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Prediction of drug-resistance in HIV-1 subtype C based on protease sequences from ART naive and first-line treatment failures in North India using genotypic and docking analysis

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ABSTRACT

Genotyping reveal emergence of drug resistance (DR)-related mutations in HIV-1 protease (PR) gene in the first-line treatment failure patients as per Stanford DR database. In order to have a subtype C specific prediction model, a three dimensional structure of local wild type C variant is created and the identified mutations were introduced to assess the mutational effects on protease inhibitors (PI) in a homology model.

We estimated viral load, CD4 count and conducted DR genotyping in HIV isolates from 129 therapy naive and 20 first-line treatment failure individuals. Several genotypic variations, as compared to subtype B sequence in the Stanford gene database were detected in HIV-1 subtype C isolates from treatment naive individuals. Among these, nine mutations (12S, 15V, 19I, 36I, 41K, 63P, 69K, 89M, 93L) occurred in more than 60% of the isolates and were considered as local wild type for molecular modelling studies. No major mutations were seen in the PR sequences in isolates from treatment-naive individuals, although isolates from two patients had T74S mutation, known to be associated with reduced susceptibility to nelfinavir (NFV) and a combination of M36I, H69K and L89M mutations found in isolates from 77 patients (59.7%), considered to be conferring resistance to tipranavir (TPV) according to ANRS algorithm. Among the first-line treatment failures, an isolate from one patient showed L33F, I47T, M46G, and G48E mutations conferring intermediate resistance to saquinavir (SQV) and lopinavir (LPV). Though the docking energy scores are in agreement with this interpretation for SQV, it, however, indicated these mutations to be causing intermediate to high level resistance to atazanavir (ATV) and tipranavir (TPV) but making it susceptible to LPV. The patient finally responded to a second-line regimen containing 3TC, AZT and LPV with significant viral suppression.

All the DR genotyping studies analyse the results using available databases which are all based on subtype B specific sequences. The proposed homology model in this study is unique, as it may predict subtype C specific susceptibility criteria for the available PIs.

1. Introduction

Drug resistance (DR) is inevitable consequence of incomplete suppression of Human Immunodeficiency virus (HIV) replication. The rapid turnover of HIV-1 RNA and its genetic variability leads to the production of many variants with decreased drug susceptibility (Ho et al., 1995; Perrin and Telenti, 1998). With the emergence of failure to first-line treatment consisting a combination of Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) in India, National AIDS Control Organisation (Government of India) introduced second-line treatment in 2009 which includes one protease inhibitor (PI) and two NRTIs in triple drug combination therapy.

Protease inhibitors (PIs), originally designed and tested against the subtype B viruses are currently also made available in other parts of the world including Indian subcontinent where the epidemic is dominated by subtype C (Arora et al., 2008; Deshpande et al., 2004; Sen et al., 2007). Importantly, the Stanford DR mutation database is mainly subtype B sequence based, and some reports including data (unpublished) from our own laboratory have indicated that protease (PR) gene in subtype C displays a reasonable level of sequence variability from subtype B and so rise the question of whether the DR mutations mentioned in the Stanford database will behave in a similar fashion for subtype C PR as well (Kinomoto et al., 2005)? Further, the mutations like M36I and I15V

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when present together are responsible for shortening the side chain in PR, which reduces the extent and strength of van der Waals force and hence loss of hydrophobic pocket as also seen with L63P. This could alter the local structure of this region (Kandathil et al., 2009). These polymorphisms are commonly detected in the majority of HIV-1 subtype C isolates from treatment-naive individuals in India (Arora et al., 2008; Deshpande et al., 2004; Gupta et al., 2010; Rajesh et al., 2009; Sen et al., 2007; Soundararajan et al., 2007).

