Research Letter

AIDS 2014, **28**:2319–2322

Novel tetra-peptide insertion in Gag-p6 ALIX-binding motif in HIV-1 subtype C associated with protease inhibitor failure in Indian patients

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A novel tetra-peptide insertion was identified in Gag-p6 ALIX-binding region, which appeared in protease inhibitor failure Indian HIV-1C sequences (odds ratio=17.1, $P < 0.001$) but was naturally present in half of untreated Ethiopian HIV-1C sequences. The insertion is predicted to restore ALIX-mediated virus release pathway, which is lacking in HIV-1C. The clinical importance of the insertion needs to be evaluated in HIV-1C dominating regions wherein the use of protease inhibitor drugs are being scaled up.

Subtype-specific differences have been observed in Gag-p6 motifs PTAPP and LYPx_nLxxL. Among them, HIV-1 subtype C (HIV-1C) has a higher frequency of duplications in the PTAPP-motif after antiretroviral therapy (ART)-failure [1,2]. Also, a natural deletion of L483Y484 residues was observed in the LYPxnLxxL motif in more than 95% of the sequences, which abrogates the ALIX-mediated particle release in absence of PTAP/TSG101 pathway in HIV-1C [3]. Considerable evidence suggests that ART-induced changes in the Gag-p6 region may modulate the therapy response and the viral fitness [4]. Studies have shown that selective drug pressure leads to accumulations of substitutions and insertions in Gag-p6, at sites distal from the mutations that render the virus highly resistant to protease inhibitors [5]. One key example is PTAPP-duplication in TSG101-binding motif in the Gag-p6, which affects the virological response to protease inhibitor drugs [6].

In this study, we investigated sequence variations of Gag-p6, using HIV-1C sequences of clinical isolates from India (HIV-$1C_{IN}$; $n = 158$), Ethiopia (HIV-$1C_{ET}$; $n = 73$) and Germany (HIV-$1C_{DE}$; $n = 125$) and their potential clinical impact. Therapy-naive and failure patients who attended several clinical cohorts from India [7,8], Germany [9,10] and Ethiopia [11] were included. From Ethiopia, only therapy-naive sequences are available. Among the patients, 61% (215/356) were therapy-naive. Data were pooled with Gag-p6 sequences ($n = 8589$) of major non-C subtypes and recombinants from the Los Alamos HIV Database.

Gag-p6, protease and partial reverse transcriptase were amplified and sequenced from the plasma viral RNA, as described [12–15]. Primary and acquired drug resistance mutations were evaluated using WHO mutations list 2009 [16] and International AIDS Society list 2013 [17], respectively. Subtyping was performed using three automated bioinformatics tools, as described by us recently [18]. Multiple-template homology models of the p6-ALIX complex were built in MODELLER 9v12 [19] using crystal structure of human ALIX/AIP1 in complex with a peptide fragment of the SIVmac239 and HIV-1 Gag-p6 proteins (PDB codes 2XS1 and 2R02) [20,21]. The models were analysed for accuracy using the DOPE statistical potential score [22]. The efficacy of the binding was analysed by computing the electrostatics using the Adapted Poisson-Boltzmann Solver software [23] and visualized using Chimera [24]. Statistical analysis was performed using SPSSv22.0 (IBM Corp, Armonk, New York, USA). The study was approved by ethical review committees in India, Germany, Sweden and Ethiopia. Written informed consent was obtained from the participants.

Cohort characteristics are presented in supplementary digital content (SDC) 1, http://links.lww.com/QAD/A560. The multiple sequence alignments of the Gag-p6 of representative strains are shown in SDC 2, http://links.lww.com/QAD/A560. Duplications of three to 13 amino acids in the TSG101-binding site were observed more frequently in the HIV-$1C_{DE}$ (29%) and HIV-$1C_{ET}$ (25%) sequences than in the HIV-$1C_{IN}$ (12%) sequences from therapy-naive individuals (SDC 2 and Fig. 1a). When therapy-naive and therapy-failure patients were compared, the duplications occurred more frequently in the therapy-failure Indians (12% vs. 25%; $P < 0.05$) but not in the German cohort (29% vs. 33%; $P = 0.69$) (Fig. 1a). Interestingly, there were subtype-specific differences in the accumulation of the PTAPP-duplication after therapy failure. The duplication occurred in greater frequency in HIV-1C (54%) than in HIV-1B (9.3%) and HIV-1F1 (17.6%) [2]. Previous controversial findings have reported that duplications of PTAPP were associated with ART in one group of population, but not in others [2,6,25–28]. In our study, we observed intra-HIV-1C specific preferential duplication in therapy-failure patients compared with therapy-naive individuals.

