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Genotypic predictors of human immunodeficiency virus type 1 drug resistance

Soo-Yon Rhee*, Jonathan Taylor[†], Gauhar Wadhera*, Asa Ben-Hur^{‡§}, Douglas L. Brutlag[‡], and Robert W. Shafer*[¶]

Division of Infectious Diseases, Departments of *Medicine, [†]Statistics, and [‡]Biochemistry, Stanford University, Stanford, CA 94305

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Understanding the genetic basis of HIV-1 drug resistance is essential to developing new antiretroviral drugs and optimizing the use of existing drugs. This understanding, however, is hampered by the large numbers of mutation patterns associated with cross-resistance within each antiretroviral drug class. We used five statistical learning methods (decision trees, neural networks, support vector regression, least-squares regression, and least angle regression) to relate HIV-1 protease and reverse transcriptase mutations to *in vitro* susceptibility to 16 antiretroviral drugs. Learning methods were trained and tested on a public data set of genotype–phenotype correlations by 5-fold cross-validation. For each learning method, four mutation sets were used as input features: a complete set of all mutations in ≥ 2 sequences in the data set, the 30 most common data set mutations, an expert panel mutation set, and a set of nonpolymorphic treatment-selected mutations from a public database linking protease and reverse transcriptase sequences to antiretroviral drug exposure. The nonpolymorphic treatment-selected mutations led to the best predictions: 80.1% accuracy at classifying sequences as susceptible, low/intermediate resistant, or highly resistant. Least angle regression predicted susceptibility significantly better than other methods when using the complete set of mutations. The three regression methods provided consistent estimates of the quantitative effect of mutations on drug susceptibility, identifying nearly all previously reported genotype–phenotype associations and providing strong statistical support for many new associations. Mutation regression coefficients showed that, within a drug class, cross-resistance patterns differ for different mutation subsets and that cross-resistance has been underestimated.

antiviral therapy | HIV | linear regression | machine learning

Twenty antiretroviral drugs are approved for treating HIV-1 infection: eight protease inhibitors (PIs), seven nucleoside and one nucleotide reverse transcriptase (RT) inhibitors (NRTIs), three nonnucleoside RT inhibitors (NNRTIs), and one fusion inhibitor. Resistance to these drugs is caused by mutations in their molecular targets. Understanding the genetic basis of cross-resistance is essential for designing new antiviral drugs and for using genotypic drug resistance testing to select optimal therapy. Despite the large number of PIs and RT inhibitors, therapy is challenging because drug resistance arises from complex patterns of mutations and because of the high degree of cross-resistance within each drug class.

Approaches for using HIV-1 drug resistance mutations to predict changes in drug susceptibility have included decision trees (1), linear regression (2), linear discriminant analysis (3), neural networks (4), and support vector regression (SVR) (5). Here, we compare five statistical learning methods each using four different sets of input mutations to develop quantitative models associating HIV-1 protease and RT mutations with changes in susceptibility to 16 antiretroviral drugs. The analyses are performed on a curated publicly available data set (6) generated with a highly reproducible drug susceptibility assay (7, 8). The results provide insight into the performance of different statistical learning methods at predicting phenotypic characteristics of highly polymorphic proteins and into the genetic mechanisms of HIV-1 antiretroviral cross-resistance.

Results

Drug Susceptibility Results, Input Mutations, and Learning Methods.

For each of the three drug classes, we created four mutation sets that included (i) a complete set of all mutations present in ≥ 2 sequences, (ii) an expert panel mutation set (9), and (iii) a set of nonpolymorphic treatment-selected mutations (TSMs) derived from a database linking protease and RT sequences to the treatment histories of persons from whom the sequenced viruses were obtained (10) (Table 1). A control set of the 30 most common mutations in the data set was also created (see *Supporting Text*, which is published as supporting information on the PNAS web site). Predictions using these 30 mutations were consistently inferior to those using the other three mutation sets (data not shown).

