The effect of primary drug resistance on CD4$^+$ cell decline and the viral load set-point in HIV-positive individuals before the start of antiretroviral therapy

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Objective: To evaluate the effect of primary resistance and selected polymorphic amino-acid substitutions in HIV reverse transcriptase and protease on the CD4$^+$ cell count and viral load set point before the start of antiretroviral treatment.

Design: Prospective cohort study.

Methods: A total of 6180 individuals with a resistance test prior to starting antiretroviral treatment accessing care in HIV clinics across Europe who had at least one viral load and one CD4$^+$ test available were included in the analysis. The impact of amino-acid substitutions variants on viral load and CD4$^+$ trends was investigated using linear mixed models. Clusters of mutations were studied using principal component analysis.

Results: Overall, the detection of any primary resistance was not associated with either the speed of CD4$^+$ cell decline or the viral load set point. However, transmitted nucleoside reverse transcriptase inhibitor and protease inhibitor resistance appeared to be weakly associated with lower viral load set points, as were the polymorphic G16E or Q92K protease mutations. There was some evidence suggesting that these effects varied according to HIV subtype, with the effects of transmitted nucleoside reverse transcriptase inhibitor and protease resistance being particularly marked among individuals with a subtype B virus. A cluster of five polymorphic protease substitutions at position 20, 13, 36, 69 and 89 was associated with less steep CD4$^+$ cell declines and lower viral load set points.

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Conclusion: Although we found little evidence for an association between primary resistance and $CD4^+$ speed of decline and viral load set point, the potential role of polymorphic protease (alone or in clusters) and their interplay with HIV subtype needs to be further evaluated.

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Background

Approximately 10% of individuals newly diagnosed with HIV in Europe carry transmitted drug resistance mutations (TDRM) [1–3], and the prevalence of TDRM appears to be either stable [1,3] or decreasing slightly over time in some countries [2]. TDRM can compromise the response to therapy unless genotypic resistance testing is used to construct a regimen that is fully suppressive [4], hence European guidelines recommend that individuals are tested for transmitted drug resistance (TDR) before the initiation of antiretroviral treatment (ART) [5]. However, TDRM may also affect disease progression before the start of ART.

Most drug resistance mutations (DRM) negatively affect the replicative capacity of HIV in the absence of treatment and therefore tend to revert to wild-type relatively quickly before treatment is started [6]. However, studies have also shown that some TDRM can persist for several years [6–8]. This could either be because the effect of the TDRM on the replicative capacity is very small, as is the case for nonnucleoside reverse transcriptase inhibitor (NNRTI) mutations such as K103N, or because a reversion would cause an initial further reduction of the replicative capacity [9]. Differences in fitness between viruses carrying distinct types of DRM and wild-type strains could also result in differences in virulence, and thus influence the natural history of HIV [10]. DRM that strongly affect fitness have been speculated to result in lower viral load set point and higher $CD4^+$ cell counts, and consequently a slower disease progression [11]. However, it is also possible that the presence of DRM with low fitness costs, or even potential fitness benefits [12], could lead to an increased $CD4^+$ cell decline and a more rapid disease progression [10].

Although all individuals should start treatment as soon as possible after being diagnosed with HIV [5,13,14], any such difference in replicative capacity could influence the outcomes of undiagnosed HIV-infected patients and the transmission dynamics of the HIV epidemic at a population level [15,16]. This could have important implications for mathematical models of the disease and consequently the development of public health policies [16,17]. Accurately determining any such impact of TDRM on disease progression is of growing importance, given the observed rise in TDRM following the roll-out of ART in sub-Saharan Africa [18,19]. Previous research has found that the detection of any TDRM can lead to a more rapid disease progression in the first year after infection [10], but the impact of specific mutations has not been comprehensively evaluated. The aim of this analysis was therefore to investigate the effect of primary drug resistance on virulence as estimated by the viral load set point and the $CD4^+$ cell decline before the start of ART. Our hypothesis was that classes of mutations or individual mutations which cause a reduction in viral fitness would be associated with a lower viral set point as well as a reduced rate of $CD4^+$ cell decline.

Methods

Data source and study population

The European Transmitted Drug Resistance collaboration database was obtained by merging the databases of two European collaborative consortiums on antiretroviral drug resistance (the Virolab Consortium and the EuResist Consortium) with data from the EuroSIDA cohort and three additional centres caring for HIV-positive patients (St. Mary's Hospital, Imperial College London; Royal Free Hospital, NHS Foundation Trust in London and 'Policlinico' hospital, University of Bari). Details of the contributing data sources are shown in Appendix I, http://links.lww.com/QAD/B374.

We included treatment-naive individuals who were aged over 18 at their first visit date and had a resistance test as well as at least one viral load and one $CD4^+$ measurement available before the start of ART.

