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Bayesian network analyses of resistance pathways against efavirenz and nevirapine

Koen Deforche^a, Ricardo J. Camacho^b, Zehave Grossman^c, Marcelo A. Soares^d, Kristel Van Laethem^a, David A. Katzenstein^e, P. Richard Harrigan^f, Rami Kantor^g, Robert Shafer^e, and Anne-Mieke Vandamme^a on behalf of the non-B workgroup

^aRega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

^bMolecular Biology Laboratory, Centro Hospitalar de Lisboa Ocidental, Lisbon, Portugal

^cChaim Sheba Medical Center, Ministry of Health, Tel-Aviv, Israel

^dDepartamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

^eDivision of Infectious Diseases, Stanford University, Stanford, California, USA

^fResearch Labs, BC Centre for Excellence in HIV/AIDS, Vancouver, Canada

^gDivision of Infectious Diseases, Brown University, Providence, Rhode Island, USA

Abstract

Objective—To clarify the role of novel mutations selected by treatment with efavirenz or nevirapine, and investigate the influence of HIV-1 subtype on nonnucleoside reverse transcriptase inhibitor (nNRTI) resistance pathways.

Design—By finding direct dependencies between treatment-selected mutations, the involvement of these mutations as minor or major resistance mutations against efavirenz, nevirapine, or coadministered nucleoside analogue reverse transcriptase inhibitors (NRTIs) is hypothesized. In addition, direct dependencies were investigated between treatment-selected mutations and polymorphisms, some of which are linked with subtype, and between NRTI and nNRTI resistance pathways.

Methods—Sequences from a large collaborative database of various subtypes were jointly analyzed to detect mutations selected by treatment. Using Bayesian network learning, direct dependencies were investigated between treatment-selected mutations, NRTI and nNRTI treatment history, and known NRTI resistance mutations.

Results—Several novel minor resistance mutations were found: 28K and 196R (for resistance against efavirenz), 101H and 138Q (nevirapine), and 31L (lamivudine). Robust interactions between NRTI mutations (65R, 74V, 75I/M, and 184V) and nNRTI resistance mutations (100I, 181C, 190E and 230L) may affect resistance development to particular treatment combinations. For example, an interaction between 65R and 181C predicts that the nevirapine and tenofovir and

lamivudine/emtricitabine combination should be more prone to failure than efavirenz and tenofovir and lamivudine/emtricitabine.

Conclusion—Bayesian networks were helpful in untangling the selection of mutations by NRTI versus nNRTI treatment, and in discovering interactions between resistance mutations within and between these two classes of inhibitors.

Keywords

Bayesian network; efavirenz; HIV-1 antiviral resistance; nevirapine

Introduction

Genotypic interpretation systems predict the therapy response for various drugs [1,2] based on the presence of mutations at positions associated with drug resistance. For many mutations observed after treatment failure in clinical isolates, their role is not sufficiently known and the impact of the large natural variation of HIV-1 is still debated. Different prevalences of known resistance-associated mutations and new mutations are seen in different subtypes [3 – 9], and with a few exceptions, these differences in prevalence could not be explained by different evolutionary distance because of different codon usage [10]. In previous work [11], we used Bayesian network learning to explain subtype differences for resistance development to nelfinavir from interactions between polymorphisms and resistance mutations. Such studies are useful to improve expert genotypic interpretation systems, in particular to make them more applicable to non-B subtypes by including resistance mutations that occur rarely in subtype B. Some rules for the Rega interpretation system [2] have been derived from Bayesian network learning (for example inclusion of 89I/V in RegaV7.1). By learning the relationship between resistance mutations, in-vitro experiments may be designed to study treatment-selected mutations in a relevant context of other resistance mutations or polymorphisms.

In recent years, treatment is finally reaching many HIV-infected individuals in low and middle-income countries [12]. At the end of 2005, around 1.3 million people in these countries were receiving antiretroviral treatment. Most countries have standardized on first-line and second-line treatments that were recommended by the WHO, and as a consequence the nonnucleoside reverse transcriptase inhibitors (nNRTIs) nevirapine (NVP), and to a lesser extent efavirenz (EFV) are predominantly used in first-line treatment regimens. Unlike the Western epidemic, which is dominated by HIV-1 subtype B, the large majority of the worldwide epidemic is caused by HIV-1 of diverse subtypes (and mainly subtype C). Therefore, a better understanding of mechanisms and mutations involved in antiviral resistance to these drugs is needed for these non-B subtypes.