Table 2 shows the number of isolates for which sequences and susceptibility results were available. Thirty-seven to 60% of isolates had reduced drug susceptibility to one or more PIs. Thirty-one to 70% had reduced drug susceptibility to one or more NRTIs. Thirty-three to 41% had reduced drug susceptibility to one or more NNRTIs. The distribution in the number of nonpolymorphic TSMs and expert panel mutations per isolate is shown in Fig. 2, which is published as supporting information on the PNAS web site.

We applied five statistical learning methods [decision trees, neural networks, least-squares regression (LSR), SVR, and least angle regression (LARS)] to classify isolates as susceptible, low/intermediate, or highly resistant to the drugs used for testing. The regression methods were used to predict the level of reduced drug susceptibility for each isolate.

Classification. The mean prediction accuracy (5 methods \times 3 mutation sets) was highest for the NNRTIs (83.0%) compared with the PIs (78.2%; $P < 0.001$) and NRTIs (75.9%; $P < 0.001$) (Table 3). Among the PIs, the highest accuracy was for ritonavir (85.4%) and the lowest was for atazanavir (70.6%), the PI with the fewest test results. Among the NRTIs, the highest accuracies were for lamivudine (88.6%) and the lowest were for tenofovir (TDF) (67.7%), the NRTI with the fewest test results. There was minimal variation in prediction accuracy among the NNRTIs.

When the prediction accuracies of the possible combinations of drug and learning method were averaged over the different mutation sets, the mean accuracies of the learning methods ranged from 76.1% (neural networks) to 79.7% (LARS). The

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Abbreviations: AZT, zidovudine; LARS, least angle regression; LSR, least-squares regression; MSE, mean-squared error; PI, protease inhibitor; RT, reverse transcriptase; NRTI, nucleoside RT inhibitor; NNRTI, nonnucleoside RT inhibitor; SVR, support vector regression; TDF, tenofovir; TSM, treatment-selected mutation.

[§]Present address: Department of Computer Science, Colorado State University, Fort Collins, CO 80523.

[¶]To whom correspondence should be addressed at: Division of Infectious Diseases, Room S-169, Stanford University, Stanford, CA 94305. E-mail: rshafer@stanford.edu.

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Table 1. Sets of protease and RT mutations used as input features for predicting drug susceptibility

Mutation set	Description	Mutations
Complete	Mutations occurring ≥ 2 times in the data set	PIs: 225 mutations at 70 positions. NRTIs: 371 mutations at 132 positions.
Expert panel	IAS-USA mutation panel (9). The complete list of mutations for each drug class is shown here. The subset of mutations associated with each drug in the primary publication was used for prediction.	PIs: 10F/I/R/V; 16E; 20M/R/I; 24I; 30N; 32I; 33F/I/V; 36I/L/V; 46I/L; 47V/A; 48V; 50V/L; 53L; 54V/M/L/A/T/S; 60E; 62V; 63P; 71V/T/I/L; 73S/A/C/T; 74P; 77I; 82A/F/T/S; 84V; 85V; 88D/S; 90M; 93L NRTIs: 41L; 44D; 62V; 65R; 67N; 69ins; 70R; 74V; 75I; 77L; 115F; 116Y; 118I; 151M; 184V/I; 210W; 215Y/F; 219Q/E NNRTIs: 100I; 103N; 106M/A; 108I; 181C/I; 188L/C/H; 190S/A; 225H; 230L; 236L
Nonpolymorphic TSMs	Nonpolymorphic mutations significantly more common in treated compared with untreated persons (10)	PIs: 10F/R; 11I; 20I/T/V; 23I; 24I; 30N; 32I; 33F; 34Q; 35G; 43T; 46I/L/V; 47A/V; 48M/V; 50L/V; 53L/Y; 54A/L/M/S/T/V; 55R; 58E; 66F; 67F; 71I; 73A/C/S/T; 74A/P/S; 76V; 79A; 82A/F/S/T; 84A/C/V; 85V; 88D/S/T; 89V; 90M; 92R; 95F NRTIs: 41L; 43E/N/Q; 44A/D; 62V; 65R; 67E/G/N; 69D/N/S/ins; 70R; 74I/V; 75I/M/T; 77L; 98G; 115F; 116Y; 151M; 184I/V; 203K; 208Y; 210W; 215F/I/V/Y; 218E; 219E/N/Q/R; 223Q; 228H/R NNRTIs: 100I; 101E/N/P; 103N/S; 106A/M; 108I; 138Q; 181C/I/V; 188C/H/L; 190A/E/Q/S; 221Y; 225H; 227L; 230L; 236L; 238T