Classification of drug resistance

Two different systems for drug resistance classification were used in the present analysis: Surveillance DRM (SDRM) from the WHO 2009 list [20], and a wider list of treatment-associated DRM, including all substitutions listed as changes conferring resistance in at least one of the four main resistance classification systems: National Agency for AIDS Research, International AIDS Society, Stanford HIVdb and Rega. Because minor compensatory mutations, particularly in the protease gene, are also likely to influence the fitness of a given strain [6,15] we also selected nonpolymorphic protease mutations associated

with protease inhibitor (PI) exposure, but not necessarily with drug resistance, from the Stanford HIVdb (Appendix II, http://links.lww.com/QAD/B374). This selection process resulted in a full list of 129 reverse transcriptase and 147 protease substitutions. Out of this complete list, we evaluated 41 substitutions which met a prespecified prevalence threshold in our dataset (1%) for their effect on CD4$^+$ cell counts and the viral load (Appendix II, http://links.lww.com/QAD/B374).

CD4$^+$ cell decline and viral load levels among individuals with at least one DRM, at least one class-specific DRM and individual mutations were compared with that among individuals with no resistance, defined as no NRTI, NNRTI or major PI mutations included in the complete list. Drug resistance was assumed to be present throughout the duration of the follow-up and the results of multiple tests considered cumulatively.

We also studied the impact of mutational patterns by conducting a principal component analysis (PCA) to identify clusters of mutations in the reverse transcriptase and protease gene (Appendix III, http://links.lww.com/QAD/B374). After identifying the clusters, each individual in the dataset was assigned a score indicating how closely their mutational pattern matched that described by each cluster. For simplicity, the scores were dichotomized using the 3rd quartile (Q3) as a cut-off point. This allowed us to categorize individuals into those whose mutation pattern was similar to that described by a given cluster (above Q3) and those whose mutation pattern was not (below Q3).

Statistical methods

We used linear mixed models with a random intercept and slope to estimate the effect of resistance on the CD4$^+$ cell count decline and on the viral load set point. CD4$^+$ cell decline according to the detection of resistance was estimated by including an interaction term between time and an indicator variable for the resistance exposure in a mixed model using CD4$^+$ cell counts as the outcome. The effect of resistance on viral load was estimated by considering the effect of resistance on the intercept from a mixed model using viral load as the outcome. The rationale for using only the intercept for the viral load outcome is due to the relative stability of viral load over the course of the natural history of HIV [21]. Potential confounders (HIV risk group, viral subtype, calendar year of genotyping and cohort) were included on the basis of clinical judgement, previous publications, and the data available in the cohorts (Model 1). We additionally present results adjusting for viral load (CD4$^+$ outcome model) and CD4$^+$ cell counts (viral load outcome model) (Model 2). We corrected for multiple testing using the Benjamini–Hochberg method for controlling the false discovery rate only for the analyses of individual mutations, as these were selected on the basis of their prevalence and not a-priori reasoning. As the described mutations lists have been generated for HIV subtype B, the analyses were repeated stratified according to subtype.

Results

Characteristics of the study population

A total of 6180 individuals were included in the analysis. The majority of individuals were male (77%), most had acquired their HIV through sex with another man (46%) and 64% were infected with a subtype B virus. Individuals contributed a median of five CD4$^+$ measurements [interquartile range (IQR) = 3–9] and four viral load measurements (IQR = 2–8) over a median of 1.4 (IQR = 0.1–3.8) years. The median time between the date of the resistance test and the first CD4$^+$/viral load measurement was 0 (IQR = −5; 0) months. The baseline median CD4$^+$ cell count was 420 (IQR = 289–583) cells/μl, and the baseline median viral load was relatively high at 4.5 $\log_{10}$ copies/ml (IQR = 3.9–5.0). The mean viral load set point as estimated from univariable mixed models was 4.4 $\log_{10}$ copies/ml [95% confidence interval (CI) = 4.34–4.4], and CD4$^+$ cell counts declined with an estimated 54 (95% CI = 56–52) cells/μl/year.

Resistance prevalence

The prevalence of SDRM was 10%. NRTI resistance was most commonly detected, at 7.1%, followed by NNRTI (3.2%) and PI (2.6%) resistance. Using the wider DRM list, resistance prevalence was 54%, with PI resistance most common (31.3%) followed by NNRTI (25.3%) and NRTI resistance (11.1%). The combined prevalence of major and minor compensatory protease mutations was very high, at 95.2%. The most common mutations were protease mutations, with L63P present in 40.8% of individuals. The most common reverse transcriptase mutation was V179I (6.5%).

Clusters of mutations

The PCA identified two reverse transcriptase and two protease clusters (Appendix III, http://links.lww.com/QAD/B374). We found that the first reverse transcriptase cluster contained a large number of reverse transcriptase mutations that conferred both NRTI and NNRTI resistance: the 151M complex (substitutions in position 151, 115, 116, 75, 77 and 62) together with substitutions in position 74 and 65 as well as substitutions in position 100, 188, 179 and 230 (Appendix III, http://links.lww.com/QAD/B374). The second reverse transcriptase cluster contained the thymidine analogue mutations, and included substitutions in position 41, 67, 219, 215, 210 and 70 as well as a polymorphic substitution in position 44, the 184 substitution and a substitution in position 181 (Appendix III, http://links.lww.com/QAD/B374).

