experimentations or because of doubtful genotypes during analysis and they were excluded from our analysis (SNPs in bold and in italics in Table S1 Supplementary data online).

3.2. SNPs associations with AIDS progression

Table S1 (Supplementary data online) summarizes the frequencies of each SNP obtained in the three populations and their position in the gene. All SNP allelic frequencies obtained in CTR samples generally matched those provided by HapMap for Caucasian populations. The distribution of all SNP alleles respected the Hardy-Weinberg equilibrium in all three populations, except for PLA2G4A_38933, PLA2G4A_70916 and PLA2G4A_106065 in the SP group.

The GRIV case−control study is based on the comparison of the allelic distributions between the seropositive extreme patients (SP and RP) and the CTR population. Fisher's exact tests were carried out and we found 11 significant signals ($p \leq 0.05$) and 5 borderline signals ($0.05 < p < 0.1$). These associations were found by comparing the SP and the CTR populations, except for PLA2G2A_8156 found by comparing RP and CTR groups, and for PLA2G6_−14136 found both by comparing the SP and the CTR groups and by comparing RP and CTR groups. All these associations are summarized in Table 2. None of these associations involved an exonic polymorphism as a tagSNP or a SNP in LD. The promoter tagSNP PLA2G6_−14136 is in LD with another promoter SNP (rs4821754) and with an intronic SNP (rs2267371), and the promoter borderline tagSNP PLA2G12A_−5765 is in LD with another promoter SNP (rs13117504). No SNPs are known to be in LD with the borderline promoter tagSNP PLA2G2D_−9229, or with the intronic tagSNPs PLA2G4A_21440, PLA2G4A_27474 and PLA2G12B_267, or with the 3′gene region tagSNPs PLA2G2A_8156, PLA2G2D_8228, PLA2G2D_8358, PLA2G2D_16131 and PLA2G2E_11193. On the other hand, the 3′UTR tagSNP PLA2G2D_6679 is in LD with another 3′UTR SNP (rs578459), and the 3′gene region PLA2G12A_22072 tagSNP describes three intronic SNPs (rs10004377, rs6831917 and rs6533451). The intronic tagSNPs PLA2G4A_38933, PLA2G4A_106065 and PLA2G6_4689 are in LD, respectively, with nine intronic SNPs (rs4650708, rs1474590, rs12720557, rs12720662, rs3736741, rs4651343, rs12720541, rs12144159 and rs10911953), two intronic SNPs (rs6683515 and rs12128551) and 20 intronic SNPs (data not shown).

3.3. Haplotype associations with AIDS progression

For each gene, haplotypes were estimated using the ISHAPE2 algorithm taking into account (1) all polymorphisms and (2) only promoter polymorphisms. Table 3 presents the seven haplotypes with frequency greater than 2% that exhibited significant differences ($p \leq 0.05$) between the groups: PLA2G2A_all_H0, PLA2G2A_all_H6 and PLA2G2D_all_H8 comparing the SP and CTR populations, PLA2G2E_all_H2, PLA2G3_all_H4, PLA2G3_prom_H1 and PLA2G6_all_H1 comparing

