Extent and Impact of APOBEC3 Editing on HIV-1 Proviral DNA Drug Resistance

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570

BACKGROUND

- APOBEC3-mediated G-to-A hypermutation in HIV-1 proviral DNA complicates drug-resistance interpretation.
- Available tools can detect hypermutations but cannot distinguish mutations driven by drug pressure from those caused by APOBEC3-mediated editing.
- Optimal interpretation and use of hypermutated proviral sequences for drug resistance remain an open question.

OBJECTIVE

- Assess the potential utility of hypermutated proviral HIV sequences for clinical HIV drug resistance interpretation.

METHODS

- We generated near-full-length (nFL) proviral genomes from suppressed persons in care in Rhode Island.
- 18 drug resistance mutations (DRMs) susceptible to APOBEC3 editing[1] were identified by Stanford tools across protease (PR), reverse transcriptase (RT), and integrase (INT).
- Among these, mutations were classified as APOBEC3-associated or not based on codon encoding (e.g., for M184I, ATA reflects a G-to-A mutation from ATG, whereas ATT or ATC do not), guiding inclusion/exclusion of DRMs.
- Hypermutation rate, defined as the proportion of APOBEC3-mediated G-to-A mutations per sequence length, was identified with Hypermut v3 in nFL genomes and individual genes.
- Proportions of hypermutated sequences within susceptible and associated DRMs were computed to assess number of DRMs that could still be included in resistance interpretation.

Figure 1. Proposed algorithm for integrating proviral DNA sequences into routine HIV-1 drug resistance testing

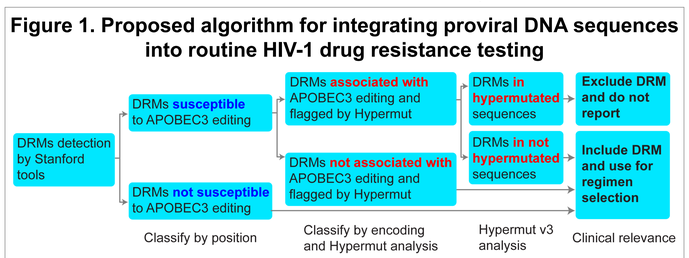
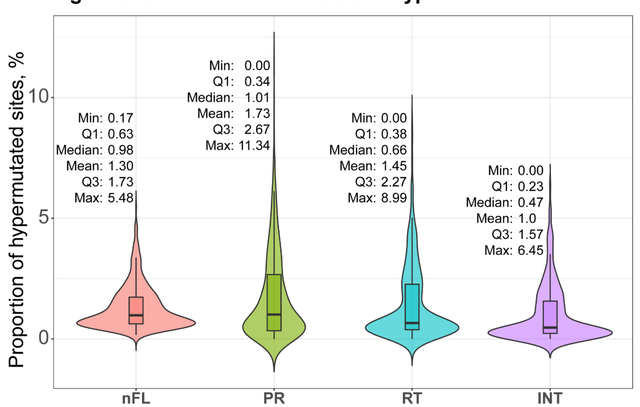


Figure 2. Distribution of APOBEC3 hypermutation rates



Evaluating susceptibility to and association with APOBEC3-mediated editing of gene-specific drug resistance mutations can guide integration of hypermutated proviral sequences into routine HIV drug resistance testing.

RESULTS

- Proviral nFL sequences were obtained from 487 participants, predominantly male (81%), median 34 years, 58% men who have sex with men, 94% with subtype B, all diagnosed between 1983 and 2022.
- At least one DRM was detected in 64% (312/487) of persons.
- Table 1 summarizes DRMs by susceptibility to APOBEC3 editing, and Table 2 further stratifies susceptible DRMs by association with APOBEC3-mediated editing.
- Hypermut v3[2] identified 43% of nFL genomes, 20% of PR, 34% of RT, and 27% of INT sequences as hypermutated based on a Fisher exact test p-value <0.05.
- At least one hypermutated site was detected in all nFL genomes, 91% PR, 100% RT, and 93% INT.
- The APOBEC3 hypermutation rates distribution in proviral DNA sequences was right-skewed (Figure 2).
- Figure 3 shows the distribution of DRMs that were susceptible to and associated with APOBEC3-mediated editing, as well as those observed in hypermutated sequences, stratified by antiretroviral class.
- Overall, 50% of persons had DRMs not susceptible to APOBEC3-mediated editing, while the remainder 50% had APOBEC3-susceptibile mutations; of these, 5% were not associated with APOBEC3 editing and may therefore be informative for drug resistance interpretation (Figure 1).
- All DRMs susceptible to and associated with APOBEC3 editing were flagged by Hypermut v3.

