

Genotypic Prediction of Human Immunodeficiency Virus Type 1 CRF02-AG Tropism[∇]

Stéphanie Raymond,^{1,2,3} Pierre Delobel,^{1,4} Maud Mavigner,¹ Michelle Cazabat,^{1,3} Corinne Souyris,^{1,3} Stéphanie Encinas,^{1,3} Karine Sandres-Sauné,^{1,2,3} Christophe Pasquier,^{1,2,3} Bruno Marchou,^{2,4} Patrice Massip,^{2,4} and Jacques Izopet^{1,2,3*}

INSERM, U563, Toulouse F-31300, France¹; Université Toulouse III Paul-Sabatier, Faculté de Médecine Toulouse-Purpan, Toulouse F-31300, France²; CHU de Toulouse, Hôpital Purpan, Laboratoire de Virologie, Toulouse F-31300, France³; and CHU de Toulouse, Hôpital Purpan, Service des Maladies Infectieuses et Tropicales, Toulouse F-31300, France⁴

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We assessed the performance of genotypic algorithms for predicting the tropism of human immunodeficiency virus type 1 coreceptor usage in 52 patients infected with the CRF02-AG subtype. The combined criteria of the 11/25 and net charge rules accurately detected CXCR4-using CRF02-AG viruses, whereas the Geno2pheno tool lacked sensitivity and the position-specific scoring matrix (PSSM) tool WebPSSM lacked specificity.

Human immunodeficiency virus type 1 (HIV-1) enters target cells through the sequential binding of the envelope glycoprotein (gp120) to CD4 and a chemokine receptor, CCR5 or CXCR4 (1). HIV-1 coreceptor usage must be identified before treatment with CCR5 antagonists, as they can only be used for patients harboring R5 viruses alone (7). The “gold standard” for characterization of HIV-1 tropism is a recombinant virus phenotypic entry assay, but genotypic methods based on the V3 sequence could be easier. We have previously shown that the V3 genotype accurately predicts the phenotype of HIV-1 coreceptor usage for subtype B viruses (5, 13). However, the V3-based genotypic algorithms could be unsuitable for predicting the tropism of non-B viruses because they were built using data sets of genotype-phenotype correlations from subtype B viruses (9). Indeed, the Geno2pheno and WebPSSM algorithms were not designed to be predictive for non-B viruses, except for a recent version of the position-specific scoring matrix (PSSM) designed for subtype C viruses (11, 12). It is thus necessary to study subtype-specific genotypic determinants of HIV-1 tropism. The CRF02-AG recombinant subtype predominates in West Africa (10) and accounts for an increasing proportion of cases in Western Europe, notably in France (6, 14). Various proportions of CXCR4-using viruses have been reported in subtype CRF02-AG-infected patients (2, 15, 16), but little is known about the genotypic determinants of HIV-1 tropism for subtype CRF02-AG viruses. Genotype-phenotype correlation studies are thus needed before genotypic algorithms can be used to predict the tropism of this particular HIV-1 subtype.

We characterized both genotypically and phenotypically the tropism of 52 HIV-1 CRF02-AG-infected individuals, recruited at the Department of Infectious Diseases of Toulouse University Hospital, France. These patients had a median plasma HIV-1 RNA load of 4.95 log copies/ml (interquartile

range, 4.18 to 5.34), and a median CD4⁺ T-lymphocyte count of 210 cells/mm³ (interquartile range, 115 to 391). All viruses were identified as the HIV-1 CRF02-AG subtype by *pol* and *env* sequence analysis using the HIVseq program (<http://hivdb.stanford.edu/>) and the NCBI genotyping tool (<http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi>). We confirmed that these viruses belonged to the CRF02-AG subtype by neighbor-joining phylogenetic analysis of the sequences studied here, together with HIV-1 subtype reference sequences from the Los Alamos National Laboratory (<http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html>).

A region spanning gp120 and the ectodomain of the gp41 *env* gene of plasma HIV-1 RNA was amplified by reverse transcription-PCR. Two separate PCR amplifications were performed in parallel for each patient and pooled to prevent sampling bias of the assessed virus population. The V3 region from the *env* PCR product was bulk sequenced, blinded to the phenotype, as previously described (13). Bulk sequencing allows the detection of minor variants when present at a frequency of at least 20% in the viral population. The phenotype of HIV-1 coreceptor usage was determined using a recombinant virus entry assay (13). The sensitivity of the assay has been enhanced to detect minor amounts of CXCR4-using virus when they accounted for 0.5 to 1% of the virus population (data not shown).

