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HIV-1 Subtype Is an Independent Predictor of Reverse Transcriptase Mutation K65R in HIV-1 Patients Treated with Combination Antiretroviral Therapy Including Tenofovir

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Subtype-dependent selection of HIV-1 reverse transcriptase resistance mutation K65R was previously observed in cell culture and small clinical investigations. We compared K65R prevalence across subtypes A, B, C, F, G, and CRF02_AG separately in a cohort of 3,076 patients on combination therapy including tenofovir. K65R selection was significantly higher in HIV-1 subtype C. This could not be explained by clinical and demographic factors in multivariate analysis, suggesting subtype sequence-specific K65R pathways.

Clinical relevance has been attributed to HIV-1 genomic diversity, classified in distinct groups and subtypes, since genetic variation can affect mutational pathways to drug resistance and possibly effectiveness of combination antiretroviral treatment (cART) (1, 2). The likelihood and rate of developing the lysine-to-arginine resistance mutation at position 65 (K65R) of reverse transcriptase (RT) has been argued to vary depending on the viral subtype (3). In particular, studies have proposed lower and higher propensities to develop K65R mutation for subtypes A and C, respectively. However, studies reporting subtype dependency of K65R selection were based mainly on observations from cell culture experiments or from patient populations with limited sample size or subtype distribution (3–5). Other studies failed to detect similar associations (6, 7). While many clinical predictors of K65R selection have been identified to date (8, 9), a clear consensus on the role of genomic diversity is presently lacking. A large majority of K65R cases observed in recent years resulted from the administration of tenofovir (TDF) (10), which is currently the most widely used nucleoside RT inhibitor (NRTI) for first-line cART. Given the rising prevalence of non-B subtypes in Europe and treatment availability in other parts of the world, improved knowledge on subtype-specific K65R resistance pathways can be important in the planning of cART for HIV-1 non-B-infected patients.

This study aimed specifically to evaluate the reported subtype-dependent selection of K65R rather than to identify novel predictors of its presence. We retrospectively investigated the emergence of K65R across different subtypes in a large TDF-experienced patient population, by integrating clinical and viral sequence data from eight countries (Belgium, Germany, Israel, Italy, Luxembourg, Portugal, Spain, and Sweden) in collaboration with the EuResist consortium. Viral isolates were obtained from genotypic resistance testing, recommended in case of therapy failure, while on treatment with a viral load high enough to allow successful sequencing. Together with information on demographics, treatment experience, and genotypic resistance profiles, we collected for each patient the most recent viral isolate while receiving TDF-based therapy, with a minimum of 30 days between the beginning of this therapy and the sampling date. When consecutive viral sequences were available, the first viral sequence containing K65R was selected. Patients were excluded if K65R was detected before the initiation of TDF therapy. The final analysis retained patients who were treated with a drug regimen consisting of two NRTIs and either a protease inhibitor (PI) or a nonnucleoside reverse transcriptase inhibitor (NNRTI) and who were infected with a subtype occurring in more than 1% of the patients. A subtype-dependent impact on K65R selection was evaluated in univariate analysis using a chi-square test of independence. To correct for possible confounders that explain various K65R prevalence across subtypes, we performed multivariate logistic regression of K65R presence, which included factors known to be predictive of K65R selection (8, 9). A population selection rate of K65R was calculated as the number of K65R cases observed divided by the time of tenofovir experience in the current regimen and modeled using a Poisson distribution. Odds ratios (OR), 95% confidence intervals (CI), and *P* values were calculated using the statistical package R (11). The level of statistical significance was set at 5%. Viral subtype was determined using

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TABLE 1 Distribution of patient characteristics included in the study population[a]

Variable	Value
No. of patients	3,076
Median yr of sampling (IQR)	2006 (2004–2007)
No. (%) of males	2,121 (69.58)
Median age in yrs (IQR)	47 (42–52)
Median length in days on current therapy (IQR)	324 (167–599)
Median no. of previous cART regimens (IQR)	3 (1–7)
No. (%) with prior exposure to TDF therapy	1,309 (42.55)
No. (%) with prior exposure to ABC or ddI therapy	1,790 (58.19)
No. (%) with second drug in combination with TDF	
ABC	161 (5.23)
ddI	424 (13.78)
AZT	135 (4.39)
d4T	222 (7.21)
3TC	866 (28.15)
FTC	1,277 (41.51)
No. (%) with third drug in combination with TDF	
Boosted PI	1,729 (56.21)
Unboosted PI	252 (8.19)
NNRTIs	1,095 (35.59)
EFV	737 (23.95)
NVP	358 (11.63)
No. (%) with co-occurrence of RT mutations	
K65R	285 (9.27)
TAM 1	894 (30.28)
TAM 2	907 (30.71)
NAMs	136 (4.61)
M184V	1,186 (40.16)
NNRTI mutations	1,090 (36.89)