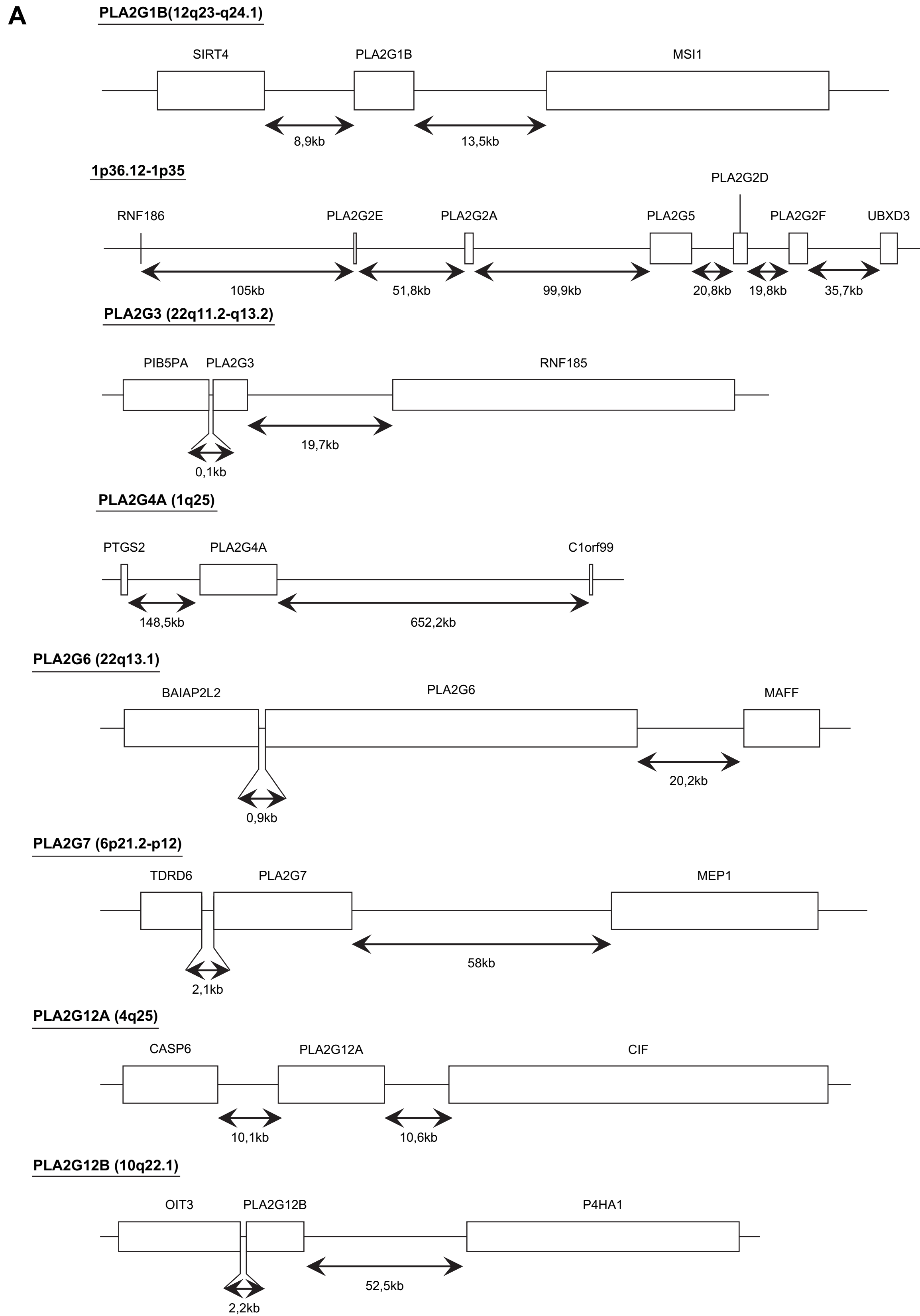

Fig. 1. (A) Chromosomic location of *PLA2* genes. (B) Genetic maps of *PLA2* genes. Exons and UnTranslated Regions (UTR) are symbolized, respectively, by full rectangle and empty rectangle. The scale is specified for each gene. All the SNPs covered by our study (tagSNPs and SNPs in LD) are represented, except for the two cytosolic PLA2s (PLA2G4A and PLA2G6): due to the size of these two genes the number of covered SNPs is too high to draw a clear map, and we only show the tagSNPs.