In this work, we analyzed clinical data to study resistance pathways to nNRTIs. We investigated a possible impact of subtype and interactions with mutations conferring resistance to nucleoside analogue reverse transcriptase (NRTIs). Resistance development to NRTIs and nNRTIs is confounded by the use of combination therapy, which typically combines use of several inhibitors from the two different classes with resistance mutations in the same region. As we will show, Bayesian networks offer a direct benefit over other

statistical approaches to determine whether treatment-associated mutations are related to NRTI or nNRTI resistance pathways, and to investigate possible interactions between NRTI and nNRTI resistance mutations.

Methods

Protease and partial reverse transcriptase (up to position 250) sequences of three HIV-1 populations were pooled from five clinical databases: Portugal, Belgium, Israel, Brazil and an international database containing sequences from subtypes other than subtype B (the non-B workgroup). The populations were defined by respectively 3837 sequences from nNRTI-naïve patients, sequences from patients treated with only experience to EFV (462 sequences) or NVP (533 sequences) as nNRTI. For 10% of RT sequences, which covered RT starting around position 35, the missing sequence fragment was treated as missing values. At most, one treated sequence and one naïve sequence per patient were included and identical sequences were removed. For each sequence, the detected polymorphic amino acids and nNRTI treatment-associated mutations, as well as known key NRTI mutations [13], with prevalence over 0.5%, and NRTI treatment experience were included in the data set for Bayesian network learning.

The analyses followed the method previously described [11], briefly summarized here. Subtyping was done on the nucleotide sequences using the Rega HIV-1 subtyping tool v2.0 [14]. Wild-type polymorphisms were detected based on a prevalence greater than 15% in untreated patients and treatment-associated mutations by testing for independence from treatment using a Cochran–Mantel–Haenszel test [correcting for multiple testing using Benjamini and Hochberg with a false discovery rate (FDR) of 0.05]. After stratifying the data sets for same subtype distribution in treated versus untreated patients, consensus Bayesian networks were learned from the data based on a nonparametric bootstrap [15]. A Bayesian network is a probabilistic model that describes statistical independencies between multiple variables. Using Bayesian network learning, a Bayesian network is searched that explains a maximum of the observed correlations in the data using a minimum number of direct influences.

The network structure was used to determine the role of new treatment-associated mutations. Mutations were considered novel for a drug, when not included in the International AIDS Society (IAS), USA list of resistance mutations of 2007 [13] or in one of the publicly available expert interpretation systems (Rega 6.4 HIVDB 4.2.9, or ANRS 2006.07) for that drug. Novel mutations whose prevalence was dependent on key nNRTI resistance mutations only were considered to be novel nNRTI resistance mutations, and similarly for NRTI resistance mutations. In addition, mutations whose prevalence was determined by presence of other mutations rather than by treatment were considered minor resistance mutations, and mutations that were directly dependent on treatment and for which Conditional Probability Distributions showed a significant prevalence in the absence of other major mutations, were considered major mutations.

Results

Novel efavirenz, nevirapine, and nucleoside reverse transcriptase inhibitor resistance mutations

A comparison of sequences isolated from EFV and NVP-treated patients to sequences from nNRTI-naïve patients, showed the association of 27 mutations with EFV treatment and 24 mutations with NVP treatment, excluding known NRTI resistance mutations (Fig. 1).

The consensus Bayesian networks that were learned from the data sets are shown in Figs 2 and 3. As in the EFV network, the novel minor mutations 28K, 90I, 196R, (currently not considered by expert genotypic interpretation systems) and 221Y (present only in the Rega 7.1 interpretation system, and added to this version based on findings in this manuscript) were associated with EFV treatment or with known EFV resistance mutations; they are potentially involved in resistance to EFV. Likewise, in the NVP network, the novel mutations 101H, 138Q, and 221Y were associated with NVP treatment or with known NVP resistance mutations and are thus potentially involved in resistance to NVP. The novel mutation 28K was mostly confined to subtype G, and within this subtype, was present in 25 out of 189 (13%) patients treated with EFV. The involvement of 28K in NRTI resistance through an interaction with mutations at position 215 (bootstrap support 38% in the NVP network) could not be excluded.

Despite the apparent interaction between mutation 188F and 190S, the mutation 188F is not likely to be directly involved in resistance, but instead a ‘transitional’ mutation in the two-nucleotide change required for the wild-type 188Y to mutate to 188L, as in the data sets, it appeared in 14 of 15 samples in a mixture with 188Y or 188L.

