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Genetic analyses of HIV *env* associated with uveitis in antiretroviral-naïve individuals

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Abstract

OBJECTIVE—To analyze and compare human immunodeficiency virus type-1 (HIV) *env* sequences from the eye to those from the blood of individuals with uveitis attributed to HIV with the goal of gaining insight into the pathogenesis of HIV-associated eye disease.

DESIGN—A prospective case series of five HIV-infected antiretroviral (ARV)-naïve individuals with uveitis negative for other pathogens.

METHODS—RNA from blood plasma and ocular aqueous humor was reverse transcribed using random hexamers. HIV *env* C2–V5 (HXB2: 6990–7668) sequences were generated by single-genome-amplification (SGA) using nested polymerase-chain-reaction (PCR) followed by bi-directional Sanger sequencing. Sequence analyses by Geneious, Geno2Pheno, N-GLYCOSITE, DIVEIN, and HyPhy evaluated relationships between HIV in plasma and aqueous humor.

RESULTS—A median of 20 (range: 13–22) plasma and 15 (range: 9–18) aqueous humor sequences were generated from each individual. The frequencies of sequences with predicted-N-linked-glycosylation-sites (PNLGS) and C-X-C chemokine receptor type 4 (CXCR4) were comparable in aqueous humor and plasma of all five subjects. Aqueous humor sequences had lower median genetic diversity compared to plasma across all subjects, but similar divergence, in 4

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of 5 subjects. Aqueous humor HIV sequences were compartmentalized from plasma across subjects by Critchlow correlation coefficient, Slatkin and Maddison, nearest-neighbor statistic, and Fixation index.

CONCLUSIONS—Among ARV-naïve individuals with uveitis attributed to HIV, the universal compartmentalization and decreased diversity of eye compared to blood sequences suggests time-limited passage of a small subset of variants from each subject's viral population into the eye tissues, followed by limited immune selection despite the inflammatory uveitis.

Keywords

human immunodeficiency virus type-1 (HIV); uveitis; aqueous humor; virus compartmentalization; Single genome amplification (SGA); Predicted N-linked glycosylation sites (PNLGS); CXCR4 and CCR5 co-receptor usage

Introduction

Genetic diversification of human immunodeficiency virus type-1 (**HIV**) allows for immune escape and therefore is essential in the pathogenesis of HIV disease [1]. Genetic “compartmentalization” defined as limited gene interchange between tissues has been documented between HIV in the blood and cerebral spinal fluid, male genital track specimens [2–11], and variably, the female genital tract [12–14]. Several reports suggest that HIV within these tissues may have diminished exposure to antiretrovirals [15–17], which could promote selection of drug-resistant viruses that may disseminate to other tissues [18, 19].

The uvea within the eye, is an immune privileged site similar to central nervous system and possesses a blood-ocular barrier analogous to brain-blood barrier [20–22]. Previous studies have shown that HIV could be recovered from multiple ocular tissues, such as the retina, conjunctiva, cornea, and iris of patients with AIDS [23, 24]. We documented the presence of intraocular HIV RNA in 32% of consecutive HIV-infected patients with intraocular inflammation. The quantity of HIV RNA in the aqueous humor was positively associated with that in plasma among ARV-naïve individuals [25], with occasional patients having higher intraocular than plasma concentrations [25–27]. In instances of uveitis with a positive ocular-to-plasma HIV RNA ratio, we hypothesized that HIV was replicating in the uvea. However, it was unclear whether the inflammatory process would promote interchange of virus between the aqueous fluid and blood plasma, or if the blood-eye barrier would limit gene flow and result in compartmentalization of ocular viruses [27].

To gain insight into the pathogenesis of HIV-associated uveitis, HIV RNA sequences were derived from contemporaneously collected aqueous humor and blood plasma of individuals with uveitis with elevated intraocular-to-plasma HIV RNA ratios. Sequences representing the major variants in the quasispecies from each person's tissues were compared for genetic diversity; predicted N-linked glycosylation sites (**PNLGS**), CXCR4 and CCR5 co-receptor usage and viral compartmentalization.

Methods

Study Population

HIV infected subjects with uni- or bilateral uveitis from the Uveitis Clinic of Chiang Mai University Hospital were invited to participate in the study as approved by the Ethics Committee. Available specimens were selected for this analysis based on the subject having a positive aqueous humor to blood plasma HIV RNA ratio, negative tests for ocular opportunistic infections, and improvement of uveitis with initiation of ART [25–26].

