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Potential for immune-driven viral polymorphisms to compromise antiretroviral-based preexposure prophylaxis for prevention of HIV-1 infection

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Additional authors: AIDS imposes a limit of 25 authors per paper on the title page. An additional seven authors contributed to this study, as part of the International HIV Adaptation Collaborative. They are: Maribel Soto-Nava, Claudia García-Morales (Centre for Research in Infectious Diseases, National Institute of Respiratory Diseases, Mexico City, Mexico), Ivette Lorenzana (Universidad Nacional Autónoma de Honduras, Tegucigalpa, Honduras), Helen Byakwaga (Mbarara University of Science and Technology, Mbarara, Uganda), David Bangsberg (Oregon Health & Science University and Portland State School of Public Health, Portland, Oregon), Roger Shapiro (Department of Immunology and Infectious Diseases, Harvard T H Chan School of Public Health, Boston, MA, USA), and John Frater (Nuffield Department of Medicine, Medical Sciences Division, University of Oxford, Oxford, UK). These authors collected, analyzed and/or provided access to data.

Conflicts of interest

There are no conflicts of interest.

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Abstract

Objective: Long-acting rilpivirine is a candidate for preexposure prophylaxis (PrEP) for prevention of HIV-1 infection. However, rilpivirine resistance mutations at reverse transcriptase codon 138 (E138X) occur naturally in a minority of HIV-1-infected persons; in particular those expressing human leukocyte antigen (HLA)-B*18 where reverse transcriptase-E138X arises as an immune escape mutation. We investigate the global prevalence, B*18-linkage and replicative cost of reverse transcriptase-E138X and its regional implications for rilpivirine PrEP.

Methods: We analyzed linked reverse transcriptase-E138X/HLA data from 7772 antiretroviral-naive patients from 16 cohorts spanning five continents and five HIV-1 subtypes, alongside unlinked global reverse transcriptase-E138X and HLA frequencies from public databases. E138X-containing HIV-1 variants were assessed for in-vitro replication as a surrogate of mutation stability following transmission.

Results: Reverse transcriptase-E138X variants, where the most common were rilpivirine resistance-associated mutations E138A/G/K, were significantly enriched in HLA-B*18-positive individuals globally ($P = 3.5\ 10^{-20}$) and in all HIV-1 subtypes except A. Reverse transcriptase-E138X and B*18 frequencies correlated positively in 16 cohorts with linked HIV/HLA genotypes (Spearman's $R = 0.75$; $P = 7.6\ 10^{-4}$) and in unlinked HIV/HLA data from 43 countries (Spearman's $R = 0.34$, $P = 0.02$). Notably, reverse transcriptase-E138X frequencies approached (or exceeded) 10% in key epidemic regions (e.g. sub-Saharan Africa, Southeastern Europe) where B*18 is more common. This, along with the observation that reverse transcriptase-E138X variants do not confer in-vitro replicative costs, supports their persistence, and ongoing accumulation in circulation over time.

Conclusions: Results illustrate the potential for a natural immune-driven HIV-1 polymorphism to compromise antiretroviral-based prevention, particularly in key epidemic regions. Regional reverse transcriptase-E138X surveillance should be undertaken before use of rilpivirine PrEP.

Keywords

E138X; escape mutation; human leukocyte antigen-B*18; replication fitness; rilpivirine

Introduction

Treatment of HIV-1 infection with combination antiretroviral therapy (ART) has significantly reduced HIV-related morbidity and mortality [1,2] and can also significantly reduce the risk of onward HIV-1 transmission [3–5]. Despite ongoing efforts to expand combination ART access globally, however, over two million new HIV-1 cases continue to be reported each year [6]. The expanded use of antiretrovirals by HIV-uninfected persons for prevention of HIV-1 infection [termed preexposure prophylaxis (PrEP)] has gained traction in recent years, after seminal studies demonstrated that oral administration of the HIV-1 nucleotide reverse transcriptase inhibitor tenofovir disoproxil fumarate combined with the