A homology model of PR was generated after introducing the genetic variations, commonly found in subtype C isolates as detected by us as well as reported by others from India, and we calculated the binding energy for various PIs. Our results indicated that in HIV-1 subtype C isolated from one of the infected individuals had major (L33F, I47T) and minor (M46G, G48E) mutations; Whereas the Stanford database indicated potentially low level resistance to atazanavir and tipranavir with these mutations, our proposed model predicted intermediate to high level resistance to these two PIs. Although computational PR models are mostly in agreement with most PI resistance predications for the rest of PIs as per Stanford database, this additional information will allow us to predict the best PI for a given set of mutations in subtype C infected individuals.

2. Materials and methods

2.1. Patient selection and sample collection

Mutation studies included 129 patients naive for antiretroviral therapy and 20 patients who had already experienced first-line ART consisting a combination of NNRTIs and NRTIs for 4–5 years and had shown clinical as well as virological signs of treatment failure (HIV-1 RNA viral load >1000 copies/ml). The study was approved by the Institutional Ethics Committee. After informed consent, 3 ml of venous blood was drawn in a K_3EDTA vacutainer (Becton Dickinson, USA) for CD4 count, viral load and genotyping for DR mutation analysis.

2.2. CD4 count and plasma viral load

The CD3/CD4+ cells were enumerated by flowcytometry using Tritest CD4 kit (BD Biosciences Immunocytometry Systems, San Jose, CA) and plasma viral load was estimated using Cobas amplicor HIV-1 monitor test, version 1.5 (Roche, Branchburg, NJ) as per the manufacturer's guidelines.

2.3. Drug resistance genotyping

For DR genotyping, the viral RNA was isolated from plasma using QIAamp viral RNA isolation kit (QIAGEN, Valencia, CA) and reverse transcribed to make complementary DNA (cDNA) copies using a cDNA synthesis kit (MBI Fermentas, Vilnius, Lithuania). The PR region (5–99 codon) was amplified using PR specific nested primers as previously described (Arora et al., 2008). The PCR products were sequenced in an automated DNA sequencer (ABI PRISM 3730 version 3.0, Applied Biosystems, Foster city, CA). All sequences were subjected to quality assessment and determination of DR mutation profile using the Stanford DR Database.

2.4. Generation of wild-type and mutant HIV-1 subtype C protease structure models

Protease sequences of HIV-1 subtype C found in this geographical region of India were compared with sub-type B using Stanford Database. More than 60% of PR sequences in HIV-1 subtype C isolates from ART-naïve individuals showed polymorphism at codon 12S, 15V, 19I, 36I, 41K, 63P, 69K, 89M, 93L. Highly identical structures of reported sequences were retrieved from the protein data bank (PDB) using BLASTP program. The PDB IDs of best hits with co-crystallized ligands were: 2O4K (atazanavir [ATV]), 3LZS (darunavir [DNV]), 3N3I (saquinavir [SQV]), 2R5P (indinavir [IDV]), 2Q5K (lopinavir [LPV]), 2R5Q (nelfinavir [NFV]) and 2O4N (tipranavir [TPV]). The multiple sequence alignment of HIV-1 subtype C sequence was performed with these protein PDB IDs using ClustalX 2.0 program. The molecular modelling and docking studies of wild subtype C and mutated proteins were carried out using the protein modelling module in Accelrys Discovery Studio 2.5 and AutoDock 4.2 program, respectively (Morris et al., 1998). Homology model of the wild HIV-1 subtype C protease was built using the crystal structure 3LZS as a template (Bandaranayake et al., 2010) which is having the maximum identity. The final generated model was optimized and checked by Ramachandran plot. Mutational models for major, minor and combined (major and minor) mutations were also built from developed model and Ramachandran plots were checked for optimization. The docking studies of wild type and mutated models of HIV-1 subtype C protease were performed for all the co-crystallized ligands and free binding energies were calculated. The AutoDock program is based on a linear regression analysis and AMBER force field for free-energy scoring functions. It uses a master equation for the free energy calculation, which includes six parameters of model viz. dispersion/repulsion (ΔG_{vdw}), electrostatic interactions (ΔG_{elec}), hydrogen bonding (ΔG_{hbond}), deviation from covalent geometry ($\Delta G_{conform}$), internal ligand torsional constraints (ΔG_{tor}), and desolvation effects (ΔG_{sol}):$\Delta G = \Delta G_{vdw} + \Delta G_{elec} + \Delta G_{hbond} + \Delta G_{conform} + \Delta G_{tor} + \Delta G_{sol}$

