Variability of HIV-1 V2 Env Domain for Integrin Binding: Clinical Correlates

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Background & aims

- The HIV-1 V2 179-181 tripeptide (HXB2 numbering) mediates binding to α4β7 integrin, which is responsible for GALT homing (*1*).
- Even if the V2 region is not essential for viral entry, it is involved in CD4 binding, influences co-receptor tropism (CRT) and its deletion renders the virus highly susceptible to antibody neutralization. However, its exact role in viral transmission and replication has not been thoroughly defined yet, and possible clinical correlates are still unclear (*2, 3*).
- Recent evidences show that anti-α4β7 antibodies are effective in obtaining viral control in non-human primates (*4*).
- We aimed to assess the variability of V2 region in naive HIV-1 infected subjects and its association with clinical and viro-immunological features.

Methods

- Gp120 sequences were obtained from 322 newly diagnosed subjects with HIV infection.
- V2 regions were evaluated for:
  - Length, potential N-linked glycosylation sites (PNGs), net charge and tripeptide motif at residues 179-181.
- A possible association was explored between V2 features and:
  - HIV subtype; R5/X4 CRT (geno2pheno, false positive rate 10%); duration of HIV infection (based on ambiguous nucleotides frequency in RT/PR, e.g. ≤0.2% ambiguity=recent infection); patients' variables (sex; age; risk factor; stage; baseline viro-immunological data).

Results

- The main clinical and viro-immunological features of the study population are presented in **Table 1**.
- A significant diversity according to length, net charge and PNGs of V2 region was observed between B and non-B subtypes, as well as a wide variability of the $^{179\text{-}181}$tripeptide. Overall, LDV was the most common triplet (41.2%), followed by LDI (24.5%). Asp^{180} was highly conserved (99% of strains). The LDI/V mimotope was significantly (p <0.001) more conserved in non-B variants than in B subtype (**Table 2**).
- A correlation of V2 length and net charge with CD4 cell count was demonstrated by multivariate analysis (**Table 3**).

Table 1: baseline features of the study population

FEATURES	TOTAL (N 322)	B SUBTYPE (N 214, 66%)	NON-B SUBTYPE (N 108, 34%)	p value
Male, nr. (%)	275 (85)	190 (88)	85 (79)	**0.03**
Age, median (IQR)	35.5 (27.5 - 44.5)	38 (29 - 46)	29 (25 - 40)	**<0.001**
AIDS presenters, nr. (%)	47 (14.9)	35 (16.5)	12 (11.5)	0.31
Risk factor, nr. (%)				
Heterosexual	123 (38)	74 (34)	49 (45)	
MSM	173 (53)	117 (55)	55 (51)	
Unknown/other	28 (9)	24 (11)	4 (4)	**0.03**
R5 CRT, nr. (%)	266 (82)	165 (77)	100 (93)	**<0.001**
CD4 nr, median (IQR)	371 (172 - 525)	353 (161 - 525)	411 (213 - 576)	0.27
CD4 %, median (IQR)	21 (12 - 28)	20 (11 - 28)	21 (13 - 28)	0.69
CD4+/CD8+ ratio, median (IQR)	0.21 (0.01-0.39)	0.22 (0.01-0.37)	0.21 (0.01-0.42)	0.85
HIV-RNA cp/ml, median (IQR)	59000 (12000 - 210000)	78000 (17000 - 280000)	29000 (4700 - 120000)	**<0.001**

IQR: interquartile range; MSM: men who have sex with men; CRT: coreceptoral tropism

- Patients with Leu^{179} were more likely to have a less recent infection, whereas subjects with Val^{181} were mainly MSM. These correlations were confirmed with multivariate analysis (**Table 4**).