The novel mutation 31L was associated with 184V (bootstrap support 83%) and is therefore likely involved in lamivudine (3TC) resistance. Mutations 203K and 228H, and to a lesser extent mutation 228R, were associated with the thymidine analogue mutations (TAMs) 219Q/E, and thus likely involved in these TAM pathways of resistance to zidovudine (ZDV) and stavudine (D4T).

The involvement of 228R in nNRTI resistance through an interaction with 181Y (bootstrap support 61%) could however not be excluded.

The role for novel mutations 135M, 162H, and 175Y could not be clarified as robust associations were lacking for these mutations in the Bayesian networks.

Efavirenz resistance pathways—For resistance to EFV, next to the predominant use of 103N, HIV-1 escapes through pathways marked by mutations 188H/L or 190E/S/A in a minority of sequences, as indicated by the connectivity around the experience with EFV (eEFV) node in the network. These pathways are not exclusive, as combinations of these major mutations were also commonly found, showing that they reinforce each other when present together. The 106M mutation, which was found almost exclusively in subtype C sequences, confirming earlier findings [16], was almost never observed without 103N or 190E/S/A. Even though it can therefore be considered a minor mutation, it was connected

directly to the eEFV node because it was common to several different pathways. Mutation 227L showed a robust interaction with mutation 106M and was also found exclusively in subtype C.

The network indicated that the 103N pathway is further associated with mutations 90I, 100I, 101P, 108I, 181C, 221Y, 225H, 238T, and 190A. The 188L/F pathway was further associated with mutations 179D (in subtypes B and C), 179E (in subtype G), and the 103R mutation. The 190E/S/A pathway was further associated with mutations 28K, 101E/Q, 103N, 181C, and 221Y.

Several interactions between EFV and NRTI resistance mutations were indicated by the network. Mutation 190E, which is selected rarely by EFV, was indicated to strongly interact with NRTI resistance mutations 74V, 75M, and 219N. Mutation 100I showed a robust interaction with NRTI resistance mutations 74V and 219N, mutations 179D/E and 103N showed an interaction with NRTI resistance mutation 219Q, and mutation 230L showed a robust interaction with NRTI resistance mutations 184I/V.

Nevirapine resistance pathways—For resistance to NVP, the network suggested 103N, 190A, and 181C/I as major mutations, which were connected directly to the experience with NVP (eNVP) node. Similar to resistance to EFV, these major mutations do not mark exclusive pathways, but reinforce each other when present together. Mutation 106A was almost never seen without 103N or 190A, indicating that it was connected to the eNVP node as a minor mutation not specific to one particular pathway. As with 106M for resistance to EFV, it was further associated with mutation 227L.

The network indicated that the 103N pathway was further associated with mutations 190A/S, and 238T and possibly 108I. The 190A pathway was further associated with mutation 101E and possibly also mutations 101Q and 188L. The 181C pathway was further associated with mutation 221Y. The novel mutations 28K and 175Y could not be assigned clearly to be selected by NVP or an NRTI.

As for EFV, NRTI resistance mutations at position 74 and 75 interacted with NVP resistance mutations, but differently. Mutation 181C showed a strong interaction with NRTI resistance mutations 65R and 74V, and mutations 188L and 190A showed a strong interaction with NRTI resistance mutations 215Y/F.

Common features in both efavirenz and nevirapine Bayesian networks—The networks showed a remarkable resemblance of interactions between the resistance mutations that were in common for resistance to NVP and EFV. For example, both networks showed that 108I and 238T were associated with 103N, 101E was associated with 190A/S, 227L was associated with 106A/M, 221Y was associated with 181C, and 28K was associated with 101E.

The networks also confirmed current knowledge on the role of many resistance mutations in relation to NRTI treatment. For example, both networks indicated that 184V was the major resistance mutation selected by 3TC, 115F was the major resistance mutation selected by abacavir (ABC), 65R was the major resistance mutation selected by tenofovir (TDF), and

215F/Y were the major resistance mutations selected by ZDV. Also mutations from the multi-NRTI resistance 151 complex (62V, 77L, 116Y and 151M) clustered clearly together in both networks. Rare antagonistic effects between NRTI resistance mutations that have been reported before were also indicated, between 65R and 215Y/F, and between 151M and 215Y (the latter only in the EFV network).