IAS-USA, International AIDS Society–USA.

superiority of LARS was due to its accuracy in using the complete mutation set (80.3%), which was significantly higher than decision trees (77.2%; $P = 0.02$), SVR (76.1%; $P < 0.001$), neural networks (74.6%; $P < 0.001$), and LSR (72.3%; $P < 0.001$). Regression methods (79.9%) had higher accuracy for the PIs than the decision tree and neural network methods (75.5%; $P < 0.001$).

Averaged over the different learning methods and drugs, the nonpolymorphic TSM set had the highest prediction accuracy (80.1%) followed by the expert panel (77.5%; $P < 0.001$) and complete mutation set (76.1%; $P < 0.001$). However, the use of all of the expert panel mutations for a drug class (pooling the mutations associated with all of the drugs of a class) increased the accuracy from 77.5% to 79.3%.

Table 4, which is published as supporting information on the PNAS web site, shows the number of highly discordant results according to the mutation data set and learning method. For 10,624

predictions obtained by applying LARS to the nonpolymorphic TSM data set, only 33 (0.31%) were highly discordant with the measured phenotype (Table 5, which is published as supporting information on the PNAS web site).

Regression. The TSM set had the highest correlation coefficients (r^2) between actual and predicted susceptibility compared with the complete and expert panel mutation set (Table 6, which is published as supporting information on the PNAS web site). The r^2 values for the TSM set determined by SVR, LSR, and LARS ranged from 0.83 to 0.84 averaged over the PIs, 0.76 to 0.77 averaged over the NRTIs, and 0.77 to 0.79 averaged over the NNRTIs.

Averaged over each of the 16 drugs and the three regression methods, the mean-squared errors (MSEs) were 0.22 for the TSMs and 0.32 for the expert panel mutations (Table 7, which is published as supporting information on the PNAS web site). However, the use of the complete set of expert panel mutations for a drug class reduced the overall MSE from 0.32 to 0.26. The MSEs of the regression methods using the TSMs were inversely proportional to the number of samples used for testing and training (Fig. 3, which is published as supporting information on the PNAS web site).

Regression coefficients for each of the PI, NRTI, and NNRTI TSMs determined by the different regression methods were highly correlated (Tables 8–10 and Fig. 4, which are published as supporting information on the PNAS web site). For the PIs, the mean r^2 between the LSR and SVR coefficients was 0.98 and between LSR and LARS was 0.96. For the NRTIs, the mean r^2 between LSR and SVR was 0.94 and between LSR and LARS was 0.91.

PI-Resistance Mutations. Fig. 1A shows the LSR coefficients for 35 PI TSMs occurring in ≥ 10 sequences and significantly associated with decreased susceptibility to one or more PIs (regression coefficient > 3.0 standard deviations above or below 0). The substrate cleft mutations G48V and I84V; the flap mutations I54V and Q58E; and the mutations L24I, G73S, and L90M were associated with decreased susceptibility to all seven PIs. The substrate cleft mutations V32I, I50V, and V82A/T/F; the flap mutations K43T, M46I/L, I47V, F53L, and I54M/L; and the mutations L10F, K20I/T, G73T, T74S, and N88D/S were associated with decreased susceptibility to four or more PIs.

The TSM coefficients provided quantitative confirmation of each nonpolymorphic expert panel mutation. In addition, six non-expert-panel TSMs (V11I, K43T, Q58E, T74S, L76V, and L89V) were significantly associated with decreased susceptibility to one or more PIs.