HIV RNA quantification and single genome amplification

Plasma and aqueous humor samples from one eye were collected and HIV RNA quantified using the COBAS AmpliPrep/COBAS Taqman HIV-1 Test (Roche Molecular Systems, Inc., Branchburg, New Jersey, USA) [25].

Frozen aliquots of plasma and aqueous humor had RNA extracted and reverse transcribed with random hexamers using the SuperScript® III First-Strand Synthesis System (Invitrogen, Corp., Waltham, Massachusetts, USA). Viral sequences of the C2–V5 region of *env* (HXB2: 6990–7668) were derived by nested single-genome-amplification (SGA) using MyTaq™ DNA Polymerase (Bioline, London, England, UK) and first round primers ED31X (5′-CAGGCCTGTCCAAAAGTATCCTTTGAGCCAATTCC-3′; HXB2 coordinates 6834 to 6865) and BH2 (5′-CCTTGGTGGGTGCTACTCCTAATGGTTCA-3′; coordinates 7697 to 7725), second round primers DR7 (5′-TCAACTCAACTGCTGTAAATGGCAGTCTAGC-3′; coordinates 6990 to 7021), ES7 (5′-TGTAACGACGGCCAGTCTGTAAATGGCAGTCTAGC-3′; coordinates 6983 to 7021), DR8 (5′-CACTTCTCCAATTGTCCCTCATATCTCCTCC-3′; coordinates 7638 to 7668), ES8 (5′-CAGGAAACAGCTATGACCCACTTCTCCAATTGTCCCTCA-3′; coordinates 7648 to 7686). Bi-directional Sanger sequencing was performed using second round PCR primers by Genewiz, Inc. (South Plainfield, New Jersey, USA).

Data Assembly and Analyses

Geneious version 6.1.3 was used to assemble sequence data files, align subjects' sequences, and create generalized time reversible maximum-likelihood phylogenetic trees [28, 29]. Sequence assemblies with large deletions or containing more than a single mixed nucleotide were omitted from further analysis.

The number of PNLGS in plasma and aqueous humor sequences was assessed using the N-GLYCOSITE program [30]. HIV co-receptor usage and subtype were determined using Geno2Pheno [31]. Genetic diversity and divergence from an imputed most recent common ancestor of infection (MRCA) were calculated using DIVEIN [32]. Evolutionary relationships between plasma and aqueous humor HIV were examined using two tree-based methods: the Critchlow correlation coefficient rb compares the number of nodes within and between tissues [33] and Slatkin and Maddison (SM) assess deviation from normality in the distribution of sequences over a predicted tree structure [34]; three distance-based methods: the Critchlow correlation coefficient r compares genetic distances within and between tissues [33], nearest neighbor (S_{nn}) compares genetic distances of sequences within and

between tissues independent of phylogeny [35], and Fixation index measures genetic variance as a function of population structure (F_{ST}) [36] using the HyPhy software [37].

Nucleotide sequence accession numbers

The HIV *env* sequences determined in this study were deposited in GenBank under accession numbers KY508809 - K508960.

Results

Specimens were analyzed from 5 out of 6 HIV-infected subjects with uveitis negative for intraocular co-pathogens that did not respond to topical ocular corticosteroids but improved on initial ART, and whose ocular-to-plasma HIV RNA ratios were >1 [26]. The mean HIV RNA load in the aqueous humor was $6.95 \log_{10}$ c/mL, which was on average $1.7 \log_{10}$ c/mL higher compared to the mean plasma load of 5.19 ($p=0.012$) (Table 1). The median CD4 cell count was 249 cells/uL (range 5–342). The median duration of uveitis was ~12 months but varied across subjects, with symptoms lasting from one to 24 months prior to collection of aqueous humor specimens.

A median of 20 (range 13–22) HIV C2–V5 *env* sequences were generated by SGA from each subject's blood plasma and a median of 15 (range 9–18) from each subject's aqueous humor. The HIV subtype was CRF01_AE by *pol* genotyping in all five individuals (Table 1). However, the C2–V5 *env* sequences revealed two subjects with AE/B recombinant subtypes (Table 1, Subjects 2 and 3), while the others were concordant for AE in both *env* and *pol*.

