Methods for HIV Resistance Determination and Interpretation

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The HIVdb System for HIV-1 Genotypic Resistance Interpretation

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HIV-1 · Genotypic resistance · Drug resistance mutations · Online resource

Abstract
The Stanford HIV Drug Resistance Database hosts a freely available online genotypic resistance interpretation system called HIVdb to help clinicians and laboratories interpret HIV-1 genotypic resistance tests. These tests are designed to assess susceptibility to nucleoside and nonnucleoside reverse transcriptase inhibitors (NRTI and NNRTI), protease inhibitors and integrase inhibitors. The HIVdb genotypic resistance interpretation system output consists of (1) a list of penalty scores for each antiretroviral (ARV) resistance mutation in a submitted sequence, (2) estimates of decreased NRTI, NNRTI, protease and integrase inhibitor susceptibility, and (3) comments about each ARV resistance mutation in the submitted sequence. The application's strengths are its convenience for submitting sequences, its quality control analysis, its transparency and its extensive comments. The Sierra Web service is an extension that enables laboratories analyzing many sequences to individualize the format of their results. The algorithm specification interface compiler makes it possible for HIVdb to provide results using a variety of different HIV-1 genotypic resistance interpretation algorithms.

Introduction

Interpreting the results of HIV-1 genotypic resistance tests is one of the most difficult tasks facing health care providers. There are many different HIV-1 drug resistance mutations with diverse effects on antiretroviral drugs (ARVs) belonging to each of the ARV classes. These mutations often occur in complex patterns and may interact to cause varying levels of HIV-1 replication and ARV susceptibility. The Stanford HIV Drug Resistance Database was created in 1999, and hosts a freely available online genotypic resistance interpretation system called HIVdb to assist clinicians and laboratories in interpreting HIV-1 genotypic resistance tests. These tests are designed to assess susceptibility to nucleoside and nonnucleoside reverse transcriptase inhibitors (NRTI and NNRTI, respectively), protease inhibitors and integrase inhibitors [1, 2]. In this article, we describe HIVdb and several related programs including HIVseq, HIValg and the Web service Sierra.

User Interface and Sequence Submission

The HIVdb html interface accepts either nucleic acid sequences or lists of mutations. The *sequence analysis* form allows users to paste one or more HIV-1 protease,

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Table 1. HIVdb criteria for classifying RT, protease and integrase mutations

RT mutations	NRTI	NNRTI	Other
	Mutations that reduce susceptibility to one or more NRTIs	Mutations that reduce susceptibility to one or more NNRTIs	Mutations that are not associated with drug resistance. Highly polymorphic mutations that may be weakly associated with drug resistance, but that are primarily accessory, are also placed in this category. It may also include rare nonpolymorphic NRTI- and NNRTI-selected mutations that have not been studied for their effects on drug susceptibility
PR and IN mutations	Major	Minor	Other
	Nonpolymorphic mutations that by themselves reduce susceptibility to one or more inhibitors and that commonly occur during virological failure	Nonpolymorphic or minimally polymorphic mutations that contribute to decreased susceptibility in combination with major drug resistance mutations. Highly unusual and poorly characterized mutations and major drug resistance positions are also usually in this category	Mutations that are not associated with drug resistance. Highly polymorphic mutations that may be weakly associated with drug resistance, but that are primarily accessory, are also placed in this category. It may also include rare nonpolymorphic PI- or INI-selected mutations that have not been studied for their effects on drug susceptibility

IN = Integrase; INI = integrase inhibitors; PR = protease; PI = protease inhibitors.

RT and/or integrase sequences into a text box or to upload a text file containing the same. The *mutation list* form allows users to type in lists of RT, protease, and/or integrase mutations or to select ARV resistance mutations from a drop-down menu.