DOI:10.1097/QAD.0000000000000419

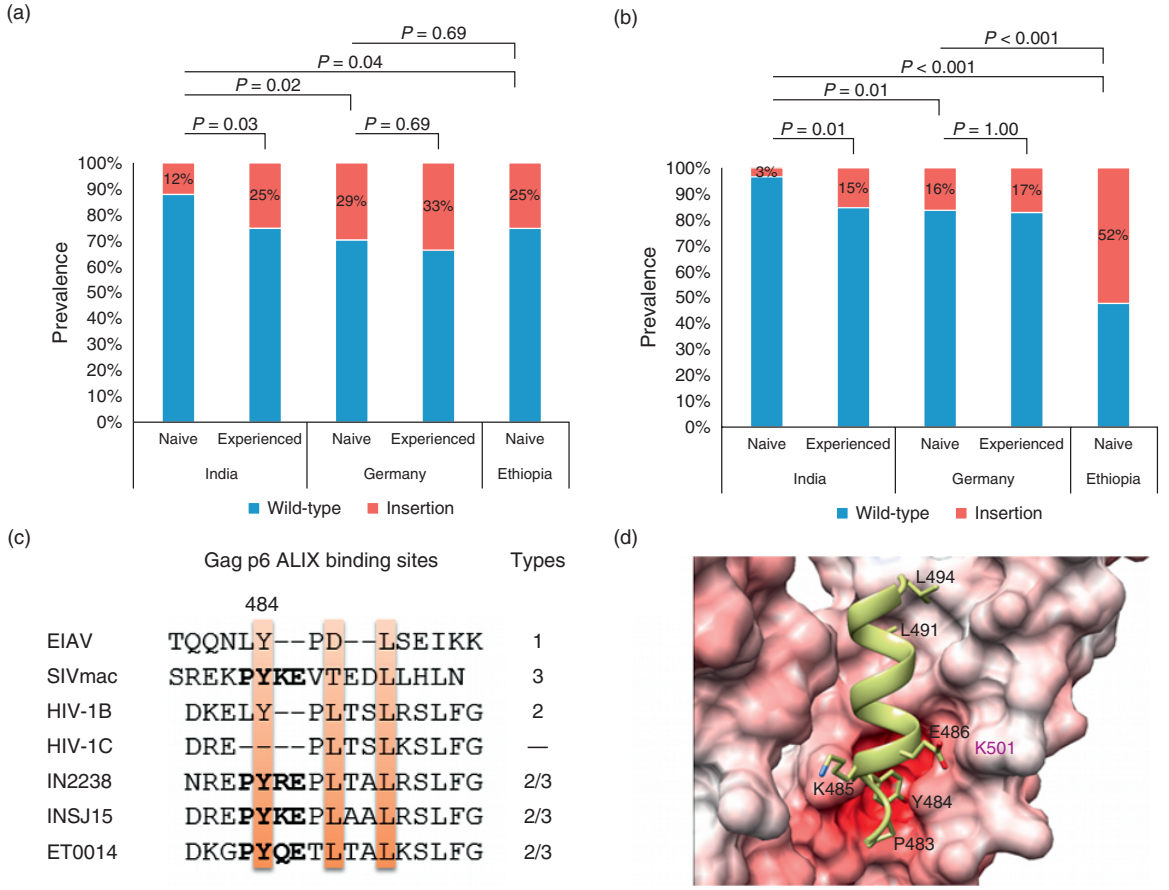

Fig. 1. Duplications and insertions in the Gag-p6. Prevalence of (a) duplications in the TSG101-binding PTAPP motif and (b) insertions in the ALIX-binding LYPx$_n$LxxL motif. (c) Different types of ALIX-binding sites in lentiviral Gag-p6 region, as described by Zhai *et al.* [20]. Representative clinical isolates with the PYxE insertion from the cohort are presented. All the strains had the key residues conserved (highlighted). In the 3D-molecular models of the Gag-p6-ALIX complex (d), ALIX is shown in surface representation, interacting with the Gag-p6 protein PYKE shown in ribbon and stick (side chain only) representation. The residues that belong to the ALIX-binding motif and the insertions are all labelled in black. The residues in ALIX that are crucial to mediate the interaction with Gag-p6 are labelled in magenta. The surface of the ALIX is coloured according to the electrostatic potential, with blue indicating positively charged regions and red indicating negatively charged regions. The intensity of the colour reflects charge intensity.