Table 2: features of V2 region

V2 Features	Overall (N 322)		B Subtype (N 214)		Non-B Subtype (N 108)		p value
Length (median, IQR)	41 (39 - 45)		41 (39 - 43)		43 (40 - 48)		**<0.001**
PNGs (median, IQR)	2.12 (2.12 – 3)		2.12 (2.12 – 3.5)		3.5 (2 – 3.5)		**<0.001**
Net Charge (median, IQR)	0.9 (-0.1 – 1.9)		0.1 (-0.1 – 1)		1 (0 – 2)		**<0.001**
Tripetide (179-181)	**Type**	**Nr**	**Type**	**nr**	**Type**	**nr**	
	LDV	133 (41.18%)	LDV	75 (34.88%)	LDV	58 (54.21%)	**<0.001**
	LDI	79 (24.46%)	LDI	50 (23.26%)	LDI	28 (27.10%)	0.56
	SDI	17 (5.26%)	SDI	17 (7.91%)	SDI	0 (0%)	**0.001**
	SDV	10 (3.1%)	SDV	9 (4.19%)	SDV	1 (0.93%)	0.11
	DDV	7 (2.17%)	DDV	6 (2.79%)	DDV	1 (0.93%)	0.28
	IDV	6 (1.86%)	IDV	5 (2.33%)	IDV	1 (0.93%)	0.38
	LDL	6 (1.86%)	LDL	4 (1.86%)	LDL	2 (1.87%)	0.99
	QDV	5 (1.55%)	QDV	0 (0%)	QDV	5 (4.67%)	**0.003**
	HDV	4 (1.24%)	HDV	4 (1.86%)	HDV	0 (0%)	0.30
	PDI	4 (1.24%)	PDI	3 (1.4%)	PDI	1 (0.93%)	1
	QDI	4 (1.24%)	QDI	0 (0,00%)	QDI	4 (3.74%)	**0.01**
	VDV	4 (1.24%)	VDV	3 (1.4%)	VDV	1 (0.93%)	1
	FDV	3 (0.93%)	FDV	3 (1.4%)	FDV	0 (0%)	0.55
	DDI	3 (0.93%)	DDI	3 (1.4%)	DDI	0 (0%)	0.55
	MDI	3 (0.93%)	MDI	3 (1.4%)	MDI	0 (0%)	0.55
	NDL	3 (0.93%)	NDL	3 (1.4%)	NDL	0 (0%)	0.55
	TDI	3 (0.93%)	TDI	3 (1.4%)	TDI	0 (0%)	0.55
	Other	29 (8.97%)	Other	24 (11.16%)	Other	4 (3.73%)	**0.03**

Table 3: correlation between V2 features and clinical / viroimmunological variables

		Univariate Analysis			Multivariate Analysis		
V2 Features	**Clinical charateristics**	**Coefficient**	**C.I. (95%)**	**P-value**	**Coefficient**	**C.I. (95%)**	**p value**
Length	Age	-0,07	-.102 to -.038	<0.001			
	Sex, Female	-0,51	-1.635 to .594	0,36			
	Risk factor, MSM	0,73	-.105 to 1.574	0,086			
	AIDS presenter	-1,33	-2.459 to -.209	0,019			
	Estimated duration of infection (>1 year)	-0,465	-1.264 to .335	0,254			
	Absolute CD4+ cell count	0,2	.054 to .357	0,008	0.16	-0.01 to 0.32	**0.06**
	CD4+/CD8+ ratio	0,93	.132 to 1.733	0,021			
	Log10 HIV-RNA	-0,5	-.945 to -.072	0,023			
	CRT, X4	-1,36	-2.398 to -.337	0,009	-0.75	- 1.80 to 0.29	0.157
Net Charge	Age	0,004	-.009 to .017	0,532			
	Sex, Female	-0,06	-.501 to .369	0,765			
	Risk factor, MSM	-0,13	-.461 to .196	0,43			
	AIDS presenter	0,703	.273 to 1.133	0,001			
	Estimated duration of infection (>1 year)	0,15	-.161 to .463	0,342			
	Absolute CD4+ cells count	-0,05	-.11 to .009	0,096	-0.09	-0.15 to -0.03	**0.006**
	CD4+/CD8+ cells ratio	-0,16	-.479 to .146	0,296			
	Log10 HIV-RNA	0,1	-.069 to .274	0,239			
	CRT, X4	0.162	-.568 to .234	0.432	-0.02	-0.42 to 0.38	0.92
V2 Feature	**Clinical charateristics**	**Odds Ratio**	**C.I. (95%)**	**P-value**	**Odds Ratio**	**C.I. (95%)**	**p value**
PNGs*	Age	0,97	.951 to .99	0,003			
	Sex, Female	1,26	.68 to 2.371	0,453			
	Risk factor, MSM	1,05	.652 to 1.7	0,833			
	AIDS presenter	0,52	.26 to 1.055	0,07			
	Estimated duration of infection (>1 year)	0,944	.599 to 1.489	0,805			
	Absolute CD4+ cells count	1,04	.958 to 1.14	0,321	1.03	0.93 to 1.14	0.57
	CD4+/CD8+ cells ratio	1,34	.813 to 2.22	0,248			
	Log10 HIV-RNA	0,62	.484 to .819	0,001			
	CRT, X4	0,91	.501 to 1.658	0,762	1.24	0.66 to 2.34	0.50