The binding energy calculations were performed by placing the protein inside user defined grid parameters. All the drug molecules were built using SYBYL7.1 molecular modelling package. The molecules were minimized using Tripos force field, Gasteiger Huckel (Gasteiger and Marsili, 1980), partial atomic charges and Powell's conjugate gradient method (Powell, 1977). Water molecules were removed from protein structures. Also, polar hydrogen molecules were added and non-polar hydrogen molecules were merged. Finally, AutoDock atom type and Kollman united atom charge was added. Grid parameters for all proteins were set in such a way that it includes active site and a significant surrounding surface. The Lamarckian genetic search algorithm was employed for docking studies and docking run was set to 30. All other parameters were set to default value: maximum number of energy evaluation 2,500,000 per run; maximum number of generations was increased to 27,000.

3. Results

3.1. Demographic details of the population

Of the 129 HIV-1 positive drug naïve individuals, recruited into the study, during the period spanning 2008–2009, a total of 120 (93%) individuals revealed a history of heterosexual transmission, while in five cases transmission was suspected to be from mother to child and two each revealed history of injection drug use (IDU) and homosexual exposures. All individuals were from north India with the majority of participants being males (58%). In male subjects, the median CD4 count was 250.8 cell/μl, while in female subjects it was 342.3 cells/μl. The age ranged from 7 to 60 years.

Twenty patients receiving first-line treatment for 3–4 years (median time of therapy 45 months; interquartile range 30–48 months) and showing clinical signs of treatment failure with baseline median CD4 count of 123 cells/μl (interquartile

range 102–199 cells/μl) and viral loads ranging from 2882 to 1.83×10^6 copies/ml, were also enrolled in the study (Table 1).

3.2. Pattern of mutations in HIV-1 subtype C protease gene

3.2.1. Therapy Naive

Several genotypic variations, as compared to subtype B sequence in Stanford DR database and termed as polymorphism, were detected in HIV-1 subtype C isolates from treatment-naive individuals recruited in our study. Among these, nine mutations (12S (63.2% (67/106)), 15V (63.4% (71/112)), 19I (63.6% (75/118)), 36I (71.3% (92/129)), 41K (90.6% (117/129)), 63P (61.2% (79/129)), 69K (98.4% (127/129)), 89M (78.3% (101/129)), 93L (94.6% (123/129))) occurred in more than 60% of the HIV-1 isolates and were considered as local wild type for molecular modelling studies.

No major HIV DR mutations were seen in the PR sequences from plasma samples of treatment-naive individuals, although isolates from two patients had a T74S mutation known to be associated with reduced susceptibility to NFV and a combination of M36I, H69K and L89M mutations found in isolates from 77 patients (59.7%), considered to be conferring resistance to TPV according to ANRS algorithm. Other mutations associated with low level resistance to PI drugs detected were: L10(I/M)(n = 3/105, 2.85%), I13V(n = 10/107, 9.35%), K20R(n = 4/119, 3.36%), E35(D/N)(n = 19/129, 14.73%), M36I(n = 60, 73.17%), M36(V/L)(n = 13, 15.86%), R41(K/H)(n = 75, 91.46%), K43R(n = 1, 1.22%), I62V(n = 3, 3.66%), L63(P/T/S/A/C/H/N)(n = 77, 93.9%), I64(V,M,L)(n = 14, 17.07%), H69K(n = 82, 100%), A71V(n = 1, 1.22%), T74S(n = 2, 2.44%), and V82I(n = 6, 7.32%).

