Performance of Genotypic Tools for Prediction of Tropism in HIV-1 Subtype C V3 Loop Sequences

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Key Words

HIV-1 subtype C · Genotypic tropism testing · R5-tropic · X4-tropic · V3 loop · Phenotype

Abstract

Currently, there is no consensus on the genotypic tools to be used for tropism analysis in HIV-1 subtype C strains. Thus, the aim of the study was to evaluate the performance of the different V3 loop-based genotypic algorithms available. We compiled a dataset of 645 HIV-1 subtype C V3 loop sequences of known coreceptor phenotypes (531 R5-tropic/non-syncytium-inducing and 114 X4-tropic/R5X4-tropic/syncytium-inducing sequences) from the Los Alamos database (<http://www.hiv.lanl.gov/>) and previously published literature. Coreceptor usage was predicted based on this dataset using different software-based machine-learning algorithms as well as simple classical rules. All the sophisticated machine-learning methods showed a good concordance of above 85%. Geno2Pheno (false-positive rate cutoff of 5–15%) and CoRSeqV3-C were found to have a high predicting capability in determining both HIV-1 subtype C X4-tropic and R5-tropic strains. The current sophisticated genotypic tropism tools based on V3 loop perform well for tropism prediction in HIV-1 subtype C strains and can be used in clinical settings.

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CCR5 antagonists bind to the CCR5 coreceptor and block the entry of R5-tropic (using the CCR5 coreceptor only) strains. However, these drugs are ineffective against HIV-1 strains that use CXCR4 coreceptors (X4-tropic) or both CCR5 and CXCR4 coreceptors (dual-tropic) for their entry into the host cell [1]. Thus, the major caveat associated with initiating CCR5 antagonist-based therapy is to perform HIV coreceptor tropism prior to its use. HIV-1 coreceptor tropism can be determined by both phenotypic and genotypic methods. Although phenotypic methods are considered to be more reliable, they are limited by higher cost and turnaround time, and more specialized infrastructure [2]. Further, even this 'gold standard' has shortcomings for clinical validation [2].

On the other hand, genotypic tropism testing based on V3 loop sequencing combined with bioinformatic tools is well suited for routine clinical laboratories [3]. There are several genotypic tropism methods available, ranging from simple methods, such as the 11/25 rule, a charge rule based on the amino acid side chains, to more complex statistical models like position-specific scoring matrices (PSSM) and support vector machines (Geno2Pheno; distant segments kernel) [4]. All these machine-learning methods are based on trained datasets available, and most of them have been developed based on the data available from genetic characteristics of HIV-1 subtype B [5]. In

Table 1. Site-specific amino acid frequencies in the V3 loop of the 531 R5-tropic/NSI and 114 X4/R5X4-tropic/SI HIV-1 subtype C database sequences

