Journal of Molecular Genetics 2 (3-4): 41-44, 2010
ISSN: 2070-4267

Low Prevalence of Non-Subtype B HIV-1 Strains in the Texas Prisoner Population

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Abstract: Genotypic drug resistance testing is standard of care in HIV management. The ViroSeq HIV-1 Genotyping System is commonly used in detecting HIV genomic mutations that confer resistance to specific types of antiretroviral drugs as an aid in treating HIV infection. Of note, the assay is FDA approved to detect only HIV-1 Group M Subtype B viral resistance. Subtype B was the most prevalent strain in developed counties when the assay was introduced into clinical care approximately 10 years ago. However, previously thought less common strains in the United States have gained in prevalence. The response to medications by non-B subtypes compared to B subtypes as well as whether the targeted mutations in non-B subtypes contribute to drug resistance the same as B subtypes need to be further studied. The purpose of this study was to identify whether HIV-1 viruses routinely tested using the ViroSeq assay in UTMB Molecular Diagnostics Laboratory are Group M Subtype B. HIV sequences generated between January and September of 2008 were aligned with HIV-1 subtype reference strains and analyzed by phylogenetic methods. About 16 of 588 HIV sequences (2.7%) examined were identified as non-B subtypes including 3 of 416 (0.7%) prisoner and 13 of 172 (7.6%) regular patient samples.

Key words: HIV-1, subtype B, non-B subtype, ViroSeq HIV-1 Genotyping System, mutation, USA

INTRODUCTION

Human Immunodeficiency Virus (HIV) is the etiologic agent responsible for the development of Acquired Immune Deficiency Syndrome (AIDS), a chronic disease leading to immunodeficiency and susceptibility to opportunistic infections. HIV is a member of the genus *Lentivirus* belonging to the family Retroviridae. HIV is transmitted as single-stranded, positive-sense, enveloped RNA virus. There are two species, *HIV-1* and *HIV-2* that are thought to have separate introductions into human populations arising from zoonotic cross-species transmissions from nonhuman primates to humans. HIV-1 is the cause of the great majority of HIV infections worldwide. Sequence similarity further classifies HIV-1 into groups, subtypes and sub-subtypes. Three groups of HIV-1 have been identified including M (main), O (outlier) and N (non-M/non-O) (Robertson *et al*., 2000). The HIV-1 Group O contains a large amount of genetic diversity although the majority of infections are found in Cameroon (Roques *et al*., 2002). Very few isolates of the HIV-1 group N have been identified and sequenced. Of the three groups of HIV-1, group M dominates the global epidemic and is further classified into subtypes A, B, C, D, F, G, H, J and K. In addition, Circulating Recombinant Forms (CRFs), mosaic viruses formed between subtypes during co-or super-infection have also been recognized (http://www.hiv.lanl.gov/).

Although, subtype B is predominant in North America and Europe, non-B variants represent >90% of HIV-1 circulating globally. In recently years the prevalence of non-B subtypes and CRFs in the U.S. is steadily increasing due to increased international travel and immigration (Brennan *et al*., 2009; Lin *et al*., 2006; Peeters *et al*., 2010). Drug resistance testing is recommended for the management of patients with HIV-1 infection (http://www.aidsinfo.nih.gov/guidelines/). The ViroSeq HIV-1 Genotyping System (Celera Diagnostics, Alameda, CA and USA): ABI PRISM 3100 genetic analyzer (Applied Biosystems, Carlsbad, CA, USA) (ViroSeq) is Food and Drug Administration (FDA) approved. The assay produces 1302 nucleotide sequences comprising codons 1-99 of the *protease* gene and 1-335 of the *reverse transcriptase* gene and is routinely used clinically in detecting mutations in the protease and Reverse Transcriptase (RT) that confer resistance to specific types of antiretroviral drugs. Although, the assay was designed specifically for HIV-1

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group M subtype B, it can amplify and sequence HIV-1 non-B subtype sequences and has been used to provide important information about the epidemiology of HIV-1 subtypes (Brennan *et al*., 2009; Eshleman *et al*., 2004; Palma *et al*., 2007).

In this study it is analyzed sequences generated in the Molecular Diagnostics Laboratory at the University of Texas Medical Branch (UTMB) using the ViroSeq system for detection of HIV-1 drug resistance mutations to establish HIV-1 virus subtypes through phylogenetic analysis.

MATERIALS AND METHODS

Specimens and ViroSeq assay: The ViroSeq HIV-1 Genotyping results were generated in 588 patients referred to the Molecular Diagnostics Laboratory at UTMB between January and September of 2008. In addition to free-world patients, UTMB serves the southeast region of Texas Department of Criminal Justice units (West of Beaumont, South of Huntsville and East of San Antonio). RNA isolation, Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and sequencing were performed according to the manufacturer's instructions (Yang *et al*., 2008). The study was approved by the UTMB Institutional Review Board (IRB).