3.2.2. First-line treatment failure

The analysis of PR gene sequences of isolates from 20 first-line ART-failure patients showed one isolate with L33F, I47T mutations depicted as major PI resistance mutations and M46G, G48E as minor PI resistance mutations as per the Stanford DR database. The L33F was selected by FPV, DRV, LPV, ATV, and TPV, and conferred resistance to these drugs. While I47V has been listed by the Stanford DR database to decrease the susceptibility to FPV, ATV, IDV, LPV, TPV, and DRV; I47A usually occurs with V32I and in this setting causes high-level LPV and FPV resistance and possibly decreases DRV susceptibility.

The I47T detected in one isolate in our study is a highly unusual mutation at this position. On the other hand M46I/L is known to decrease susceptibility to IDV, NFV, FPV, LPV, and ATV as per the Stanford DR database when present with other mutations. M46V is an uncommon PI-selected mutation at this position, while M46G detected in our isolate is a highly unusual mutation. Another mutation G48V is known to cause high-level resistance to SQV, intermediate resistance to ATV and NFV, and low-level resistance to IDV and LPV. While G48M/A/S/T/Q are uncommon PI-selected mutations of uncertain effects, the G48E is a highly unusual mutation at this position. Other mutations likely to affect PI drug susceptibility found in our study isolates were L10I and T74S. The T74S is associated with reduced NFV susceptibility and L10I/V/F/R/Y is associated with resistance to most PIs when present with other mutations.

3.3. Molecular modelling and docking studies of DR mutations in protease–inhibitor complexes

The homology model was built for wild-type HIV-1 subtype C protease using template 3LZS (wild-type subtype B protease) (Bandaranayake et al., 2010). Fig. 1 is showing the sequence alignment of HIV-1 subtype C and template sequence. Ramachandran plot was checked (Fig. 2) for the quality of developed model. It was found that 92.4% of the total residues were located in the allowed core region and 7.6% in the additionally allowed region in the model (Fig. 2). There were no residues in the disallowed region, so the developed model was best fit to Ramachandran plot. This model was further used for mutational studies of HIV-1 subtype C protease using minor and major mutations. The mutational models for minor, major and combined mutations were built. Ramachandran plot showed good fitting of all the mutated models. Fig. 3 displays superimposed view of the template and mutated model (major and minor). The crystal structure of HIV-1 protease-DRV complex binding pattern is presented in Fig. 4, which clearly shows that active site is hydrophobic in nature. This inhibitor interacted with residues L23, D25, G27-D30, V32, I47-I50, T80-V82, I84, L23′, D25′, G27′-D30′, V32′, I47′-I50′, T80′-V82′, and I84′ in the active site. Some residues, i.e. D25, G27, D29, I50, D25, A28, and D30, consistently made extensive contacts with the inhibitors, while others made minimal contacts. Active site residues D25, D29 and D30 play important role in binding with the inhibitor. Structural

Table 1
Genotypic resistance results in 20 patients on ART with virological failure (HIV-1 RNA > 1000 copies/mL).