Discussion

Novel resistance mutations

On the basis of higher prevalence in sequences from treated versus untreated patients, together with a robust association with a drug or a known resistance mutation, we confirmed the selection of many known mutations, but also identified selection of mutations currently not considered by expert interpretation system. In particular, mutations 28K, 90I, 196R, and 221Y are most likely involved in resistance to EFV, and mutations 101H, 138Q, and 221Y in resistance to NVP. Although the novel EFV mutations 28K and 90I were also associated with NVP treatment, they did not associate in a robust way with known NVP resistance mutations in the Bayesian network, probably owing to the small prevalence numbers. Of these novel mutations, most notably 221Y was selected at high levels by both NVP and EFV treatment, and at levels higher than some mutations currently included in the IAS list of resistance mutations such as 108I or 230L. Also, mutation 28K was selected in subtype G, more than most IAS listed mutations (for EFV, only with the exception of 103N and 100I, and for NVP only with the exception of 103N and 181C). The interaction of this mutation with 101E, indicated by both networks, may in part be explained by an association between these mutations in the p51/p66 interface of the reverse transcriptase heterodimer, as residue 28 in p51 and residue 101 in p66 are closely together with opposing charge and likely connected with a salt bridge (Frederico Gago and Joeri Auwerx, personal communication).

The clinical impact of most of these novel mutations on the activity of NVP or EFV may be limited, as they accumulate only after major resistance mutations (such as 103N or 181C) which on their own already reduce the activity of these low genetic barrier drugs such that a treatment change is necessary even in absence of the new mutations. Their selection by existing commonly used drugs may however impact cross-resistance with novel nNRTIs such as etravirine.

Some of the treatment-associated mutations were suggested to be involved in NRTI resistance rather than nNRTI resistance. The NVP treatment-associated 31L was robustly associated with 184V, and therefore likely involved in 3TC resistance. Given the high impact of 184V on replication capacity, perhaps this mutation partially restores this adverse effect of 184V. Other mutations likely involved in resistance to TAMs were 203K and 228H (and perhaps also 228R).

Of these novel mutations, mutations 28K, 101H, 138Q, and 196R were not previously reported as resistance mutations. Mutations 203K, 228H/R and 208Y were reported in other studies to be involved in NRTI resistance [17–20], and 90I and 221Y in nNRTI resistance [17,20,21], in agreement with our results. Contrary to our results, however, mutation 31L has been reported to be involved in nNRTI resistance [22] based on an association between

the presence of this mutation and a reduced phenotypic susceptibility to nNRTIs, in univariable analyses in isolates without known nNRTI resistance mutations. However, this association could not be confirmed in their multivariable analyses, suggesting that the univariable association with nNRTI phenotypic resistance was not causal. Multivariable analyses for association with phenotypic susceptibility (not only to nNRTIs but also to 3TC) are needed to confirm that mutation 31L is involved in resistance to 3TC rather than nNRTIs.

Also contrary to our results, mutations 228H/R have been reported to be involved in nNRTI resistance in two studies [17,21]. In contrast, we found that mutation 228H is involved in NRTI resistance (connected to mutations at position 219 with bootstrap support over 90%), whereas the role of 228R is less certain and perhaps dual. These conclusions were based on the association of these mutations with decreased susceptibility to nNRTI resistance, in a univariable analysis, and with an increased prevalence in nNRTI-treated patients compared with treatment-naïve patients. It may not be excluded that the association found between presence of 228H/R and decreased susceptibility to nNRTIs may have been caused by the presence of other nNRTI mutations in the viral sequence, and no multivariable analyses supported their conclusions. As their data showed that in patients naïve to nNRTIs, but experienced with NRTIs, the mutation 228H had a prevalence over 5%, compared with being virtually absent in treatment-naïve patients, this is in agreement with a role for this mutation in NRTI resistance.

Subtype differences

One of the objectives was to look for subtype-dependent differences that could be explained by interactions with wild-type polymorphisms. Therefore, we included polymorphisms (some of which linked with subtype) in the analyses. In contrast with protease resistance mutations, very few interactions between background polymorphisms and nNRTI resistance mutations were found, indicating that observed differences are to be explained mostly by the impact of different codon usage on genetic barrier. The subtype dependence of 106M was previously explained in this way [8,16], as is the absence of 108I in subtype G (requiring two transitions), and the preference for 179E instead of 179D, also in subtype G [23]. Instead, robust interactions between polymorphisms and resistance mutations involved NRTI resistance mutations, but because data sets were not stratified according to subtype with respect to NRTI treatment, these associations are likely artifacts given that they were different in the EFV versus NVP network. The synergistic interaction between the polymorphism 135T and resistance mutation 103N [21] could not be confirmed in the Bayesian network analysis (although the association was present in the data set, Fisher's exact test, $P < 0.01$), and the Bayesian network could not indicate a possible cause for the confinement of the novel mutation 28K to subtype G.