The co-receptor usage was homogenous or nearly so in the aqueous humor specimens of all five subjects, with CXCR4-usage observed in two subjects' sequences (Table 1). Whereas, the plasma had CXCR4 co-receptor usage detected in four of the five subjects (Table 1). Glycosylation sequence motifs (N-X-[S or T]) were observed at multiple sites within C2–V5, but the mean prevalence was comparable in aqueous humor (9.1, 11.9, 9.1, 10.8, 11.2) and plasma (10.8, 11.4, 11.8, 11.3, 10.3) sequences (Table 1).

Aqueous humor viruses were compartmentalized from the plasma variants as indicated by the phylogenetic tree of each subject's HIV C2–V5 *env* SGA sequences (Figure 1); and multiple statistical analysis (two tree-based and three distance-based methods) (**data not shown**), with all comparisons remaining statistically significant after Bonferroni correction.

The mean genetic diversity of each subject's aqueous humor sequences (2.7%, 5.5%, 3.7%, 0.3%, 3.3%) was lower compared to those of their blood (11.3%, 10%, 6%, 7.5%, 10.7%) ($p<0.001$) (Supplementary Figure 1a). The divergence of aqueous humor and plasma sequences from imputed MRCA within each subject was comparable in four of five subjects, with Subject 5 having a more divergent aqueous humor population ($p<0.001$) (Supplementary Figure 1b).

Discussion

The eye, similar to the brain, possesses a blood-ocular barrier that generally limits immune activation in the ocular tissues [20–22]. Whether HIV infection of the eye mirrors that of the

brain, with genetically distinct HIV variants that utilize the CCR5 co-receptor and forms a distinct viral compartment from the blood [22], or whether viruses in the eye are in perpetual interchange with variants in the blood, has not been investigated.

This study of five HIV-infected subjects with uveitis found that viral sequences from the aqueous humor were less genetically diverse compared to those in plasma in each subject, and were compartmentalized from those derived from the plasma. The relatively low diversities of aqueous humor sequences suggest that a limited subset of each individual's viral quasispecies entered their eye. The divergence of aqueous humor sequences from the individual subject's MRCA was similar to plasma viruses in all but one subject, which in this cross-sectional study does not allow us to ascertain when the eye first became infected.

The similar frequencies of viruses predicted to use the CXCR4 co-receptor and with PNLGS across the plasma and aqueous humor specimens indicate that HIV co-receptor usage and HIV-specific humoral immune responses were unlikely to have had a discriminating role between the plasma and ocular viruses. The observation of homogenous HIV co-receptor usage within each subject's aqueous humor, in contrast to heterogeneous mixtures of CXCR4 or CCR5 viruses across the subjects' plasma specimens, likely reflects the limited size of the eye's founder virus population. The observation of both CXCR4 and CCR5 viruses in the aqueous humor specimens is similar to other findings that show virus isolated from the brain can use CCR5, CXCR4, or both coreceptors [39]. While our sample size was limited, it appears that co-receptor usage of aqueous humor viruses correlates with the prevalent plasma variants suggests that uveitis may result from a vascular accident or inflammatory infiltration of lymphocytes rather than infection of an existing niche resident cell within the eye.

The variable timespan between the onset of uveitis and the cross-sectional sampling of aqueous humor and blood plasma specimens are weaknesses of this study that limit our ability to evaluate the evolution of virus in the eye. Longitudinal sampling of both aqueous humor and blood could provide additional data to confirm or refute persistent compartmentalization between the virus in the aqueous humor and blood. While only a single viral variant appears to have passed into the aqueous humor some subjects (e.g. Subjects 1, 3 and 4), serial samples could reveal if others (e.g. Subjects 2 and 5) had multiple episodes of gene flow, which would suggest a range of compromise to the blood-ocular barrier.

