AIDS 2026, **40**:530–538

OPEN

HIV-1 subtype diversity trends in a Northern California cohort

Kaiming Tao[a], Philip L. Tzou[a], Malaya K. Sahoo[c], Charles B. Hare[b], Benjamin A. Pinsky[a,c] and Robert W. Shafer[a]

Between 2000 and 2019, the Stanford Healthcare Clinical Virology Laboratory performed HIV-1 resistance testing for 9199 individuals in Northern California. Nonsubtype B viruses were identified in 3.5%, most often CRF01_AE (1.0%), subtype C (1.0%), CRF02_AG (0.46%), and subtype A (0.45%). Non-B viruses, particularly CRF01_AE and CRF02_AG, increased significantly over time. Although subtype B remained dominant, the rising presence of non-B subtypes reflects growing viral diversity within the U.S. epidemic.

HIV type 1 (HIV-1) subtype B was introduced into the United States from the Caribbean approximately 1970 [1]. The proportion of viruses belonging to non-B subtypes have increased in the United States but only gradually, with prevalence rates remaining below 10% [2,3]. In Europe, higher prevalences of nonsubtype B viruses have been reported with prevalences reaching up to 15–30% in newly diagnosed persons with HIV-1 (PWH) [4,5]. Although HIV-1 subtype B remains predominant in the United States, nonsubtype B infections are increasingly observed. These non-B infections, though still uncommon, they indicate the need for ongoing surveillance for mapping regional HIV-1 transmission patterns. Drawing from 20 years of sequence data in Northern California, we describe the modest but consistent increases in non-B subtypes in a region that was one of the early focal points of the U.S. HIV epidemic.

The Stanford Healthcare Clinical Virology Laboratory performed HIV-1 genotypic resistance testing for routine clinical care for a large Northern California clinic population (Kaiser Permanente Northern California, KPNC) from 1997 to 2019. During this period, nearly all sequences from KPNC clinics were analyzed in our laboratory. After 2019, testing volumes declined sharply when clinicians were able to order tests from multiple other laboratories. We previously reported that between July 1997 and June 2000, 99.4% of 2246 PWH undergoing genotypic resistance testing at Stanford had subtype B viruses [6]. In a subsequent study of 4253 ART-naive individuals from KPNC between 2003 and 2016, 95.3% of PWH had subtype B viruses [7]. To evaluate subsequent trends and to include previously treated as well as ART-naive PWH, we analyzed the prevalence of HIV-1 subtypes in the entire KPNC population from 2000 through 2019. Approval for this study, including a waiver of informed consent, was obtained from the Stanford University and Kaiser Permanente Northern California institutional review boards.

Between 2000 and 2019, a total of 9199 individuals had 13 214 samples submitted for genotypic resistance testing. Overall, 75.4% of individuals had a single sample, 14.7% had two samples, 5.6% had three samples, and 4.3% had four or more samples. The median number of individuals tested per year was 446 (IQR: 434–469; range: 374–730), calculated by assigning each individual to the first year a sample was obtained. Overall, a downward trend was observed, with the three highest annual counts (730, 565, and 486) occurring between 2000 and 2002, and two of the lowest counts (374 and 388) occurring in 2018 and 2019.

The median age at the time of the first sample was 42 years (IQR: 33–50). About 85.8% were men, 9.6% were women, and for 4.6% the sex was not available. Reported race/ethnicity was 46.4% White, 20.7% Black, 18.4% Hispanic, 6.9% Asian, 0.4% Native American, and 7.1% unspecified.

Of the 13 214 samples, 93.9% (12 414) included the protease and reverse transcriptase region; 5.1% (671) included protease, reverse transcriptase, and integrase; and 1.0% (129) included integrase alone. At the time of sequencing, 45.0% of individuals were ART-naive, 32.0% were ART-experienced, and 6.3% had samples obtained both before and after ART initiation. Treatment history was unavailable for 16.7% of individuals undergoing genotypic resistance testing.