We used a genotypic rule based on amino acid residues at positions 11 and 25 and the overall net charge of V3 to predict HIV-1 tropism from the V3 genotype (3, 4, 8). One of the following criteria is required for predicting CXCR4 coreceptor usage: (i) R or K at position 11 of V3 and/or K at position 25, (ii) R at position 25 of V3 and a net charge of $\geq +5$, or (iii) a net charge of $\geq +6$ (13). The V3 net charge was calculated by subtracting the number of negatively charged amino acids (D and E) from the number of positively charged ones (K and R). We have previously shown that these combined criteria are better for predicting HIV-1 coreceptor usage of subtype B viruses than the 11/25 and net charge rules used separately (5, 13). We have now assessed the performance of these combined criteria for predicting the tropism of subtype CRF02-AG vi-

* Corresponding author. Mailing address: CHU de Toulouse, Hôpital Purpan, Laboratoire de Virologie, Toulouse F-31300, France. Phone: 33 5 67 69 04 24. Fax: 33 5 67 69 04 25. E-mail: izopet.j@chu-toulouse.fr.

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TABLE 1. Comparison of genotypic prediction of HIV tropism and the observed phenotype and performances of the V3 genotype for predicting CXCR4 usage of the HIV-1 CRF02-AG subtype

Prediction method and genotype	No. of patients with virus phenotype:		Performance ^a			
	R5	R5X4/X4	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Combined 11/25 and net charge rule						
R5	41	3	70	98	88	93
X4	1	7				
Geno2pheno ^b						
R5	38	6	40	90	50	86
X4	4	4				
WebPSSM _{X4/R5}						
R5	32	2	80	76	44	94
X4	10	8				
WebPSSM _{SI/NSI}						
R5	38	3	70	90	64	93
X4	4	7				

^a PPV, positive predictive value; NPV, negative predictive value.

^b Geno2Pheno was used with a false-positive rate of 10%.

ruses and those of the bioinformatic tools Geno2pheno (false-positive rate of 10%) and WebPSSM with the SI/NSI and X4/R5 matrices (WebPSSM_{SI/NSI} and WebPSSM_{X4/R5}, respectively). Geno2pheno is available at <http://coreceptor.bioinf.mpi-sb.mpg.de/cgi-bin/coreceptor.pl> (September 2008). WebPSSM is available at <http://ubik.microbiol.washington.edu/computing/psm/> (September 2008).

The phenotypic assay revealed 42 virus populations with an R5 phenotype and 10 virus populations with a dual/mixed R5X4 phenotype but no virus population with a pure X4 phenotype. The genotypic classifications based on the combined criteria from the 11/25 and net charge rules and the Geno2pheno and WebPSSM tools were compared to the phenotype of the subtype CRF02-AG viruses (Table 1). The combined criteria from the 11/25 and net charge rules misclassified only four of the samples from the 52 patients (global concordance, 92%), while Geno2pheno misclassified 10 samples (global concordance, 81%), PSSM_{X4/R5} misclassified 12 samples, and PSSM_{SI/NSI} misclassified 7 samples (global concordance, 77 to 87%). The combined 11/25 and net charge rule criteria successfully detected CRF02-AG subtype CXCR4-using viruses with a sensitivity of 70% and a specificity of 98%. Geno2pheno lacked sensitivity (40%), while PSSM_{X4/R5} was sensitive (80%) but less specific (76%).

A recent study reported that the genotypic algorithms currently used lack sensitivity for detecting CXCR4-using viruses among non-B subtypes, but no details were given of their performance for particular subtypes (9). Subtype-specific genotype-phenotype correlations should be assessed because the genotypic determinants of coreceptor usage for some particular subtypes may be different. We found that the Geno2pheno tool lacked sensitivity for predicting the CXCR4 usage of subtype CRF02-AG viruses, although it performs well for subtype B viruses (13). In contrast, the combined 11/25 and net charge

rule criteria were equally good at predicting the CXCR4 usage of both subtype CRF02-AG and subtype B viruses (13). Bulk sequencing is less sensitive than the phenotypic assay at detecting minor CXCR4-using variants in the virus population, but the impact of such minor variants on the clinical response to CCR5 antagonists remains to be determined. Multicenter studies analyzing the correlations between the genotypic determination of HIV-1 tropism and clinical response to CCR5 antagonists are needed to validate this approach in clinical practice.

In conclusion, the combined criteria from the 11/25 and net charge rules performed well for predicting the tropism of HIV-1 subtype CRF02-AG, while the Geno2pheno bioinformatic tool did not. Simple genotypic methods could make the clinical use of CCR5 antagonists easier and cheaper than using phenotypic assays. Additional studies are needed to assess the performances of the various genotypic algorithms for predicting the tropism of other HIV-1 non-B subtypes.

Nucleotide sequence accession numbers. The sequences reported here were given GenBank accession no. FJ652327 to FJ652378.

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