	AA position												
	1	2	3	4	5	6	7	8	9	Ins	10	11	12
R5/NSI	C ₁₀₀	T _{74.2} I _{18.5} V _{2.6} A _{2.5}	R ₁₀₀	P _{98.7}	N _{55.9} G _{32.6} S _{5.5} H _{2.1} D _{1.7}	N _{98.1}	N _{99.8}	T _{96.8} I _{1.3}	R _{97.6} S _{1.9}	–	K _{72.9} R _{13.8} Q _{6.2} T _{4.5} E _{2.1}	S _{97.2} G _{2.3}	I _{44.4} V _{37.5} M _{14.5} T _{1.3} Y _{1.1}
X4/R5X4/SI	C ₁₀₀	T _{75.4} I _{7.9} M _{6.1} A _{3.5} V _{2.6} G _{1.8}	R ₁₀₀	P _{99.1}	N _{47.4} G _{36.8} D _{5.3} Y _{5.3} S _{3.5}	N ₈₆ Y _{5.3} S _{3.5} I _{2.6} K _{2.6}	N _{72.8} K _{10.5} T ₇ H _{2.6} S _{1.8}	T _{70.2} I _{11.4} K _{11.4} R _{5.3}	R _{78.9} I _{7.9} S _{4.4} E _{1.8} K _{1.8} N _{1.8}	M _{5.3}	K _{66.7} R _{28.9} T _{2.6} Q _{1.8}	S _{58.8} R _{26.3} G ₇ N _{3.5} H _{1.8}	I ₄₉ V _{38.6} M _{5.3} L _{4.4} A _{1.8}
	AA position												
	13	14	Ins	Ins	15	16	17	18	19	20	21	22	23
R5/NSI	R _{96.2} G _{1.5}	I _{99.2}	–	–	G _{99.6}	P _{99.6}	G _{99.6}	Q _{98.1} R _{1.3}	T _{69.9} A _{23.9} V _{3.8} S _{1.9}	F _{96.6} Y _{1.3} L _{1.1}	Y _{84.7} F _{14.7}	A _{93.8} T _{3.8} – _{1.3}	T _{93.6} N _{2.8} M _{1.1} A _{1.1}
X4/R5X4/SI	R _{79.8} G _{10.5} K _{8.8}	I _{93.9} L _{3.5}	G _{22.8}	I _{21.1} V _{1.8}	G ₁₀₀	P _{71.1} R _{24.6}	G _{94.7} R _{4.4}	Q _{52.6} R _{23.7} H _{13.2} L _{4.4} Y _{3.5} K _{1.8}	A _{44.7} T _{43.9} S _{6.1} V _{2.6}	F _{89.5} V _{3.5} I _{2.6} L _{1.8}	Y _{67.5} F ₁₄ H _{7.9} V _{6.1} L _{1.8}	A _{75.4} T _{10.5} R _{1.8} V _{1.8} – _{10.5}	T _{69.3} R _{6.1} K _{5.3} M _{3.5} A _{1.8} H _{1.8} N _{1.8} – _{3.5}
	AA position												
	24	25	26	27	Ins	28	29	30	31	32	33	34	35
R5/NSI	G _{78.3} N _{11.9} T _{2.5} D _{1.9} – _{2.6}	D _{56.9} E _{22.6} G _{7.5} A _{6.6} R _{1.9} N _{1.1} – _{1.1}	I ₉₄ V _{5.7}	I ₉₃ T _{3.8} V _{2.6}	–	G _{99.4}	D _{77.2} N _{21.1}	I _{98.9}	R _{99.2}	Q ₇₅ E _{11.5} K _{9.6} R _{2.3}	A _{99.6}	H _{77.2} Y _{20.2} S _{1.1}	C ₁₀₀
X4/R5X4/SI	G ₅₇ N _{15.8} K _{12.3} R _{4.4} E _{3.5} D _{2.6} T _{2.6}	K _{20.2} A _{14.9} G ₁₄ R _{12.3} D _{8.8} N _{6.1} T _{6.1} E _{5.3} Q _{5.3} H _{2.6} – _{2.6}	I _{78.9} V _{13.2} T _{2.6} M _{1.8} – _{2.6}	I ₆₄ K ₇ T ₇ V _{6.1} R _{5.3} G _{3.5} L _{3.5}	G _{3.5}	G _{92.1} R _{5.3} D _{1.8}	D _{82.5} N ₁₄ E _{1.8} I _{1.8}	I _{95.6} L _{1.8} R _{1.8}	R _{98.2} Q _{1.8}	Q _{82.5} K _{13.2} A _{2.6} R _{1.8}	A _{94.7} S _{1.8} T _{1.8}	H _{71.9} Y ₁₄ F _{7.9} S _{2.6} C _{1.8}	C ₁₀₀

Amino acids with frequencies less than 1 are not included. AA = Amino acid; Ins = insertion; – = deletion.

Table 2. Performance of genotypic tools in predicting X4 tropism in HIV-1 subtype C V3 loop sequences with known phenotypes

	Phenotype		Concordance	κ value	Sensitivity	Specificity
	X4/R5	X4/SI (n = 114)				
Geno2Pheno _{5%}	X4	95	13	95	0.83	83.3
	R5	19	518			97.6
Geno2Pheno _{10%}	X4	99	28	93.3	0.78	86.8
	R5	15	503			94.7
Geno2Pheno _{15%}	X4	100	48	90.2	0.7	87.7
	R5	14	483			91
Geno2Pheno _{20%}	X4	104	77	86.5	0.6	91.2
	R5	10	454			85.5
CoRSeqV3-C	X4	103	42	91.8	0.75	90.4
	R5	11	489			92.1
C-PSSM _{SINSI}	X4	99	70	86.8	0.62	86.8
	R5	15	461			86.8
B-PSSM _{X4R5}	X4	87	33	90.7	0.68	76.3
	R5	27	498			93.8
B-PSSM _{SINSI}	X4	77	33	89.2	0.62	67.5
	R5	37	498			93.8
ds kernel	X4	82	2	94.7	0.8	71.9
	R5	32	529			99.6
11/25 rule	X4	55	12	89	0.55	48.2
	R5	59	519			97.7
11/24/25 rule	X4	66	18	89.8	0.61	57.9
	R5	48	513			96.6
Net charge $\geq +5$	X4	106	159	74.1	0.42	93
	R5	8	372			70.1
Net charge $\geq +6$	X4	86	22	92.2	0.73	75.4
	R5	28	509			95.9
Raymond's rule ^a	X4	92	30	91.9	0.73	80.7
	R5	22	501			94.4
Garrido's rule ^b	X4	106	162	73.6	0.41	93
	R5	8	369			69.5

