

A. Vasilescu · S. C. Heath · G. Diop · H. Do ·
T. Hirtzig · H. Hendel · S. Bertin-Maghit ·
J. Rappaport · A. Therwath · G. M. Lathrop ·
F. Matsuda · J.-F. Zagury

Genomic analysis of *Fas* and *FasL* genes and absence of correlation with disease progression in AIDS

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Abstract Apoptosis has been suggested as a major mechanism for the CD4⁺ T-lymphocyte depletion observed in patients infected with human immunodeficiency virus 1 (HIV-1). To evaluate the impact of genetic variations to apoptosis during progression of acquired immunodeficiency syndrome (AIDS), we have performed an extensive genetic analysis of *Fas* and Fas ligand (*FasL*) genes. The coding regions and promoters of these genes were resequenced in a cohort of 212 HIV-1-seropositive patients presenting extreme disease phenotypes and 155 healthy controls of Caucasian origin. Overall, 33 single nucleotide polymorphisms (SNPs) with an allele frequency >1% were identified and evaluated for their association with disease progression. Among them, 14 polymorphisms were newly characterized. We did not find any statistically significant association of *Fas* and

FasL polymorphisms and haplotypes with AIDS progression.

Keywords *Fas/FasL* genes · AIDS progression · Single nucleotide polymorphism · Haplotype · GRIV cohort

Apoptosis, or programmed cell death, is a key mechanism allowing multicellular organisms to tightly regulate cell growth, preventing pathological processes such as auto-reactivity, cancer and immunodeficiency. In the pathogenesis of acquired immunodeficiency syndrome (AIDS), apoptosis is considered to play an important role in human immunodeficiency virus 1 (HIV-1)-dependent CD4⁺ T-lymphocyte depletion, induced and accelerated by the virus itself (Ameisen and Capron 1991; Gougeon et al. 1991; Laurent-Crawford et al. 1991; Terai et al. 1991; Westendorp et al. 1995; Zinkernagel 1995). Indeed, earlier studies showed a great fragility of CD4⁺ T cells leading to their apoptosis in patients infected with HIV-1 (Ameisen and Capron 1991; Groux et al. 1992). It was shown later that the degree of CD4⁺ T-cell apoptosis in patients prior to T-cell depletion tightly correlates with disease progression (Gougeon et al. 1996; Liegler et al. 1998).

Several different extrinsic pathways are hypothesized to induce death receptor-associated apoptosis in AIDS. Among them, accumulating evidences strongly indicate the direct involvement of the Fas/Fas ligand (*FasL*) pathway to the T-lymphocyte depletion. In T lymphocytes of HIV-1-positive individuals, the expression of *Fas* and *FasL* is significantly increased (Debatin et al. 1994; Mitra et al. 1996) and Fas-mediated apoptosis is accelerated by antibody cross-linking (Katsikis et al. 1995). Upregulated expression of *FasL* in macrophages infected with HIV-1 can induce apoptotic death of normal peripheral blood T cells in vitro, suggesting another death-signal transmission through macrophages in T-cell depletion (Badley et al. 1996). Moreover, a study using anti-Fas antibodies showed a significant decrease of Fas-induced cell death in T cells of patients with extremely slow progression as