**PNGs ≤ 2*

Table 4: correlation between 179-181 tripeptide and clinical / viroimmunological variables

		Univariate Analysis			Multivariate Analysis		
Position	**Clinical charateristics**	**Odds Ratio**	**C.I. (95%)**	**P-value**	**Odds Ratio**	**C.I. (95%)**	**p value**
Leu^{179}	Age	0,995	.976 to 1.014	0,597			
	Sex, Female	1,161	.594 to 2.275	0,661			
	Risk factor, MSM	1,133	.685 to 1.873	0,626	0.99	0.57 to 1.73	0.974
	AIDS presenter	0,595	.316 to 1.122	0,108			
	Estimated duration of infection (>1 year)	0,519	.32 to .845	0,008	0.52	0.29 to 0.91	**0.022**
	Absolute CD4+ cell count	1,059	.966 to 1.162	0,221	0.97	0.86 to 1.09	0.589
	CD4+/CD8+ cells ratio	1,299	.703 to 2.4	0,404			
	Log10 HIV-RNA	0,862	.665 to 1.12	0,266			
	CRT, X4	1,123	.603 to 2.096	0,712	1.57	0.80 to 3.05	0.187
	Subtype, Non-B	3,003	1.705 to 5.29	<0.001	3.11	1.70 to 5.76	<0.001
Val^{181}	Age	0,975	.956 to .993	0,007			
	Sex, Female	0,69	.374 to 1.278	0,238			
	Risk factor, MSM	2,368	1.473 to 3.807	<0.001	2.39	1.43 to 3.98	**0.001**
	AIDS presenter	0,4	.211 to .762	0,004			
	Estimated duration of infection (>1 year)	0,66	.423 to 1.03	0,068	0.93	0.56 to 1.55	0.771
	Absolute CD4+ cell count	1,106	1.014 to 1.208	0,023	1.04	0.93 to 1.16	0.496
	CD4+/CD8+ cells ratio	1,381	.779 to 2.451	0,268			
	Log10 HIV-RNA	0,842	.66 to 1.077	0,171			
	CRT, X4	0,788	.445 to 1.399	0,416	0.99	0.54 to 1.83	0.981
	Subtype, Non-B	1,562	.974 to 2.51	0,064	1.62	0.97 to 2.70	0.067

Conclusions

- A certain variability was observed in HIV-1 V2 structure, including the α4β7-binding tripeptide, in both B and non-B subtypes. Our study suggests a possible association between V2 features and viro-immunological characteristics.
- Further knowledge regarding the virus/integrin interaction could have important therapeutic implications, given the current availability of anti-α4β7 monoclonal antibodies (*5*).

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