In addition to its html interface, HIVdb can be accessed via the Web service Sierra (http://hivdb.stanford.edu/pages/webservices/). Sierra is a computer-to-computer programmatic interface designed for research and clinical laboratories that typically upload large numbers of sequences and therefore require automated extraction of HIVdb's output. Sierra allows users to submit 1,000 simultaneous sequences. Sierra returns the results as an XML report that is easy to parse, making it unnecessary to manually inspect large numbers of html results. For several reasons, sequences submitted to HIVdb either via the Web interface or Sierra are not stored on local servers.

Sequence Analysis and Mutation Classification

Submitted nucleotide sequences are aligned to a consensus HIV-1 subtype B polymerase amino acid sequence (http://hivdb.stanford.edu/pages/asi/releaseNotes/#consensusbsequences) using a nucleotide-to-amino-acid-sequence alignment algorithm. Sequences undergo a quality control analysis to assess the likelihood of a regional or sequence-wide technical artifact that may confound sequence interpretation. The quality control analysis identifies (1) positions with stop codons or frame shifts, (2) positions with highly ambiguous nucleotides, (3) evidence for APOBEC3G and/or 3F-mediated G-to-A hypermutation [3] and (4) positions with mutations found at an extraordinarily low prevalence in the Stanford HIV Drug Resistance Database.

The alignment process generates a list of mutations from the submitted sequence defined as amino acid differences from the consensus B sequence. RT mutations are classified into 3 categories: NRTI resistance mutations, NNRTI resistance mutations and 'other' mutations. Protease and integrase mutations are also each classified into 3 categories: major resistance mutations, minor resistance mutations and 'other' mutations. Table 1 outlines the considerations for classifying RT, protease and integrase into these categories. The complete classification scheme can be found in the HIVdb Release Notes.

Genotypic Resistance Interpretation

The HIVdb genotypic resistance interpretation system output consists of (1) a list of penalty scores for each ARV resistance mutation in a submitted sequence, (2) estimates of decreased NRTI, NNRTI, protease and integrase inhibitor susceptibility, and (3) comments about each ARV resistance mutation in the submitted sequence. Genotypic resistance interpretations are implemented by a compiler – the algorithm specification interface (ASI), which we developed to encode genotypic interpretation rules [4]. The ASI comprises an XML format for specifying an algorithm and a compiler that transforms the algorithm into executable code. The goal of the ASI is to prevent the implementation of genotypic interpretation systems from becoming locked within inaccessible proprietary formats and to allow clinician experts to focus on developing, testing and modifying interpretation systems rather than on developing software to encode them.

Mutation Penalty Scores

Mutation penalty scores are developed with 2 main considerations: (1) to reflect the effect of individual mutations on drug susceptibility, and (2) to reflect how mutation penalties are combined to yield reliable estimates of ARV susceptibility for the most commonly occurring patterns of ARV resistance mutations. The first consideration is based primarily on in vitro and in vivo studies that show which mutations are selected by which ARVs and on studies that quantify the effect of mutations on in vitro ARV susceptibility. The second consideration is based on these same criteria and on clinical studies that correlate pretherapy mutations with the virological response to an ARV used as part of a salvage therapy regimen. Because many mutations act synergistically to reduce drug susceptibility scores, a subset of mutation penalty scores is 'triggered' only when certain combinations of mutations are present. Because several mutations increase susceptibility to one or more ARVs, several mutation penalty scores have negative values.

Each mutation penalty score is hyperlinked to a set of entries in the Stanford HIV Drug Resistance Database that support a mutation's association with decreased ARV susceptibility. The complete lists of mutation penalty scores and comments for each ARV class can also be examined, sorted (by drug or position) and downloaded. (http://hivdb.stanford.edu/pages/drugSummaries.html).

