

Short report

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Genetic analysis of HIV-1 Circulating Recombinant Form 02_AG, B and C subtype-specific envelope sequences from Northern India and their predicted co-receptor usage

Ujjwal Neogi¹, Vikas Sood¹, Arpita Chowdhury¹, Shukla Das²,
Vishnampettai G Ramachandran², Vijesh K Sreedhar³, Ajay Wanchu³,
Nilanjana Ghosh¹ and Akhil C Banerjea*¹

Address: ¹Virology Laboratory, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110067 India, ²Microbiology Department, UCMS and GTB Hospital, Delhi 110095 India and ³Department of Internal Medicine, PGIMER, Sector 12, Chandigarh 160012 India

Email: Ujjwal Neogi - ujjwal@nii.res.in; Vikas Sood - vikas@nii.res.in; Arpita Chowdhury - arpita@nii.res.in; Shukla Das - shukla@ucms.in; Vishnampettai G Ramachandran - Rama@ucms.in; Vijesh K Sreedhar - vijesh@pgi.in; Ajay Wanchu - Wanchu@pgi.in; Nilanjana Ghosh - Nilanjana@nii.res.in; Akhil C Banerjea* - akhil@nii.res.in

* Corresponding author

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Abstract

HIV-1 epidemic in India is largely driven by subtype C but other subtypes or recombinants have also been reported from several states of India. This is mainly due to the co-circulation of other genetic subtypes that potentially can recombine to generate recombinant/mosaic genomes. In this study, we report detail genetic characterization of HIV-1 envelope sequences from North India (Delhi and neighboring regions). Six of 13 were related to subtype C, one B and the rest six showed relatedness with CRF02_AG strain. The subtype C possessed the highly conserved GPGQ motif but subtype B possessed the GPGR motif in the V3 loop as observed earlier. While most of the sequences suggested CCR5 co-receptor usage, one subtype C sample clearly indicated CXCR4 usage. A successful mother to child transmission was established in two pairs. Thus, co-circulation of multiple subtypes (B and C) and the recombinant CRF02_AG strains in North India suggests a rapidly evolving scenario of HIV-1 epidemic in this region with impact on vaccine formulation. Since this is the first report of CRF02_AG envelope from India, it will be important to monitor the spread of this strain and its impact on HIV-1 transmission in India.

Introduction

HIV-1 displays a tremendous amount of genetic diversity. The binding of the HIV-1 to host cells is mediated by envelope glycoprotein. When the HIV-1 envelope protein binds to its primary receptor CD4, it undergoes conformational changes and it then binds to one of the coreceptors (chemokine receptor CCR5, CXCR4 or others) via its V3 loop. This tri-molecular interaction leads to the viral membrane fusion [1]. HIV-1 envelope is composed of rel-

atively conserved (C1 to C5) and variable regions (V1 to V5). The V3 region elicits neutralizing antibodies and also govern co-receptor usage [1,2]. Replacements in the V3 region with basic amino acids are associated with CXCR4 usage [2,3]. Subtypes A and C usually contain a highly conserved GPGQ amino acid motif, while GPGR is the predominant motif in the V3 loop of subtype B envelopes [4,5]. Mutational patterns in the V3 loop region are likely to be of clinical significance as they can influence their

susceptibility to known CCR5 inhibitors. Although all HIV-1 genetic subtypes originated in Africa, it is not fully understood how certain subtypes dominate different regions of the world. For e.g. subtype B predominates in US and UK but subtype C is predominant in India, some parts of Asia and Africa [6].

It is fairly well established that HIV-1 that uses CCR5 chemokine receptor (R5-tropic) is transmitted preferentially than the ones that use CXCR4 chemokine receptor [7]. Individuals with a 32 bp deletion in the CCR5 open reading frame (ORF) are largely protected against HIV-1 infection [7-9]. Approximately 50% of HIV-1 subtype B infected individuals show HIV-1 co-receptor switch from CCR5 to CXCR4 which is associated with rapid progression of HIV/AIDS [10]. This is observed mainly in US and UK where subtype B predominates. However, in India, where subtype C predominates, the coreceptor switch has not been observed [11]. Replacements of charged amino acids within the V3 region are known to alter the co-receptor usage [2,3,12]. Genetic variations in the subtype C HIV-1 envelope sequences have recently been reported from Southern India with some strains exhibiting multiple co-receptor usage, including CXCR4 chemokine receptor, present predominantly on T-helper lymphocytes [13,14]. It is noteworthy that we recently reported novel B/C LTR [15] and Vpr B/C/D sequences from North India [16].

Given the large size of India, and with increasing global travel, it is likely that subtypes other than B may also co-circulate, creating an ideal situation for the formation of recombinants. With this in mind, we genetically characterized the HIV-1 envelope sequences from HIV-1 infected individuals from Northern India and report the presence of HIV-1 CRF02_AG for the first time.