nucleoside reverse transcriptase inhibitor emtricitabine can protect against HIV-1 infection in high-risk groups, provided that high rates of adherence are maintained [7–9]. Though PrEP is now approved for use in the United States, Canada, and elsewhere, adherence limitations of daily dosing and the risk of transmission of HIV-1 strains resistant to PrEP components [10] represent two major barriers to PrEP efficacy. To address the former, long-acting injectable formulations of antiretroviral agents, notably the investigational integrase inhibitor cabotegravir and the second-generation nonnucleoside reverse transcriptase inhibitor rilpivirine, are being considered for use as PrEP [11–14]. However, whereas HIV-1 variants resistant to tenofovir disoproxil fumarate are still relatively uncommon in most areas [15,16], and integrase-resistant variants rarer still [17], rilpivirine-resistant variants, in particular those with mutations at the 138th position of HIV-1 reverse transcriptase (E138X), occur naturally at a significant frequency in some areas [16,18–20]. Indeed, reverse transcriptase-E138X was previously shown to be significantly enriched among HIV-1 subtype B-infected individuals carrying the human leukocyte antigen (HLA) class I-B*18 allele [21,22]; reverse transcriptase-E138X variants were later confirmed to be HLA-B*18-restricted $CD8^+$ cytotoxic T-lymphocyte escape mutations occurring at the second (HLA/peptide anchor) position of a B*18-restricted cytotoxic T-lymphocyte epitope spanning HIV-1 reverse transcriptase codons 137–144 [23].

As rilpivirine PrEP will presumably fail to protect against infection by reverse transcriptase-E138X-containing strains, establishing their regional prevalence is paramount. However, no previous study has characterized reverse transcriptase-E138X prevalence and its association with HLA-B*18 frequency across HIV-1 subtypes and global populations. In light of recent studies demonstrating ongoing adaptation of HIV-1 to HLA class I [24], we combine in-vitro experiments with analyses of host and viral genetic data to test the hypothesis that differential global reverse transcriptase-E138X frequencies reflect the B*18-mediated selection and subsequent stable transmission of these variants in human populations, and interpret our observations in context of the regional implications for the use of rilpivirine as PrEP.

Methods

Cohorts and data sources

Reverse transcriptase-E138X and human leukocyte antigen-B*18 frequencies in global cohorts (linked analysis)—Plasma HIV-1 RNA-derived reverse transcriptase sequences linked to high-resolution HLA-B typing data from 7647 chronically HIV-infected, ART-naive individuals from 15 established contemporary cohorts in Canada [22], United States [22], Mexico [25,26], Belize [27], Guatemala [28], Honduras [29], Nicaragua [30], Panama [31], United Kingdom [24], Uganda [32], Botswana [24], South Africa [24], Japan [23], Vietnam [33], and Australia [22], and one published study including 125 HLA-B locus typed, chronically HIV-infected, ART-naive individuals from France [20] (retrieved via a PubMed search of English language articles published between 2010 and 2015 using keywords ‘HIV’ and ‘resistance’ that yielded 6803 articles, of which only this one featured linked reverse transcriptase-E138X and B*18 frequency data) were analyzed with respect to reverse transcriptase-E138X and HLA-B*18 carriage. In total, the analyzed data were

derived from 7772 HIV-infected ART-naïve individuals and these data comprised HIV-1 group M subtypes A (n = 233), B (n = 5641), C (n = 1063), D (n = 186), circulating recombinant form (CRF)01_AE (n = 438), and others/ recombinants (n = 86) (subtype data were not available for the French study). Study participants from all of our 15 cohorts gave written informed consent for their participation and/or specimens were anonymized by institutional review board (IRB)-approved procedures. This study was approved by all relevant IRBs.

Reverse transcriptase-E138X and human leukocyte antigen-B*18 frequencies in global databases (unlinked analysis)—Unlinked reverse transcriptase-E138X and HLA-B*18 frequencies were additionally retrieved from the Stanford University HIV Drug Resistance [34] and Allele Frequencies [35] databases, respectively, using custom Python web scripts [36]. Briefly, HLA frequencies at allele-level resolution were retrieved for all published studies of N = 100 or greater, yielding 27 480 449 HLA alleles from 85 countries. Similarly, a total of 44 934 HIV-1 sequences from unique therapy-naive study participants were retrieved from a total of 132 countries (mean 123 sequences/country, interquartile range = 19–279). Pairwise alignment of the translated sequences against the HIV-1 subtype B reference strain HXB2 was performed using *HyPhy* as described in [36]. Analyses of reverse transcriptase-E138X and HLA-B*18 frequencies were limited to countries with a minimum of 100 HIV-1 sequences; 43 countries were thus represented in both reverse transcriptase-E138X and HLA data sets. Here, analysis was performed using ‘traditional’ allele frequencies, where the denominator is the number of alleles in the diploid human genome (2N).