Phylogenetic analysis: A phylogenetic tree was constructed using a total of 605 sequences including the 588 patient sequences together with homologous sequences from 17 HIV-1 subtype reference strains obtained from the Los Alamos database (http://www.hiv.lanl.gov/).

The 17 reference strains included an HIV-1 group O out-group, 13 HIV-1 group M subtype strains and 3 CRFs (NCBI accession numbers: AF082395, AF084936, AF190128, AF193276, AF259955, AF286238, AJ249235, AF249238, AY169812, AY835766, FJ389367, K03454, K03455, L39106, U46016, U51190 and U54771). The sequences were aligned with ClustalW implemented in MACVECTOR version 11.1 (Accelrys, San Diego, CA and USA).

Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method (18) implemented in the PAUP version 4.0a109 software package (Kumar and Gadagkar, 2000; Swofford, 2002). For NJ analysis, a distance matrix was calculated from the aligned sequences using the Hasegawa, Kishino and Yano (HKY85) formula by allowing transitions and transversions to occur at different rates and also allowing base frequencies to vary. The phylogenetic tree was visualized with the FigTree program (http://tree.bio.ed.ac.uk). Non-B subtypes were confirmed by a second NJ analysis after aligning putative non-B subtypes with 17 reference sequences. The data were sampled using 1000 bootstrap replicates to determine the confidence indices within the phylogenetic tree (Felsenstein, 1988). Non-B-subtypes with bootstrap values >80% were considered significant (Lin *et al*., 2006).

Statistical analysis: The Fisher's exact test was used to detect the difference in prevalence of HIV-1 non-B infection between the prison population and those in the free-world.

RESULTS AND DISCUSSION

Analysis of the sequences revealed the presence of two major clades (Fig. 1) subtype B and non-subtype B. A total of 16 of the 588 samples (2.7%) were HIV-1 non-B subtypes (10×A/CRF01, 2×C, 1×H, 1×D and 2×CRFO1) (Fig. 2 and 3). Further analysis revealed a 10 fold difference in prevalence of non-B infection between the prison population (0.7%, 3 of 416) and those in the free-world (7.6%, 13 of 172); the difference is statistically significant (Fisher's Exact test, p = 0.0000). The significant difference in the prevalence of HIV-1 non-B infections in prisoners and free-world patients warrants further investigation. Information about the country of origin and source of HIV-1 infection should help such analysis. For example, since non-B HIV-1 strains are not prevalent in the U.S., it is possible that HIV clinics at UTMB that serve free-world patients provide care to a greater proportion of patients that were born or traveled to countries/regions that have high prevalence of non-B

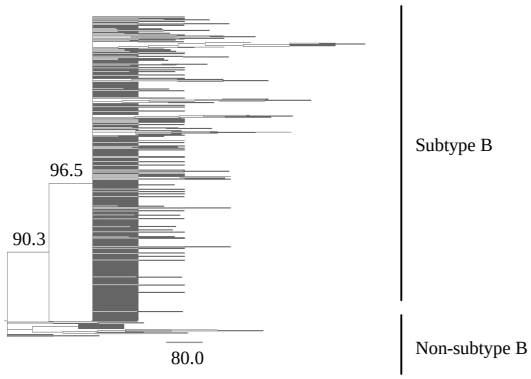

Fig. 1: Phylogenetic relationships of HIV-1 sequences based on nucleotide sequences of protease and RT regions of the *Pol* gene. Phylogenetic analyses were carried out using the NJ method. Distances were measured using the HKY85 formula. The bootstrap method with a NJ search was carried out with the same options. Groups with a frequency of 50% were retained. Group O was used as the out-group to root the tree. Numbers adjacent to each branch represent the percentage bootstrap support calculated for 1000 replicates