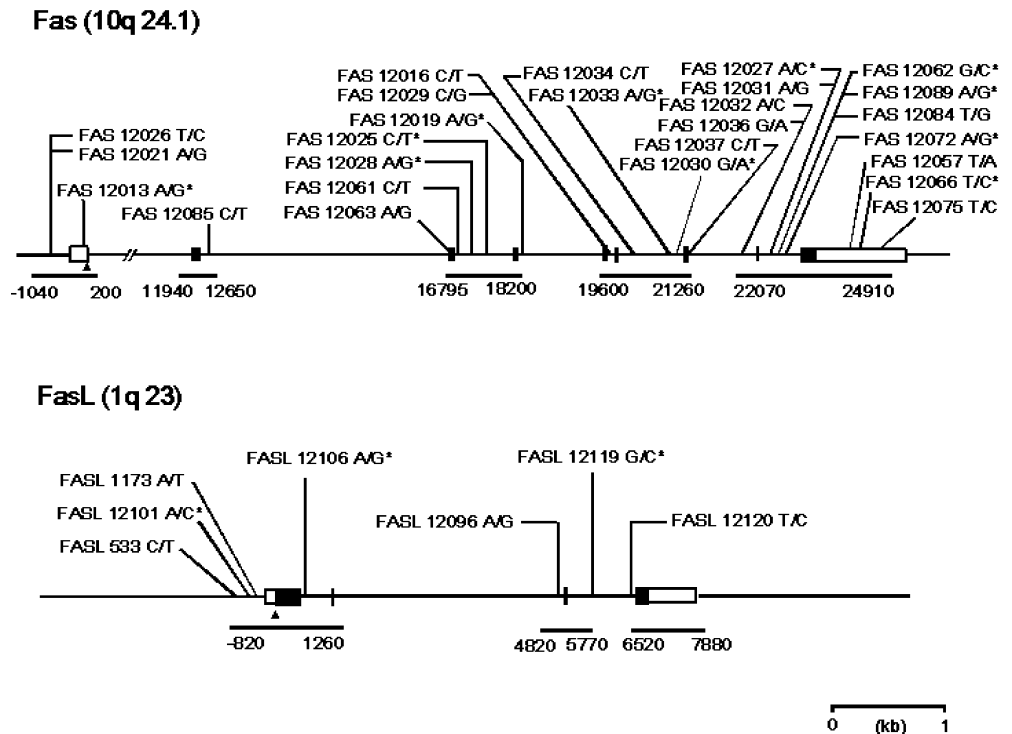
A. Vasilescu · S. C. Heath · G. Diop · H. Do · G. M. Lathrop ·
F. Matsuda (✉)
Centre National de Génotypage,
2 rue Gaston Crémieux, 91000 Evry, France
e-mail: fumi@cng.fr
Tel.: +33-1-60878338
Fax: +33-1-60878383

A. Vasilescu · G. Diop · H. Do · T. Hirtzig · H. Hendel ·
A. Therwath · J.-F. Zagury (✉)
Equipe Génomique,
Bioinformatique et Pathologies du système immunitaire,
INSERM EMI 0355-15,
rue de l'École de Médecine, 75006 Paris, France
e-mail: jfz@ccr.jussieu.fr
Tel.: +33-1-40468473
Fax: +33-1-42346993

S. Bertin-Maghit · J. Rappaport · J.-F. Zagury
Center for Neurovirology,
Temple University,
Philadelphia, PA 19122, USA

A. Therwath
Laboratoire d'Oncologie Moléculaire,
Université Paris VII,
75005 Paris, France

Fig. 1 Organization of *Fas* and *FasL* genes. Coding and untranslated regions of each gene are, respectively, indicated by *solid rectangles* and *open rectangles*. Positions of the 33 SNPs with frequencies of more than 1% are indicated with the name and nucleotide changes. The *asterisks* correspond to SNPs that were newly characterized in this study. Sequenced regions are shown by the *horizontal line* below each gene with start and end positions according to the first nucleotide of the initiation codon as +1 (*black triangle*). The genomic sequences used for the alignment are: *Fas*, NT_008769.11 and *FasL*, NT_029874.5. For experimental details, see Vasilescu et al. (2003)



well as in one of their uninfected parents (Bottarel et al. 2001). This indicates the inherited alteration of the Fas-signaling pathway resulting in the quantitative difference in the degree of apoptosis, and thus the disease progression.

In the present study, we focused on genetic variations of *Fas* and *FasL* genes to clarify whether genetic variations of these apoptosis-related genes could influence disease onset and progression. To perform this study, we employed the GRIV cohort consisting of two sub-populations of Caucasian HIV-1-positive individuals with extreme phenotypes: 154 asymptomatic individuals (SP) with a CD4⁺ cell count above 500/mm³ for 8 or more years after seroconversion and 58 patients with rapid progression (RP) showing a drop in their CD4⁺ cell count below 300/mm³ in less than 3 years after the last seronegative test (for more details, see Flores-Villanueva et al. 2003; Huber et al. 2003; Rappaport et al. 1997). We also employed 155 seronegative control (CTR) subjects from the same ethnic origin.