Viral replication assessment—To delineate the effect of reverse transcriptase-E138X on viral replication capacity, recombinant HIV-1’s were produced in the subtype B NL4–3 reference backbone (HIV-$1_{NL4–3}$) [23]and single-strain HIV-1 replication assays were performed as described in [37]. Briefly, MT-2 cells (1×10^5) were exposed to a standardized viral inoculum (500 blue-cell-forming units in MAGIC-5 cells) for 2 h, washed twice with phosphate-buffered saline (PBS), and cultured in 1 ml of complete medium. The culture supernatants were harvested every other day, and p24 Gag amounts were determined using a chemiluminescence enzyme immunoassay (Fuji-Rebio, Tokyo, Japan). Replication assays were performed in triplicate and repeated three times using independently generated virus preparations. To further delineate the effect of reverse transcriptase-E138X, competitive HIV-1 replication assays were also performed [37]. Freshly prepared H9 cells (3×10^5) were exposed to mixtures of paired virus preparations at various ratios for 2 h, washed twice with PBS, and cultured. On day 1, one-third of the infected H9 cells were harvested and washed twice with PBS, and proviral DNAwere sequenced (denoted passage 0). The viral culture which best approximated a 50 : 50 mixture on day 1 was further propagated. Culture supernatants were transferred weekly to new uninfected H9 cells for 18 additional passages. DNA extracted from cells harvested at the end of each passage was subjected to PCR amplification of the HIV-1 reverse transcriptase region followed by direct DNA sequencing; the relative proportions of each viral population were estimated by their relative chromatogram peak heights.

Statistical analysis

The association between reverse transcriptase-E138X and B*18 carriage in all study cohorts, overall and stratified by HIV-1 subtype, was assessed using Fisher's exact test. Sequences containing amino acid mixtures at reverse transcriptase codon 138 were counted as variants. The correlation between reverse transcriptase-E138X prevalence and HLA-B*18 frequency was evaluated using Spearman's rank correlation. Differences in p24 Gag production by wild-type versus reverse transcriptase-E138X-containing HIV-1 strains in the viral replication assay was assessed using student's *t*-test. All tests of significance were two-sided with a defined as $P<0.05$. All statistical analyses were performed with SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Analysis of linked viral/HLA genotypes from 7772 antiretroviral-naive HIV-infected patients from 16 cohorts spanning five continents revealed a highly significant overall enrichment of reverse transcriptase-E138X among HLA-B*18-positive compared with HLA-B*18-negative individuals, with 11.9 and 2.1% of B*18-positive and B*18-negative individuals, respectively, harbouring reverse transcriptase-E138X ($P= 3.5\ 10^{-20}$, Fig. 1a). The most common variants at this position, comprising 97.6% of all those observed, were E138A, followed by E138G and E138K (Fig. 1b, c), all major rilpivirine resistance-associated mutations [38]. On average therefore, 11.6% of HLA-B*18-positive patients worldwide naturally harbour rilpivirine-resistant HIV-1 variants.

Stratified by cohort, E138X was enriched in B*18-expressing individuals in all countries except Belize (where no E138X variants were observed), Vietnam, and the United Kingdom (where overall E138X frequencies were <1%). In the remaining 13 countries, E138X frequencies ranged from 5.6 to 21% in B*18-expressing persons compared with only 0.37–10% in non-B*18-expressing persons (Fig. 1d). Moreover, the enrichment of reverse transcriptase-E138X variants in B*18-expressing persons was statistically significant in seven of these 13 countries (Japan, Mexico, Guatemala, South Africa, United States, Uganda, and Canada). These observations confirm reproducible selection of reverse transcriptase-E138X by HLA-B*18 in human populations globally.