The first protease cluster contained a number of major PI resistance-associated substitutions (position 46, 82, 47,

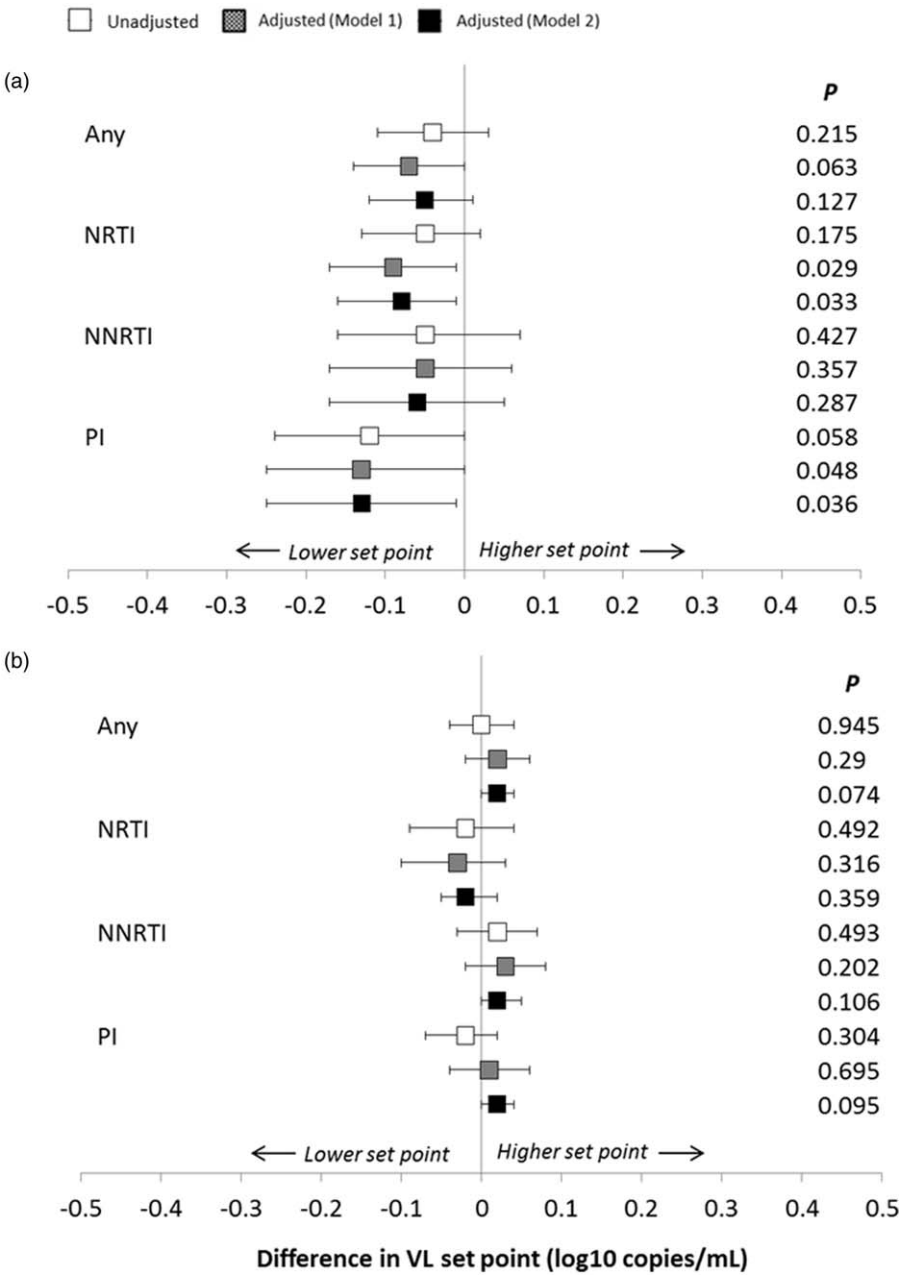

Fig. 1. The effect of any and class of surveillance drug resistance mutations (a) and drug resistance mutations (b) on estimated viral load set point.

30, 32, 84, 48, 90, 50, 54 and 88) as well as a few minor PI mutations in position 73, 53 and 24. The second PI cluster contained five minor/polymorphic protease substitutions in position 20, 13, 36, 69 and 89 (Appendix III, http://links.lww.com/QAD/B374).

Effect of surveillance drug resistance mutations and drug resistance mutations on the viral load set point

Associations between the detection of SDRM, DRM and the viral load set point can be seen in Fig. 1a,b. The estimated viral load set point did not seem to vary according to the detection of SDRM after adjustment for the prespecified confounders (difference = −0.05 $\log_{10}$ copies/ml, $P = 0.13$). There was some weak evidence that the set point was slightly lower among individuals with NRTI and PI SDRM compared with those with no resistance ($P = 0.03$ and 0.04, respectively), but the magnitude of the difference was relatively small (difference = −0.08 $\log_{10}$ copies/ml, 95% CI = −0.16; −0.01 and −0.13 $\log_{10}$ copies/ml, 95% CI = −0.25; −0.01, respectively).