Interactions between nonnucleoside reverse transcriptase inhibitor and nucleoside reverse transcriptase inhibitor resistance mutations

Interactions between nNRTI and NRTI resistance pathways have been repeatedly observed. For example, as early as in 1994, it was observed that in absence of ZDV, mutation 181C was the most prevalent NVP-selected mutation, whereas coadministration with ZDV

prevented this mutation [24]. Using Bayesian network learning, it was possible to confirm that the mechanism for this observation is not an effect of ZDV directly, but rather an interaction of nNRTI resistance mutations with mutations at position 215, a major resistance position for resistance to ZDV [25]. The interaction between 190E and 74V or 75I was reported previously and verified with in-vitro experiments [26]. A number of novel interactions between NRTI and nNRTI resistance mutations were identified. The interaction between 184I/V and 230L may potentially be explained using the three-dimensional (3D) structure of the enzyme [27], by a direct steric interaction between these residues, which are closely located ($<6 \text{ \AA}$). The 184I/V mutations have been demonstrated to have a clinical effect due to lowered replication capacity [28], and have also been reported to increase reverse transcriptase fidelity [29]. Therefore, it may be interesting to investigate how the interaction between 230L and 184V influences these effects. The interactions between mutation 219N and mutations 190E and 100I, between mutations 100I and 74V, and between mutations 181C and 65R warrant more investigations. Some of these interactions could be involved in the re-sensitization by certain NRTI resistance mutations of susceptibility to nNRTIs [30,31] or vice versa. On the basis of the observed interactions and considering the difference in preferred mutations selected by NVP versus EFV, one could argue that certain treatment combinations are more likely to fail more quickly than other treatments. Most notably, the suggested interaction between 181I/C and 65R could indicate that a treatment including TDF and NVP will lead to a more rapid failure than a treatment including TDF and EFV.

Virological failure on combination therapy may be associated with resistance to one, two or all drugs in the combination. Typically, resistance to one drug will accelerate the development of resistance to the other drugs, as the inhibition of virus replication is weakened, and therefore the virus may actively replicate during selective pressure of the remaining active drugs in the therapy. Therefore, associated prevalence of NRTI and nNRTI resistance mutations may not necessarily imply a biochemical interaction. Still, some of the interactions that were found were previously described, or are plausible given the 3D structure of the enzyme. For interactions that involve mutations which are not the most common resistance mutations selected by specific drugs, a biological reason is the most likely explanation, in particular when the observed unconditional dependencies in the networks were found highly robust and involved similar positions in both networks. However, as it cannot be excluded that the analyses were confounded, these interactions should be confirmed with in-vitro experiments.

Limitations

Our analysis was limited in two important ways. First, because only a fragment of reverse transcriptase is routinely sequenced, we were unable to find mutations outside this region that were involved in drug resistance development such as the mutations recently reported in the RNase H and connections domains [32]. Secondly, for computational reasons, only polymorphisms with a prevalence over 15% in nNRTI-naïve patients were included. It is conceivable that polymorphisms with a lower prevalence may still explain some subtype dependencies that could not be explained (such as the occurrence of 28K in subtype G).

Conclusion

Bayesian network learning proved a valuable tool to untangle the simultaneous selection of mutations in reverse transcriptase by two classes of inhibitors, and contributed to identify novel mutations likely involved in EFV and NVP resistance. Unlike for protease, subtype dependencies in selection of resistance mutations are less common and seldom explained by interaction with polymorphic residues. The Bayesian networks indicated both known and novel interactions between nNRTI and NRTI resistance mutations that explain observed interactions seen in different treatment combinations.

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K.D. conceived the study, performed the analyses and contributed to the manuscript. R.C., Z.G., K.V.L., M.A.S., D.A.K., P.R.H., R.K., R.S., A.-M.V., and the non-B workgroup contributed clinical and virological data. A.-M.V. supervised the work. All authors contributed to the design of the study, interpretation and discussion of the results, and to the manuscript.