Consistent with recent reports of population-level HIV-1 adaptation to HLA class I alleles in human populations [24], E138X prevalence correlated significantly with HLA-B*18 frequency in the 16 antiretroviral-naive study cohorts. This remained true regardless of, whether E138X frequencies were computed in the overall population (Spearman's $R = 0.75$; $P = 7.6 \times 10^{-4}$, Fig. 2a) or only among the B*18-negative population subset (Spearman's $R = 0.66$; $P = 0.0055$, Fig. 2b). The positive relationship between reverse transcriptase-E138X and B*18 population frequencies was further corroborated by an analysis of unlinked reverse transcriptase-E138X and HLA-B*18 data from 43 countries (see methods; Spearman's $R = 0.34$; $P = 0.02$, Fig. 3). Overall, these data strongly suggest that reverse transcriptase-E138X variants are accumulating in regions where the restricting HLA-B*18 allele is more common. Strikingly, the areas where population reverse transcriptase-E138X frequencies are most elevated (e.g. approaching or exceeding 10% in some countries) tend to

be ‘key’ epidemic regions in terms of high HIV prevalence (e.g. sub-Saharan Africa) or concentrated HIV-1 epidemics (e.g. Southeastern Europe).

Although relative enrichment of E138A in HIV-1 subtype C compared with subtype B has previously been reported [39], reverse transcriptase-E138X distribution is incompletely characterized in other major HIV-1 subtypes though the data in Fig. 3 suggests that it arises in all major HIV-1 subtypes and CRFs globally. We, therefore, stratified reverse transcriptase-E138X prevalence by subtype in our 15 cohorts with linked HIV/HLA data. Reverse transcriptase-E138X was most common in subtype C (6.7%), followed by D (4.3%), A (3.9%), B (1.9%), and CRF01_AE (0.68%; all comparisons except CRF01_AE significant at $P<0.05$ with subtype B used as the reference group). These frequencies corroborated those from 60 518 unique HIV-infected reverse transcriptase sequences from reverse transcriptase inhibitor-naive patients retrieved from the Stanford HIV drug resistance database (Spearman’s $R = 0.90$; $P = 0.037$ and Supplemental Figure 1, http://links.lww.com/QAD/B122), indicating that our cohorts are not unrepresentative of the pandemic. Also consistent with our overall analyses (Fig. 1b, c), rilpivirine resistance-associated mutations E138A followed by E138G and E138K accounted for more than 95% of reverse transcriptase codon 138 variants across HIV-1 subtypes A–G and CRFs 01_AE and 02_AG in the Stanford database (Supplemental Figure 1, http://links.lww.com/QAD/B122). These data further confirm that rilpivirine-resistance mutations arise naturally in all major HIV-1 subtypes and CRFs, and that their frequencies tend to be higher in non-B subtypes.

Visualization of reverse transcriptase-E138X distribution confirmed substantial frequency differences globally, with regions hardest hit by the epidemic, notably Sub-Saharan African nations where HIV-1 subtype C predominates, harbouring the highest reverse transcriptase-E138X burdens (Supplemental Figure 2, http://links.lww.com/QAD/B122). Reverse transcriptase-E138X frequency in published HIV-1 reverse transcriptase sequences from Zimbabwe, for example, exceeds 15%. Reverse transcriptase-E138X prevalence was also elevated in certain Southeastern European nations dominated by HIV-1 subtype F epidemics where B*18 frequency is also elevated (e.g. Romania [40]; Fig. 3 and Supplemental Figure 2, http://links.lww.com/QAD/B122). The relationship between HLA-B*18 carriage and reverse transcriptase-E138X frequency was also heretofore incompletely uncharacterized for non-B HIV-1 subtypes: but stratification of our 15 cohorts by HIV-1 subtype revealed statistically significant associations between E138X and HLA-B*18 carriage in HIV-1 subtypes B, C, and D, and a marginally significant association in CRF01_AE (Fig. 4). Together, these data indicate that regional reverse transcriptase-E138X prevalence depends on both HIV-1 subtype as well as population HLA-B*18 frequency.