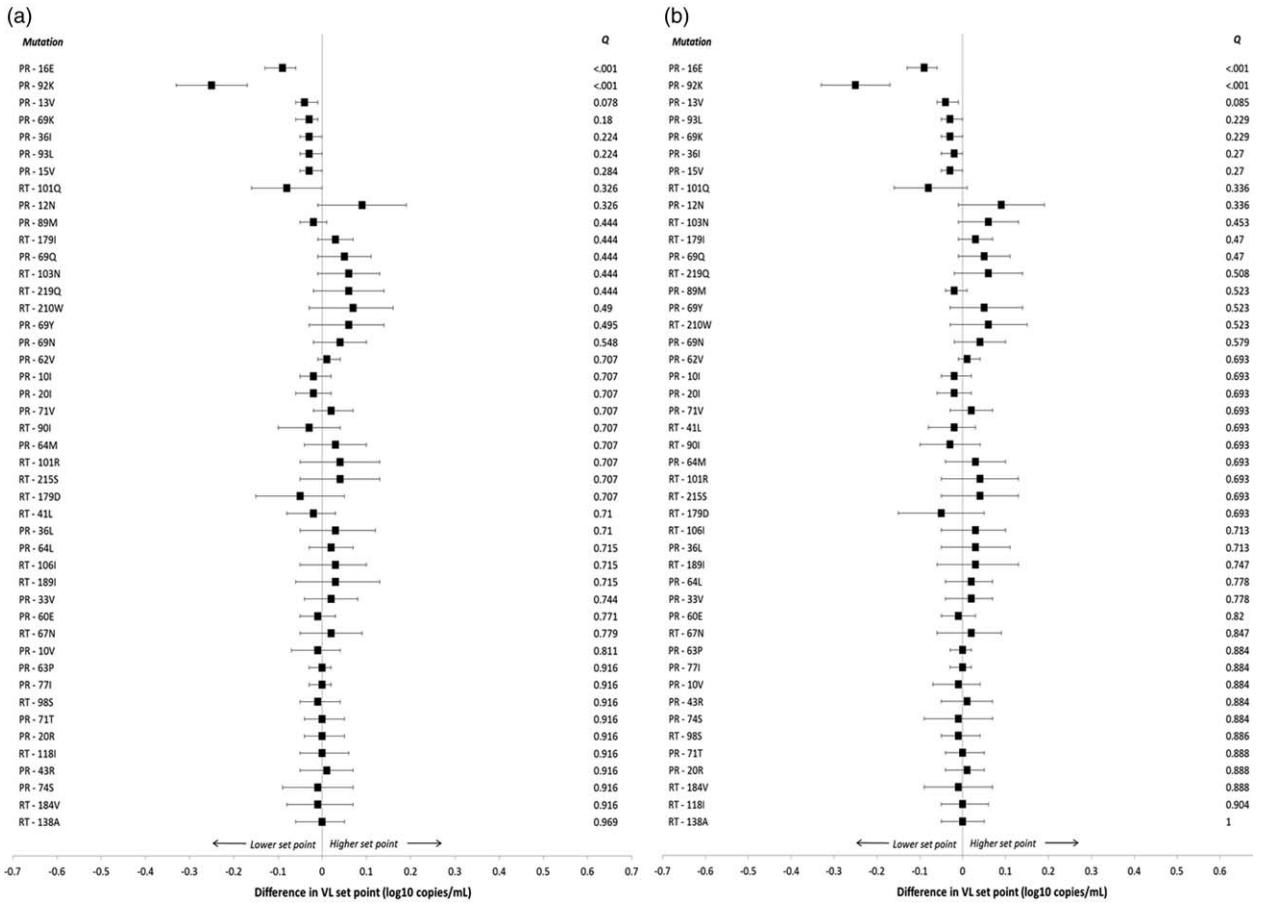

Fig. 2. Adjusted difference in the viral load set point according to the presence of specific mutations adjusted for prespecified confounders (a) and CD4$^+$ cell counts (b).

The effect of individual mutations and clusters of mutations on the viral load set point

After adjustment for all prespecified confounders, there was evidence for a lower viral load set points among individuals who carried the G16E or Q92K mutations in the protease (both $q < 0.001$, Fig. 2a,b). There was reasonable evidence suggesting that individuals whose mutation pattern aligned closely with that described by the second protease cluster, containing minor protease mutations, had lower viral load set points both in univariable and multivariable analyses (adjusted $P = 0.004$; Fig. 3a).

Effect of surveillance drug resistance mutations and drug resistance mutations on CD4$^+$ cell decline

CD4$^+$ cells decline was estimated to be 53 cells/μl per year (95% CI = −56; −49) among individuals infected with viruses without SDRM and 55 (95% CI = −63; −48) cells/μl per year among those infected with a virus carrying at least one SDRM (P value for difference = 0.47). These estimates did not change markedly upon covariate adjustments (Fig. 4a,b). There was also no evidence that the detection of SDRM of any class was associated with reduced or increased CD4$^+$ cell declines (Fig. 4a). The findings were similar when considering DRM (Fig. 4b), although there was a slightly stronger evidence suggesting that the detection of NNRTI DRM was associated with steeper CD4$^+$ cell decline when compared with people with no resistance (difference = −6 cells/μl per year, 95% CI = −12;0) $P = 0.04$ after adjustment, Fig. 4b).