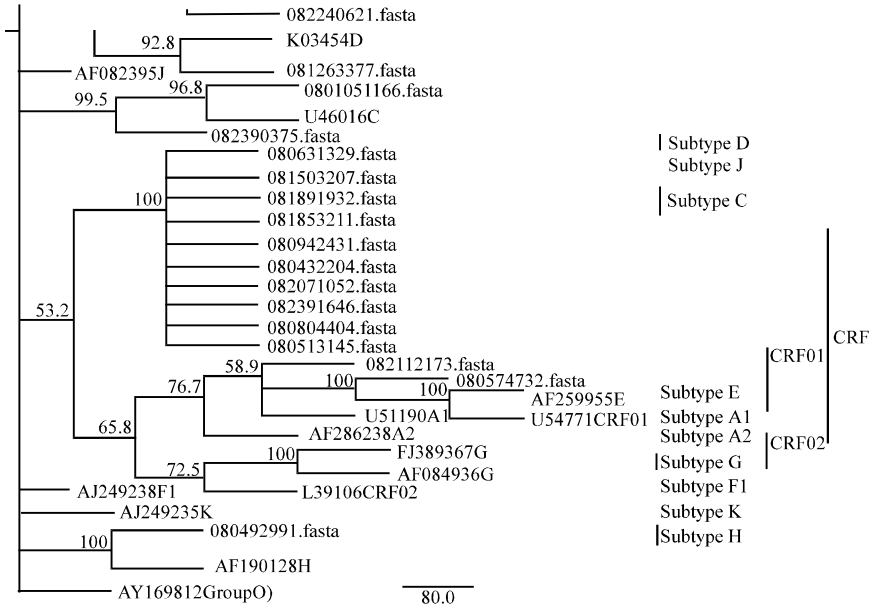

Fig. 2: Higher magnification of the NJ tree for non-subtype B sequences from Fig. 1. Numbers adjacent to each branch represent the percentage bootstrap support calculated for 1000 replicates

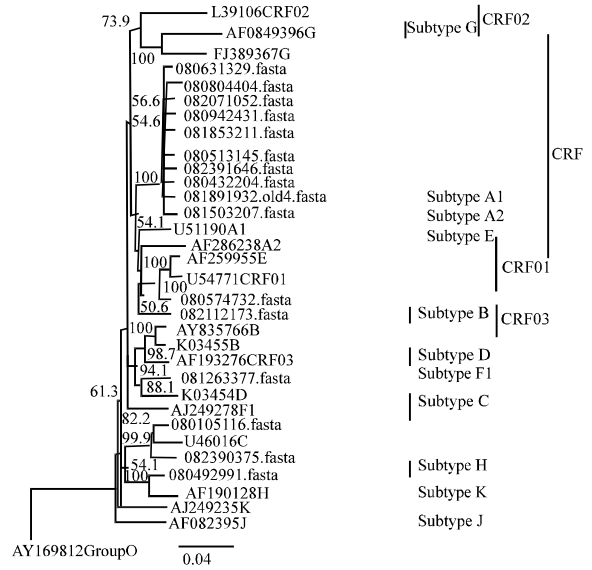

Fig. 3: Phylogenetic relationship of 16 non-subtype B sequences based on the nucleotide sequences of protease and RT regions of the *Pol* gene with reference sequences. Phylogenetic analyses were carried out using the NJ method. Distances were measured using the HKY85 formula. The bootstrap method with a NJ search was carried out with the same options. Groups with a frequency of 50% were retained. Group O was set as the out-group to root the tree. Numbers adjacent to each branch represented the percentage bootstrap support calculated for 1000 replicates

subtypes. Of note, only successfully sequenced samples were included in this study. As with other currently available molecular techniques, the performance characteristics of HIV-1 genotyping assays are subject to sequence variations existing in non-B as well as B strains. Although, there is an increasing awareness of the need to cover broader HIV strains (Church *et al*., 2006; Holguin *et al*., 2008), reagents and controls currently used in routine clinical care (including the WHO International Standard) are based on subtype B viruses. The HIV-1 ViroSeq assay was designed to identify subtype B strains. Since the assay is proprietary, the PCR and sequencing primer sequences are not available for us to perform sequence comparisons to predict annealing to various HIV-1 strains. Researchers observed sequencing failure rates of 2-8% after repeated testing.

The sequencing failure is related to viral load (2, 7 and 8% at viral loads of >1,000, 500-999 and 75-499 copies mL^{-1}, respectively) with plasma input of 500 μL according to the ViroSeq assay protocol (Yang *et al*., 2008). Viral load testing was done using the VERSANT HIV-1 RNA 3.0 Assay (bDNA, Tas in several previous publications (Church *et al*., 2006; Eshleman *et al*., 2004; Soto-Ramirez *et al*., 2008), this study provided another example of determining HIV-1 subtype prevalence from resistance genotyping sequences that were routinely obtained in a clinical diagnostics laboratory. This approach is cost effective, quick and convenient to detect and monitor the HIV-1 subtype distribution and prevalence in the patient population and geographical region in which clinics/laboratories serve. Additionally,

these sequence data are part of routine patient care; linking particular subtypes with other clinical information should help to recognize the potential impact of the growing genetic diversity of HIV-1 subtypes on patient management (Geretti, 2006; Kantor *et al*., 2005; Martinez-Cajas *et al*., 2009; Peeters *et al*., 2010).

CONCLUSION

The study showed that the great majority of HIV-1 infections identified in Galveston Texas were still Subtype B and within FDA label to perform ViroSeq HIV-1 Genotyping testing.

ACKNOWLEDGEMENTS

Researchers would like to thank members of the UTMB Molecular Diagnostics Laboratory for HIV sequencing data and Dr. David H. Walker for critical review of the manuscript.

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