HIV-1 immune escape mutations conferring little or no cost to viral fitness are the most likely to accumulate in circulation, as there is no pressure to revert to consensus following transmission to an individual lacking the restricting HLA allele. To assess the replicative cost of reverse transcriptase-E138X (as a surrogate of its potential to persist upon transmission) we constructed recombinant HIV-1 variants harbouring E138A, E138G, and E138K and assessed their in-vitro replicative competence compared with wild-type HIV-1 (E138). E138X variants exhibited no significant differences in replicative kinetics with respect to wild-type HIV-1 in a 10-day monoculture assay (Fig. 5a) nor in a competition

assay spanning 18 passages of 1 week each (Fig. 5b–d). The lack of in-vitro replicative costs, combined with the significant positive correlation between reverse transcriptase-E138X and HLA-B*18 frequencies in all regions (Figs. 2 and 3) and across most major HIV-1 subtypes (Fig. 4) strongly suggest that, once selected in HLA-B*18-positive individuals, E138X mutations are stably maintained even after transmission to HLA-B*18-negative individuals.

Discussion

Regardless of whether antiretrovirals are used for HIV-1 treatment or prevention, the presence of drug resistance mutations will undermine their efficacy. Reverse transcriptase-E138K is the most commonly identified mutation upon virologic failure of rilpivirine-containing ART [41], conferring two to three-fold decreased susceptibility to this drug when present alone [38]; E138A and E138G confer the same level of rilpivirine resistance as E138K [23]. Using large global datasets of linked HIV/HLA genotypes, we demonstrate that E138X variants (most commonly E138A, E138G, or E138K) naturally occur in persons expressing HLA-B*18 allele in the majority of global regions and in most major HIV-1 group M subtypes and CRFs. Notably, regional reverse transcriptase-E138X frequency correlated positively and significantly with HLA-B*18 frequency in over 40 countries – with subtype C epidemics in sub-Saharan Africa (e.g. Zimbabwe) and subtype F epidemics in Southeastern Europe (e.g. Romania) exhibiting the highest E138X frequencies globally (>15% and nearly 10%, respectively). This observation is unlikely to be explained by confounding by regional rilpivirine use (as use of this drug is negligible in resource-limited settings) or by cross-resistance to other nonnucleoside reverse transcriptase inhibitors (as E138X specifically confers resistance to rilpivirine [23,38]). Instead, our observations, combined with the lack of in-vitro replicative costs of E138X (Fig. 5) are consistent with the B*18-mediated selection and subsequent stable persistence of reverse transcriptase-E138X variants upon transmission, leading them to accumulate in circulating HIV-1 sequences to a degree that mirrors regional HLA-B*18 frequencies [24].

It is also notable that the regions where long-acting PrEP has the potential to make the greatest impact on reducing HIV-1 incidence are also the areas where reverse transcriptase-E138X prevalence is highest. HIV-1 subtype C, by virtue of its dominance in sub-Saharan Africa and other regions bearing a disproportionate HIV-1 infection burden, is the most prevalent subtype globally. It also features the highest natural burden of reverse transcriptase-E138X (6.4% in nearly 10 000 unique treatment-naive sequences examined, with frequencies exceeding 15% in published HIV-1 sequences from Zimbabwe). Long-acting rilpivirine is currently being evaluated as PrEP in some areas including sub-Saharan Africa [12,13,42], but data from this current and previous studies [39] strongly suggest that rilpivirine will fail to protect against infection by the substantial minority of reverse transcriptase-E138X-containing strains circulating in these areas.

Critically, whereas HIV-1 genotypic resistance testing can be used to identify transmitted or de novo selected resistance mutations in HIV-infected persons (thus avoiding the prescription of antiretrovirals for which the autologous virus harbours resistance), no analogous personalized screening can be performed in the context of HIV-1 prevention.