Effect of individual mutations and clusters of mutations on CD4$^+$ cell decline

No individual mutation was associated with the CD4$^+$ slope (Fig. 5a,b). The strongest signals were found for the A71T, L10V in protease region and K101Q in the reverse transcriptase, which were all associated with a steeper CD4$^+$ cell decline albeit not significantly after correcting for multiple testing. The first reverse transcriptase and protease clusters did not seem to have any marked effect on CD4$^+$ cell decline ($P = 0.37$ and 0.17, respectively, Fig. 5a,b). In contrast, the second protease cluster was strongly associated with less steep CD4$^+$ cell decline ($P < 0.001$, Fig. 3b). CD4$^+$ cell counts declined of nine cells (95% CI = 4−15) less per year among individuals whose mutation pattern was similar to that described by

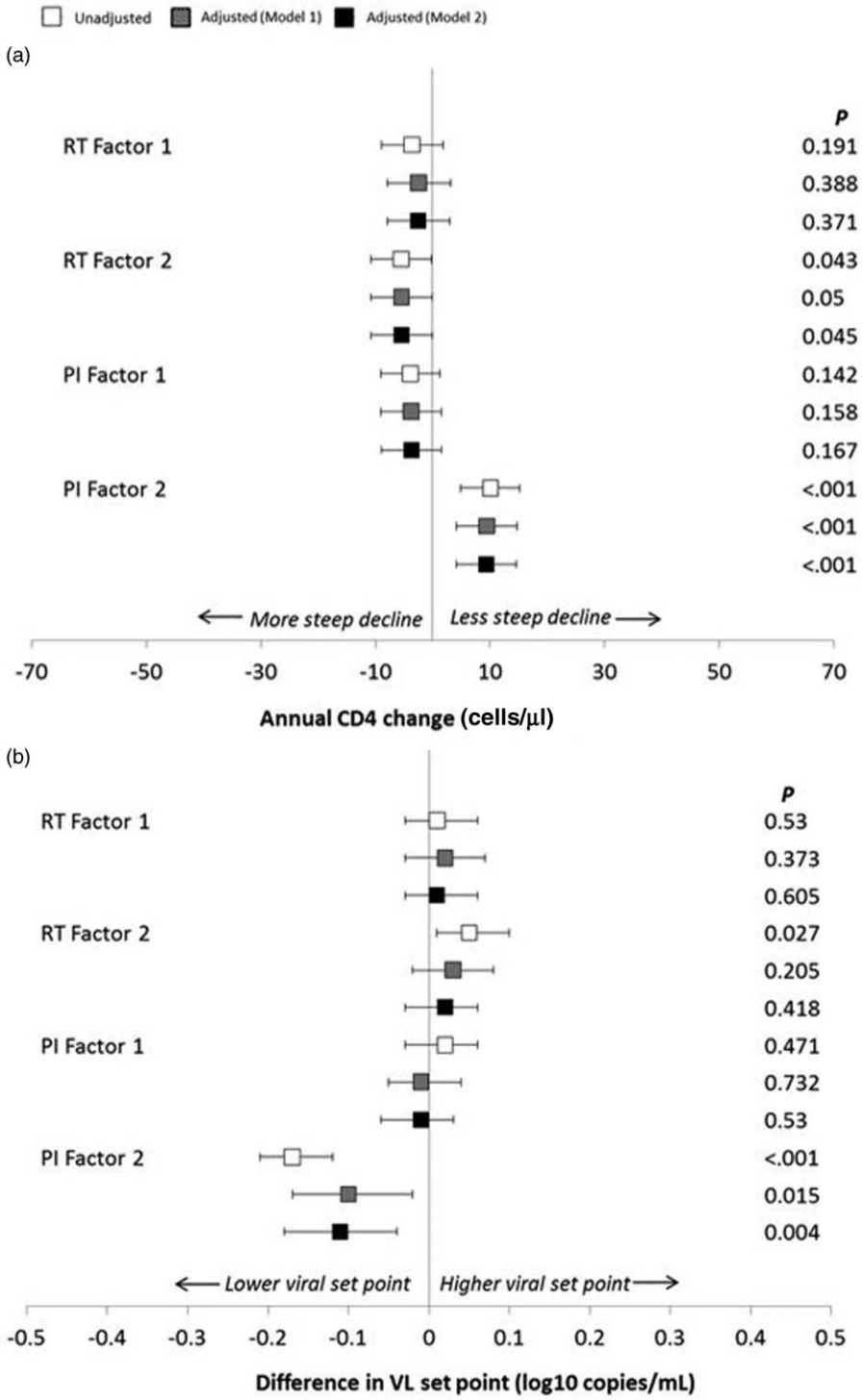

Fig. 3. The effect of clusters of mutations on $CD4^+$ cell decline (a) and the estimated viral load set point (b).

this cluster. The second reverse transcriptase cluster was marginally associated with a slightly steeper $CD4^+$ cell decline ($P = 0.05$).

