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Performance of genotypic algorithms for predicting tropism of HIV-1CRF02_AG subtype

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1 ABSTRACT

2 **Background:** Several genotypic rules for predicting HIV-1 non-B subtypes tropism are
3 commonly used, but there is no consensus about their performances.

4 **Objectives:** Three genotypic methods were compared for CRF02_AG HIV-1 tropism
5 determination.

6 **Study design:** V3 *env* region of 178 HIV-1 CRF02_AG from Pitié-Salpêtrière and Saint-
7 Antoine Hospitals was sequenced from plasma HIV-1 RNA. HIV-1 tropism was determined
8 by Geno2Pheno algorithm, false positive rate (FPR) 5% or 10%, the 11/25 rule or the
9 combined criteria of the 11/25 and net charge rule.

10 **Results:** A concordance of 91.6% was observed between Geno2pheno 5% and the combined
11 criteria. The results were nearly similar for the comparison between Geno2pheno 5% and the
12 11/25 rule. More mismatches were observed when Geno2pheno was used with the FPR 10%.
13 A lower nadir CD4 cell count was associated with a discordance of tropism prediction
14 between Geno2pheno 5% and the combined criteria or the 11/25 rule (p=0.02 and p=0.03,
15 respectively). A lower HIV-1 viral load was associated with some discordance for the
16 comparison of Geno2pheno 10% and the combined rule (p=0.02).

17 **Conclusion:** Geno2pheno FPR 5% or 10% predicted more X4-tropic viruses for this set of
18 CRF02_AG sequences than the combined criteria or the 11/25 rule alone. Furthermore,
19 Geno2pheno FPR 5% was more concordant with the 11/25 rule and the combined rule than
20 Geno2pheno 10% to predict HIV-1 tropism. Overall, Geno2pheno 5% could be used to
21 predict CRF02_AG tropism as well as other genotypic rules.

22 Keywords: HIV tropism; non B subtype; genotypic prediction.

23 **BACKGROUND**

24 In the natural history of HIV disease, R5-tropic viruses were evidenced in the earlier
25 stages of the disease and X4-tropic viruses emerged in later stages. The presence of X4-tropic
26 HIV may have clinical impact on the disease progression.¹⁻³ Furthermore, a class of
27 antiretroviral, the CCR5 inhibitors like maraviroc, is only active in patients harbouring
28 exclusively R5-tropic viruses which implies a tropism determination before their prescription.
29 Then, the determination of tropism is useful in clinical practise.

30 First genotypic methods used very simple rules, such as the 11/25 rule, predicting X4
31 on the basis of the presence of basic residues 11 or 25 of the V3-loop. The combined criteria
32 of the 11/25 and net charge rules were also use to determine HIV tropism.⁴ Several
33 bioinformatics methods for prediction of HIV coreceptor usage have been proposed over the
34 years, several are available as online tools and the most used of them was Geno2pheno
35 [coreceptor].⁵ The system is restricted to using the V3-loop as viral sequence information
36 input and used support vector machines. The values have been achieved on clonal sequences
37 data.

38 Usually, the bioinformatics tools available to determine HIV tropism were built on
39 phenotypic-genotypic correlation based on HIV B subtype samples. The genotypic

40 determination is now commonly used to determine tropism for HIV-1 B subtype, but the
41 performances of various genotypic rules or algorithms for predicting tropism of HIV-1 non-B
42 subtypes are still discussed.^{4,6,7} It is of importance, because the prevalence of non B subtypes
43 is increasing across Europe and the CRF02_AG recombinant is the predominant non B
44 subtype in France.⁵

45

46 **OBJECTIVES**

47 The aim of this study was to compare 3 genotypic methods on a set of 178 HIV-1
48 CRF02_AG recombinants to evaluate the concordance between these measures of HIV-1
49 tropism.

50

51 **STUDY DESIGN**

52 This study has enrolled 178 HIV-1 patients infected by CRF02_AG recombinant from
53 two HIV clinical centers (Pitié-Salpêtrière and Saint-Antoine Hospitals) between September
54 2009 and June 2014. All viruses were identified as HIV-1 CRF02_AG recombinant by
55 Smartgene algorithm (Smartgene®, Switzerland) or by phylogenetic analyses, by estimating
56 the relationships among RT sequences and reference sequences of HIV-1 genetic subtypes
57 and circulating recombinant forms (CRF) obtained from the Los Alamos Database.

58 For the comparison of sequences, 180 HIV-1 patients infected by B subtype were
59 studied in the same period in the two clinical centers.