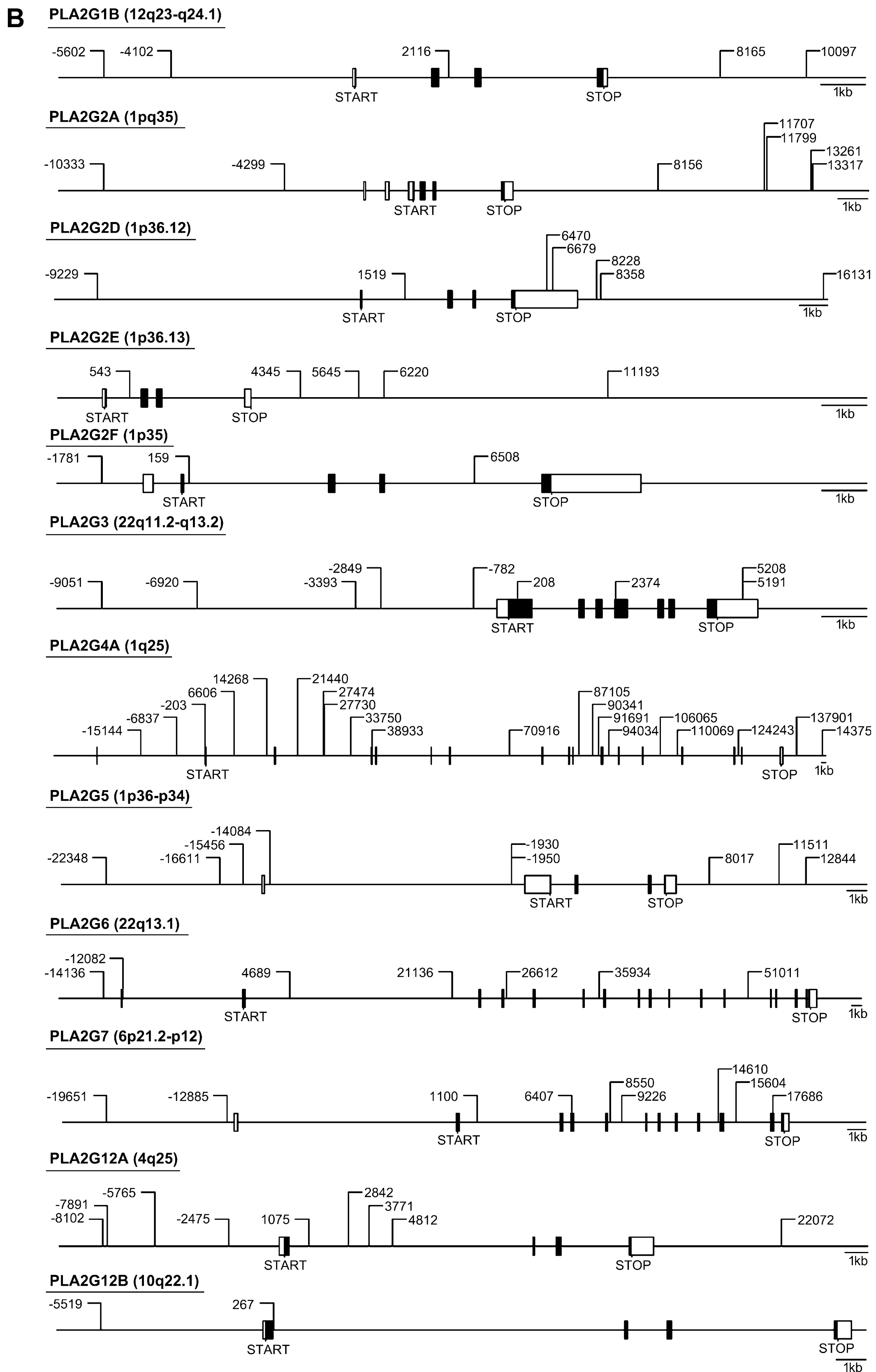

Fig. 1. (*continued*).

Table 1
Summary of the genomic study presenting the candidate genes (gene size, accession number, number of exons, protein size)

Gene	Gene size (pb)	Accession number	Number of exons	Protein size (aa)	Total number of tagSNPs	Number of tagSNPs experimented	Number of SNPs covered	Total number of SNPs
PLA2G1B	6479	NM_000928	4	148	6	3	5	13
PLA2G2A	5779	NM_000300	6	144	14	4	7	27
PLA2G2D	8377	NM_012400	4	145	19	6	7	23
PLA2G2E	4111	NM_014589	4	142	22	3	5	32
PLA2G2F	11,854	NM_022819	5	168	11	3	3	14
PLA2G3	6477	NM_015715	7	509	16	7	9	21
PLA2G4A	160,809	NM_024420	18	749	38	20	62	108
PLA2G5	21,616	NM_000929	5	138	22	6	9	50
PLA2G6[a]	71,060	NM_003560	17	806	11	7	57	79
PLA2G7	31,625	NM_005084	12	441	14	5	9	25
PLA2G12A	17,322	NM_030821	4	189	7	4	9	13
PLA2G12B	20,373	NM_032562	4	195	3	2	2	3

For each gene, we indicate the total number of tagSNPs, the number of tagSNPs studied, the number of SNPs covered and the total number of SNPs. Gene size includes the 5′ and the 3′UTR when they are known. The human genome version used is the NCBI B35 assembly.