In summary, our detection of aqueous humor HIV RNA with rather limited genetic diversity and high-level of compartmentalization from the blood plasma viruses suggests that a limited subset of each subject's HIV quasispecies entered the eye, and that the duration of rampant virus replication was limited in duration compared to the blood. Precisely, when and how often the eye was seeded with virus is not clear from our studies, as no pathogens except HIV were detected across multiple PCR assays [26]. However, given that time-ordered evolution is typical of untreated HIV infection [38], the observation that most subjects' aqueous humor viruses were not on the "tips" of the phylogenetic trees suggests that their eye was seeded with a variant that evolved earlier during infection, preceding the onset of uveitis. Given the high aqueous humor HIV RNA load, with little viral diversity in

each case, and that uveitis symptoms improved with initiation of ART, we speculate that dysfunctional HIV-specific immune response in the aqueous humor may have caused the uveitis. While uveitis symptoms improved with ART [26], we did not ascertain whether HIV replication was suppressed in the eye. Whether the eye has the potential to serve as “viral sanctuary” separate from other central nervous system tissues, protected from HIV-specific immune control or from certain antiretrovirals is not known, and requires further study as part of efforts to cure HIV infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ganosov VV, Goonetilleke N, Liu MK, Ferrari G, Shaw GM, McMichael AJ, et al. Fitness costs and diversity of the cytotoxic T lymphocyte (CTL) response determine the rate of CTL escape during acute and chronic phases of HIV infection. *J Virol.* 2011; 85(20):10518–28. [PubMed: 21835793]
2. Blasi M, Carpenter JH, Balakumaran B, Cara A, Gao F, Klotman ME. Identification of HIV-1 genitourinary tract compartmentalization by analyzing the env gene sequences in urine. *AIDS.* 2015; 29(13):1651–7. [PubMed: 26372275]
3. de Almeida SM. Cerebrospinal fluid analysis in the HIV infection and compartmentalization of HIV in the central nervous system. *Arq Neuropsiquiatr.* 2015; 73(7):624–9. [PubMed: 26200059]
4. Sturdevant CB, Joseph SB, Schnell G, Price RW, Swanstrom R, Spudich S. Compartmentalized replication of R5 T cell-tropic HIV-1 in the central nervous system early in the course of infection. *PLoS Pathog.* 2015; 11(3):e1004720. [PubMed: 25811757]
5. Karris MA, Smith DM. Tissue-specific HIV-1 infection: why it matters. *Future Virol.* 2011; 6(7): 869–882. [PubMed: 23946764]
6. Schnell G, Price RW, Swanstrom R, Spudich S. Compartmentalization and clonal amplification of HIV-1 variants in the cerebrospinal fluid during primary infection. *J Virol.* 2010; 84(5):2395–407. [PubMed: 20015984]
7. Harrington PR, Schnell G, Letendre SL, Ritola K, Robertson K, Hall C, et al. Cross-sectional characterization of HIV-1 env compartmentalization in cerebrospinal fluid over the full disease course. *AIDS.* 2009; 23(8):907–15. [PubMed: 19414991]
8. Diem K, Nickle DC, Motoshige A, Fox A, Ross S, Mullins JI, et al. Male genital tract compartmentalization of human immunodeficiency virus type 1 (HIV). *AIDS Res Hum Retroviruses.* 2008; 24(4):561–71. [PubMed: 18426336]
9. Pillai SK, Pond SL, Liu Y, Good BM, Strain MC, Ellis RJ, et al. Genetic attributes of cerebrospinal fluid-derived HIV-1 env. *Brain.* 2006; 129(Pt 7):1872–83. [PubMed: 16735456]
10. Burkala EJ, He J, West JT, Wood C, Petito CK. Compartmentalization of HIV-1 in the central nervous system: role of the choroid plexus. *AIDS.* 2005; 19(7):675–84. [PubMed: 15821393]
11. Philpott S, Burger H, Tsoukas C, Foley B, Anastos K, Kitchen C, et al. Human immunodeficiency virus type 1 genomic RNA sequences in the female genital tract and blood: compartmentalization and intrapatient recombination. *J Virol.* 2005; 79(1):353–63. [PubMed: 15596829]
12. Petito CK. Human immunodeficiency virus type 1 compartmentalization in the central nervous system. *J Neurovirol.* 2004; 10(Suppl 1):21–4.