60 The V3 *env* region was sequenced from plasma HIV-1 RNA as previously described
61 and tropism was determined by Geno2pheno algorithm (False Positive Rate, FPR 5% or FPR
62 10%, <http://coreceptor.bioinf.mpi-inf.mpg.de/>), the 11/25 rule or the combined criteria of the

63 11/25 and net charge rule: CXCR4 coreceptor usage if R or K at position 11 of V3 and/or K at
64 position 25, or R at position 25 of V3 and a net charge of ≥ 5 , or a net charge of ≥ 6 . The V3
65 net charge was calculated by subtracting the number of negatively charged amino acids (D
66 and E) from the number of positively charged ones (K and R).⁸ A consensus sequence was
67 built with the predominant amino acid at each position of the V3 loop of gp120.

68 Comparisons between groups were performed using the non-parametric Mann-
69 Whitney or chi-squared tests. Statview software v5.0 was used. Cohen Kappa coefficient is
70 used to measure the inter-rate agreement for HIV tropism prediction.

71

72 **RESULTS**

73 The CRF02_AG HIV-1 patients had a median plasma HIV-1 viral load of 4.39 log
74 copies/mL (range 1.58-7), a median CD4 cell count of 366 cells/mm³ (range 5-1392) and a
75 median of nadir CD4 cell count of 214 cells/mm³ (range 1-1129). Approximately, half of
76 them were receiving an antiretroviral treatment without CCR5 antagonists. The different
77 genotypic algorithms predicted R5-tropic viruses as follows: 89.3%, 83.1%, 93.2%, and
78 91.0% for Geno2pheno FPR 5%, Geno2pheno FPR 10%, 11/25 rule and 11/25 rule combined
79 with net charge rule, respectively.

80 For CRF02_AG recombinant tropism prediction, a concordance of 91.6% (163/178)
81 was observed between Geno2pheno FPR 5% and the combined criteria (Figure 1A). Of the
82 178 samples, 154 (86.5%) and 9 (5.1%) were identified by both rules as R5- and X4-tropic
83 viruses, respectively. Then, 5 (2.8%) were identified as R5 by Geno2pheno FPR 5% but as X4
84 by the combined criteria, and 10 (5.6%) were identified as X4 by Geno2pheno FPR 5% but
85 R5 by the combined rule. The results are similar for the comparison between Geno2pheno

86 FPR 5% and the 11/25 rule in terms of R5 prediction (154 and 5 sequences were identified R5
87 by both rules and only by Geno2pheno FPR 5%, respectively) (figure 1A). The prediction of
88 X4 tropic viruses slightly differed between the 11/25 rule and the combined criteria as
89 revealed by the specificity score: 42.1% vs 47.2%.

90 Of the 178 samples, 143 (80.3%) and 11 (6.2%) were identified together by
91 Geno2pheno FPR 10% and the combined criteria rule as R5- and X4-tropic viruses,
92 respectively. Then, 24 sequences were not predicted for the same tropism by these two rules:
93 19 were identified as R5-tropic viruses by Geno2pheno FPR 10% and X4-tropic viruses by
94 the combined rule; 5 were identified as X4-tropic viruses by Geno2pheno FPR 10% and R5-
95 tropic viruses by the combined rule (figure 1B). For the comparison of Geno2pheno FPR 10%
96 with the 11/25 rule, a discordance was observed 21 and 3 sequences predicted X4- and R5-
97 tropic by Geno2pheno FPR5%, respectively (figure 1B). The specificity score was lower for
98 the comparison with the 11/25 rule than the combined criteria (32.2% vs 36.6%) as
99 demonstrated with Geno2pheno FPR 5% (figure 1). Overall, the Cohen Kappa coefficient
100 evidenced a better correlation between Geno2pheno FPR 5% and the combined criteria
101 (figure 1).

102 A lower nadir CD4 cell count was associated with a discordance of the tropism
103 prediction between geno2pheno FPR5% and the combined criteria or the 11/25 rule
104 ($p=0.0211$ and $p=0.0305$, respectively). A lower HIV-1 viral load was associated with the
105 discordance for the comparison of geno2pheno 10% and the combined rule ($p=0.0235$).

106 A consensus sequence was established with the main amino acid at each of the 35
107 positions of the V3 loop for B subtypes and CRF02_AG subtypes (table 1A). There was no
108 difference between both groups of patients (B *versus* CRF02_AG) in terms of CD4
109 ($p=0.0948$), plasma viral load ($p=0.0696$) and antiretroviral treatment ($p=0.2397$). Overall

110 polymorphisms were the same for both subtypes. We studied in particular the position 11 and
111 25 involved in the 11/25 and combined rule; the presence of an arginine codon was
112 statistically different between B and CRF02_AG subtypes ($p=0.0385$ and $p=0.0452$ for 11 and
113 25 position, respectively) (table 1B).

