High Concordance between the Position-Specific Scoring Matrix and Geno2pheno Algorithms for Genotypic Interpretation of HIV-1 Tropism: V3 Length as the Major Cause of Disagreement[▽]

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The agreement between the position-specific scoring matrix (PSSM) and geno2pheno as tools for genotypic interpretation of HIV-1 tropism using 800 clinical specimens was assessed. There was an overall concordance of 88%. Disagreement was found mostly in specimens with short V3 lengths (<35 amino acids). Thus, consideration of V3 lengths should improve the predictability of HIV-1 tropism using genotypic algorithms.

The approval of the CCR5 antagonist maraviroc (MVC), a drug with specific inhibitory antiviral activity against HIV-1 R5-tropic variants, has pushed the determination of HIV-1 coreceptor usage in clinical settings. The phenotypic assay Trofile has been the most widely used tropism test, given that this assay was employed in the screening of patients enrolled in the pivotal MVC clinical trials. However, this method displays logistical and technical limitations that make it far from convenient in routine clinical practice, particularly in sites outside the United States. Given that HIV-1 tropism is largely determined by V3 sequences, several genotypic methods have been developed for estimating viral tropism. They are generally more feasible and progressively have replaced phenotypic testing in many places (6, 11).

Early studies evaluating the reliability of genotypic tools to determine HIV-1 tropism in clinical samples suggested that there was an overall low sensitivity of these methods for detection of X4 variants in comparison with Trofile (3). However, a *post hoc* reanalysis of MVC clinical trials using improved genotypic tools demonstrated that their performance is comparable to Trofile and ES-Trofile (enhanced sensitivity Trofile assay) in predicting the virologic response to MVC (4, 5), even though their sensitivity to detect X4-tropic variants was sometimes lower. Based on this information, recent guidelines support the use of genotypic methods to guide the clinical use of CCR5 antagonists (6, 11).

Two genotypic algorithms, the position-specific scoring matrix (PSSM) and geno2pheno, are currently the most widely used for the assessment of HIV-1 tropism in the clinical setting. The aim of our study was to evaluate the agreement between both tools in a large number of HIV-1-infected patients who underwent genotypic tropism testing to guide the therapeutic use of MVC. Viral and host factors potentially responsible for disagreement were examined.

All HIV-1-infected patients on regular follow-up at several Spanish clinics who underwent genotypic tropism determination between January 2009 and June 2010 were identified. Characterization of HIV-1 tropism had been performed in plasma samples based on single-V3 genotyping, as described elsewhere (7), and using geno2pheno and both of the PSSMs ($PSSM_{X4/R5}$ and $PSSM_{SI/NSI}$ [SI/NSI, syncytium inducing/nonsyncytium inducing]). For geno2pheno, a false-positive rate (FPR) of 10% was used for detecting X4 variants, as recommended by the Spanish and European guidelines for HIV-1 tropism determination (6, 11). In a recent reanalysis of the MOTIVATE and A4001029 trials, tropism predictions using V3 genotyping were shown to be comparable based on single or triplicate testing using a FPR of 10% (10). For the PSSMs, the original cutoff values were used for both matrices (2). V3 sequences harboring nucleotide mixtures were interpreted using the value provided by the geno2pheno website (http://coreceptor.bioinf.mpi-inf.mpg.de/) or the maximum score from the expanded sequences when using the PSSM.

Several genotypic characteristics of V3 sequences were analyzed, such as amino acid length, and the presence of glycosylation sites or nucleotide mixtures. HIV-1 subtype analysis was performed by phylogenetic analysis of *pol* and *env* gene sequences (ViroSeq HIV-1 genotyping system; Abbott Diagnostics, Madrid, Spain). In addition, clinical parameters, including CD4 counts, plasma HIV-1 viral load, and antiretroviral (ARV) exposure, were recorded for each patient at the time of HIV-1 tropism determination using a case report form.