We systematically screened the *Fas* and *FasL* genes for polymorphisms by resequencing the exons, flanking regions and promoter regions. We identified 46 and 30 polymorphisms in *Fas* and *FasL* genes, respectively (Fig. 1). Out of these 76 polymorphisms identified, 33 single nucleotide polymorphisms (SNPs) have an allele frequency of 1% or greater in at least one of SP, RP and CTR groups. Fourteen among these 33 SNPs are newly identified in this study. As shown in Fig. 1, 6 SNPs are located in exons. The SNPs *Fas*_12063 and *Fas*_12037 introduce synonymous changes, while the other four are located either in the 5'UTR (*Fas*_12013) or the 3'UTR

(*Fas*_12057, *Fas*_12066 and *Fas*_12075). Table 1 summarizes the frequency of each SNP in the SP, RP and CTR populations, their association with AIDS progression and the relevant information known to date for each SNP. The SNP distributions in cases and controls were in Hardy-Weinberg equilibrium.

We performed statistical analysis to test whether these polymorphisms or deduced SNP haplotypes are associated with disease progression. We failed to obtain any significant association between polymorphisms in *Fas* and *FasL* genes and AIDS progression by Fisher's exact test (the lowest *P* value is 0.096 in *FasL*_533 between RP and SP). Similarly, the same test using estimated haplotypes (Fig. 2) did not find any significant association between particular haplotypes of *Fas* or *FasL* genes and AIDS progression (Table 2). The lowest *P*-value was 0.102 for the haplotype 3 of the *Fas* gene obtained by comparing RP with SP.

A general case-control study using 283 HIV-positive patients and 111 control subjects (Cascino et al. 1998) on two SNPs in the *Fas* gene (*Fas*_12063 and *Fas*_12037) did not show significant association between combination of two SNPs and AIDS. The more specific study shown here using two sub-populations of AIDS patients with extreme phenotypes did not provide any further genetic evidence that *Fas* and *FasL* genes are major genetic determinants in the onset and pathogenicity of HIV-1 infection.

Increased apoptosis of T cells has been reported after Epstein-Barr virus infections (Akbar et al. 1993; Uehara et al. 1992), varicella-zoster infections (Akbar et al. 1993) and infections induced by *herpesviridae* (Razvi and