Among the 9199 individuals sequenced, 96.5% had subtype B viruses according to the COMET subtyping tool [8]. Non-B subtypes included CRF01_AE (1.0%), subtype C (1.0%), CRF02_AG (0.46%), subtype A (0.45%), subtype D (0.17%), subtype G (0.09%), subtype F1 (0.09%), CRF07_BC (0.07%), as well as seven other circulating recombinant forms, four unique recombinant forms, and subtype F2 (0.17% combined) (Fig. 1). Two cases of superinfection with CRF01_AE were identified in individuals who previously had subtype B viruses. In general, linear logistic regression models with year as a continuous predictor, the proportion of non-B viruses increased significantly over time ($P < 0.000001$), as did

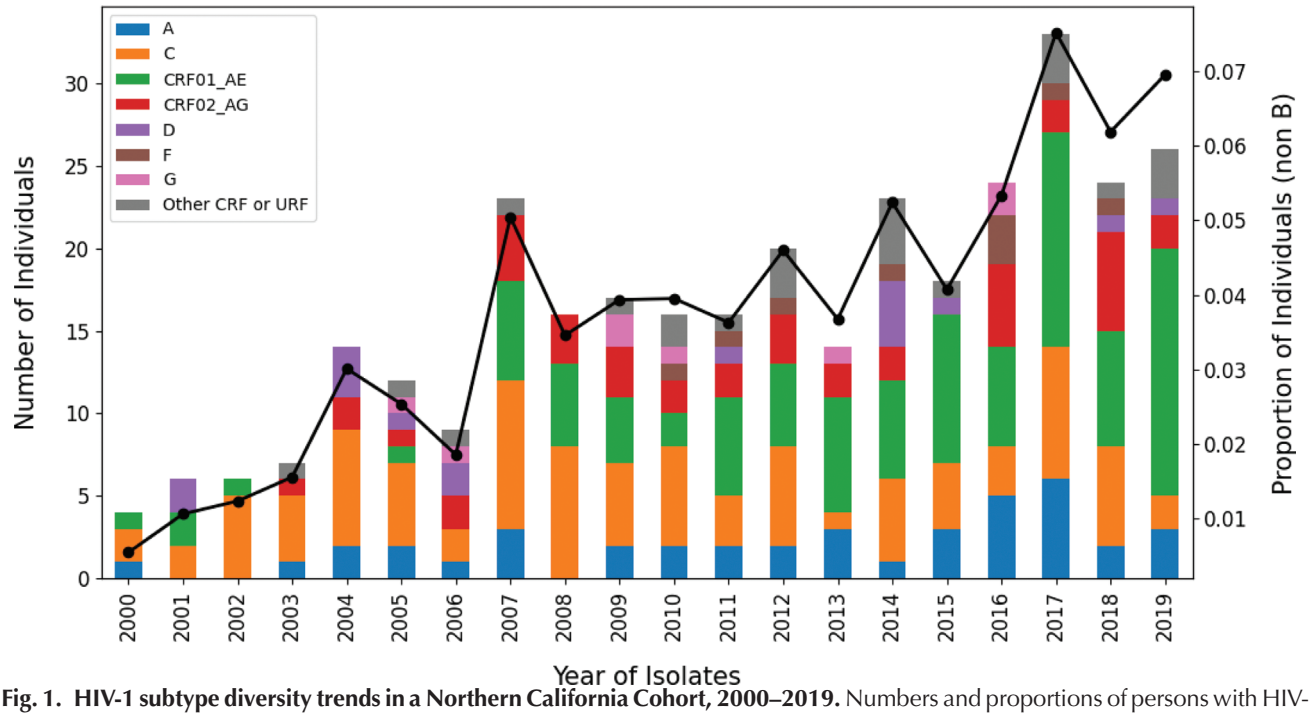

Fig. 1. HIV-1 subtype diversity trends in a Northern California Cohort, 2000–2019. Numbers and proportions of persons with HIV-1 with nonsubtype B viruses per year between 2000 and 2019. The histograms indicate the number of cases per year. The black line indicates the proportion of cases per year.

the proportion of CRF01_AE ($P < 0.000001$) and CRF02_AG ($P = 0.0008$) viruses. No significant increase was observed for subtypes A or C. Compared to individuals with subtype B, those with non-B viruses were more likely to be Black and female. CRF01_AE infections were concentrated among Asian individuals (58.0% vs. white 27.5%, $P < 0.001$ Fisher's Exact Test).