Instead, regional molecular epidemiologic surveillance of circulating HIV-1 variants represents the only means to inform the regional selection of antiretrovirals used as PrEP (as well as to inform the regional selection of first-line antiretroviral regimens in settings where drug resistance genotyping is not routinely available [26]). Given that rilpivirine PrEP will presumably fail to protect against infection by reverse transcriptase-E138X containing strains, and that these strains occur at appreciable frequencies in areas where HIV-1 prevention efforts are most needed, we strongly advocate that regional molecular epidemiologic surveillance of HIV-1 reverse transcriptase sequence variation be undertaken prior to the selection of antiretrovirals used as HIV-1 prevention, and that rilpivirine not be used as single-agent PrEP in areas with elevated E138X frequencies. The combination of cabotegravir and rilpivirine is now also being evaluated for HIV maintenance therapy [43,44]. Given that reverse transcriptase-E138X does not affect cabotegravir susceptibility, and assuming that there are no common naturally occurring HIV integrase polymorphisms that confer decreased susceptibility to cabotegravir, it would be sensible to consider this combination as dual PrEP if safe and well tolerated. Regardless, PrEP combining at least two antiretroviral agents, ideally from different drug classes, would be preferable over single-agent PrEP, particularly in areas with a high burden of transmitted HIV-1 drug resistance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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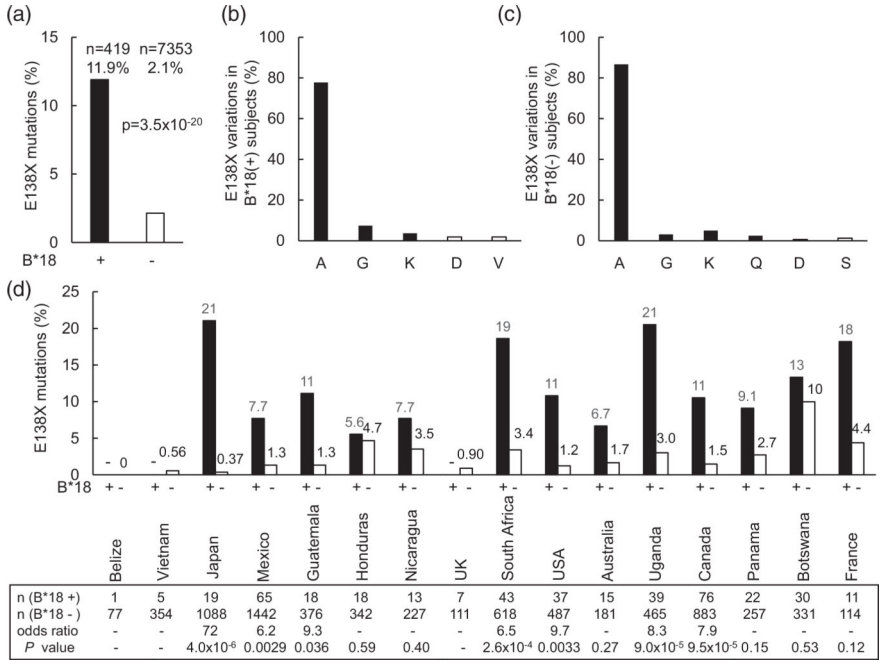

Fig. 1. RT-E138X mutations are significantly enriched in HLA-BM*18-positive individuals globally.

HLA, human leukocyte antigen; RT, reverse transcriptase. (a) Prevalence of RT-E138X mutations in HLA-B*18-positive (black bar) and B*18-negative (white bar) individuals. Ns, percentages of E138X mutations, and the Fisher's exact test *P* value are shown. (b, c) Variations of E138X mutations in $N = 50$ HLA-B*18-positive patients (b) and $N = 157$ HLA-B*18-negative patients (c), shown as percentages. Black bars indicate E138X mutations listed as primary rilpivirine resistance mutations; white bars indicate other E138X mutations. (d) Prevalence of RT-E138X mutations in HLA-B*18-positive (black bars) and -negative (white bars) patients, stratified by cohort. Percentages of E138X mutations, numbers of study participants, odds ratios and *P* values (calculated for cohorts with a minimum of $N = 10$ HLA-B*18-positive patients) are shown. Odds ratios were not calculated for *P* values >0.05. Adapted with permission [38].