13. Bull M, Learn G, Genowati I, McKernan J, Hitti J, Lockhart D, et al. Compartmentalization of HIV-1 within the female genital tract is due to monotypic and low-diversity variants not distinct viral populations. *PLoS One*. 2009; 4(9):e7122. [PubMed: 19771165]
14. Bull ME, Heath LM, McKernan-Mullin JL, Kraft KM, Acevedo L, Hitti JE, et al. Human immunodeficiency viruses appear compartmentalized to the female genital tract in cross-sectional analyses but genital lineages do not persist over time. *J Infect Dis*. 2013; 207(8):1206–15. [PubMed: 23315326]
15. Dahl V, Gisslen M, Hagberg L, Peterson J, Shao W, Spudich S, et al. An example of genetically distinct HIV type 1 variants in cerebrospinal fluid and plasma during suppressive therapy. *J Infect Dis*. 2014; 209(10):1618–22. [PubMed: 24338353]
16. Craig JK, Gupta P. HIV-1 in genital compartments: vexing viral reservoirs. *Curr Opin HIV AIDS*. 2006; 1(2):97–102. [PubMed: 19372791]
17. Lorenzo-Redondo R, Fryer HR, Bedford T, Kim EY, Archer J, Kosakovsky Pond SL, et al. Persistent HIV-1 replication maintains the tissue reservoir during therapy. *Nature*. 2016; 530(7588):51–6. [PubMed: 26814962]
18. Vázquez-Santiago F, García Y, Rivera-Román I, Noel RJ Jr, Wojna V, Meléndez LM, et al. Longitudinal Analysis of Cerebrospinal Fluid and Plasma HIV-1 Envelope Sequences Isolated From a Single Donor with HIV Asymptomatic Neurocognitive Impairment. *J Virol Antivir Res*. 2015; 4(1)
19. Christo PP, Greco DB, Aleixo AW, Livramento JA. Analysis of correlation between cerebrospinal fluid and plasma HIV-1 RNA levels in patients with neurological opportunistic diseases. *Rev Inst Med Trop Sao Paulo*. 2011; 53(4):193–6. [PubMed: 21915461]
20. Wekerle H, Sun DM. Fragile privileges: autoimmunity in brain and eye. *Acta Pharmacol Sin*. 2010; 31(9):1141–8. [PubMed: 20818330]
21. Benhar I, London A, Schwartz M. The privileged immunity of immune privileged organs: the case of the eye. *Front Immunol*. 2012; 3:296. [PubMed: 23049533]
22. Che X, Fan XQ, Wang ZL. Mechanism of blood-retinal barrier breakdown induced by HIV-1 (Review). *Exp Ther Med*. 2014; 7(4):768–772. [PubMed: 24660027]
23. Cantrill HL, Henry K, Jackson B, Erice A, Ussery FM, Balfour HH Jr. Recovery of human immunodeficiency virus from ocular tissues in patients with acquired immune deficiency syndrome. *Ophthalmology*. 1988; 95(10):1458–62. [PubMed: 2852338]
24. Stewart MW. Human immunodeficiency virus and its effects on the visual system. *Infect Dis Rep*. 2012; 4(1):e25. [PubMed: 24470932]
25. Pathanapitoon K, Riemens A, Kongyai N, Sirirungsi W, Leechanachai P, Ausayakhun S, et al. Intraocular and plasma HIV-1 RNA loads and HIV uveitis. *AIDS*. 2011; 25(1):81–6. [PubMed: 21099669]
26. Kunavisarut P, Sirirungsi W, Pathanapitoon K, Rothova A. Clinical manifestations of human immunodeficiency virus-induced uveitis. *Ophthalmology*. 2012; 119(7):1455–9. [PubMed: 22542726]
27. Rothova A, Schneider M, de Groot-Mijnes JD. Human immunodeficiency virus-induced uveitis: intraocular and plasma human immunodeficiency virus-1 RNA loads. *Ophthalmology*. 2008; 115(11):2062–4. [PubMed: 18562005]
28. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*. 2012; 28(12):1647–49. [PubMed: 22543367]
29. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol*. 2003; 52(5):696–704. [PubMed: 14530136]
30. Zhang M, Gaschen B, Blay W, Foley B, Haigwood N, Kuiken C, et al. Tracking global patterns of N-linked glycosylation site variation in highly variable viral glycoproteins: HIV, SIV, and HCV envelopes and influenza hemagglutinin. *Glycobiology*. 2004; 14(12):1229–46. [PubMed: 15175256]
31. Lengauer T, Sander O, Sierra S, Thielen A, Kaiser R. Bioinformatics prediction of HIV coreceptor usage. *Nat Biotechnol*. 2007; 25(12):1407–10. [PubMed: 18066037]