Patient ID	ART regimen started	Months	Viral load	Subtype	Major mutation	Minor mutation	Protease mutations
204	ZDV + 3TC + NVP	27	2880	C			Q7X, T12S, K14R, I15V, L19I, M36I, R41K, H69K, L89M, I93L
205	ZDV + 3TC + EFV	36	94,700	C			Q7X, T12S, I15V, L19I, M36I, R41K, L63P, H69K, I93L
207	D4T + 3TC + NVP	47	715,000	C			Q7X, T12S, I15V, L19I, M36I, R41K, L63P, H69K, I93L
208	D4T + 3TC + NVP	36	6.78×10^4	C			I15V, L19T, M36I, R41K, L63T, H69K, I93L
210	D4T + 3TC + NVP	46	62,000	C			T12S, I15V, L19T, M36I, R41K, L63P, H69K, I93L
211	ZDV + 3TC + EFV	35	94,700	C			T12S, K14R, I15V, G16E, L19I, M36I, L63S, H69K, L89M, I93L
212	D4T + 3TC + NVP	50	89,900	C			I15V, L19M, M36I, R41K, L63P, H69K, L89M, I93L
213	ZDV + 3TC + NVP	26	17,900	C			Q7X, L19I, M36I, N37S, R41K, L63P, H69K, L89M, I93L
214	D4T + 3TC + NVP	25	4.82×10^5	C			T12S, K14R, L19I, M36I, N37D, L63P, H69K, L89M, Q92R, I93L
215	D4T + 3TC + NVP	60	99,600	C			Q7X, T12S, I15V, L19V, M36I, R41K, L63S, H69K, L89M, I93L
217	D4T + 3TC + NVP	45	8.61×10^5	C			Q7X, T12S, K14R, I15V, L19I, M36I, N37D, L63P, H69K, K70R, L89M, I93L
218	D4T + 3TC + NVP	42	1.92×10^5	C			T12S, I15V, L19T, M36I, R41K, L63P, H69K, I93L
219	D4T + 3TC + NVP	45	2.6×10^5	C		**L10I**	Q7X, T12S, I13V, I15V, L19I, N37T, R41K, L63P, I64M, H69K, L89M, I93
221	D4T + 3TC + NVP	29	4.01×10^4	C			T12S, I15V, G16A, L19I, M36I, R41K, L63P, H69K, I93L
222	ZDV + 3TC + NVP	51	4.55×10^5	B			Q7X, T12P, L19S, K20R, E35D, R41K, D60E, L63P, I93L
223	D4T + 3TC + NVP	41	1.83×10^6	B			K14R, L33V, N37C, I64V
226	ZDV + 3TC + EFV	48	1.13×10^4	C	**L33F, I47T**	**M46G, G48E**	Q7X, I15V, L19I, E35K, M36V, P39X, P44L, K45D, G49E, R57K, I62V, L63P, H69K, V82I, R87K, L89M, I93L, G94E
228	ZDV + 3TC + EFV	82	1.74×10^4	C			Q7X, I15V, K20R, M36I, R41K, I62V, L63P, H69K, T74A, L89M, I93L
229	D4T + 3TC + NVP	42	2.46×10^5	C			T12S, I15V, L19I, M36I, R41K, L63P, H69K, I93L
230	D4T + 3TC + NVP	45	1.79×10^4	C		T74S	Q7X, T12S, K14R, I15V, L19T, M36I, R41K, L63T, H69K, L89M, I93L

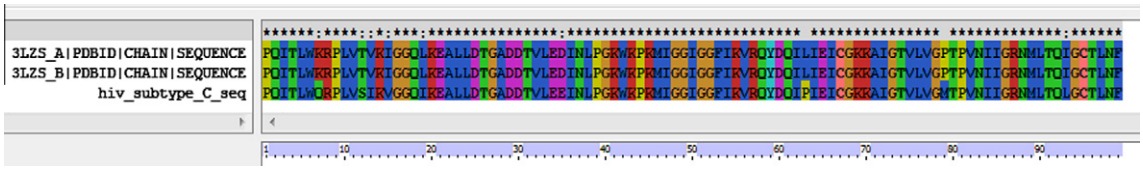

Fig. 1. Sequence alignment with 3D crystal structure of HIV-1 protease (PDB ID 3LZS) and sequenced data of HIV-1 subtype C found in North Indian patients showing >95% similarity.