Figure 3. Proportions of DRMs my APOBEC3 Susceptibility, Association and Hypermutation Across Drug Classes

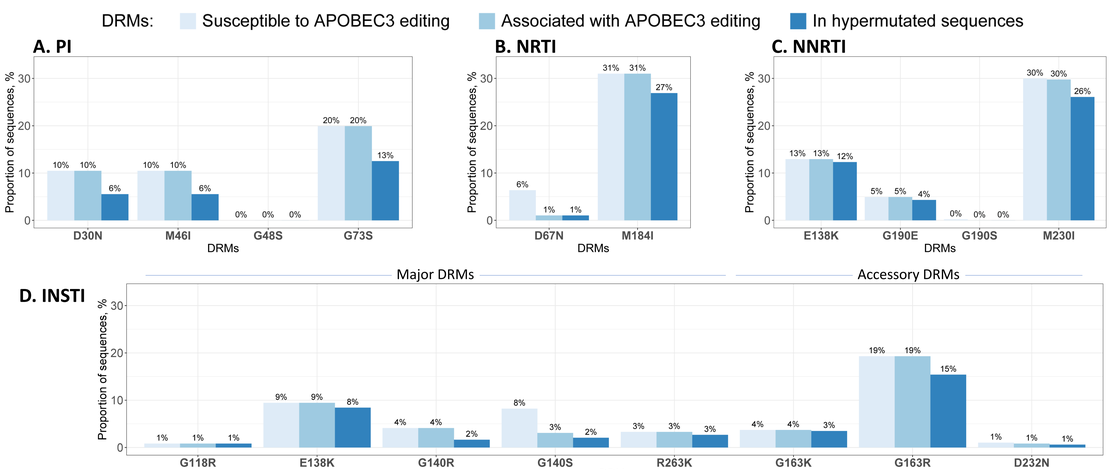


Table 1
Proviral Sequences With DRMs by Susceptibility to APOBEC3-Mediated Editing

Variables	Proviral sequences, n (%)	
	With susceptible DRMs	With not susceptible DRMs
Any DRM	207 (43%)	245 (50%)
PI Major	77 (16%)	12 (2%)
PI Accessory	97 (20%)	11 (2%)
NRTI	168 (34%)	141 (29%)
NNRTI	173 (36%)	145 (30%)
INSTI Major	101 (21%)	5 (1%)
INSTI Accessory	113 (23%)	75 (15%)

Table 2
Susceptible DRMs by Association to APOBEC3-Mediated Editing

Variables	Proviral sequences, n (%)	
	With associated* DRMs	With not associated* DRMs
Any DRM	197 (95%)	10 (5%)
PI Major	77 (100%)	0 (0%)
PI Accessory	97 (100%)	0 (0%)
NRTI	151 (90%)	17 (10%)
NNRTI	172 (99%)	1 (1%)
INSTI Major	85 (84%)	16 (16%)
INSTI Accessory	113 (100%)	0 (0%)

Note: * associated or not associated with APOBEC3-mediated editing.

CONCLUSIONS

- We characterized proviral HIV-1 sequences from a large Rhode Island cohort according to their susceptibility to and association with APOBEC3-mediated hypermutation.
- This approach may enable inclusion of proviral DNA sequences with DRMs that are not susceptible to or not associated with APOBEC3-mediated editing in resistance interpretation – sequences that might otherwise be excluded - and may facilitate integration of proviral sequences into routine HIV-1 drug resistance testing.
- Further validation of this approach in clinical care is needed.

References

1. Wensing et al. Utilizing HIV Proviral DNA to Assess for the Presence of HIV Drug Resistance. Clin Infect Dis. Apr 3 2025.
2. Lapp et al.. Hypermut 3: identifying specific mutational patterns in a defined nucleotide context that allows multistate characters. Bioinform Adv. 2025;5(1):vbaf025.

For questions and clarifications, please contact Vlad Novitsky at vnovitsky@brownhealth.org

Acknowledgements

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