114

115 **DISCUSSION**

116 The HIV tropism determination can be slightly different according to the algorithm
117 used (Geno2pheno FPR 5%, Geno2pheno FPR 10%, 11/25 rule, 11/25 rule combined to net
118 charge rule) for this set of CRF02_AG recombinant sequences. The Geno2pheno algorithm
119 FPR 5 or 10% predicted more X4-tropic viruses than the 11/25 rule alone or combined to net
120 charge. Then, Geno2pheno seems to be more careful concerning the prediction of tropism in
121 regards to the prescription of CCR5 antagonists.

122 The bioinformatics tools available to determine HIV tropism were built on
123 phenotypic-genotypic correlation using HIV-1 B subtype samples. Thus, HIV genetic
124 variability between B subtype and CRF02_AG recombinant can have an impact on the
125 tropism determination by genotypic algorithms. Indeed, the V3 region of gp120 gene is
126 known to be highly variable in non B subtypes.⁹ It is also known that sensitivity rates of
127 genotypic approaches in non-B subtypes seem to vary between studies, probably reflecting
128 differences in panel of viruses included.^{4,8,10,11}

129 Even the presence of an arginine codon involved in the 11/25 rule was statistically
130 different between B and CRF02_AG subtypes in the present study, the sensitivity of
131 predictive tools was not improved by using simple rules such as 11/25 or 11/25 combined to
132 net charge in comparison with Geno2pheno. Then, the geno2pheno algorithm could be used to
133 predict tropism of CRF02_AG recombinants.

134 A potential impact of HIV-1 subtype on MVC activity was suggested in the MERIT
135 and MOTIVATE studies.¹²⁻¹⁴ It is still unclear if this could be due to different intrinsic
136 maraviroc activity between B and non-B subtypes or lack of sensitivity or specificity in the
137 methods used for the determination of tropism. Whatever, it is necessary to determine tropism
138 in all HIV subtypes with simple and economic methods. It would be interesting to study the
139 clinical response of an antiretroviral treatment including maraviroc regarding the used
140 algorithm to determine HIV tropism.

141 In summary, Geno2Pheno predicted more X4-tropic viruses for this set of CRF02_AG
142 sequences than the combined criteria or the 11/25 rule alone. Furthermore, Geno2pheno FPR
143 5% was more concordant with the 11/25 rule and the combined rule than Geno2pheno FPR
144 10% to predict HIV-1 tropism. Overall, in clinical practice, Geno2pheno FPR 5% could be
145 used to predict CRF02_AG tropism as for B subtype.

