

GAG VARIABILITY IN DIFFERENT HIV-1 SUBTYPES AND UNDER ANTIRETROVIRAL THERAPY MAY INFLUENCE VIRAL BUDDING

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INTRODUCTION

HIV and other retroviruses exit infected cells by budding from the plasma membrane.

The budding is promoted by short and highly conserved motifs in the HIV-1 P6^{gag} proteins. They constitute interaction sites for the host cellular proteins Tsg101 and AIP1, both required for the release of HIV.

The tetrapeptide P(T/S)AP motif (residues 7-10) within P6^{gag} is crucial for recruiting TSG101, and for the detachment of viruses from the cell surface and from each other.

HIV-1 P6^{gag} residues 32-46 are sufficient for AIP1 binding site. LYP and LXXLF domains (residues 35-37 and 41-45, respectively) form part of AIP1 primary binding site.

Non-B clades and recombinant viruses are responsible for more than 90% of the 40 million HIV-1 infections worldwide.

Most data on the genetic mechanisms of HIV-1 biology are from subtype B viruses, prevalent in North America and Western European countries.

This study provides the first genetic evidence suggesting that non-B subtypes may have different interactions with host cells involved in viral budding, being also influenced by antiretroviral (ARV) exposure.

OBJECTIVE

TO STUDY THE EFFECT OF HIV-1 SUBTYPES AND ANTIRETROVIRAL THERAPY ON THE VARIABILITY OF P6^{gag} DOMAINS INVOLVED IN VIRAL BUDDING

RESULTS

Table 1. Distribution of HIV-1 subtypes in the study population and exposure to antiretroviral therapy.

Genetic region	Subtypes	Drug-naïve (n=82)	ARV-experienced (n=40)	Total (n=122)
<i>gag</i>	non-B	49	19	68
		(35A, 1C, 1F, 9G, 1H, 2U)	(15A, 1C, 3G)	
	B	33	21	54

PATIENTS and METHODS

Plasma specimens collected since 1993 to 2003 from a total of 122 HIV-1 infected subjects living in Spain. 81 were drug-naïve and 41 were under ARV regimens including PI.

48% were Africans, coming from 14 different countries and mainly from West Africa. Native Spaniards: 19% of cases, 13% came from other 9 European countries, 12% from South America, and 1.6% from Asia. The origin of 6 foreigners was unknown.

Subtypes were defined by phylogenetic analysis in *gag* gene (Table 1).

The chi-square or Fischer's exact tests were used to detect significant differences between proportions. Comparisons were conducted using Epi Info version 6.02 (CDC, Atlanta, GA, USA). Only *p* values below 0.05 were considered as significant.

Table 2. Effect of the HIV-1 subtype and the exposure to ARV treatment in the rate of subjects harboring residues modifications at p6^{gag} motifs involved at HIV-1 budding.

Motif within P6 ^{gag}	Positions in P6 ^{gag}	Modifications	54 subtype B		<i>p</i>	68 non-B subtypes (50A, 2C, 1F, 12G, 1H, 2U)		<i>p</i>	HIV-1 subtype		
			33 Naive	21 Treated		49 Naive	19 Treated		54 B	68 non-B	<i>p</i>
PT/SAP	7-10	Ins near motif	8/33 (24.2%)	5/21 (23.8%)	NS	0/49 (0%)	0/19 (0%)	NS	13/54 (24.1%)	0/68 (0%)	<0.0001
		Subs ¹	1/33 (3%)	0/21 (0%)	NS	1/49 (2%)	0/19 (0%)	NS	1/54 (1.8%)	1/68 (1.5%)	NS
LXXLF	41-45	R42K	18/33 (54.5%)	9/21 (42.8%)	NS	45/49 (92%)	16/19 (84%)	NS	27/54 (50%)	61/68 (89.7%)	<0.0001
		Subs ²	13/33 (39.4%)	2/21 (9.5%)	0.01	18/49 (36.7%)	4/19 (21%)	NS	15/54 (27.8%)	22/68 (32.3%)	NS
LYP	35-37	P37 duplication	3/33 (9.1%)	1/21 (4.8%)	NS	32/49 (65.3%)	14/19 (73.7%)	NS	4/54 (7.4%)	46/68 (67.6%)	<0.0001

Subs, amino acid substitutions. Ins, amino acid insertions. ¹A9T, and P10L. ²L35Q/M/K/P/G/I/H/S/T/R/V, Y36P/L/A/D/C/H/V/N/T, and P37Q.