Stratified analyses according to viral subtype

There were some notable differences in the stratified analyses. First of all, the effect of any PI SDRM on $CD4^+$ cell was more marked among individuals infected with a subtype B virus (adjusted difference = +15 cells/μl per years, 95% CI = 0–30, $P = 0.05$), as was the effect of NNRTI DRM (adjusted difference = −10 cells/μl per year, 95% CI = −17; −3, $P = 0.005$). In contrast, NNRTI DRM was not associated with $CD4^+$ cell decline among individuals infected with a non-B virus

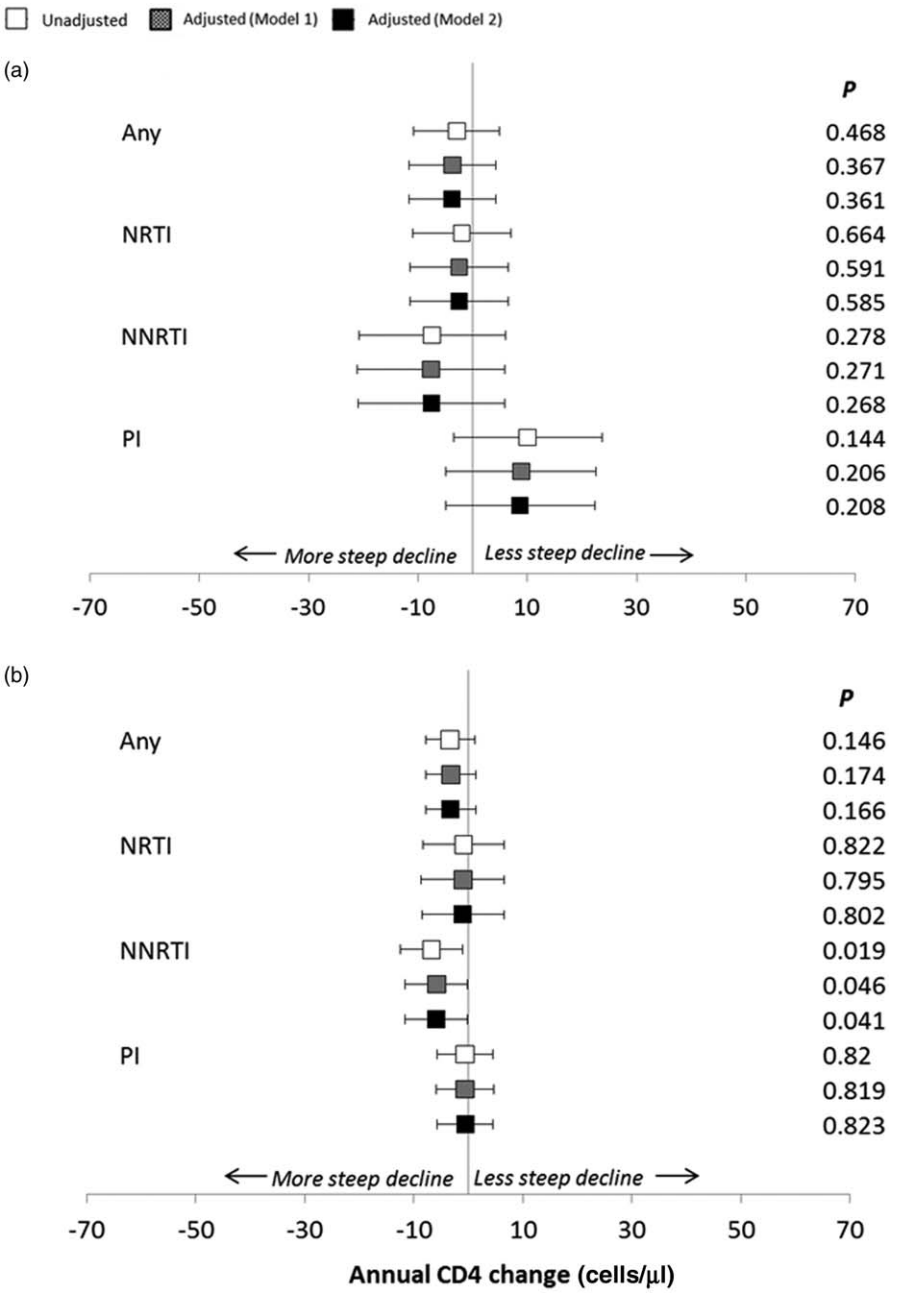

Fig. 4. The effect of any and class of surveillance drug resistance mutations (a) and drug resistance mutations (b) on CD4$^+$ cell decline.

(−2.17, 95% CI = −15.94; 11.60, $P = 0.757$). There was reasonable evidence that these effects varied significantly according to subtype (P interaction = 0.02 for both). The effect of both the second reverse transcriptase and protease cluster on CD4$^+$ cell decline grew more extreme with wider CIs among individuals with subtype B viruses, but interaction tests indicated that only the effect of the second protease cluster was likely to vary significantly according to subtype ($P = 0.007$).

In terms of individual DRM, the evidence supporting an effect of the A71T and K101Q mutations on CD4$^+$ cell decline grew slightly stronger when restricting the analyses to individuals infected with a subtype B virus (both adjusted $P = 0.06$), although interaction tests were only marginally significant ($P = 0.07$ and 0.08, respectively). The point estimates for both the G16E and Q92K substitutions also moved towards zero when restricting to this patient population, but grew more extreme among individuals infected with non-B viruses. Tests for interaction indicated that the effect of at least the G16E mutation differed significantly according to subtype ($P = 0.001$ for G16E and $P = 0.11$ for Q92K).