[a] *PLA2G6* gene codes for two isoforms a and b. Isoform a is presented in this table. Isoform b RNA (NM_001004426) is composed of 16 exons and codes for a 752-amino acid protein.

the RP and CTR populations. We also estimated haplotypes derived from neighbouring tagSNPs exhibiting low association *p*-values. These haplotypes, called Haplo_2SNPs and Haplo_3SNPs, also led to positive signals (Table 3).

The composition of all haplotypes with a global frequency ≥2% is given in Table S2 (Supplementary data online).

3.4. Correlation with biological markers

The effects of *PLA2* SNPs on standard markers of HIV disease (CD4 T-cell counts, viral load, etc.) were assessed in the SP patients (see Section 2). The correlations described in Table 4 were in agreement with the genetic associations previously found for tagSNPs (Table 2) and haplotypes (Table 3).

4. Discussion

We have undertaken a preliminary genomic study of the *PLA2* family genes in the GRIV cohort to look for genetic factors involved in AIDS disease progression. We used the SNPlex™ method with tagSNPs derived from the HapMap project. The associations were done by comparing the SP group versus the CTR group and the RP group versus the CTR group, which is the basis of this case–control study.

We identified 11 tagSNPs exhibiting a positive signal ($p \leq 0.05$) and 5 tagSNPs exhibiting a borderline signal ($0.05 < p < 0.1$). These associations reflected comparison between CTR and RP or SP groups with the exception of the PLA2G6_−14136-A allele where carriage was increased in both populations. For this tagSNP, the frequency of AA

Table 2
Significant and borderline tagSNPs results

Gene	SNP position	Reference to dbSNP	A1	A2	Allelic frequency (A1) %			Location	Nominal *p*-values for statistical tests			
					CTR	RP	SP		SP vs CTR		RP vs CTR	
									AF	D/R	AF	D/R
PLA2G2A	8156	rs1891320	C	T	75.78	70.37	79.22	3′ gene region	0.24		0.23	**0.0277 (R-T)**
PLA2G2D	−9229	rs12722987	C	A	85.45	81.48	81.63	5′ gene region	0.12	0.0947 (R-C)	0.31	
PLA2G2D	6679	rs617180	C	G	48.43	38.68	42.86	3′UTR	0.1	**0.00479 (R-C)**	0.08	
PLA2G2D	8228	rs7551550	C	A	53.99	49.04	46.54	3′ gene region	**0.03**	**0.00548 (D-A)**	0.4	
PLA2G2D	8358	rs7515774	A	T	59.42	58.33	55.33	3′ gene region	0.23	**0.0380 (R-A)**	0.83	
PLA2G2D	16131	rs492738	C	A	33.85	34.26	40.06	3′ gene region	0.05	0.0533 (D-A)	0.91	
PLA2G2E	11193	rs3738122	C	G	73.94	66.98	78.79	3′ gene region	0.1	**0.0288 (R-C)**	0.16	
PLA2G4A	21440	rs2223307	C	T	21.41	26.85	29.17	intron 3-4	**0.01**	**0.01 (D-C)**	0.21	0.0511 (R-T)
PLA2G4A	27474	rs1980444	T	C	12.68	16.67	19.16	intron 3-4	**0.01**	**0.0111 (D-T)**	0.28	
PLA2G4A	38933	rs7519192	A	G	76.8	78.3	75.89	intron 4-5	0.75	**0.0177 (R-G)**	0.81	
PLA2G4A	106065	rs12125857	C	T	79.58	80.56	78.82	intron 14-15	0.81	**0.0114 (R-T)**	0.9	
PLA2G6	−14136	rs4820321	T	A	70.49	63.89	64.67	5′ gene region	0.06	**0.0485 (R-A)**	0.18	**0.0485 (R-A)**
PLA2G6	4689	rs4376	T	C	88.78	88.89	91.96	intron 2-3	0.13	0.0957 (R-T)	1	
PLA2G12A	−5765	rs7439493	G	A	54.76	55.77	59.23	5′ gene region	0.18	0.0787 (R-A)	0.92	
PLA2G12A	22072	rs1541373	C	T	41.5	43.4	35.88	3′ gene region	0.09	**0.0413 (R-C)**	0.75	
PLA2G12B	267	rs3829126	G	T	89.97	88.68	92.81	intron 1-2	0.17	0.0663 (R-G)	0.73	

For each polymorphism, we indicate the allelic frequency in the different populations (CTR, RP and SP), the dbSNP rs code, the location and the *p*-values from Fisher's exact tests. A1 represents the nucleotide which is identical to reference sequences. The calculation modes for Fisher's exact tests are indicated (AF: allelic frequency, D: dominant, R: recessive). For dominant and recessive modes, the allele involved is specified. Significant *p*-values ($p \leq 0.05$) are in bold.