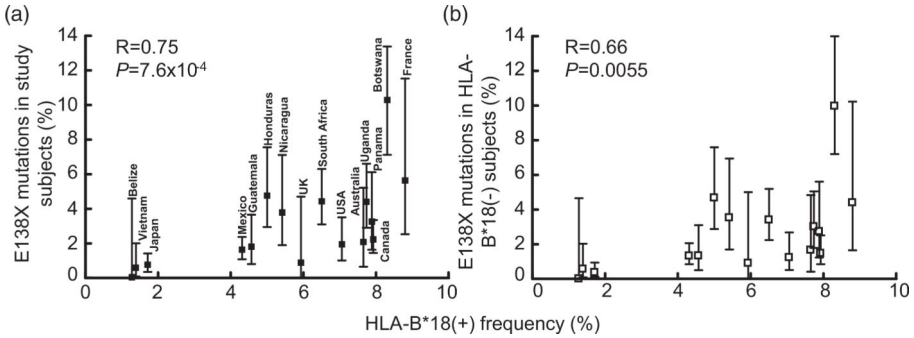

Fig. 2. RT-E138X frequency generally mirrors regional HLA-B*18 frequency.

HLA, human leukocyte antigen; RT, reverse transcriptase. (a) Correlation between RT-E138X prevalence in the overall population and HLA-B*18 frequency in the 16 study cohorts. (b) Correlation between E138X prevalence in HLA-B*18-negative patients and HLA-B*18 frequency in the 16 study cohorts. Error bars represent 95% confidence limits, obtained using a binomial error distribution. Spearman's *R* and *P* values are shown.

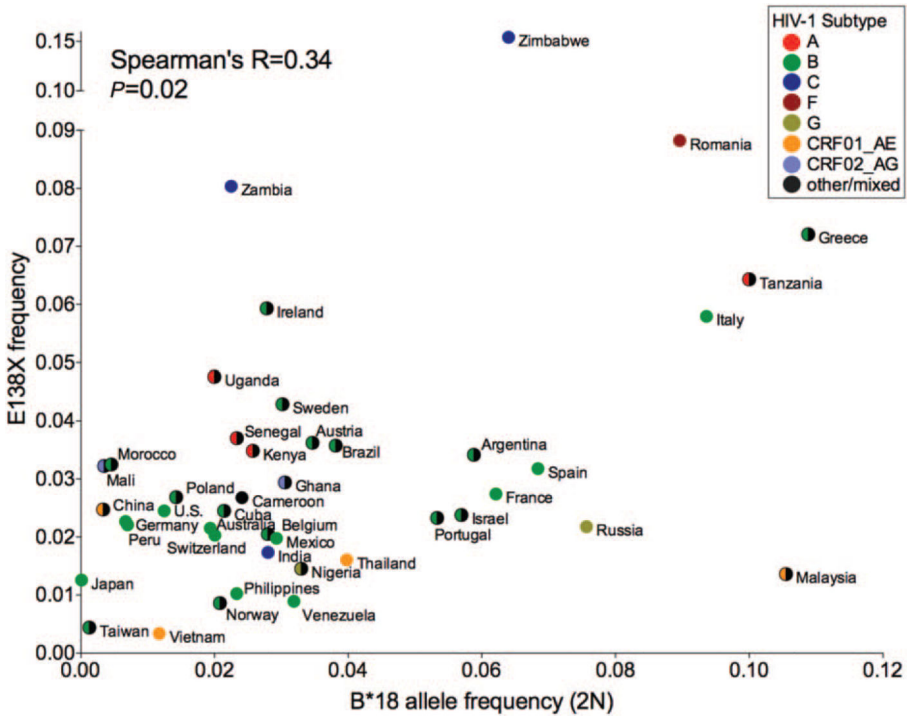

Fig. 3. Correlation between E138X prevalence and HLA-B*18 frequency in 43 countries for which unlinked HIV-1 and HLA frequencies were retrieved from public databases.

CRF, circulating recombinant form; HLA, human leukocyte antigen. Colours denote the predominant HIV-1 subtype in that country (defined as that observed in >75% of sequences in the Los Alamos HIV database; https://www.hiv.lanl.gov/components/sequence/HIV/geo/geo.comp); countries where the predominant subtype was less than 75% are additionally denoted with a black-filled semicircle. Cameroon's complex mixed-subtype epidemic is indicated with a full black circle.