Table 1 Summary of the polymorphisms of *Fas* and *FasL* genes

Gene	SNP			Freq A1			P-values for test statistics ^a		References to previous studies of the variant and new IDs
	ID	A1	A2	CTR	SP	RP	CvRvS	RvS	
<i>Fas</i>	12026	T	C	0.87	0.90	0.89	0.617	0.746	NCBI (rs2234768)
	12021	A	G	0.53	0.51	0.53	0.942	1.000	(Huang et al. 1997)
	12013	A	G	0.98	0.97	1.00	1.000	1.000	NEW (ss 20399338)
	12085	C	T	0.58	0.64	0.61	0.316	0.643	NCBI (rs2296603)
	12063	A	G	0.96	0.94	0.97	0.352	0.304	(Cascino et al. 1998)
	12061	C	T	0.89	0.89	0.88	0.970	0.851	(Bolstad et al. 2000)
	12028	A	G	0.96	0.94	0.97	0.312	0.428	NEW (ss 20399339)
	12025	T	C	0.57	0.62	0.57	0.373	0.440	NEW (ss 20399340)
	12019	A	G	0.99	0.97	1.00	0.141	0.353	NEW (ss 20399341)
	12016	C	T	0.98	0.98	1.00	0.787	0.566	NCBI (rs3218620)
	12029	C	G	0.58	0.63	0.55	0.302	0.192	(Bolstad et al. 2000)
	12034	C	T	0.28	0.34	0.30	0.435	0.588	NCBI (rs1571020)
	12033	A	G	0.96	0.93	0.95	0.403	0.791	NEW (ss 20399342)
	12030	G	A	0.84	0.87	0.91	0.199	0.421	NEW (ss 20399343)
	12037	T	C	0.30	0.31	0.31	0.992	1.000	(Cascino et al. 1998)
	12036	A	G	0.58	0.64	0.58	0.291	0.345	NCBI (rs3752986)
	12032	A	C	0.54	0.58	0.53	0.627	0.560	NCBI (rs3752985)
	12031	A	G	0.56	0.57	0.56	0.981	0.908	NCBI (rs1571019)
	12027	C	A	0.89	0.89	0.88	0.953	0.848	NEW (ss 20399344)
	12062	G	C	0.96	0.93	0.95	0.264	0.613	NEW (ss 20399345)
	12089	A	G	1.00	1.00	0.99	0.965	0.265	NEW (ss 20399346)
	12084	T	G	0.56	0.64	0.60	0.232	0.545	NCBI (rs1977389)
	12072	G	A	0.98	0.97	0.96	0.468	0.741	NEW (ss 20399347)
	12057	A	T	0.90	0.88	0.84	0.267	0.381	NCBI (rs1051070)
	12066	T	C	0.96	0.94	0.97	0.274	0.303	NEW (ss 20399348)
12075	C	T	0.89	0.88	0.88	0.853	1.000	NCBI (rs1468063)	
<i>FasL</i>	533	C	T	0.63	0.59	0.70	0.226	0.096	(Nolsoe et al. 2002)
	12101	A	C	1.00	1.00	0.99	0.964	0.260	NEW (ss 20399349)
	1173	A	T	0.84	0.84	0.84	0.988	1.000	(Nolsoe et al. 2002)
	12106	A	G	0.90	0.89	0.93	0.482	0.253	NEW (ss 20399350)
	12096	A	G	0.84	0.83	0.84	0.940	0.879	(Bolstad et al. 2000)
	12119	G	C	0.54	0.54	0.52	0.956	0.902	NEW (ss 20399351)
	12120	T	C	0.00	0.01	0.01	0.959	1.000	NCBI (rs2639653)

^a Since the nominal P-values results were not significant, we did not perform the Bonferroni corrections

		Fas																										
Freq		12026	12021	12013	12085	12063	12061	12028	12025	12019	12016	12029	12034	12033	12030	12037	12036	12032	12031	12027	12062	12089	12084	12072	12057	12066	12075	
H1	0.305	T	A	A	T	A	C	A	C	A	C	G	T	A	G	C	G	C	G	C	G	A	G	G	A	T	C	
H2	0.131	T	G	A	C	A	C	A	T	A	C	C	C	A	G	T	A	A	A	C	G	A	T	G	A	T	C	
H3	0.097	T	G	A	C	A	C	A	T	A	C	C	C	A	G	T	A	A	A	C	G	A	T	G	T	T	C	
H4	0.085	T	G	A	C	A	T	A	T	A	C	C	T	A	G	C	A	A	A	A	G	A	T	G	A	T	T	
H5	0.078	C	A	A	C	A	C	A	T	A	C	C	T	A	A	C	A	A	A	C	G	A	T	G	A	T	C	
H6	0.063	T	A	A	C	A	C	A	T	A	C	C	C	A	G	T	A	A	A	C	G	A	T	G	A	T	C	
H7	0.042	T	G	A	C	G	C	G	T	A	C	C	T	G	G	C	A	C	G	C	C	A	T	G	A	C	C	
H8	0.036	T	G	A	T	A	C	A	C	A	C	G	T	A	G	C	G	C	G	C	G	A	G	G	A	T	C	
H9	0.018	C	A	A	C	A	C	A	T	A	C	C	T	A	A	C	A	A	C	G	A	T	A	A	A	T	C	
H10	0.014	T	G	G	T	A	C	A	C	A	C	G	T	A	G	C	G	C	G	C	G	A	G	G	A	T	C	
H11	0.014	T	G	A	C	A	C	A	T	A	C	C	T	A	A	C	A	A	A	C	G	A	T	G	A	T	C	
H12	0.011	T	G	A	T	A	C	A	C	A	T	G	T	A	G	C	G	C	G	A	C	G	A	G	G	A	T	C
H13	0.011	T	A	A	C	A	C	A	T	A	C	C	T	A	A	C	A	A	A	C	G	A	T	G	A	T	C	
H14	0.011	T	G	A	C	A	C	A	T	G	C	C	C	A	G	T	A	A	A	C	G	A	T	G	A	T	C	