Table 3
Haplotypes with significant results ($p \leq 0.05$)

Allele	Haplotype	Haplotype details (position: allele)	Frequence CTR	Frequence SP	Frequence RP	SP-CTR AF	SP-CTR D/R	CTR-RP AF	CTR-RP D/R
PLA2G2A_all	H0	−10333: T; −4299: T; 8156: C; 11707: C	24.57	28.01	23.15	0.252	**0.0432 (R)**	0.81	
PLA2G2A_all	H6	−10333: T; −4299: A; 8156: C; 11707: C	3.18	0.6	0	**0.00793**	**0.00727 (D)**	0.0595	
PLA2G2D_all	H8	−9229: C; 1519: T; 6679: C; 8228: C; 8358: A; 16131: A	3.2	0.92	4.72	**0.0297**	**0.0277 (D)**	0.39	
PLA2G2D_2SNPs	H0	6679: G; 8228: A	44.96	52.85	48.08	0.242	**0.00296 (D)**	0.597	
PLA2G2D_2SNPs	H1	6679: C; 8228: C	46.74	41.14	34.62	0.101	**0.0236 (R)**	**0.0259**	**0.0495 (R)**
PLA2G2D_2SNPs	H2	6679: G; 8228: C	7.27	5.38	14.42	0.339		**0.0202**	**0.0138 (D)**
PLA2G2D_3SNPs	H0	6679: C; 8228: C; 8358: A	46.43	41.08	34.62	0.13	**0.0310 (R)**	**0.0261**	
PLA2G2D_3SNPs	H1	6679: G; 8228: A; 8358: T	39.58	45.86	39.42	0.0713	**0.00418 (D)**	1	
PLA2G2D_3SNPs	H2	6679: G; 8228: C; 8358: A	7.29	5.41	14.42	0.338		**0.0204**	**0.0139 (D)**
PLA2G2E_all	H2	543: G; 5645: T; 11193: G	17.61	14.33	27.36	0.208		**0.023**	**0.0424 (D)**
PLA2G3_all	H4	−9051: G; −6920: G; −2849: G; 2374: A	6.36	6.36	12.5	1		**0.0383**	**0.0317 (R)**
PLA2G3_prom	H1	−9051: G; −6920: G; −2849: G	22.78	24.67	27	0.515		0.375	**0.00579 (R)**
PLA2G4A_2SNPs	H0	21440: T; 27474: C	74.72	67.37	70.37	**0.0144**	**0.00853 (R)**	0.346	
PLA2G4A_2SNPs	H2	21440: C; 27474: T	8.9	15.57	13.89	**0.00201**	**0.0107 (D)** **0.00522 (R)**	0.113	
PLA2G6_all	H1	−14136: A; −12082: G; 4689: T; 21136: G; 26612: C; 51011: G	27.62	32.73	35.85	0.105		0.0846	**0.00799 (R)**

For each haplotype, we indicate the frequency in each population (SP, RP or CTR) and the p-value (Fisher's exact tests) for the comparisons SPvsCTR and CTRvsRP. The calculation modes AF, D, and R correspond, respectively, to the allelic frequency, the dominant, and the recessive modes.

homozygotes was 8.9% in the CTR population, 15% in the SP population and 18.5% of the RP population. These findings suggest a role in susceptibility to infection by HIV-1. However, the frequency of the AA homozygotes in the 60 HapMap Caucasian subjects is 15%. This may represent sampling error or genetic differences between the French Caucasian population sampled for GRIV and the Caucasians residing in the USA, who are mainly of Dutch descent, sampled for HapMap. This is the only *PLA2* tagSNP exhibiting a positive signal associated with HIV disease that is located in a promoter region. Two borderline associations are also observed comparing SP and CTR groups for PLA2G2D_−9229 and PLA2G12A_−5765 promoter tagSNPs: these tagSNPs or SNPs in LD could be involved in slow progression to AIDS. For these tagSNPs located in promoter region, functional studies are warranted.