Methods

Genomic DNA isolation and Polymerase chain reaction

Genomic DNA was isolated from fresh peripheral blood collected in EDTA using a kit from Qiagen (QIAamp Blood Minikit) as described before by us [8,9]. All requisite ethical clearances were obtained before initiating this study. All the polymerase chain reactions (PCRs) were performed with high fidelity Taq DNA polymerase (Ex-Taq, Takara, Japan) using the following primers:

Forward primer: 5'-ATGGGATCAAAGCCTAAAGCCATGTG

Reverse primer: 5'-AGTGCTTCCTGCTGCTCCCAAGAACC-CAAG

Approximately 1.25 Kb DNA fragment corresponding to V1 to V5 region was amplified initially. Thereafter, 700 bp

fragment (V3 to V5) was amplified using two internal sets of primers with following sequences:

Forward primer: CTGTTAAATGGCAGTCTAGC

Reverse primer: CACTTCTCCAATTGTCCCTCA

The cycling conditions for amplifying both the fragments were: 35 cycles at 98°C for 15 sec, 55°C for 30 sec and 72°C for 1 min with a final extension at 72°C for 10 min. PCR-amplified DNA was cloned into pGem-T expression vector (Promega Biotech, WI, USA) and sequenced in both directions using T7 and SP6-specific primers. The sequence from one representative clone from each sample was used to carry out phylogenetic analysis and sequence comparisons. The final concentration of MgCl₂ was 20 mM for both the PCRs. Mother and child samples were processed separately to avoid cross contamination.

Patient population and genetic analysis

We carried out genetic analysis of 13 HIV-1 envelope sequences from Northern India. Nine unrelated and 2 mother-child pairs (Pair 1, D & E 57 and Pair 2, D & E 58) were selected randomly from two locations (one from GTB Hospital, Delhi - Samples ND1 to 5, all from commercial sex workers-CSW) and the rest were from Punjab/Haryana region. Primers were designed to carry out nested PCR as described earlier. It is noteworthy that we were unable to amplify envelope sequences from several samples which may be due to extreme genetic variability and therefore difficult to draw conclusions about the frequency of any genetic subtype from this study. Alternatively, since most of the HIV-1 infected individuals were on antiretrovirals, the amounts of proviral DNA may have been too small to amplify. Sequences were compared with reference strains (figure 1) (Los Alamos-<http://www.hiv.lanl.gov>). At least 4 independent clones were analyzed from each sample and only one representative clone from each sample was genetically analyzed. Multiple sequence analysis was performed in ClustalW 1.8.3 obtained from DNA data bank of Japan (DDBJ) website <http://clustalw.ddbj.nig.ac.jp/top-e.html>. The phylogenetic analysis was carried out using MEGAA 4.1 (beta) software. Genotyping was carried out using viral genotyping tools located at NCBI <http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi>, REGA subtyping tool ver 2.0 <http://www.bioafrica.net/subtypetool/html> and Recombination Identification Program (RIP) 3.0. <http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html>. Potential N-glycosylation sites were calculated using N-GlycoSite program <http://www.hiv.lanl.gov/content/sequence/GLYCOSITE/glycosite.html>.

Results and discussion

All of the HIV-1 infected individuals were infected through heterosexual route (except mother-child pair)