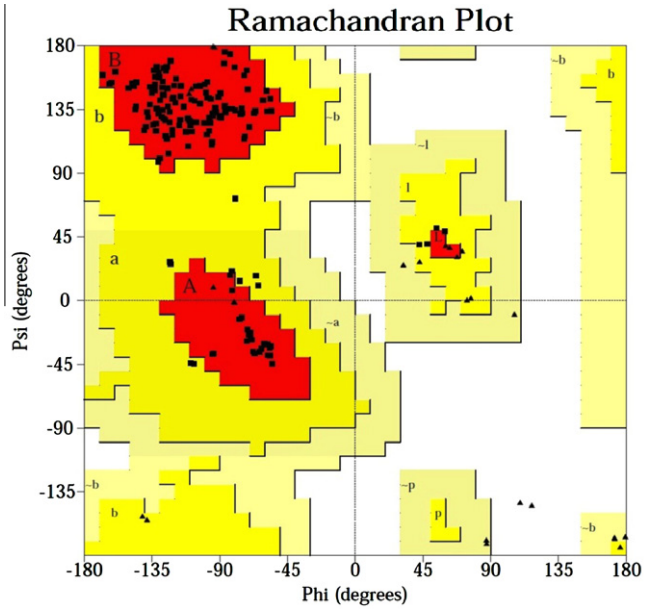

Fig. 2. Ramachandran plot of modelled protein HIV-1 subtype C was obtained by PROCHECK validation package. The 92.4% of the total residues were located in the allowed core region and 7.6% in the additionally allowed region, while no residue was restrained to the disallowed region.

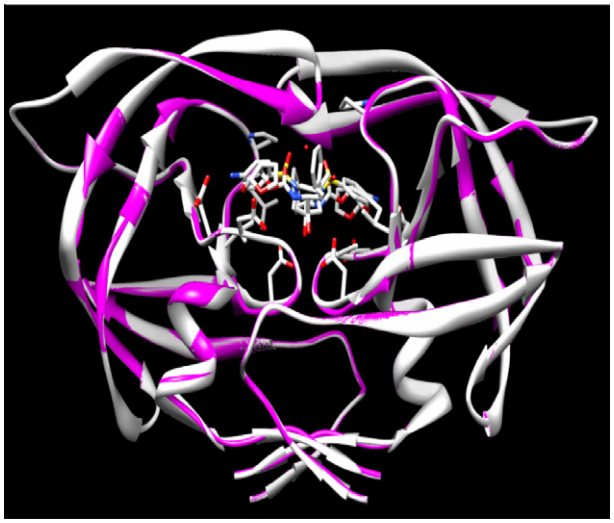

Fig. 3. Superposition of the crystal structures of HIV-1 protease PDB ID 3LZS (subtype B, white colour) in complex and with mutated model subtype C (magenta) with mutations 12S, 15V, 19I, 36I, 41K, 63P, 69K, 89M and 93L when compared with subtype B, shows that most of the core protease structure extensively overlaps. The ligand shows two conformations in the active site of crystal structure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analysis of proteins that contain resistance mutations indicated that mutations at drug-binding sites usually alter the tight packing

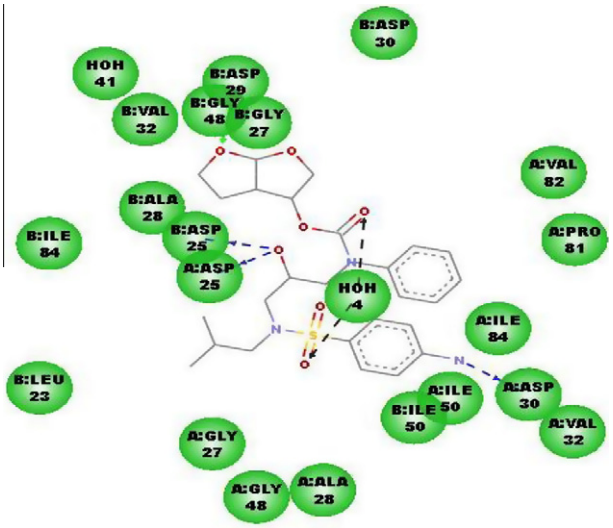

Fig. 4. The crystal structure of HIV-1 protease complex with Darunavir shows the binding interaction into the active site residues: D25, G27-D30, V32, I47-I50, T80-V82, I84, L23′, D25′, G27′-D30′, V32′, I47′-I50′, T80′-V82′, and I84′ (green) of HIV-1 protease. The dotted lines are showing H-bonding the active site residues D25, D30, D25′ and D29′. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between the binding drug and its protein without substantial change in overall conformation (Ala et al., 1997; Erickson and Burt, 1996).