The other SNPs exhibiting a positive or borderline signal lie in the 3′UTR region (PLA2G2D_6679) or intronic or 3′gene region (the remaining SNPs). Neither the 3′UTR tagSNP PLA2G2D_6679 nor the 3′UTR SNP in LD (rs578459) are

Table 4
Correlations with biological and disease markers

Allele	Biological marker	Percentile	Calculation mode	Percentage of patients in the lowest percentile (n)	Percentage of patients in the highest percentile (n)	Nominal p-value
PLA2G2E_11193-C	CD4 T-cells count	25	R	50 (20)	74.29 (26)	0.04
PLA2G2E_11193-C	CD4 T-cells count	33	R	51.92 (27)	69.39 (68)	0.05
PLA2G2D_2SNPs-H0	CD4 T-cells count	25	D	80 (32)	97.22 (35)	0.03
PLA2G2D_3SNPs-H0	CD4 T-cells percentage	33	AF	50 (48)	35.42 (68)	0.02
PLA2G2D_3SNPs-H0	CD4/CD8 T-cells ratio	33	AF	48.84 (42)	34.66 (61)	0.03
PLA2G2D_3SNPs-H0	CD4/CD8 T-cells ratio	33	R	23.26 (10)	9.09 (8)	0.03
PLA2G4A_2SNPs-H2	CD4 T-cells count	25	AF	1.28 (1)	13.24 (9)	0.01
PLA2G4A_2SNPs-H2	CD4 T-cells count	25	D	2.56 (1)	23.53 (8)	0.01
PLA2G4A_2SNPs-H2	CD4 T-cells count	33	AF	1 (1)	7.89 (15)	0.01
PLA2G4A_2SNPs-H2	CD4 T-cells count	33	D	2 (1)	14.74 (14)	0.02
PLA2G4A_2SNPs-H2	CD4 T-cells percentage	25	AF	2.7 (2)	11.76 (8)	0.05

Only significant correlations are presented ($p \leq 0.05$). For each allele, biological markers, percentile, calculation mode (AF: allelic frequency, D: dominant, R: recessive), number of patients (n) and p-value are informed.

in a known polyadenylation site. The associations involving 3′UTR SNPs, intronic SNPs, or 3′gene region SNPs could influence gene expression, mRNA stability, mRNA regulation, or mRNA splicing but they are more difficult to interpret experimentally.

Most of the *p*-values obtained for the SNP associations were comprised within the [0.01–0.05] interval except for the alleles PLA2G2D_6679-C in the recessive mode, PLA2G2D_8228-A in the dominant mode, and the SNPs PLA2G4A_21440 and PLA2G4A_27474 in the allelic frequency mode (Table 2). Of note, PLA2G2A_8156, PLA2G2D_6679, PLA2G2D_8228, PLA2G2D_8358 and PLA2G2E_11193 are tagSNPs located within neighbouring *PLA2* genes of the 1p36-p35 locus (Fig. 1A) and exhibit positive signals, but they are not in LD with each other.

We also computed haplotypes and found 15 haplotypes associated with HIV disease (Table 3). The haplotypes over the whole genes are slightly artificial because some tagSNPs were not genotyped in this study. The PLA2G2D_all_H8 haplotype association observed between the SP and CTR groups ($p = 0.03$) is less strong than the associations found from the individual SNPs composing this haplotype (positive or borderline signals, including two strong signals ($p < 0.01$)). Thus, this haplotype may not be involved directly in HIV-1 infection. PLA2G3_prom_H1 haplotype is a subhaplotype of PLA2G3_all_H2 and PLA2G3_all_H4 (Table S2 Supplementary data online). The association found for PLA2G3_prom_H1 haplotype with rapid progression ($p = 0.006$) is stronger than that found for PLA2G3_all_H4 haplotype ($p = 0.03$). Furthermore, PLA2G3_all_H2 haplotype does not exhibit a positive signal comparing the RP and CTR groups. This suggests that only the promoter haplotype could influence rapid progression of AIDS disease. The significant *p*-values found for the PLA2G2D_2SNPs and PLA2G2D_3SNPs haplotypes (Table 3) are roughly the same than the individual *p*-values found for PLA2G2D_6679, PLA2G2D_8228 and PLA2G2D_8358 (Table 2). The association of the *PLA2G2D* gene with slow progression may thus involve the individual SNPs or their haplotypes. The remaining haplotypes are more complex to interpret. Of note, seven *p*-values obtained on haplotype associations were lower than 0.01 (Table 3).

