Identification and Implications of HIV-1 CRF01_AE Subtype in Hawai‘i

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Abstract

Human Immunodeficiency Virus has a high propensity for genetic variation, demonstrated by its complex phylogeny and multiplicity of subtypes. Subtype B is predominant in North America as well as in Hawai‘i while CRF01_AE is found in over 50% of cases in the Philippines and Southeast Asia. In a small collaborative study between the Hawai‘i Center for AIDS and Philippines General Hospital, molecular phylogenetic subtyping was conducted on HIV+ participants. Two of 15 (13%) participants from the Hawai‘i cohort and 12 of 21 (57%) participants from the Philippines cohort were identified as having CRF01_AE subtype of HIV-1, with remaining participants identified as subtype B. While one individual in Hawai‘i with CRF01_AE had emigrated from the Philippines, the other participant from Hawai‘i with CRF01_AE subtype was a local individual, born and raised in Hawai‘i. The authors report that HIV subtype diversity may be increased in Hawai‘i and discuss its potential clinical and public health implications.

Keywords

HIV, CRF01_AE, Hawai‘i, Philippines

Abbreviations

ART = Antiretroviral Therapy
CRF = Circulating Recombinant Form
HICFA = Hawai‘i Center for AIDS
HIV = Human Immunodeficiency Virus
IRB = Internal Review Board
PBMC = Peripheral Blood Mononuclear Cells
PCP = Pneumocystis Pneumonia
PCR = Polymerase Chain Reaction
PI = Protease Inhibitor
R5 = CCR5
URF = Unique Recombinant Form
X4 = CXCR4

Introduction

Human Immunodeficiency Virus (HIV) is a single stranded, positive sense RNA lentivirus responsible for the HIV pandemic. The virus is characterized by its infection of CD4+ T cells as its main reservoir in the presence of co-receptor CCR5 (R5) or CXCR4 (X4). HIV has a high propensity for mutation, a result of a high reverse transcription error rate and increased viral production in established infection.[1] The virus is divided into 2 distinctive viral types, HIV-1 and HIV-2.[2] HIV-2 circulates primarily in West Africa compared to HIV-1's global prevalence, and HIV-2 is associated with decreased pathogenicity compared to HIV-1.[2] HIV-1 is further divided into groups (M-P) followed by subtypes.[2] Subtypes are primarily aggregated according to geographic location, however, the presence of HIV infection worldwide has given rise to circulating recombinant forms (CRF) composed of portions of subtypes mixed into a genetically distinguishable novel subtype. Unique recombinant forms (URF) are isolated cases of recombination not yet in primary circulation.[3] Subtype B dominates in North America, however nationwide studies have yet to extensively characterize HIV subtype makeup in all 50 states.[2] Hawai‘i does not have data on subtypes of HIV infections. It is likely that the state follows the subtype B dominance found in the continental US, however it is possible that emigration from Southeast Asia, a subtype CRF01_AE dominant area, may influence's HIV subtype demographics. Such information may be important as an increase in the presence of the CRF01_AE subtype compared to subtype B within a population may have potential public health and clinical ramifications. The purpose of this study, therefore, was to determine the percentage of individuals with CRF01_AE and B subtypes in a cohort of HIV+ individuals in the Philippines and in Hawai‘i.

Methods

Study Participants

The Hawai‘i Center for AIDS (HICFA) launched a collaborative study with the Philippines General Hospital in Manila to compare clinical and neurologic differences by site between HIV-positive and negative participants. This study recruited 50 HIV+ and 50 HIV- participants in the Philippines and 17 HIV+ and 20 HIV- participants in Hawai‘i. The Philippine cohort participants were recruited from patients of the STD-AIDS Guidance Intervention Prevention Clinic at the Philippines General Hospital. The Hawai‘i participants were recruited through advertisement in the Clint Spencer Clinic and by word of mouth through an established network of clinic/physician practices. The study protocol was approved by the internal review boards (IRB) of the University of Hawai‘i (IRB#: 2016-31099) and Philippines General Hospital (IRB#: UPMREB 2017-201-01) and all participants signed approved informed consent documents. Blood samples and clinical data were collected from study participants in both cohorts. Plasma and peripheral blood mononuclear cells (PBMCs) were separated using standard Ficoll-Paque density gradient separation from whole blood, cryopreserved, and transferred to the HICFA biorepository for further downstream analysis.

Subtype Determination

Proviral DNA was isolated from cryopreserved PBMCs and amplified by polymerase chain reaction (PCR) using in-house primers targeting the *gag* and *pol* regions of HIV-1. PCR products were then purified and subsequently sequenced using Applied Biosystems DNA Analyzer (Life Technologies, Carlsbad, CA). Samples were processed at the Genomic Core Facility at the John A. Burns School of Medicine and University of Hawai'i Advance Studies in Genomics, Proteomics and Bioinformatics Center. A detailed protocol for subtype determination was previously described. [4] Phylogenetic subtyping was completed with phylogenetic analysis using molecular evolutionary genetics analysis (MEGA) version 10 software (Penn State University, State College, PA).