NRTI- and NNRTI-Resistance Mutations. Fig. 1B shows the LSR regression coefficients for 23 NRTI TSMs occurring in ≥ 10 se-

Table 2. Summary of HIV-1 isolates with genotype and phenotype correlations according to drug tested and level of resistance

Drug	Isolate phenotypes*			Total
	Susc, %	Low/int, %	High, %	
Protease inhibitors				
Amprenavir (APV)	61	29	10	768
Atazanavir (ATV)	48	30	22	329
Indinavir (IDV)	51	28	21	827
Lopinavir (LPV)	45	23	32	517
Nelfinavir (NFV)	40	25	35	844
Ritonavir (RTV)	48	20	32	795
Saquinavir (SQV)	60	18	22	826
Nucleoside RT inhibitors				
Lamivudine (3TC)	32	15	53	633
Abacavir (ABC)	30	45	25	628
Zidovudine (AZT)	52	23	25	630
Stavudine (D4T)	57	28	15	630
Didanosine (DDI)	56	37	7	632
Tenofovir (TDF)	70	18	12	353
Nonnucleoside RT inhibitors				
Delavirdine (DLV)	64	14	21	732
Efavirenz (EFV)	64	16	22	734
Nevirapine (NVP)	56	7	37	746

*Susc, susceptible; Low/int, low/intermediate; High, high-level. The cut-offs (levels of fold-decreased susceptibility) for distinguishing susceptible from low/intermediate resistance and low/intermediate from high-level resistance are described in *Methods*.

Table 3. Predictive accuracy of LSR, SVR, LARS, decision trees, and neural networks using the nonpolymorphic TSMs, complete set (Comp), and expert panel mutations (Expert)

Drug*	LSR			SVR			LARS			Decision trees			Neural networks			Drug mean [†]
	TSM	Comp	Expert	TSM	Comp	Expert	TSM	Comp	Expert	TSM	Comp	Expert	TSM	Comp	Expert	
Protease inhibitors																
APV	0.84	0.81	0.8	0.84	0.82	0.8	0.84	0.81	0.8	0.78	0.77	0.77	0.76	0.74	0.76	0.80
ATV	0.77	0.68	0.73	0.76	0.69	0.73	0.77	0.76	0.7	0.65	0.71	0.65	0.7	0.64	0.65	0.71
IDV	0.79	0.78	0.75	0.79	0.77	0.76	0.79	0.77	0.75	0.75	0.75	0.75	0.76	0.73	0.71	0.76
LPV	0.81	0.79	0.82	0.81	0.8	0.83	0.81	0.83	0.81	0.74	0.77	0.73	0.76	0.76	0.73	0.79
NFV	0.82	0.79	0.74	0.82	0.79	0.76	0.81	0.8	0.73	0.8	0.76	0.8	0.77	0.73	0.73	0.78
RTV	0.89	0.86	0.85	0.89	0.86	0.86	0.88	0.88	0.86	0.85	0.84	0.84	0.82	0.81	0.82	0.85
SQV	0.84	0.81	0.76	0.85	0.81	0.77	0.82	0.82	0.74	0.8	0.75	0.77	0.8	0.76	0.75	0.79
Avg [‡]	0.82	0.79	0.78	0.82	0.79	0.79	0.82	0.81	0.77	0.76	0.76	0.76	0.77	0.74	0.74	0.78
Nucleoside RT inhibitors																
3TC	0.89	0.83	0.87	0.88	0.84	0.87	0.87	0.88	0.88	0.92	0.9	0.92	0.92	0.9	0.92	0.89
ABC	0.77	0.63	0.77	0.77	0.65	0.77	0.78	0.77	0.78	0.74	0.69	0.75	0.71	0.66	0.71	0.73
AZT	0.78	0.64	0.74	0.78	0.7	0.75	0.77	0.76	0.72	0.71	0.7	0.72	0.73	0.71	0.71	0.73
D4T	0.8	0.66	0.78	0.8	0.68	0.78	0.79	0.78	0.79	0.76	0.75	0.77	0.75	0.72	0.76	0.76
DDI	0.77	0.61	0.73	0.77	0.67	0.73	0.77	0.75	0.74	0.75	0.74	0.75	0.72	0.71	0.71	0.73
TDF	0.76	0.46	0.66	0.76	0.69	0.66	0.72	0.7	0.65	0.69	0.68	0.67	0.69	0.73	0.63	0.68
Avg [‡]	0.80	0.64	0.76	0.79	0.71	0.76	0.78	0.77	0.76	0.76	0.74	0.76	0.75	0.74	0.74	0.75
Nonnucleoside RT inhibitors																
DLV	0.82	0.73	0.82	0.81	0.78	0.78	0.8	0.84	0.82	0.84	0.84	0.82	0.84	0.78	0.82	0.81
EFV	0.86	0.78	0.82	0.86	0.82	0.82	0.85	0.87	0.8	0.85	0.84	0.8	0.84	0.77	0.8	0.83
NVP	0.87	0.74	0.87	0.86	0.78	0.85	0.88	0.87	0.86	0.91	0.91	0.89	0.91	0.81	0.89	0.86
Avg [‡]	0.85	0.75	0.84	0.84	0.79	0.82	0.84	0.86	0.83	0.87	0.86	0.84	0.86	0.79	0.84	0.83