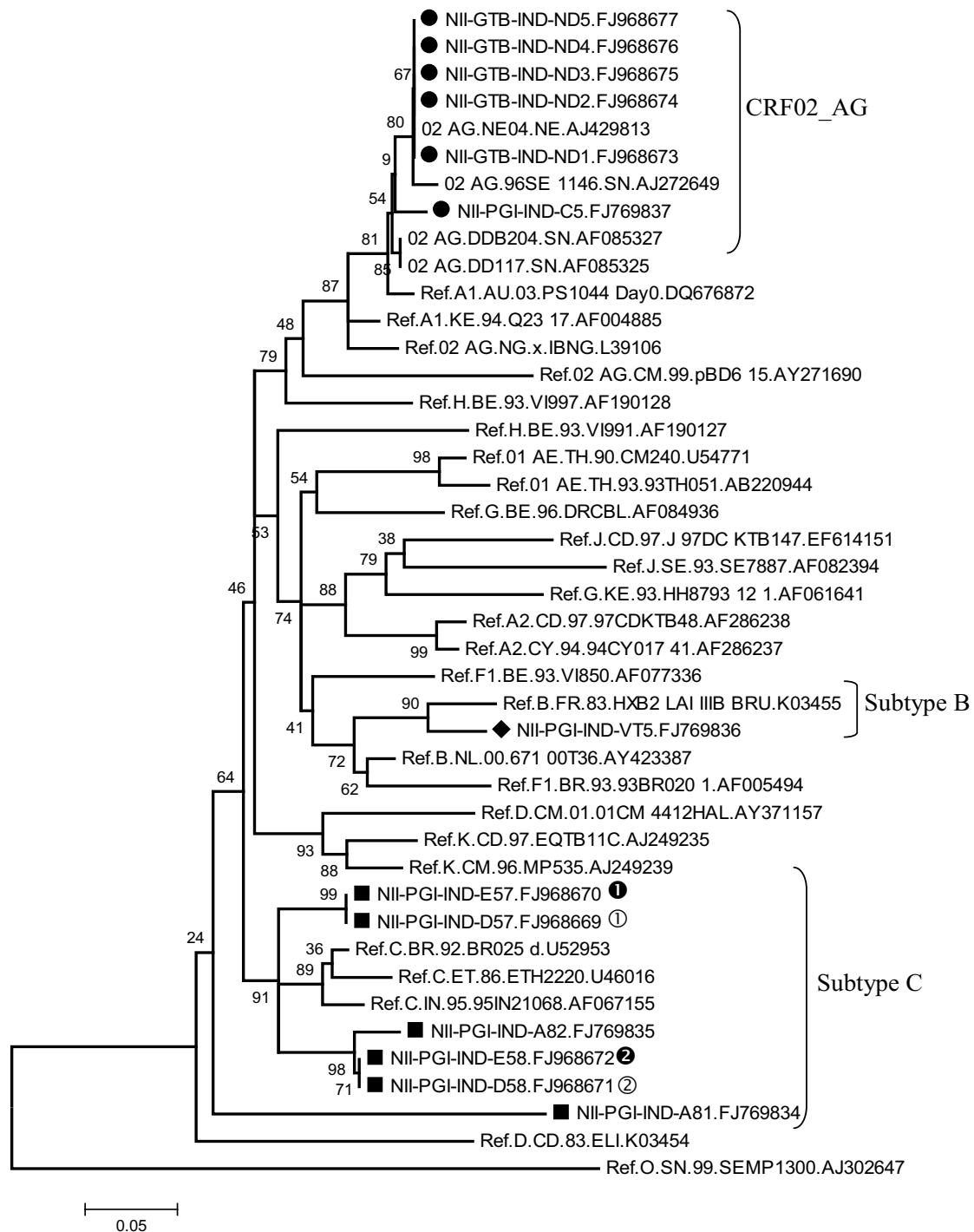


Figure 2
Phylogenetic analysis of the HIV-1 envelope sequences from North India. All the reference sequences from M-group & outlier group were retrieved from Los Alamos Data Base and used for constructing neighbor joining phylogenetic tree. Indian CRF02_AG strains are represented as 'Black Circles'; 'Black Square' for subtype C; and 'Black Diamond' as subtype B. The evolutionary history was created using Neighbor-Joining Method in MEGA4. Similar evolutionary pattern was detected when Maximum Likelihood and UPGMA methods were used (data not shown). Mother-child pairs were shown by filled and empty circles by numbers (1 and 2). Empty circles denote maternal envelope sequences while filled circles denote infant envelope sequences.

Ghana and Cameroon etc.) followed by Korea, Spain and France. The potential glycosylation sites present in V3 to V5 region varied from 7 (A81) to 15 (ND5 (data not shown)). This is important because in some instances hypo-glycosylated forms of envelope have been associated with better transmission and in their ability to interact with neutralizing antibody [18].

It was remarkable that sample A81 clearly showed CXCR4 coreceptor usage by both the programs (WebPSSM and Geno2Pheno) designed to predict HIV-1 coreceptor usage. This is important because earlier studies with Indian subtype C envelope showed exclusive use of CCR5 co-receptor [11]. It is important to note that Samples A82 and C5 showed discrepancy in their predicted coreceptor usage and this is because the two programs use different parameters [19,20].

Successful transmission of virus (judged by high bootstrap values) was observed in both the mother-child pair samples. It is important to study the functional implications of the changes in the viral gene sequences between mother-infant pairs to understand the molecular basis of successful transmission [21]. VT5 (subtype B) sample, as expected, showed CXCR4 usage and all of the CRF02_AG strains showed CCR5 usage.

In summary, we show for the first time presence and transmission of CRF02_AG HIV-1 strain in India (Delhi - Capital of India) and presence of subtypes B and C in North India. These observations will impact on the T-cell epitope based vaccine. The existence of multiple HIV-1 genetic subtypes in this region is likely to generate novel and complex recombinants.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

UN, VS, NG and AC carried out the experiments. SD, VGR, VKS, AW were responsible for providing the blood samples and their clinical characteristics. ACB is the principal investigator responsible for designing the work and writing the manuscript. VS and UN contributed equally to this work.

Additional material

Additional file 1

Identification of HIV-1 subtypes. RIP tool available in Los Alamos HIV Database was used to type four representative query sequences (HXB2 - panel A; FJ769836 - panel B; FJ968673 - panel C and FJ968672 - panel D) as indicated at the top of each square. Similarity of the sequences was compared with various subtypes with a window size 400 bp having significant threshold (0.9). It is noteworthy that HXB2 (Accession no.

K03455, panel A) and FJ769836 (NII-PGI-IND-VT5) (Panel B) were identified as subtype B (lemon green); FJ968673 (NII-GTB-IND-ND1) as 02_AG (dark green, panel C) and FJ968672 (NII-PGI-IND-E58) as subtype C (blue, panel D).

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