Concordance, sensitivity and specificity of >90% are highlighted in italics. ds = Distant segments.

^a One of the following criteria is required for predicting CXCR4 coreceptor usage: (1) 11 R/K and/or 25 K in V3; (2) 25 R in V3 and a net charge of $\geq +5$ and (3) a net charge of $\geq +6$.

^b 11/25 or a net charge of $\geq +5$.

spite of the presence of several methods that may be used to determine HIV-1 tropism, there still seems to exist a considerable discrepancy among these tropism tools. Furthermore, though the validity of these tests is well studied in subtype B strains, data are lacking for subtype C strains which account for more than 60% of the infection worldwide; subtype C is, moreover, the most predominant subtype in India. Thus, there is no clear consensus on the appropriate tool for routine clinical use in subtype C-predominant settings.

Thus, we aimed to evaluate the performance of the different V3 loop-based genotypic algorithms available in predicting coreceptor usage in a dataset of HIV-1 subtype C V3 loop sequences with known biological phenotypes.

We compiled a dataset of 645 HIV-1 subtype C V3 loop sequences of known coreceptor phenotypes based on coreceptor-transfected cell lines for CCR5 or CXCR4 coreceptor usage or MT-2 cells for non-syncytium-inducing (NSI) and syncytium-inducing (SI) properties, from the Los Alamos database (<http://www.hiv.lanl.gov/>) and from previously published literature [6–9]. Sequenc-

es containing #, \$ or * were eliminated from the dataset. Multiple sequences from a single patient were included only when the amino acid sequences were unique. 531 R5-tropic/NSI V3 sequences (from 365 patients) and 114 X4-tropic/R5X4-tropic/SI sequences (from 69 patients) were included. The frequency of amino acid substitutions at each site of the V3 loop for both the R5-tropic and X4-tropic dataset is shown in table 1. The coreceptor usage was predicted on this dataset using the different software-based algorithms, which comprised Geno2Pheno_{coreceptor} (<http://coreceptor.bioinf.mpi-inf.mpg.de/>) at false-positive rate cutoffs of 5, 10, 15 and 20%, PSSM (<http://indra.mullins.microbiol.washington.edu/webpssm/>), both X4/R5 and SI/NSI matrices for subtype B (B-PSSM_{X4R5} and B-PSSM_{SI/NSI}, respectively) and SI/NSI matrices for subtype C (C-PSSM_{SI/NSI}), distant segments kernel (<http://genome.ulaval.ca/hiv-dskernel/>), CoRSeqV3-C (<http://tools.burnet.edu.au/coreceptor/>) specific for subtype C, as well as classical models like the V3 loop net charge rule, the 11/25 rule and the 11/24/25 rule (based on positive charge at these positions) and a combination of simple rules like Garrido's rule (11/25 or a net charge of $\geq +5$) [10] and Raymond's rule (any of the following criteria: 11 R/K and/or 25 K in V3; 25 R in V3 and a net charge of $\geq +5$ or a net charge of $\geq +6$) [11].