The non-B workgroup consists of Rami Kantor of the Division of Infectious Diseases, Brown University, Providence, Rhode Island, USA; David A. Katzenstein, and Robert W. Shafer of the Division of Infectious Disease, Stanford University, Stanford, California, USA; Ricardo J. Camacho and Ana Patricia Carvalho of the Molecular Biology Laboratory, Centro Hospitalar de Lisboa Ocidental, Lisbon, Portugal; Brian Wynhoven and P. Richard Harrigan of the BC Centre for Excellence in HIV/AIDS, Vancouver, Canada; Patricia Cane of the Health Protection Agency Antiviral Susceptibility Reference Unit, Birmingham, UK; John Clarke and John Weber of the Wright Fleming Institute, Imperial College, St Mary's Hospital, London, UK; Sunee Sirivichayakul and Praphan Phanuphak of the Chulalongkorn University, Bangkok, Thailand; Macelo A. Soares of the Departamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; Amilcar Tanuri of the Universidade Federal do Rio de Janeiro, Rio de Janeiro – Rio de Janeiro, Brazil; Joke Snoeck and Anne-Mieke Vandamme of the Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium; Lynn Morris of the National Institute of Communicable Diseases, Johannesburg, South Africa; Hagit Rudich and Zehava Grossman of the Central Virology, PHL, Ministry of Health, Tel-Hashomer, Israel; Jonathan M Schapiro of the National Hemophilia Center, Tel-Hashomer, Israel; Rosangela Rodrigues and Luis F Brigido Instituto of the Adolfo Lutz, Sao Paulo, Brazil; Africa Holguin and Vincent Soriano of the Hospital Carlos III, Madrid, Spain; Koya Ariyoshi and Wataru Sugiura of the National Institute of Infectious Diseases, Tokyo, Japan; Maria Belen Bouzas and Pedro Cahn of the Fundación Huesped, Buenos Aires, Argentina; Deenan Pillay of the Department of Infection, University College London, London, United Kingdom; Terese L. Katzenstein and Louise Bruun Jørgensen of the Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark.

References

1. Shafer RW. Genotypic testing for human immunodeficiency virus type 1 drug resistance. *Clin Microbiol Rev.* 2002; 15:247–277. [PubMed: 11932232]
2. Van Laethem K, De Luca A, Antinori A, Cingolani A, Perno CF, Vandamme AM. A genotypic drug resistance interpretation algorithm that significantly predicts therapy response in HIV-1 infected patients. *Antivir Ther.* 2002; 7:1359–6535.
3. Frater AJ, Beardall A, Ariyoshi K, Churchill D, Galpin S, Clarke JR, et al. Impact of baseline polymorphisms in RT and protease on outcome of highly active antiretroviral therapy in HIV-1-infected African patients. *AIDS.* 2001; 15:1493–1502. [PubMed: 11504981]