Table 3. Main found aa insertions and deletions within P6^{gag}

P6 ^{gag}	Insertions	Flanking positions (NS)	ARV	Clade
Near PT/SAP motif	EPRP	6-7	Y	B
	APP	11-12	N	B
	APP	11-12	N	B
	APP	11-12	N	B
	APP	11-12	N	B
	APP	11-12	N	B
	APP	11-12	N	B
	APP	11-12	N	B
	APP	11-12	N	B
	APP	11-12	N	B
	APP	11-12	N	B
	APP	11-12	N	B
Near KQE	NSLPPNAPP	11-12	Y	B
	APP	11-12	N	B
	P	22-26	N	B
	APP	11-12	N	B
	ORS	28-29	N	A
	ORMS	32-33	N	B
	APP	11-12	N	B
	KE*	34-35	Y	G
	KE*	34-35	Y	G
	KE*	34-35	Y	B
	KE*	34-35	Y	B
	Near LYP motif	EADV	36-37	N
A1*		N	A	C
A1*		N	H	H
A1*		N	C	C
A1*		N	C	C
A1*		N	B	B
A1*		N	B	B
A1*		N	B	B
A1*		N	B	B
A1*		N	B	B
A1*		N	B	B
Within LYP motif		A31-37	N	A
	A31-36	Y	B	B
	A31-35	Y	A	A
	A31-34	N	F	F
	A31-33	N	F	F
	A31-32	N	F	F
	A31-31	N	B	B
	A31-30	N	B	B
	A31-29	N	B	B
	A31-28	N	B	B
	A31-27	N	B	B
	others	A12-15	N	A
A12-14		N	A	A
A12-13		N	A	A
A12-12		N	A	A
A12-11		N	A	A
A12-10		N	A	A
A12-9		N	A	A
A12-8		N	A	A
A12-7		N	A	A
A12-6		N	A	A
A12-5		N	A	A

ARV: Y, ARV-experienced; N, drug-naïve subject. PT/SAP, KQE; LYP: residues 7-10, 27-29, and 35-37 within P6^{gag}, respectively. * Samples with KEKEKE, upstream of LYP motif.

Table 4. Alignment of the critical Tsg101 and minimal AIP1 binding sites in P6^{gag} sequences from different subtypes carrying substitutions within these motifs

No.	HIV-1 P6 ^{gag}									
	Critical Tsg101 Binding Site					Minimal AIP1 Binding Site				
1	P	T	S	A	P	D	A	K	L	V
2	P	T	S	A	P	D	A	K	L	V
3	P	T	S	A	P	D	A	K	L	V
4	P	T	S	A	P	D	A	K	L	V
5	P	T	S	A	P	D	A	K	L	V
6	P	T	S	A	P	D	A	K	L	V
7	P	T	S	A	P	D	A	K	L	V
8	P	T	S	A	P	D	A	K	L	V
9	P	T	S	A	P	D	A	K	L	V
10	P	T	S	A	P	D	A	K	L	V
11	P	T	S	A	P	D	A	K	L	V
12	P	T	S	A	P	D	A	K	L	V
13	P	T	S	A	P	D	A	K	L	V
14	P	T	S	A	P	D	A	K	L	V
15	P	T	S	A	P	D	A	K	L	V
16	P	T	S	A	P	D	A	K	L	V
17	P	T	S	A	P	D	A	K	L	V
18	P	T	S	A	P	D	A	K	L	V
19	P	T	S	A	P	D	A	K	L	V
20	P	T	S	A	P	D	A	K	L	V
21	P	T	S	A	P	D	A	K	L	V
22	P	T	S	A	P	D	A	K	L	V
23	P	T	S	A	P	D	A	K	L	V
24	P	T	S	A	P	D	A	K	L	V
25	P	T	S	A	P	D	A	K	L	V
26	P	T	S	A	P	D	A	K	L	V
27	P	T	S	A	P	D	A	K	L	V
28	P	T	S	A	P	D	A	K	L	V
29	P	T	S	A	P	D	A	K	L	V
30	P	T	S	A	P	D	A	K	L	V
31	P	T	S	A	P	D	A	K	L	V
32	P	T	S	A	P	D	A	K	L	V
33	P	T	S	A	P	D	A	K	L	V
34	P	T	S	A	P	D	A	K	L	V
35	P	T	S	A	P	D	A	K	L	V
36	P	T	S	A	P	D	A	K	L	V
37	P	T	S	A	P	D	A	K	L	V
38	P	T	S	A	P	D	A	K	L	V
39	P	T	S	A	P	D	A	K	L	V
40	P	T	S	A	P	D	A	K	L	V
41	P	T	S	A	P	D	A	K	L	V
42	P	T	S	A	P	D	A	K	L	V
43	P	T	S	A	P	D	A	K	L	V
44	P	T	S	A	P	D	A	K	L	V
45	P	T	S	A	P	D	A	K	L	V

A clade G sample with change A9T within PT/SAP had L35Q substitution at LYP motif.

A clade B virus carried P10L substitution, disrupting the Tsg101 interaction, maintained unaltered LYP motif and had a PTAPP insertion following the changed residue P11E. This insertion could represent an additional binding site for the Tsg101 protein.

CONCLUSIONS

Aa sequences in viral P6^{gag} domains involved in the interaction with host cell proteins necessary for HIV budding may vary according to HIV-1 subtype as well as under ARV pressure.

Variability (aa substitutions, deletions, insertions) at PT/SAP, LYP and LXXLF domains could probably lead to a different Tsg101 and AIP1 binding affinity among different clades, maybe favoring alternative budding pathways.

P6^{gag} variability and exposure to ARV may also affect the viral production efficacy, contributing to the variability of replication capacities or fitness of different HIV-1 isolates.