Univariate (chi-square and Mann-Whitney tests) and multivariate (logistic regression) analyses were conducted to identify variables associated with disagreement in HIV-1 tropism reports using the two genotypic algorithms. Parameters included in the multivariate analyses were CD4 counts, plasma HIV-1 viral load, ARV exposure, viral subtype, and several V3 characteristics, including amino acid length and the presence of glycosylation sites or nucleotide mixtures. All results were expressed as percentages or medians with interquartile ranges (IQR). All statistical tests were two tailed, and only P values of <0.05 were considered signifi-

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TABLE 1. Viral and host factors associated with disagreement in HIV-1 tropism prediction using geno2pheno and PSSM

Characteristic	Value for:							
	Geno2pheno$_{FPR = 10\%}$ vs. PSSM$_{X4/R5}$				Geno2pheno$_{FPR = 10\%}$ vs. PSSM$_{SI/NSI}$			
	Concordant	Discordant	P value[a]		Concordant	Discordant	P value[a]	
			Univariate	Multivariate			Univariate	Multivariate
CD4 count [IQR] (no. of cells/mm^3)	315 [197–504]	304 [161–475]	0.496	0.647	316 [189–511]	304 [207–451]	0.486	0.704
Plasma HIV-RNA [IQR] (no. of log copies/ml)	4.20 [3.21–4.91]	4.28 [3.41–4.94]	0.612	0.580	4.24 [3.22–4.92]	4.23 [3.40–4.81]	0.825	0.786
Viral subtype (% non-B)	11.3	19.6	**0.018**	0.867	11.6	17.7	0.099	0.696
ARV exposure (% naïve)	47.2	56.3	0.132	0.150	52.2	46.8	0.395	0.333
Amino acid length (% without 35 aa)	17.5	38.3	**<0.001**	**<0.001**	17.3	41.7	**<0.001**	**<0.001**
Nucleotide mixture (% presence)	69.4	69.8	0.652	0.988	70.0	67.7	0.637	0.564
Glycosylation site (% absence)	2.9	4.7	0.364	0.707	3.0	4.2	0.528	0.724

[a] Significant P values are highlighted in boldface type.

cant. Statistical analyses were performed using the SPSS v17.0 software (SPSS Inc., Chicago, IL).

A total of 921 HIV-1 patients underwent genotypic tropism determination in plasma samples during the study period. V3 amplicons were obtained from 845 (91.7%) of them. Another 45 (5.3%) sequence samples were excluded due to the presence of 9 or more nucleotide mixtures within the V3 sequences. Of the remaining 800 V3 sequences (GenBank accession numbers JF511660 to JF512459), 87.6% (701/800) belonged to individuals carrying HIV-1 subtype B, and 48.5% (388/800) to ARV-naïve patients.

Most V3 sequences had 35 amino acids (aa) (79.8%; 638/800), 17% (134/800) had 34 aa, and only 3.2% (26/800) had any other length (ranging from 31 to 38 aa). Almost all V3 sequences presented at least one potential glycosylation site (96.9%). A total of 30.3% (242/800) of V3 sequences did not harbor any nucleotide mixture, 42.5% (340/800) had 1 to 3, and 27.3% (218/800) had 4 to 8. At the time of tropism determination, the median CD4 count was 314 cell/mm^3 (IQR, 196 to 500 cell/mm^3), and the median plasma HIV-1 RNA was 4.24 log copies/ml (IQR, 3.25 to 4.91 log copies/ml).

Patients infected with non-B subtypes were more frequently ARV naïve than individuals infected with clade B (65.8% versus 46.0%; $P = 0.001$). Interestingly, V3 sequences with lengths other than 35 aa were more frequently recognized in non-B subtypes than in clade B viruses (32.3% versus 18.5%; $P = 0.01$). No significant differences were noticed with respect to CD4 counts, plasma HIV-1 viral load, and presence of glycosylation sites or nucleotide mixtures within the V3 region.

The overall prevalence of X4 viruses was 16.9%, 18.3%, and 23.0% using PSSM$_{X4/R5}$, PSSM$_{SI/NSI}$, and geno2pheno$_{FPR = 10\%}$, respectively. There was no significant difference between HIV-1 subtypes. A higher prevalence of X4 viruses was found in ARV-experienced patients than in ARV-naïve patients using either geno2pheno$_{FPR = 10\%}$ (20% versus 14%; $P = 0.043$), PSSM$_{X4/R5}$ (23% versus 14%; $P = 0.005$), or PSSM$_{SI/NSI}$ (27% versus 20%; $P = 0.029$).