[a] ABC, abacavir; ddI, didanosine; AZT, zidovudine; d4T, stavudine; 3TC, lamivudine; FTC, emtricitabine; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; EFV, efavirenz; NVP, nevirapine; IQR, interquartile range. Mutation patterns in RT were defined as thymidine analogue mutation 1 (TAM1) (M41L, L210W, or T215Y), TAM2 (D67N, K70R, 215F, or D219E/Q), M184V, other nucleoside analogue mutations (NAMs) (A62V, V75I, F77L, F116Y, or Q151M), and nonnucleoside RT inhibitor (NNRTI) mutations (L100I, K103N, or Y181C).

the Rega HIV-1 subtyping tool V2. Table 1 shows the distribution of demographic and clinical characteristics of 3,076 patients fulfilling the inclusion criteria. K65R was detected in 285 patients, resulting in an overall prevalence of 9.3% (CI, 8.3 to 10.3), consistent with earlier reports on the infrequent emergence of K65R in HIV-1 patients failing tenofovir-based cART (7, 9, 10). When the number of K65R events was normalized by the time of observation, we observed a rate of 7.5 cases per 100 patient-years (CI, 6.6 to 8.4; total of 3,822 years of observation). The study population was primarily infected with subtype B viruses (77.4%). The subtype distribution is shown in Table 2. Univariate analysis indicated a significant difference in K65R prevalence across subtypes ($P = 0.038$). For each subtype, the respective K65R prevalence and selection rate per 100 patient-years of observation are shown in Table 2. K65R prevalence and selection rate were the highest in subtype C patients. A pairwise comparison of K65R prevalence in subtype B viruses with other subtypes revealed only a higher selection in subtype C viruses ($P = 0.016$) (see Table S1 in the supplemental material for pairwise comparisons of K65R prevalence for all possible combinations of viral subtypes). Next, we performed multivariate logistic regression of K65R presence to confirm a subtype-dependent impact and corrected for factors that could influence the differential prevalence across subtypes, including differences in treatment experience and cooccurring mutations known to interact with K65R (3, 7, 8, 9, 12). Compared to subtype B, only infection with subtype C remained independently significantly associated with a higher probability of K65R emergence ($P = 0.028$, OR = 2.22) (Table 3). Phylogenetic analysis did not reveal clustering of K65R among the subtype C patients, excluding the possibility that transmission of K65R, as opposed to independent selection of the mutation by cART, may significantly influence its differential abundance (data not shown). Furthermore, the model identified the coadministration of didanosine or abacavir as a predictor of K65R presence compared to emtricitabine. The use of an NNRTI was also associated with higher selection of K65R than the use of a PI as a third agent. K65R prevalence across factors in the model is shown in Table S2 in the supplemental material. To our knowledge, this is the first large-scale study that evaluated subtype dependency of K65R emergence over prevailing subtypes A, B, C, F, G, and CRF02_AG. In a patient population at risk for K65R development, the presence of subtype C virus was significantly associated with higher rates of K65R selection than subtype B. These findings support earlier reports of a higher propensity of subtype C in developing the K65R mutation due to the molecular mechanism of RNA template pausing (3). A differential selection of K65R was not observed for other subtypes in our population, in accordance with knowledge to date, but limited sample sizes could have resulted in insufficient power to detect a true association for these subtypes. We could not confirm the lower prevalence of K65R in subtype A than in

TABLE 2 Distribution of HIV-1 subtypes and K65R across subtypes[a]