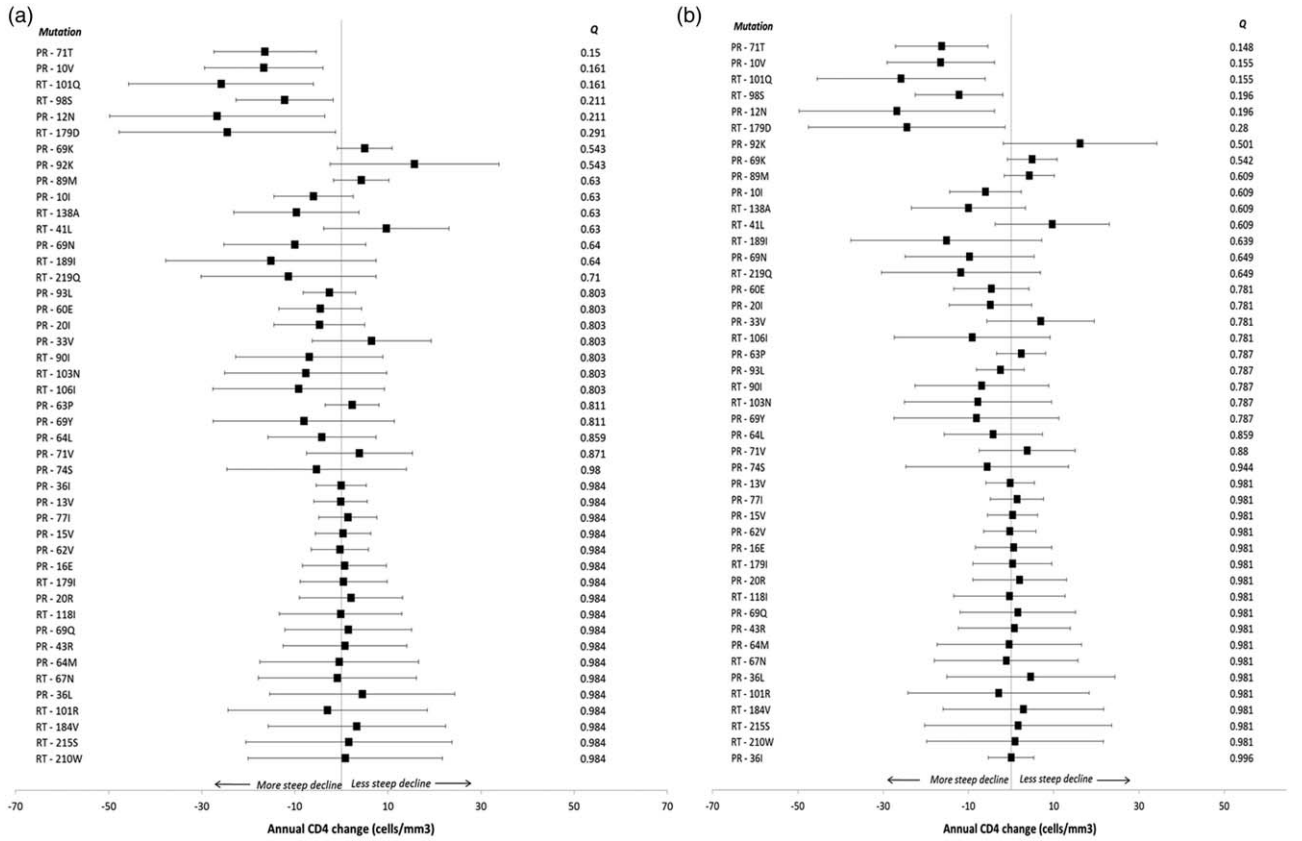

Fig. 5. Adjusted differences in CD4$^+$ cell decline according to the presence of specific mutations adjusted for prespecified confounders (a) and viral load (b).

Discussion

To our knowledge, this is the largest analysis to date describing the influence of primary HIV drug resistance on the viral load set point and CD4$^+$ cell decline before the start of ART. Overall, the detection of any SDRM or DRM was not associated with either end-point, although a number of other genetic changes in the HIV genome appeared to have a small but significant effect on both the viral load set point and CD4$^+$ cell decline.

Viral load set point

We found weak evidence that the detection of transmitted NRTI and PI resistance was associated with lower viral load set points, but the overall differences were small – around 0.1 $\log_{10}$ copies/ml. It is unclear whether a difference of this size would impact on either the transmission dynamics of HIV at population level or clinical progression. There were slightly larger differences between individuals who had the G16E and Q92K mutations, both of which were associated with lower viral load set points at least among individuals infected with non-B viruses. We also found reasonably robust evidence that a cluster of minor protease mutations, involving positions 13, 20, 36, 69 and 89, had a small but significant protective effect on both the CD4$^+$ cell decline and viral load set point, particularly among patients infected with a subtype B virus. A previous study found that the 20I substitution significantly correlates with lower viral load during primary HIV infection [22]. On the other hand, substitutions at position 36 are very common in non-B subtypes, and viruses carrying this substitution have a higher replicative capacity than subtype B wild-type viruses [23,24]. In other subtypes, such as G or CRF02-AG, substitutions at position 36 tend to appear together with substitutions at position 20, and it has been suggested that the combination of these mutations may present a selective advantage to the virus [24]. In this respect, our findings are somewhat counterintuitive as, although the cluster contained other minor protease mutations, we would expect any strain with higher replicative capacity to cause faster CD4$^+$ cell count decline and a higher viral load set point. It is possible that the effect of the 20 and 36 mutations differ depending on the detection of other substitutions that formed part of this cluster.