Patients with X4 viruses had lower median CD4 counts than those with R5 variants using geno2pheno$_{FPR = 10\%}$ (247 cells/mm^3 [IQR, 131 to 445 cells/mm^3] versus 339 cells/mm^3 [IQR,

222 to 514 cells/mm^3], respectively; $P < 0.001$), PSSM$_{X4/R5}$ (210 cells/mm^3 [IQR, 97 to 371 cells/mm^3] versus 343 cells/mm^3 [IQR, 226 to 515 cells/mm^3], respectively; $P < 0.001$), or PSSM$_{SI/NSI}$ (255 cells/mm^3 [IQR, 116 to 456 cells/mm^3] versus 333 cells/mm^3 [IQR, 218 to 504 cells/mm^3], respectively; $P = 0.001$). Median plasma HIV-1 viral loads were similar in patients harboring X4 or R5 viruses using geno2pheno$_{FPR = 10\%}$ (4.06 log HIV-RNA copies/ml [IQR, 3.11 to 4.85 log HIV-RNA copies/ml] versus 4.27 log HIV-RNA copies/ml [IQR, 3.29 to 4.93 log HIV-RNA copies/ml], respectively; $P = 0.249$) and PSSM$_{X4/R5}$ (3.90 log copies/ml [IQR, 2.98 to 4.81 log copies/ml] versus 4.27 log copies/ml [IQR, 3.35 to 4.92 log copies/ml], respectively; $P = 0.114$) but were lower for X4 viruses than for R5 viruses using PSSM$_{SI/NSI}$ (3.88 log copies/ml [IQR, 2.96 to 4.80 log copies/ml] versus 4.28 log copies/ml [IQR, 3.35 to 4.94 log copies/ml], respectively; $P = 0.031$).

Disagreement in tropism interpretation comparing PSSM$_{X4/R5}$ or PSSM$_{SI/NSI}$ with geno2pheno$_{FPR = 10\%}$ was found in 13.4% and 12% of specimens, respectively. Viral subtype and amino acid length were identified as the major drivers of disagreement (Table 1). Discordant specimens were more frequently seen in patients infected with non-B subtypes than in those infected with B subtypes (19.6% versus 11.3%, respectively; $P = 0.018$) and in samples with V3 length sequences other than 35 aa than in those with 35 aa (38.3% versus 17.5%, respectively; $P < 0.001$). In the multivariate analysis, only a short V3 length remained an independent predictor of disagreement ($P < 0.001$). The results of the multivariate analyses were reproduced when patients infected with non-B subtypes were excluded ($P < 0.001$).

In light of these results, an analysis of concordance was performed using a separate set of 201 plasma samples from HIV-1-infected patients with paired V3 genotypes and Trofile results according to V3 length (8). The genotypic algorithms performed more concordantly with Trofile using V3 sequences with 35 aa ($n = 168$) than using those with other V3 lengths (concordance values, 88/66/71% versus 52/64/67% for PSSM$_{X4/R5}$/PSSM$_{SI/NSI}$/geno2pheno$_{FPR = 10\%}$, respectively).

The results of our study demonstrate that the PSSM and

geno2pheno algorithms are highly concordant (>85% agreement) for the genotypic interpretation of HIV-1 tropism in the clinical setting. Disagreements were seen mostly in specimens from patients infected with non-B subtypes and when V3 sequences had less than 35 aa. In the multivariate analysis, a short V3 length emerged as the only cause of disagreement.

A lower sensitivity for detecting X4 viruses in non-B rather than B subtypes has been reported using several genotypic methods (1, 9). It could explain the worse concordance results between the PSSM and geno2pheno to determine HIV tropism in this set of clinical samples. In our knowledge, we report for the first time that V3 length might be a major cause of disagreement between genotypic interpretation methods assessing HIV-1 tropism. Since all genotypic algorithms analyzed are based on the analysis of each amino acid position within the V3 region, an incorrect alignment would yield different results. Although >80% of V3 sequences had 35 aa, the design and implementation of specific matrices within the PSSM and geno2pheno algorithms trained on databases with paired phenotypic data and 34-aa V3 sequences should be considered.

In summary, the PSSM and geno2pheno are highly concordant in the genotypic interpretation of HIV-1 tropism in clinical samples. Overall, disagreement was found in 12% of samples, mostly in specimens with short V3 lengths (34 aa). Therefore, improvements of the current versions of the PSSM and geno2pheno, considering this subset of short V3 samples, would be advisable.

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