Results

Subtype analyses were attempted in 50 HIV+ participants in the Philippines and 17 HIV+ participants from Hawai'i and successfully identified in 21 HIV+ participants from the Philippines and 15 HIV+ participants from Hawai'i. HIV+ blood samples from 12 participants in the Philippines (57%) and 2 in Hawai'i (13%) were identified as having the CRF01_AE subtype. The remaining participants at both sites were identified to have subtype B. Both participants with CRF01_AE subtype in Hawai'i self-identified themselves as men who have sex with men and were on combination HIV antiretroviral therapy (ART) with plasma HIV RNA undetectable at < 20 copies/mL at study entry. One participant was a Filipino man diagnosed with HIV in 2014 who emigrated to Hawai'i the following year. This participant contracted pneumocystis pneumonia (PCP), an AIDS defining illness, concurrent with HIV+ diagnosis. Nadir CD4 count was self-reported to be between 50-99 cells/uL and CD4 count at study entry was 379 cells/uL. The other participant from the Hawai'i cohort with CRF01_AE was a man of mixed Asian, White, and Native Hawaiian race who was born and raised in Hawai'i, diagnosed with HIV in 2008, and doing well on stable antiretroviral therapy (ART) composed of a ritonavir boosted protease inhibitor darunavir, and fixed dose emtricitabine and tenofovir disoproxil fumarate (Truvada®). Nadir CD4 count was 88 cells/uL with a CD4 count of 369 cells/uL at study entry. Travel history was not available for either participant.

Limitations

To the authors' knowledge this is the first study that reveals CRF01_AE subtype infection specifically in a local resident born and raised in Hawai'i. Not all HIV+ participant subtyping analyses were successful, potentially due to cell death during transport and subsequent lower DNA yield. It is also possible that primer mismatches occurred and that subtypes other than B and CRF01_AE were present in the population. If more Philippine subtype analyses were successful, it would most likely reveal an increased percentage of non-B subtypes. This would make the comparison between the Philippines and Hawai'i cohort that much more distinct. Future studies need to address these difficulties with increased quality control measures for cryopreservation and transportation of samples. Additionally, tailoring of primers to the known circulating subtypes of HIV in the Philippines might circumvent future primer mismatches.

Discussion

Here the authors describe a serologic survey in a small cohort of HIV+ individuals in Hawai'i. The authors unexpectedly identified 2 individuals with the CRF01_AE subtype of HIV-1, 1 of whom was born and raised in Hawai'i. Studies examining subtype distribution in Hawai'i have been limited to nationwide epidemiologic surveys and have included minimal samples from the state. The assumption seems to be that Hawai'i has a dominant subtype B distribution similar to the continental US. The most recent study conducted by the Centers for Disease Control and Prevention (CDC) examined the diversity of HIV-1 subtypes in the US spanning the years 2008-2016 and concluded that the most prevalent strain remains subtype B, accounting for 95.1% of cases.[5] This study characterized HIV specimens from only 17 states and did not include Hawai'i. Of note was the increasing prevalence of non-B subtypes across the study time. A study conducted by Pyne, et al in 2013 to explore HIV non-B subtype diversity in the US identified CRF01_AE in the single sample they received from Hawai'i.[2] In 2015, Germer, et al,[6] characterized HIV-1 subtypes in the US between July 2011 and June of 2012 using clinical laboratory samples from the Mayo Medical Laboratories, and noted that the highest levels of non-B subtypes were found in the West North Central region. States in this area, Minnesota, Iowa, and North and South Dakota, had an average of 20.2% non-B subtypes. A total of 13 (7%) of the total non-B subtype samples were identified as CRF01_AE. The study did not include Hawai'i or 5 other states in its analysis.

Identification of CRF01_AE and additional non-B subtypes may have potential public health and clinical ramifications. It has been suggested that genetic diversity among subtypes explains differences in transmission rate, natural history, sensitivity of wild-type strains to ART, and mechanisms of escape due to sub-standard ART regimens.[7] Co-receptor switching has been documented to occur in HIV infected individuals regardless of ART treatment status or HIV subtype.[8-11] CRF01_AE has been demonstrated to lead to a more rapid viral co-receptor switch from CCR5 (R5) tropism to CXCR4 (X4) tropism.[12,13] The increased speed at which CRF01_AE switches from R5 to X4 tropism is hypothesized to be a consequence of the subtype's dual-tropic nature.[14] The switch in tropism to CXCR4 leads to a more rapid decrease in CD4 count compared to CCR5 tropism.[15] Swifter declines in CD4+ T-cell counts can result in earlier onset of AIDS-defining illnesses and death if ART is not instituted. It may be relevant that the 2 CRF01_AE patients in the Hawai'i cohort were both diagnosed to have HIV in the later

stages of HIV with CD4 count below 200 cells/mm^3. Impaired reconstitution of CD4 count following ART institution has also been associated with CRF01_AE infections.[16] Thus, a potential public health ramification of an increased percentage of HIV infections in the state due to CRF01_AE infection may be the need to increase effort to identify and treat individuals early in their HIV disease course because of the increased risk of more rapid CD4+ T-cell decline in individuals with CRF01_AE subtype. The second potential ramification of increased HIV infection due to CRF01_AE subtype may be that CCR5 antagonists such as maraviroc, which are effective only against CCR5 utilizing viruses, can be expected to be less useful with the increased frequency of CXCR4 and dual tropic X4/R5 virus in CRF01_AE infections.[17]

In conclusion, increasing subtype diversity worldwide has been described and may become of public health and epidemiologic interest.[3] This small study suggests that non-B subtypes are present in Hawai‘i. Because of Hawai‘i’s cultural ties to Southeast Asia, the presence of CRF01_AE in the study is perhaps unsurprising. Medical personnel in Hawai‘i should be aware of the potentially increasing prevalence of non-B subtypes in Hawai‘i and its potential clinical ramifications.

Conflict of Interest

None of the authors identify a conflict of interest.

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