The analysis of laboratory markers of HIV disease among the SP patients tends to confirm one SNP association and three haplotype associations. PLAG2E_11193-C, linked to slower progression in the recessive mode (Table 2), was associated with higher CD4 T-cells count (Table 4). PLA2G2D_2SNPs-H0, linked to slower progression in the dominant mode (Table 3), was associated with higher CD4 T-cells count (Table 4). PLA2G4A_2SNPs-H2, linked to slower progression in the allelic frequency and dominant modes (Table 3), was associated with higher CD4 T-cells count and percentage (Table 4). PLA2G2D_3SNPs-H0, linked to prevention of slow progression in the recessive mode (Table 3), was associated with lower CD4 T-cells percentage and lower CD4/CD8 T-cells ratio (Table 4). This last finding is logical since weaker CD4 T-cell levels and elevated CD8 T-cell levels are a feature of advancing HIV disease.

This study is the first genomic work exploring the involvement of *PLA2* gene polymorphisms in the resistance/susceptibility towards progression to AIDS. We have obtained strong signals ($p < 0.01$) for SNP or haplotype alleles involving five genes: *PLA2G2A*, *PLA2G2D*, *PLA2G3*, *PLA2G4A* and *PLA2G6*. Other groups have previously published genetic associations in other diseases for *PLA2G2A*, *PLA2G2D*, *PLA2G4A* and *PLA2G7* genes but addressed SNP/haplotypes which were not covered in our study, except for the PLA2G7 A379V mutant (rs1051931). A study by Wootton et al. [37] has identified a 6-tagSNPs haplotype of the *sPLA2G2A* gene associated with the sPLA2G2A protein serum level in Caucasians, and associated with increased coronary artery disease risk. Similarly, in the same subjects, they identified a 7-tagSNPs haplotype of the *PLA2G5* gene exhibiting a strong association with total and LDL cholesterol levels [38]. The non-synonymous SNP rs584367 (Gly80Ser) in *sPLA2G2D* has been associated with body weight loss in Japanese patients with chronic obstructive pulmonary disease [39]. For the cytosolic PLA2G4A, an association was found among Pima Indians between the F479L variant and type II diabetes mellitus [40]. This variant is reported neither in NCBI dbSNP nor in HapMap, so it is not included in our study. The V279F sPLA2G7 variant, a loss-of-function mutation found in more than 30% of Japanese subjects, is associated with atherosclerosis risk [41,42], cardiovascular diseases [43,44], atopy and asthma [45,46]. This variant is not reported in the Caucasian population. The A379V sPLA2G7 mutant is associated with myocardial infarction in the Taiwanese population [47] and with coronary arterial disease in the German population [48]. Both PLA2G7 mutants I198T and A379V were associated with atopy and asthma in Caucasians [49]. I198T mutant (rs1805018) is not covered by our study, whereas A379V variant (rs1051931) is in total LD with the tagSNP rs1421372 which was not involved in any association in our study.

We have undertaken here the first genomic study taking an interest in the involvement of *PLA2* gene polymorphisms in the pathogenesis of HIV-1 infection using the SNPlex™ methodology. The SNPlex™ Genotyping System is based on the simultaneous genotyping of already known SNPs. Out of the 70 tagSNPs studied, several associations were found for tagSNPs and haplotypes in genes encoding secreted PLA2s, which is in line with the *in vitro* results about the host defense role of secreted PLA2 enzymes against HIV infection [11–13]. We also found several associations for tagSNPs and haplotypes in genes encoding cytosolic PLA2s suggesting that cytosolic PLA2 enzymes could also influence HIV-1 infection and AIDS progression. Our study thus indicates that the PLA2 family is a target of interest for its involvement in AIDS disease development. A more exhaustive study on all the tagSNPs already known in this gene family will have to be performed either with the same SNPlex™ method or with genotyping chips. The genetic variations of *PLA2G10*, previously shown to neutralize HIV-1 *in vitro* [13], will also have to be studied with the same method or with PCR/sequencing if it remains unexplored in the HapMap project. However, the investigation of *cPLA2* genes might be of greater

interest since it has been shown recently that HIV-1 could escape inhibition by sPLA2 via an endocytic pathway [50]. In order to consider future experimental interpretations, the associations identified in this study will need to be confirmed and validated by other genomic studies in AIDS cohorts.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biopha.2007.11.001.

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