The concordance, sensitivity and specificity of all the algorithms are depicted in table 2. The concordance between the tropism results obtained by the genotypic algorithms and the HIV-1 subtype C phenotypes ranged from 73.6 to 95%, depending on the genotypic tools used. All the sophisticated machine-learning methods showed a good concordance of above 85%. The best concordance was obtained with Geno2Pheno_{5%} (95%). Among the simple rules, net charge $\geq +6$ and Raymond's rule showed a concordance of $>90\%$. In terms of sensitivity of detecting X4 tropism, though Garrido's rule (93%) and the net charge $\geq +5$ rule (93%) were among the most sensitive tools, they severely lacked specificity (69.5 and 70.1%, respectively). Among the other tools, CoRSeqV3-C showed the highest sensitivity (90.4%), without compromising the specificity (92.1%). Geno2Pheno at the recommended false-positive rate cutoff of 10% as per the European guideline of HIV-1 tropism testing also showed a high sensitivity (86.8%) and specificity (94.7%). The other tools that showed both good sensitivity and specificity are Geno2Pheno_{5%} (83.3/97.6%), Geno2Pheno_{15%} (87.7/91%), Geno2Pheno_{20%} (91.2/85.5%), C-PSSM_{SI/NSI} (86.8/86.8%) and Raymond's rule (80.7/94.4%).

Currently, there is no consensus on the genotypic tools to be used for tropism analysis. European virologists have

suggested in their guidelines that the use of Geno2Pheno_{10%}, which is a well-validated tool, may be acceptable [2]. Other than Geno2Pheno, the most widely validated tool for subtype C is C-PSSM [12]. Prior to C-PSSM, PSSM_{X4R5} and PSSM_{SI/NSI} matrices were developed by Jensen et al. [13] based on subtype B V3 loop sequences. In view of a poor performance of the subtype B-specific PSSM in subtype C strains, a subtype C-specific C-PSSM was developed by the same group [12]. The difference in predictive performance of both subtype B- and subtype C-specific PSSM is evident in our observation. Though the overall concordance of C-PSSM was lower than that of B-PSSM, C-PSSM showed a better sensitivity in picking up X4 tropism in subtype C strains. A recent study based on database sequences has shown a 90 and 89% sensitivity of detecting X4-tropic strains by Geno2Pheno_{10%} and C-PSSM, respectively [14]. This was moderately higher than what we have observed. In our dataset, both Geno2Pheno_{5%} and Geno2Pheno_{10%} showed a much better concordance and specificity and a similar sensitivity to that of C-PSSM. Our results are highly similar to the values obtained by Raymond et al. [15] on subtype C live samples from Malawi and France. In their study, Raymond et al. [15] found the combined 11/25 and net charge rule to have a superior sensitivity (93.3%) and specificity (96.4%) compared to other tools. However, in our study, their rule lacked sensitivity. In our dataset of X4-tropic strains, a good interassay concordance of 93% between Geno2Pheno_{10%} and C-PSSM was observed.

Contrary to what had been observed in subtype C strains, earlier studies on subtype B strains observed Geno2Pheno (sensitivity ranging from 88 to 97%) to outperform B-PSSM (sensitivity ranging from 69 to 94%) in terms of sensitivity [10, 11, 16, 17]. But in these four studies on subtype B strains, Geno2Pheno (specificity ranging from 51 to 93%) lacked specificity compared to B-PSSM (81–100%). This subtype B-specific B-PSSM showed poor sensitivity ($<80\%$) when analyzed against subtype C strains as compared to its subtype C-specific counterpart (C-PSSM), as both have been trained against their respective subtypes. However, Raymond et al. [15] suggested that both HIV-1 subtype C and B viruses share the genotypic determinants of coreceptor usage mainly located in the V3 loop and, thus, algorithms trained against subtype B strains perform equally well in subtype C strains.

Very recently, another novel genotypic tool specific for subtype C strains (CoRSeqV3-C) has been evaluated against known phenotype datasets by Cashin et al. [18]. In their dataset, they observed a superior sensitivity of CoRSeqV3-C with a specificity comparable to Geno-

2Pheno and C-PSSM [18]. We made a very similar observation. For a proper clinical evaluation of tropism, it is equally important to look into the specificity of a test to rule out false X4 prediction, along with the sensitivity of determining X4 tropism. Thus, among all the tools, both Geno2Pheno (false-positive rate cutoff of 5–15%) and CoRSeqV3-C were found to have a high predicting capability in determining both HIV-1 subtype C X4-tropic and R5-tropic strains.

In conclusion, the current sophisticated genotypic tropism tools based on V3 loop perform well for tropism

prediction in HIV-1 subtype C strains and can be used for assessing the option of using CCR5 antagonists in clinical settings. However, there is a need to perform further studies to elucidate additional coreceptor determinants across all the subtypes for making coreceptor determination more robust.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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