4. Parkin NT, Schapiro JM. Antiretroviral drug resistance in non-subtype B HIV-1, HIV-2 and SIV. *Antivir Ther.* 2004; 9:3–12. [PubMed: 15040531]
5. Brindeiro PA, Brindeiro RM, Mortensen C, Hertogs K, De Vroey V, Rubini NPM, et al. Testing genotypic and phenotypic resistance in human immunodeficiency virus type 1 isolates of clade B and other clades from children failing antiretroviral therapy. *J Clin Microbiol.* 2002; 40:4512–4519. [PubMed: 12454144]
6. Ariyoshi K, Matsuda M, Miura H, Tateishi S, Yamada K, Sugiura W. Patterns of point mutations associated with antiretroviral drug treatment failure in CRF01_AE (subtype E) infection differ from subtype B infection. *J Acquir Immune Defic Syndr.* 2003; 33:336–342. [PubMed: 12843744]
7. Grossman Z, Vardinon N, Chemtob D, Alkan ML, Bentwich Z, Burke M, et al. Genotypic variation of HIV-1 reverse transcriptase and protease: comparative analysis of clade C and clade B. *AIDS.* 2001; 15:1453–1460. [PubMed: 11504976]
8. Grossman Z, Istomin V, Averbuch D, Lorber M, Risenberg K, Levi I, et al. Israel AIDS Multi-Center Study Group. Genetic variation at NNRTI resistance-associated positions in patients infected with HIV-1 subtype C. *AIDS.* 2004; 18:909–915. [PubMed: 15060438]
9. Camacho RJ, Vandamme AM. Antiretroviral resistance in different HIV-1 subtypes: impact on therapy outcomes and resistance testing interpretation. *Curr Opin HIV AIDS.* 2007; 2:123–129. [PubMed: 19372877]
10. van de Vijver DA, Wensing AMJ, Angarano G, Asjö B, Balotta C, Boeri E, et al. The calculated genetic barrier for antiretroviral drug resistance substitutions is largely similar for different HIV-1 subtypes. *J Acquir Immune Defic Syndr.* 2006; 41:352–360. [PubMed: 16540937]
11. Deforche K, Silander T, Camacho R, Grossman Z, Soares MA, Van Laethem K, et al. Analysis of HIV-1 pol sequences using Bayesian Networks: implications for drug resistance. *Bioinformatics.* 2006; 22:2975–2979. [PubMed: 17021157]
12. WHO. Towards universal access by 2010: how WHO is working with countries to scale-up HIV prevention, treatment, care and support. WHO; Geneva: 2006.
13. Johnson VA, Brun-Vénizet F, Bonaventura C, Günthard H, Kuritzkes DR, Pillay D, et al. Update of the drug resistance mutations in HIV-1: 2007. *Top HIV Med.* 2007; 15:119–125. [PubMed: 17720996]
14. de Oliveira T, Deforche K, Cassol S, Salminen M, Paraskevis D, Seebregts C, et al. An automated genotyping system for analysis of HIV-1 and other microbial sequences. *Bioinformatics.* 2005; 21:3797–3800. [PubMed: 16076886]
15. Friedman, N.; Goldszmidt, M.; Wyner, AJ. Data analysis with Bayesian networks: a bootstrap approach. *Proceedings of UAI-99*; San Francisco, CA: 1999. p. 206-215.
16. Brenner B, Turner D, Oliveira M, Moisi D, Detorio M, Carobene M, et al. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to nonnucleoside reverse transcriptase inhibitors. *AIDS.* 2003; 17:F1–F15. [PubMed: 12478089]
17. Saracino A, Monno L, Scudeller L, Cibelli DC, Tartaglia A, Punzi G, et al. Impact of unreported HIV-1 reverse transcriptase mutations on phenotypic resistance to nucleoside and non-nucleoside inhibitors. *J Med Virol.* 2006; 78:9–17. [PubMed: 16299731]
18. Svicher V, Sing T, Santoro MM, Forbici F, Rodríguez-Barrios F, Bertoli A, et al. Involvement of novel human immunodeficiency virus type 1 reverse transcriptase mutations in the regulation of resistance to nucleoside inhibitors. *J Virol.* 2006; 80:7186–7198. [PubMed: 16809324]
19. Rhee SY, Fessel WJ, Zolopa AR, Hurley L, Liu T, Taylor J, et al. HIV-1 protease and reverse-transcriptase mutations: correlations with antiretroviral therapy in subtype B isolates and implications for drug-resistance surveillance. *J Infect Dis.* 2005; 192:456–465. [PubMed: 15995959]
20. Cane PA, Green H, Fearnhill E, Dunn D, the UK collaborative group on HIV drug resistance. Identification of accessory mutations associated with high-level resistance in HIV-1 reverse transcriptase. *AIDS.* 2007; 21:447–455. [PubMed: 17301563]
21. Ceccherini-Silberstein F, Svicher V, Sing T, Artese A, Santoro MM, Forbici F, et al. Characterization and structural analysis of novel mutations in human immunodeficiency virus type 1 reverse transcriptase involved in the regulation of resistance to nonnucleoside inhibitors. *J Virol.* 2007; 81:11507–11519. [PubMed: 17686836]