3.4. Evaluation of binding free energy of inhibitors with wild and mutated HIV-I subtype C protease active site

All the inhibitors were docked into the active site of wild and mutated HIV-1 subtype C protease. The binding free energies were analyzed during each docking process. HIV-1 PR has two identical chains in which residues D25, D29 and D30 are located in both chains showing H-bonding. Other residues G49, I50, P81, V82 and I84 showed both hydrophobic and hydrogen bonding interactions with all inhibitors. Table 2 shows estimated free binding energy of various inhibitors: ATV, DNV, IDV, NFV, SQV, TPV for major (33F and 47T), minor (46G, 48E) as well as both types of mutations existing together. These results are in good agreement with the Stanford DR database except for ATV, LPV, NFV and TPV (Table 1).

4. Discussion

The present study aimed at elucidating the consequence of the emergence of DR-associated mutations and naturally occurring polymorphisms in the PR gene of HIV-1 subtype C isolates from cohorts of infected individuals from northern states of India, who have not been exposed to any antiretroviral treatment and the first-line treatment failures who have not earlier received PIs as part of the ART regimen. A three dimensional homology model of

Table 2
Minimum estimated free energy of binding (Kcal/mol) at 300 K Temperature for every docking procedure of PI inhibitor in circulating HIV-1 subtype wild type protease and the mutations in the study.

HIV protease inhibitors	HIV-1 subtype C: studies wild type Energy score	PR major: 33F, 47T Energy score	PR minor: 46G, 48E Energy score	PR Major + Minor: L33F, I47T, M46G, G48E Energy score	Stanford data base
Atazanavir	−9.17	−8.87	−8.86	−8.17	Potential low-level resistance
Darunavir	−8.95	−8.78	−8.58	−8.85	Susceptible
Indinavir	−10.52	−9.96	−10.73	−10.87	Susceptible
Lopinavir	−9.74	−9.89	−10.68	−9.73	Potential low-level resistance
Nefinavir	−11.51	−11.34	−12.03	−11.86	Low-level resistance
Saquinavir	−11.61	−10.67	−10.76	−10.76	Intermediate resistance
Tipranavir	−10.58	−9.25	−10.88	−9.48	Potential low-level resistance

HIV-1 PR of subtype C was developed to determine the effect of mutations on the drug binding site of the protein for better understanding the changes in susceptibility to PIs. This would possibly aid in determining the right treatment regimen for the HIV-1 (subtype-C)-infected individuals.

Great strides have been made over the past few years to diagnose HIV DR using in-house developed low cost kits or standard DR diagnostic kits in India (Kumarasamy et al., 2008; Deshpande et al., 2010; Gupta et al., 2010; Neogi et al., 2010). In the present study, no major HIV DR mutations were seen in the PR sequence of HIV-1 isolates from plasma samples of treatment-naive individuals. Isolates from only 2/129 (1.55%) patients had the T74S mutation which is known to be associated with reduced NFV susceptibility. This is a very low frequency and a probable explanation could be that the PIs have just been introduced recently by the Government of India in the ART program in India in the second-line treatment regimens, which is offered to only those patients who fail the first-line treatment. Thus it is now important to closely monitor the emergence of DR related mutations in the PR gene.

Among the patients failing first-line treatment (containing NRTI and NNRTI only), only one of the twenty HIV-1 isolates had 33F, 47T, 46G and 48E mutations, which are likely to confer potentially low-level resistance to PIs like ATV, FPV, LPV, TPV, low-level resistance to NFV and intermediate resistance to SQV (as per Stanford DR database).

Since the Stanford DR database is subtype B sequence-based, it was envisaged to understand if these mutations have similar effects on susceptibility to PIs in the subtype C infected individuals. Therefore, we have taken the consensus sequence of 129 therapy-naive subtype C PR sequences to construct a homology model as wild type. The mutated models created by inserting major and minor mutations, optimized and checked by Ramchandran Plot before calculation of PI binding energy revealed clearly that the modelled structures of both wild-type and mutant HIV-1 protease–drug complexes were consistent with the crystallographic structures, with root mean square deviation ranging from 0.5 to 1.2 Å (Chen et al., 2001; Shenderovich et al., 2003; Weber and Harrison, 1999), which suggests that the quality of these modelled structures reached the level useful for facilitating structure-based study and prediction of resistance.