In this large, clinic-based Northern California cohort (2000–2019), HIV-1 remained overwhelmingly subtype B, with modest but statistically significant increases in non-B lineages. The overall prevalence of non-B subtypes (3.5%) was slightly lower than in our previous study of ART-naive individuals from the same population (4.7% between 2003 and 2016) [7] likely reflecting that ART-experienced PWH, more often infected earlier in the epidemic, were enriched for subtype B. Our findings align with a San Francisco study reporting 2.8% non-B infections among 7036 cases between 2000 and 2016, primarily subtype C and CRF01_AE [9]. The prevalence we observed was lower than two CDC surveys: 6.7% in seven states between 2006 and 2013 [3] and 4.9% across 28 states between 2014 and 2018 [10]. A national reference laboratory also reported 3.3% non-B infections among 24 386 specimens collected between 2004 and 2011 [11].

Our study is limited by the absence of data on country of birth and by not performing phylogenetic analyses of non-B sequences, which we previously carried out in ART-naïve individuals [7]. However, by extending our analysis to include both ART-naïve and ART-experienced persons over a longer time period (2000–2019), this study updates our two earlier reports: the first, which showed that nearly all infections were subtype B between 1997 and 2000 [6], and the second, which documented a modest rise in non-B subtypes among ART-naïve individuals between 2003 and 2016 [7]. Together, these studies demonstrate the persistence of subtype B as the overwhelmingly predominant lineage in Northern California, while also highlighting a gradual but measurable increase in non-B subtypes.

In conclusion, the persistence of subtype B as the overwhelmingly dominant lineage in Northern California underscores the relative insularity of the U.S. epidemic, yet the gradual emergence of non-B subtypes demonstrates that diversity is increasing. Our analysis provides one of the few regional benchmarks for monitoring HIV-1 subtype diversity in the United States. It is a further demonstration of how data from laboratories performing genotypic resistance can enhance the surveillance of HIV-1 variants [11].

Acknowledgements

R.W.S. conceived of the project. K.T., P.L.T., and R.W.S. analyzed the data. B.A.P., M.K.S. generated the sequence data and contributed to the analysis. C.B.H.

contributed to data generation. K.T., B.A.P., C.B.H., and R.W.S. wrote the manuscript.

Of the 13214 virus sequences described in this study, 9633 (72.9%) were previously submitted to GenBank as a part of previous publications. The remaining 3581 (27.1%) sequences from this study have been submitted to GenBank sequences under the accession numbers PX355021 – PX358602. The complete dataset described in this study is available from the authors upon request.

Conflicts of interest

The authors report no conflicts of interest.

[a]*Department of Medicine, Division of Infectious Diseases, Stanford University, Stanford;* [b]*Department of Infectious Diseases, Kaiser Permanente Northern California, San Francisco; and* [c]*Department of Pathology, Stanford University, Stanford, California, USA.*

Correspondence to Robert W. Shafer, MD, Stanford University School of Medicine, Stanford, CA 94305, USA.
E-mail: rshafer@stanford.edu

Received: 22 September 2025; accepted: 22 October 2025.