22. Parkin NT, Gupta S, Chappey C, Petropoulos CJ. The K101P and K103R/V179D mutations in human immunodeficiency virus type 1 reverse transcriptase confer resistance to nonnucleoside reverse transcriptase inhibitors. *Antimicrob Agents Chemother*. 2006; 50:351–354. [PubMed: 16377709]
23. Vergne L, Peeters M, Mpoudi-Ngole E, Bourgeois A, Liegeois F, Toure-Kane C, et al. Genetic diversity of protease and reverse transcriptase sequences in nonsubtype-B human immunodeficiency virus type 1 strains: evidence of many minor drug resistance mutations in treatment-naïve patients. *J Clin Microbiol*. 2000; 38:3919–3925. [PubMed: 11060045]
24. Richman DD, Havlir D, Corbeil J, Looney D, Ignacio C, Spector SA, et al. Nevirapine resistance mutations of human immuno-deficiency virus type 1 selected during therapy. *J Virol*. 1994; 68:1660–1666. [PubMed: 7509000]
25. Selmi B, Deval J, Alvarez K, Boretto J, Sarfati S, Guerreiro C, et al. The Y181C substitution in 3' - azido-3'-deoxythymidine-resistant human immunodeficiency virus, type 1, reverse transcriptase suppresses the ATP-mediated repair of the 3' -azido-3'-deoxythymidine 5'-monophosphate-terminated primer. *J Biol Chem*. 2003; 278:40464–40472. [PubMed: 12902345]
26. Boyer PL, Gao HQ, Hughes SH. A mutation at position 190 of human immunodeficiency virus type 1 reverse transcriptase interacts with mutations at positions 74 and 75 via the template primer. *Antimicrob Agents Chemother*. 1998; 42:447–452. [PubMed: 9527805]
27. Ren J, Milton J, Weaver KL, Short SA, Stuart DI, Stammers DK. Structural basis for the resilience of efavirenz (DMP-266) to drug resistance mutations in HIV-1 reverse transcriptase. *Structure*. 2000; 8:1089–1094. [PubMed: 11080630]
28. Turner D, Brenner B, Wainberg MA. Multiple effects of the M184V resistance mutation in the reverse transcriptase of human immunodeficiency virus type 1. *Clin Diagn Lab Immunol*. 2003; 10:979–981. [PubMed: 14607855]
29. Hsu M, Inouye P, Rezende L, Richard N, Li Z, Prasad VR, et al. Higher fidelity of RNA-dependent DNA mispair extension by M184V drug-resistant than wild-type reverse transcriptase of human immunodeficiency virus type 1. *Nucleic Acids Res*. 1997; 25:4532–4536. [PubMed: 9358162]
30. Shulman N, Zolopa AR, Passaro D, Shafer RW, Huang W, Katzenstein D, et al. Phenotypic hypersusceptibility to non-nucleoside reverse transcriptase inhibitors in treatment-experienced HIV-infected patients: impact on virological response to efavirenz-based therapy. *AIDS*. 2001; 15:1125–1132. [PubMed: 11416714]
31. Shulman NS, Bosch RJ, Mellors JW, Albrecht MA, Katzenstein DA. Genetic correlates of efavirenz hypersusceptibility. *AIDS*. 2004; 18:1781–1785. [PubMed: 15316338]
32. Jones J, Taylor B, Wilkin TJ, Hammer SM. Advances in anti-retroviral therapy. *Top HIV Med*. 2007; 15:48–82. [PubMed: 17485787]

Fig. 1. Dataset prevalence (%) in sequences from nNRTI-naïve (left bar) and nNRTI-treated (right bar) patients of mutations significantly associated with EFV or NVP treatment (FDR = 0.05)

For both drugs, the data were stratified for the overall subtype distribution of the sequences, shown in the inset pies, to be identical for treated and untreated patients. Known NRTI resistance mutations that were associated with treatment are not shown. FDR, false discovery rate; NRTI, nucleoside reverse transcriptase inhibitor; nNRTI, nonnucleoside reverse transcriptase inhibitor; NVP, nevirapine; EFV, efavirenz.

Fig. 2. Annotated Bayesian network learned for analyzing resistance to EFV, learned autonomously from clinical sequence data

An arc represents a direct dependency between the corresponding variables and thickness is proportional to bootstrap support. Solid arcs have bootstrap support over 65% and were considered robust. Dashed arcs have bootstrap support over 35% and lack of arcs was also considered robust. Arc color indicates whether it is a direct influence between resistance mutations (black), an influence from background polymorphisms on resistance mutations (blue), a direct influence between treatment reflecting bias in treatment combinations (purple), associations between background polymorphisms (green), or other interactions such as between nNRTI and NRTI resistance mutations (gray). Arc direction does not reflect cause and effect, nor order of accumulations of mutations, but may indicate a nonadditive multivariable effect, which requires analysis of the quantitative components of the Bayesian network. Arcs were colored according to their function to improve reading the graph, but coloring is only indicative. EFV, efavirenz; NRTI, nucleoside reverse transcriptase inhibitor; nNRTI, nonnucleoside reverse transcriptase inhibitor.

Fig. 3. Annotated Bayesian network learned for analyzing resistance to NVP
Legend as in Fig. 2. NRTI, nucleoside reverse transcriptase inhibitor; nNRTI, nonnucleoside reverse transcriptase inhibitor; NVP, nevirapine.