A number of authors have described the relationship between TDRM detection and viral load values [15,25–28]. Harrison *et al.* [25] did not find any evidence that resistance to a single drug class was associated with viral

load, although the M184V mutation appeared to be associated with a lower baseline viral load. We did not find any evidence of this. However, resistance mutations that markedly impair HIV replicative capacity, such as M184V, are likely to wane over time due to reversion to wild-type and the overgrowth by more fit variants [29]. The fact that we estimated the viral load set point using more than a single viral load value could also explain the apparent discrepancy between our findings and those of Harrison *et al.*

CD4$^+$ cell count decline

Despite the large sample size, we were not able to find any definitive evidence that SDRM/DRM influenced CD4$^+$ cell decline. Nonetheless, patients infected by viruses harbouring A71T and L10V substitutions in the protease region had steeper CD4$^+$ cell declines, though these associations were not statistically significant after applying a correction for multiple testing. Significantly, previous in-vitro studies have linked changes in positions 10 and 71 with recovery of viral fitness during treatment with protease inhibitors [30–32]. In addition, an in-vivo study suggested that mutations L10I/V and A71V/T do not reduce the relative fitness of the virus once treatment is stopped [33]. A possible interpretation of these findings is that certain compensatory mutations occurring outside of the active site of the enzyme may act to stabilize the structure of the protease. Unlike major resistance mutations, these changes do not seem to impair the replicative capacity of the virus in absence of drug selective pressure and could even confer an increased fitness compared with wild-type viruses, thus possibly explaining their association with a steeper CD4$^+$ reduction in our cohort. Theys *et al.* [15] have previously found a number of polymorphic mutations, including A71T and L10V, in the protease gene to be associated with a higher viral load, lower CD4$^+$ cell count and higher estimated fitness from a fitness landscape. Although this is intriguing, neither A71T nor L10V were associated with a higher viral set point in this analysis, as would be expected if they were associated with a markedly higher replicative capacity. It is also important to note that after correction for multiple testing we could not rule out that the effects found were due to chance.

Other authors investigating the relationship between resistance and disease progression before the start of ART have tended to study associations between any TDRM on CD4$^+$ cell counts at a single point in time, and results have been conflicting [10,15,26–28,34–36]. Among those describing longitudinal CD4$^+$ cell count changes, one of the largest studies was conducted by Pillay *et al.* [10] They found evidence that CD4$^+$ cell counts declined faster among patients with TDRM, but only during the first year of infection. On the contrary, date of infection was not available in our dataset, and it is possible that the use of a dataset where persons could enter at any stage of infection masked any potential time-dependent effect that TDRM might have on CD4$^+$ cell decline.

Strengths of our analysis include the large sample size, the comprehensive evaluation of different types of resistance, individual mutations and clusters of mutations and finally the longitudinal nature of the data. However, there are also a number of limitations. The main weakness is the lack of an available date of infection. Second, most individuals did not have repeated resistance tests, meaning that we had to make assumptions regarding how long mutations persisted for. For simplicity, we assumed that resistance was present throughout follow-up. However, as median follow-up in this study was just over 1 year and TDRM can persist for several years [6], this does not seem to be an unreasonable assumption. We also selected mutations for testing based on an arbitrary prevalence threshold. Although a comprehensive Genome-wide Association Study or the estimation of human leukocyte antigen-types or cytotoxic T-lymphocyte-escape mutations from genotypic data would also be of interest, such an analysis was not possible utilising our data source due to the lack of full sequencing data from some contributing cohorts. It should also be noted that some misclassification of subtype in the dataset is possible. Very few subtype B strains (3.6%) were classed as belonging to protease cluster 2, and it could be that this cluster is a marker for subtype. Finally, we were unable to investigate the effect of mutations in the integrase gene, as no data from this region was available. Future studies investigating the effect of transmitted integrase mutations would be of great scientific interest, although current evidence indicates that the prevalence of such mutations is likely to be low.

Conclusion

Bearing these limitations in mind, our results suggests that the detection of TDR or a larger set of treatment-associated genetic changes in the reverse transcriptase or protease gene of HIV is unlikely to have a large effect on virulence or disease progression as indicated by the viral load set point and CD4$^+$ cell count decline before the start of ART. Although it is reassuring that our analyses did not find evidence of faster disease progression or more virulent disease among individuals infected with resistant HIV, limitations of the data prevent us from ruling out such an impact. Future studies should combine epidemiological analyses with basic science to provide a better understanding of the population-level impact of both resistance-associated and polymorphic changes in the HIV genome and their possible interplay with HIV subtype on disease progression.

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Ethics approval

Ethics approval was granted from local ethics committees for each of the participating clinics.

Conflicts of interest

A.S. is currently a salaried employee at Evidera, a research consultancy providing services, not related to the current work, to the pharmaceutical industry. The other authors declare no conflicts of interest.

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