Binding energy of ATV was decreased ($\Delta G = -0.3$ to -1.0 Kcal/mol) when either major (33F, 47T) or minor (46G, 48E) protease mutations were inserted. These mutations had an additional effect on decreasing the binding energy of ATV when present together ($\Delta G = -1.0$ Kcal/mol). This data agreed well with Stanford DR database which suggested a low level resistance when these mutations are present individually, but causes high resistance when all present together. In case of DNV and IDV, energy did not change significantly after introduction of mutations in the wild type sequence. However, with a major mutation, IDV exhibited low level resistance, which is contrary to Stanford DR database. The presence of minor or all combined mutations showed susceptibility to this inhibitor (See Table 1). In the case of SQV, energy difference of nearly 1.0 Kcal/mol was observed after introduction of all the four mutations together. Decrease in binding energy of this drug in the presence of these mutations, concurs with the Stanford DR database-based prediction. For LPV, binding energies were increased from −0.15 to −0.94 with major and minor mutations present separately, but no changes in binding energy occurred in the presence of all mutations together. LPV exhibited susceptibility with minor and major mutations which is contrary to the Stanford DR database prediction. Similarly, NFV exhibited susceptibility in minor and combined mutations due to the increase in binding energy which again is contrary to Stanford DR database prediction (See Table 1). In case of TPV, the presence of major or all mutations together predict intermediate to high level resistance due to increase in the binding energy. However, according to the Stanford DR database, it should have potential of low level resistance.

A combination of M36I, H69K and L89M polymorphisms was found in 63.4% of HIV-1 isolates sequenced in the study population. Accumulation of these mutations correlated with reduced virologic response to TPV, according to the ANRS algorithm (http://www.rega.kuleuven.be/cev). TPV is one of the latest approved PIs for the treatment of HIV (Poveda et al., 2008) and its susceptibility in the study population is of concern. Phenotypic studies to determine the efficacy seems important before TPV is introduced in the treatment regimen in countries where HIV-1 subtype C is prevalent. Incidentally these are the countries which carry the highest burden of HIV/AIDS in the world.

One of the HIV-1 isolates showing major and minor PI mutations effecting drug susceptibility was from a female among first-line treatment failure cases recruited in the study, whose ART was started in Nov. 2005 (first-line regimen: ZDV + 3TC + EFV). With the history of consistently low CD4 count (<250 cells/μl) over a period of 4–5 years and high viral load of 1.13×10^4 copies/ml, DR genotyping and molecular docking study was performed before shifting to second-line treatment. The patient responded to the second-line ART, consisting a combination of D4T + 3TC + ZDV_LPV, since a repeat viral load after 6 months of start of second-line treatment showed <400 copies/ml. This confirmed the validity of the proposed model which indicated susceptibility to LPV in spite of the existence of these mutations, while the Stanford DR database suggested a potentially low level resistance to this drug.

We have attempted to demonstrate a correlation between the mutation pattern in the PR gene of HIV-1 subtype C and the level of reduced PI susceptibility using docking studies of homology models and PIs. The results obtained do overlap to a large extent the DR interpretation from a well tested and universally accepted Stanford DR database. However, since the Stanford DR database is based on sequences obtained from subtype-B isolates, it always

leaves an element of discrepancy due to existence of genetic variability between the two subtypes of HIV-1. This justifies the need for a more closely related analysis and a prediction model that is based on subtype C sequences. Consequently, the homology model proposed here is a modest attempt in that direction and provides a feasibility of predicting the susceptibility or resistance to various drugs and will allow the clinicians to make a rational choice of treatment regimen especially in treatment failure cases. Yet, we believe that the validation of this model coupled with phenotypic studies using larger number of sequences will reveal its usefulness.

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