References

1. Gilbert MTP, Rambaut A, Wlasiuk G, Spira TJ, Pitchenik AE, Worobey M. **The emergence of HIV/AIDS in the Americas and beyond**. *Proc Natl Acad Sci U S A* 2007; **104**:18566–18570.
2. Kline RL, Saduvala N, Zhang T, Oster AM. **Diversity and characterization of HIV-1 subtypes in the United States, 2008-2016**. *Ann Epidemiol* 2019; **33**:84–88.
3. Oster AM, Switzer WM, Hernandez AL, Saduvala N, Wertheim JO, Nwangwu-Ike N, *et al*. **Increasing HIV-1 subtype diversity in seven states, United States, 2006-2013**. *Ann Epidemiol* 2017; **27**: 244–251. e1.
4. Abecasis AB, Wensing AM, Paraskevis D, Vercauteren J, Theys K, Van de Vijver DA, *et al*. **HIV-1 subtype distribution and its demographic determinants in newly diagnosed patients in Europe suggest highly compartmentalized epidemics**. *Retrovirology* 2013; **10**:7.
5. Beloukas A, Psarris A, Giannelou P, Kostaki E, Hatzakis A, Paraskevis D. **Molecular epidemiology of HIV-1 infection in Europe: an overview**. *Infect Genet Evol* 2016; **46**:180–189.
6. Gonzales MJ, Machekano RN, Shafer RW. **Human immunodeficiency virus Type 1 reverse-transcriptase and protease subtypes: classification, amino acid mutation patterns, and prevalence in a Northern California clinic-based population**. *J Infect Dis* 2001; **184**:998–1006.
7. Rhee S-Y, Clutter D, Fessel WJ, Klein D, Slome S, Pinsky BA, *et al*. **Trends in the molecular epidemiology and genetic mechanisms of transmitted human immunodeficiency virus Type 1 drug resistance in a large US clinic population**. *Clin Infect Dis* 2019; **68**:213–221.
8. Struck D, Lawyer G, Ternes A-M, Schmit J-C, Bercoff DP. **COMET: adaptive context-based modeling for ultrafast HIV-1 subtype identification**. *Nucleic Acids Res* 2014; **42**:e144.
9. O'Keefe KJ, Pipkin S, Fatch R, Scheer S, Liegler T, McFarland W, *et al*. **Non-B variants of HIV-1 in San Francisco, California**. *Infect Genet Evol* 2021; **90**:104677.
10. McClung RP, Oster AM, Ocfemia MCB, Saduvala N, Heneine W, Johnson JA, Hernandez AL. **Transmitted drug resistance among human immunodeficiency virus (HIV)-1 diagnoses in the United States, 2014–2018**. *Clin Infect Dis* 2022; **74**:1055–1062.
11. Pyne MT, Hackett J, Holzmayer V, Hillyard DR. **Large-scale analysis of the prevalence and geographic distribution of HIV-1 non-B variants in the United States**. *J Clin Microbiol* 2013; **51**:2662–2669.

DOI:10.1097/QAD.0000000000004399

N-acetyl-aspartyl-glutamate connects neuroinflammatory signatures to attention and working memory in people with HIV

Leah H. Rubin[a,b,c,d,*], Atiksh Chandra[a], Jesse Alt[e], Raha M. Dastgheyb[a], Hayley Romero[c], Jennifer M. Coughlin[b,f], Rana Rais[a,e,g,h], Rebecca T. Veenhuis[a,c] and Barbara S. Slusher[a,e,g,h,i,j,*]

Despite viral suppression, many people with HIV (PWH) experience persistent cognitive difficulties. We previously demonstrated that cerebrospinal fluid (CSF) *N*-acetyl-aspartyl-glutamate (NAAG) was associated with spatial attention and working memory. Here, we report that CSF NAAG also correlates with an inflammatory signature (M-CSF, IL-15, MCP-1, sCD40L, IL-18, MMP-9) that relates to spatial attention and working memory. These results suggest that CSF NAAG may serve as an immunomodulatory biomarker relevant to cognition in PWH.

Despite viral suppression, many people with HIV (PWH) experience cognitive difficulties in attention/working memory (WM) [1]. We recently reported that cerebrospinal fluid (CSF) concentrations of *N*-acetyl-aspartyl-glutamate (NAAG), a brain dipeptide that modulates glutamatergic neurotransmission via metabotropic glutamate receptor 3 (mGluR3; reviewed in [2]), were selectively associated with spatial attention/WM in 28 virally suppressed (VS)-PWH [3]. These findings extend prior magnetic resonance spectroscopy studies linking higher NAAG to better attention, WM, and executive function across neuropsychiatric conditions [4–6], including HIV [7], demonstrating that this relationship extends to CSF. However, the biological context of CSF NAAG in HIV remains poorly understood, including whether it reflects or modulates the neuroinflammatory milieu.

We extended our prior analysis by examining CSF NAAG and CSF inflammation associations in a subset of 15 VS-PWH (mean age 58.5 years [SD 10.9, range 39–76]; 67% male; 67% Black; 87% undetectable plasma viral load <20 cp/ml, two detectable at 45.6 and 65 cp/ml) from our original cohort [3] with available CSF inflammatory data. No additional selection criteria or subsampling were applied. The 15 participants were comparable to the full cohort in demographic (age, sex, race) and viral load,