32. Deng W, Maust BS, Nickle DC, Learn GH, Liu Y, Heath L, et al. DIVEIN: a web server to analyze phylogenies, sequence divergence, diversity, and informative sites. *BioTechniques*. 2010; 48(5): 405–408. [PubMed: 20569214]
33. Critchlow DE, Shuying L, Nourijelyani K, Pearl DK. Some Statistical Methods for Phylogenetic Trees with Application to HIV Disease. *Math Comp Model Dyn*. 2000; 32:69–81.
34. Slatkin M, Maddison WP. A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics*. 1989; 123(3):603–13. [PubMed: 2599370]
35. Hudson RR. A new statistic for detecting genetic differentiation. *Genetics*. 2000; 155(4):2011–4. [PubMed: 10924493]
36. Holsinger KE, Weir BS. Genetics in geographically structured populations: defining, estimating and interpreting F_{ST} . *Nat Rev Genet*. 2009; 10(9):639–650. [PubMed: 19687804]
37. Pond SL, Frost SD, Muse SV. HyPhy: hypothesis testing using phylogenies. *Bioinformatics*. 2005; 21(5):676–9. [PubMed: 15509596]
38. Shankarappa R, Margolick J, Gange S, Rodrigo A, Upchurch D, Farazdegan H, et al. Consistent Viral Evolutionary Changes Associated with the Progression of Human Immunodeficiency Virus Type 1 Infection. *J Virol*. 1999; 73(1):10489–10502. [PubMed: 10559367]
39. Gorry PR, Bristol G, Zack JA, Ritola K, Swanstrom R, Birch CJ, et al. Macrophage Tropism of Human Immunodeficiency Virus Type 1 Isolates from Brain and Lymphoid Tissues Predicts Neurotropism Independent of Coreceptor Specificity. *J Virol*. 2001; 75(21):10073–89. [PubMed: 11581376]

Figure 1. Maximum likelihood phylogenetic analyses of HIV *env* C2–V5 RNA single-genome-analysis (SGA) sequences

Generalized time reversible phylogenetic maximum likelihood trees of HIV RNA sequences, derived by SGA, from blood plasma (open squares, □), aqueous humor (closed circles, ●) and an imputed most recent common ancestor (MRCA). The scale bar indicates the horizontal branch length corresponding to 1 substitution per 100 sites.

Analysis of HIV infected subjects with uveitis

Table 1

Subject	Clinical Characteristics						Parameters of Uveitis			# (%) of sequences (+) for HIV CXCR4 tropism		Mean # of PNLGS within C2-V5		
	Sex	Age	CD4 Cells (cells /μL)	HIV env Subtype	Plasma HIV RNA Load (Log10 c/mL)	Aqueous humor HIV RNA Load (Log10 c/mL)	Duration of Uveitis at specimen collection (Months)	Laterality	Visual Acuity	Location	Plasma RNA	Aqueous humor RNA	Plasma RNA	Aqueous humor RNA
1	F	40	342#	AE§	5.23*	7.95*	~12 [‡]	Unilateral	0.6 [‡]	Anterior	2 (15.4%)	0 (0%)	10.8	9.1
2	M	50	5#	AE,B§	5.73*	6.39*	~24 [‡]	Bilateral	CF, CF [‡]	Anterior [¶]	21 (95.5%)	16 (88.9%)	11.4	11.9
3	M	35	305#	AE,B§	4.4*	6.74*	~12 [‡]	Bilateral	CF, 0.5 [‡]	Anterior	11 (52.4%)	0 (0%)	11.8	9.1
4	M	49	249#	AE§	5.03*	7.18*	1 [‡]	Unilateral	0.25	Anterior [¶]	0 (0%)	0 (0%)	11.3	10.8
5	F	34	7#	AE§	5.58*	6.5*	~24 [‡]	Unilateral	0.2 [‡]	Anterior [¶]	2 (12.5%)	12 (100%)	10.3	11.2

Antiretroviral naïve

§ CRF01_AE at pol gene, no HIV drug resistance mutations

* HIV RNA load in aqueous humor was higher than paired plasma samples (p = 0.001)

‡ Intraocular real-time PCR negative for co-pathogens (Herpes Simplex virus, Varicella Zoster virus, Cytomegalovirus, and Taxoplasma gondii)

‡ As a result of cataract and/or inflammatory cells

¶ With vitreous involvement

CXCR4: C-X-C chemokine receptor type 4

PNLGS: Potential N-linked glycosylation site