Estimates of Decreased ARV Susceptibility

Estimates of ARV susceptibility are derived by adding the mutation penalty scores for each of the mutations present within a submitted sequence. Susceptibility estimates can be treated as a continuous variable – the sum of the mutation penalty scores – or as 1 of 5 categories. (1) Susceptible: no evidence of reduced ARV susceptibility compared with a wild-type virus (total score of 0–9). (2) Potential low-level resistance: the virus encoded by the submitted sequence is likely to be fully susceptible; however, the sequence contains mutations that may indicate previous ARV exposure (total score of 10–14). (3) Low-level resistance: the virus encoded by the submitted sequence may have reduced in vitro ARV susceptibility and/or patients harboring viruses with the submitted mutations may have a suboptimal virological response to treatment with the ARV (total score of 15–30). (4) Intermediate resistance: a level of ARV resistance greater than low-level resistance but lower than high-level resistance (total score of 30–59). An ARV to which the virus has intermediate resistance should generally be used only if the ARV has a high genetic barrier to resistance (e.g. some ritonavir-boosted inhibitors) or if few other active drugs are available. (5) High-level resistance: the mutations present in the submitted sequence of the virus are similar to those observed in viruses with the highest levels of in vitro drug resistance (total score >60). Alternatively, clinical data exist demonstrating that patients infected with viruses having such mutations usually have little or no virological response to treatment with the ARV.

Mutation Comments

HIVdb output contains 4 types of comments. (1) Comments on ARV resistance mutations that receive mutation penalty scores. These comments are designed to justify the score and to provide additional information about a mutation that may be clinically relevant, depending on the clinical scenario. (2) Comments on mutations that have been potentially associated with decreased ARV susceptibility but which do not have mutation penalty scores because they are either highly polymorphic or have a minimal, if any, effect on drug susceptibility. Comments on these mutations are designed to alert the user to the presence of these mutations while at the same time justifying the absence of mutation penalty scores. (3) Comments on highly unusual mutations at known drug resistance positions. (4) Mutations associated with commonly used 'genotypic susceptibility scores' developed for tipranavir [5], darunavir [6] and etravirine [7].

Related Programs and Features

HIVseq and HIValg have similar user interfaces to HIVdb but yield different information about a sequence. HIVseq outputs a table that shows the prevalence of the mutation in viruses belonging to group M subtypes A, B, C, D, F and G, and CRFs 01_AE and 02_AG according to ARV treatment [8]. HIVseq, therefore, identifies subtype-specific polymorphisms and ARV resistance mutations. The prevalence of each mutation according to subtype and treatment is hyperlinked to the published reports of that mutation according to the specified subtype and treatment.

HIValg provides resistance interpretations using 3 algorithms: HIVdb, ANRS [9] and REGA [10]. This has been made possible due to the courtesy of researchers at the ANRS and REGA. It enables users to determine whether applying these algorithms to the same sequence yields similar estimates of resistance to each antiretroviral drug. This program is available and possible because each of the algorithms is implemented using the ASI compiler.

The Stanford HIV-1 Drug Resistance database also hosts the REGA HIV-1 Subtyping Tool [11, 12], which detects most HIV-1 circulating recombinant forms as well as many unique recombinant forms provided sufficient long sequences are submitted.

Conclusion

HIVdb is a publicly available, Web-based system to help clinicians and researchers understand the results of genotypic resistance testing by providing targeted information on the phenotypic and clinical significance of mutations in a patient's plasma HIV-1. The application's strengths are its convenience for submitting sequences, its quality control analysis, its transparency and its extensive comments aimed at educating the user. The Sierra Web service is an extension that enables laboratories analyzing many sequences to individualize the format of their results. The ASI compiler makes it possible for HIVdb to provide results using a variety of different HIV-1 genotypic resistance interpretation algorithms.

Genotypic interpretation systems do not take into account the relative potencies of different ARVs to the same extent [13, 14]. They also do not consider relevant clinical data such as previous drug resistance test results, ARV treatment history, plasma HIV-1 RNA levels, CD4 cell counts and drug toxicity. Finally, these systems do not incorporate the fundamental principles on how ARV regimens should be constructed. Clinicians using HIVdb, therefore, must have a sound understanding of the principles of ARV therapy to make the most informed treatment decisions for their patients.

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