Subtype	No. (%) of patients	K65R prevalence (%; 95% CI)	Observation time (yrs)	K65R selection rate (95% CI)
A	84 (2.7)	5 (6.0; 2.7–13.0)	105.1	4.8 (1.5–11.1)
B	2,386 (77.4)	209 (8.8; 7.7–10.0)	2,924.2	7.1 (6.2–8.1)
C	105 (3.4)	17 (16.2; 10.4–24.4)	106.4	15.9 (9.3–25.5)
F	55 (1.8)	3 (5.5; 1.9–14.9)	67.7	4.4 (0.9–12.9)
G	333 (10.8)	40 (12.0; 8.9–15.9)	490.6	8.2 (5.8–11.1)
CRF 02_AG	116 (3.8)	11 (9.5; 5.4–16.2)	127.4	8.6 (4.3–15.4)

[a] For each subtype, the table shows the number of study patients as an absolute number and as a percentage, the number of K65R cases as an absolute number and as a percentage with 95% confidence intervals (CI), the total observation time in patient-years of tenofovir experience in the current therapy, and K65R selection rate as the number of K65R events per 100 patient-years with 95% confidence intervals.

TABLE 3 Multivariate analysis of the effect of HIV-1 subtype on K65R selection[a]

Variable	OR (95% CI)	*P* value
Yr of sampling, for every additional yr	0.86 (0.76–0.98)	0.027
Median length in days on current therapy	0.99 (0.99–1.00)	0.775
Previous no. of cART regimens	0.99 (0.94–1.05)	0.470
Prior exposure to TDF	1.37 (0.94–2.02)	0.098
Prior exposure to ABC or ddI	0.94 (0.64–1.40)	0.453
Second drug in combination with TDF		
FTC	1 (Ref)	
ABC	2.50 (1.12–5.38)	0.011
ddI	5.10 (2.82–9.27)	<0.001
AZT	0.34 (0.05–1.28)	0.142
d4T	0.56 (0.15–1.67)	0.346
3TC	1.35 (0.77–2.35)	0.127
Third drug in combination with TDF		
Boosted PI	1 (Ref)	
Unboosted PI	0.95 (0.38–2.16)	0.77
EFV	4.51 (2.77–7.44)	<0.001
NVP	7.02 (4.17–11.96)	<0.001
Co-occurrence of RT mutations		
TAM 1	0.05 (0.02–0.09)	<0.001
TAM 2	0.47 (0.31–0.70)	<0.001
NAM	7.62 (4.48–12.96)	<0.001
M184V	1.47 (1.03–2.10)	0.042
NNRTI	2.31 (1.62–3.32)	<0.001
HIV-1 subtype		
B	1 (Ref)	
A	0.67 (0.21–1.69)	0.423
C	2.23 (1.07–4.45)	0.028
F	0.71 (0.14–2.52)	0.635
G	1.31 (0.83–2.05)	0.242
CRF 02_AG	1.35 (0.51–3.21	0.289

[a] A logistic regression model was used to test for differential selection of K65R in HIV-1 subtypes and was corrected for possible confounders. These additional factors included previous treatment information, drugs accompanying tenofovir, and resistance mutations co-occurring with K65R. Subtype B was used in the regression model as a reference for comparison. *P* values, odds ratios (OR), and 95% confidence intervals (CI) are shown. Ref, reference value. See the legend of Table 1 for abbreviations.

subtype B that was previously reported by Gupta et al. (4). Although Gupta et al. found a significant difference in K65R prevalence across subtypes A, B, and C, our reanalysis of their data does not support lower K65R selection in subtype A than in subtype B alone or to subtypes B and C combined (see Tables S3 and S4 in the supplemental material). This analysis was confined to patients with indications of failing cART including tenofovir, in order to reduce heterogeneity of characteristics in the study population. We and others have previously shown that, within a TDF-experienced patient population, probability of K65R selection can vary according to accompanying drugs (8–12). The analysis considered each subtype separately, in contrast to dichotomizing B versus non-B subtypes, and an association with K65R was corrected for confounding factors. The resulting regression model was consistent with knowledge on predictors of K65R development, providing strong support for the validity of our analysis and a role for the HIV-1 subtype. A limitation of this study is that we cannot exclude the presence of K65R as the minority variant, since population-based sequencing assays detect only mutations present in more than 20% of variants in the viral population. Our study confirms that the use of TDF in subtype C-infected patients should be carefully evaluated given the higher rate of K65R selection in patients failing cART. The use of TDF is recommended for first-line HIV-1 treatment, and our findings have a significant clinical relevance for management of HIV-1 infection with cART, particularly in developing countries where subtype C is predominant.

Nucleotide sequence accession numbers. Sequences have been submitted to GenBank under accession numbers KC218938 and KC222012.

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