A novel tetra-peptide insertion [PYxE, where x represents either arginine (R), lysine (K) or glutamine (Q)] was observed in the C-terminal position of the Gag-p6 in the defective HIV-1C ALIX-binding domain. This PYxE insertion was observed in 52% of the untreated Ethiopian sequences, but significantly less often in the untreated German sequences (16%; $P < 0.001$), and even more seldom in the untreated Indian sequences (3%; $P < 0.001$) (Fig. 1b). When analysing sequences of therapy-naive individuals obtained from the Los Alamos HIV Database, the frequency of the PYxE insertion was not only much less common in therapy-naive patients infected with non-C subtypes (0.1%; $n = 4263$), but also less common in HIV-1C sequences from southern Africa (1%, $n = 2295$) and eastern African (3%, $n = 61$).

The PYxE insertion restores the key Y484 residue (SDC 2 and Fig. 1c). The 3D-molecular models of the Gag-p6-ALIX complex showed a specific interaction involving the inserted Y484 residue of Gag-p6 and the ALIX (Fig. 1d). Thus, the insertion variant would restore the binding of Gag-p6 to ALIX that was lost due to the L483Y484 deletion in HIV-1C and probably restores the ALIX-mediated virus release pathway.

Among the HIV-1C$_{IN}$ sequences from therapy-failing patients, the PYxE insertion was found significantly more often in protease inhibitor failure patients (six out of 10) than in those failing a nonprotease inhibitor containing regimen (five out of 62) [odds ratio; 95% confidence interval (95% CI) 17.1; 3.6–81.4; $P < 0.001$]. Among the other clinical and demographic parameters, the median CD4$^+$ T-cell count was significantly lower among the individuals with the PYxE insertion in the ALIX-motif than in those without the insertion (73 vs. 160 cells/μl; $P < 0.001$). The finding that Indian patients with PYxE

insertion had significantly lower $CD4^+$ T-cells than those without could possibly indicate that the virus with the insertion is more pathogenic. This suggestion was further supported by the very low $CD4^+$ T-cells in the three therapy-naive Indian individuals with the insertion before initiating ART (22, 90 and 56 cells/μl). These three patients were followed for 2 years after initiation of therapy. Their gain of $CD4^+$ T-cells was then suboptimal and $CD4^+$ T-cell count never reached more than 350 cells/μl (data not shown).

In conclusion, we have for the first time identified a PYxE tetra-peptide insertion in the ALIX-binding motif, which appeared in HIV-$1C_{IN}$ infected patients with protease inhibitor therapy failure, but occurred naturally in more than half of therapy-naive HIV-$1C_{ET}$ infected patients. We therefore hypothesize that the viral genetic background might have influenced the preferential selection of these insertions. The insertion probably restores the ALIX-mediated virus release pathway, which is lacking in HIV-1C and the virus with this insertion might be more pathogenic. To better elucidate the clinical importance of this novel insertion, further investigations are needed when protease inhibitor drugs are used in countries with a high prevalence of HIV-1C.

Acknowledgements

The study was partially funded by Swedish International Developing Agency, CHAIN FP7 EU, the Swedish Civil Contingencies Agency [SWE-2009-151] and the Swedish Research Council [521-2012-3476 and 2007-7092]. M.L.E. acknowledge the funds received from R01MH067513 from the National Institute of Mental Health (NIMH) (Bethesda, Maryland, USA) for the study Examining ART Adherence Issues in Bangalore India. V.R.P. and V.R.R. wish to acknowledge support from the National Institute of Health, USA (NIH) grant 1R01MH083579 (to V.R.P.).

The study was partially funded by Swedish International Developing Agency, CHAIN FP7 EU, the Swedish Civil Contingencies Agency [SWE-2009-151], the Swedish Research Council [521-2012-3476 and 2007-7092], National Institute of Mental Health grant R01MH067513 (to M.L.E.), National Institute of Health grant 1R01MH083579 (to V.R.P.) and The Wellcome Trust/DBT India Alliance grant (to M.S.M.)

Conflicts of interest

There are no conflicts of interest.

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Received: 27 June 2014; revised: 15 July 2014; accepted: 16 July 2014.

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