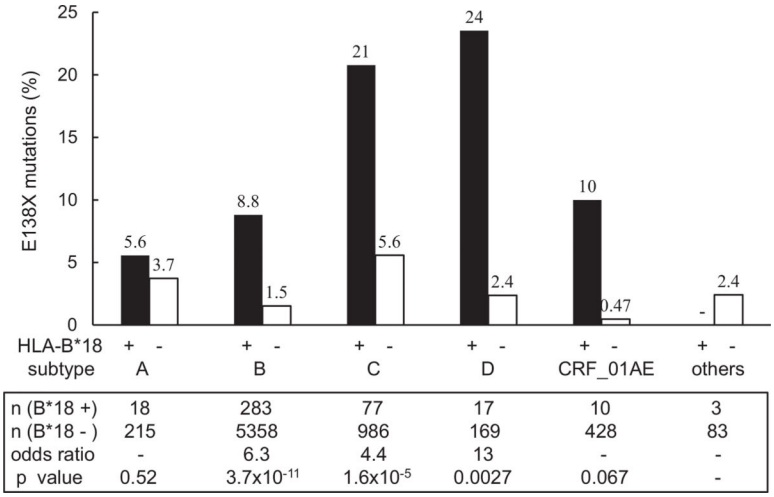

Fig. 4. Prevalence of E138X mutations in HLA-B*18-positive and -negative patients, stratified by HIV-1 subtype.

CRF, circulating recombinant form; HLA, human leukocyte antigen. ‘Other’ HIV-1 subtypes include subtype F, G, and recombinants other than CRF_01AE. Percentages of E138X mutations, numbers of study participants, *P* values (calculated for subtypes with a minimum of $N = 10$ HLA-B*18-positive patients) and odds ratios (calculated for all associations with $P < 0.05$) are indicated.

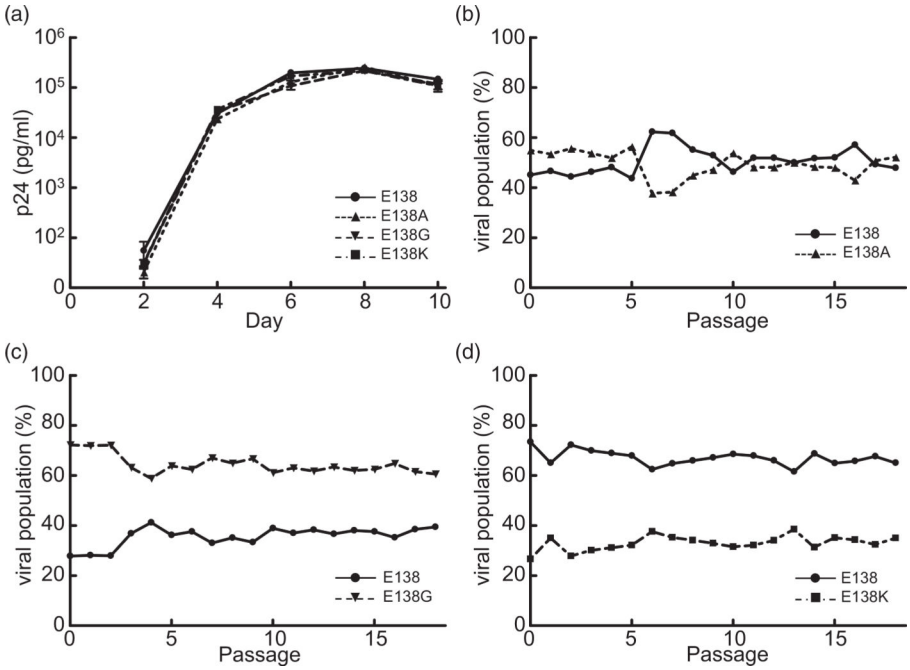

Fig. 5. Effects of E138X mutations on HIV-1 replication fitness.

(a) Replication kinetics of wild-type (E138) and E138X (E138A, E138G, E138K)-containing HIV-1. (b–d) Competitive HIV-1 replication assay.