		FasL						
Freq		533	12101	1173	12106	12096	12119	12120
H1	0.413	C	A	A	A	A	G	C
H2	0.176	C	A	A	A	A	C	C
H3	0.148	T	A	T	A	G	C	C
H4	0.108	T	A	A	A	A	C	C
H5	0.085	T	A	A	G	A	G	C
H6	0.016	T	A	A	A	A	G	C

Fig. 2 Summary of polymorphisms combinations. Haplotype frequencies using SNPs with frequencies >1% for each gene were estimated with the EM algorithm (Laird 1993). The haplotypes were sorted by estimated frequency among population

Welsh 1993). So the identification of 14 novel SNPs with relatively high frequency (>1%) and haplotype information on these genes should prove useful for the genetic study of patients with other virus infections or with

cancer. In another hand, more extensive genetic studies on additional genes related to Fas/FasL-mediated apoptosis will clarify the role of this pathway in AIDS.

Table 2 Summary of polymorphism combinations and estimated frequencies for haplotypes in the *Fas* and *FasL* genes. *CTR* Seronegative control subjects, *SP* asymptomatic individuals with a CD4⁺ cell count above 500/mm³ for 8 or more years after

seroconversion, *RP* rapid progression individuals showing a drop in their CD4⁺ cell count below 300/mm³ in less than 3 years after the last seronegative test

Gene	Haplotype ^a	CTR		SP		RP		P-values	
		Counts	Frequency	Counts	Frequency	Counts	Frequency	CvSvR	SvR
<i>Fas</i>	H1	100/286	0.350	86/268	0.321	34/102	0.333	0.732	0.804
	H2	43/286	0.150	39/268	0.146	15/102	0.147	0.989	1.000
	H3	26/286	0.091	26/268	0.097	16/102	0.157	0.178	0.102
	H4	26/286	0.091	26/268	0.097	9/102	0.088	0.983	1.000
	H5	29/286	0.101	23/268	0.086	4/102	0.039	0.140	0.178
	H6	14/286	0.049	23/268	0.086	7/102	0.069	0.240	0.675
	H7	9/286	0.031	18/268	0.067	4/102	0.039	0.157	0.460
	H8	13/286	0.045	8/268	0.030	4/102	0.039	0.627	0.742
	H9	4/286	0.014	4/268	0.015	3/102	0.029	0.595	0.398
	H10	5/286	0.017	3/268	0.011	2/102	0.020	0.754	0.618
	H11	6/286	0.021	3/268	0.011	1/102	0.010	0.688	1.000
	H12	4/286	0.014	2/268	0.007	2/102	0.020	0.575	0.303
	H13	3/286	0.010	4/268	0.015	1/102	0.010	0.707	1.000
	H14	4/286	0.014	3/268	0.011	0/102	0.000	0.690	0.565
<i>FasL</i>	H1	129/294	0.439	115/282	0.408	51/106	0.481	0.495	0.205
	H2	58/294	0.197	48/282	0.170	21/106	0.198	0.701	0.551
	H3	44/294	0.150	47/282	0.167	15/106	0.142	0.777	0.642
	H4	31/294	0.105	36/282	0.128	11/106	0.104	0.672	0.603
	H5	26/294	0.088	29/282	0.103	6/106	0.057	0.359	0.231
	H6	6/294	0.020	7/282	0.025	2/106	0.019	0.795	0.679

^aHaplotypes with frequency greater than 1% were used for the statistical analysis, which was performed as previously described (Vasilescu et al. 2003)

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