146

147

148 **ACKNOWLEDGMENT**

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150

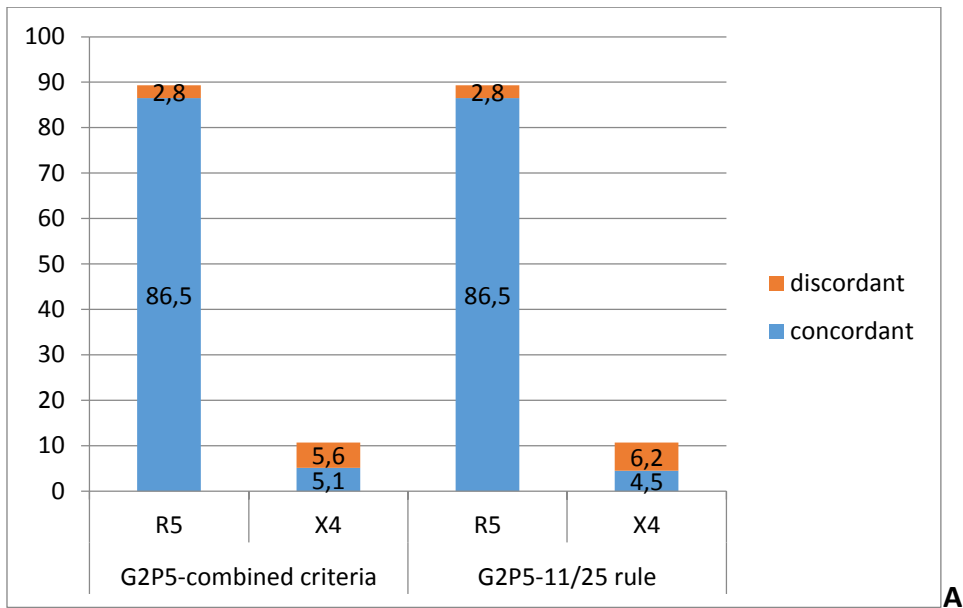
151 **CONFLICTS OF INTEREST STATEMENT**

152 None to declare.

153

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Sensitivity: 96.8%

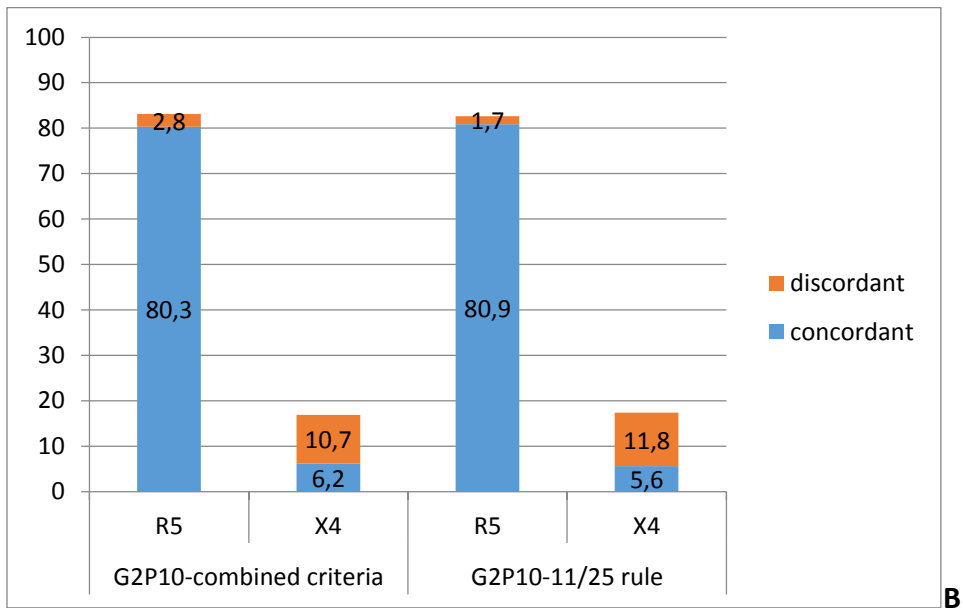
Specificity: 47.4%

Kappa=0.5002

Sensitivity: 96.8%

Specificity: 42.1%

Kappa=0.4525



Sensitivity: 96.6%

Specificity: 36.6%

Kappa=0.4090

Sensitivity: 97.9%

Specificity: 32.2%

Kappa=0.3920

Figure 1: Representation of tropism prediction by different algorithm for CRF02_AG recombinant

A: comparison between G2P5 (Geno2pheno FPR 5%) and combined criteria or 11/25 rule

B: comparison between G2P10 (Geno2pheno FPR 10%) and combined criteria or 11/25 rule

Gp120	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330
V3 loop	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
HXB2	C	T	R	P	N	N	N	T	R	K	S	I	R	I	Q	R	G	P	G	R	A	F	V	T	I	G	I	G	D	M	R	Q	A	H	C
B	C	T	R	P	N	N	N	T	R	K	S	I	H	I	G	P	G	R	A	F	Y	A	T	G	D/E	I	I	G	D	I	R	Q	A	H	C
CRF02_AG	C	T	R	P	D/N	N	N	T	R	K	S	V	R	I	G	P	G	Q	A/T	F	Y	A	T	G	D	I	I	G	D	I	R	Q	A	H	C

Table 1A: Amino acid consensus sequences of V3 loop-gp120 HIV B subtype and CRF02_AG recombinants.

	B subtype	CRF02_AG recombinant
A	0.5	
D	1.5	1.1
G	29.0	18.2
K		0.5
L	0.5	0.5
N	0.5	0.5
Q		0.5
R	7.5	2.7
S	59.5	75.4
T	0.5	
Y	0.5	0.5
11 S		
A	5.7	6.9
D	33.0	51.0
E	22.2	13.7
G	5.7	6.9
H	1.4	
I	7.1	2.4
K	2.4	2.9
N	3.8	4.9
Q	9.4	2.9
R	6.1	1.9
S	2.8	1.5
T	0.5	2.4
V		0.5
Y		0.5
25 I		
*		1.5

Table 1B: Distribution of amino acid at position 11 and 25 of V3 loop-gp120.

In bold, the major amino acid