Shown is the proportion of phenotypes for which the predicted result matched the actual result: susceptible vs. low/intermediate vs. high-level resistance.

*The list of drug abbreviations is shown in Table 2.

[†]Mean accuracy for each drug averaged over mutation set and learning method.

[‡]Mean accuracy for drug class according to mutation set and learning method.

quences and significantly associated with decreased susceptibility to one or more NRTIs. The TSM coefficients provided quantitative confirmation of each nonpolymorphic expert panel mutation. In addition, 8 non-expert-panel TSMs (K43E/Q, V75M/T, E203K, D218E, K219R, and L228H) decreased susceptibility to one or more NRTIs. As previously reported, M184V increased susceptibility to zidovudine (AZT), stavudine (d4T), and TDF; L74V increased susceptibility to AZT and TDF; and K65R increased susceptibility to AZT (11–13).

The LSR coefficients for 24 NNRTI TSMs occurring in ≥ 2 sequences and associated with decreased susceptibility to one or more NNRTIs are shown in Table 10. Most mutations decreased susceptibility to each of the NNRTIs. Seven non-expert-panel TSMs (K101E/P, K103S, Y181V, G190E/Q, and K238T) decreased susceptibility to one or more NNRTIs.

To explore the effect of NNRTI-resistance mutations on NRTI susceptibility and NRTI-resistance mutations on NNRTI susceptibility, we used LSR and the combined set of NRTI and NNRTI TSMs to predict both NRTI and NNRTI susceptibility. Two NNRTI-resistance mutations, L100I and Y181C, had significantly negative regression coefficients for AZT (-0.45 and -0.45) and TDF (-0.43 and -0.33) consistent with previous reports (12, 14). Five NRTI-resistance mutations had significantly negative regression coefficients for efavirenz (EFV) including M41L, D67N, M184V, L210W, and K219Q (range of -0.08 to -0.17), consistent with previous reports (15).

Discussion

Association studies with HIV-1 genotype are complicated by the high dimensionality of the data caused by HIV-1's high mutation rate. We used several types of external feature selection to limit the dimensionality of HIV-1 genotypic data and five statistical learning methods to create models of how genotype influences susceptibility. LARS, the only regression method that performed its own feature selection, was superior to the other regression methods at predicting decreased susceptibility in the absence of external feature selection

(i.e., using the complete set of mutations present in ≥ 2 sequences in the data set). However, LARS was not superior to the other regression methods when externally derived features were used.

The most successful approach to external feature selection was to use a set of mutations previously identified as absent in viruses from treatment-naïve individuals and occurring at significantly increased frequencies in viruses from treated individuals (10). This set of nonpolymorphic TSMs had the highest accuracy (80.1%) averaged over the five learning methods when classifying isolates as susceptible, low/intermediate, and high-level resistant, and had the lowest MSEs compared with the other mutation data sets when used for regression. The success of TSMs at predicting susceptibility follows from Darwinian principles: the TSMs emerge during therapy presumably because they facilitate virus escape from drug inhibition. Exploiting the results of an analysis on a separate data set containing genotype-treatment correlations thus proved superior to relying completely on the genotype-phenotype data set to predict phenotype.

The fact that prediction accuracy was lowest for the drugs (atazanavir and TDF) with the fewest samples suggests that accuracy depends to a large extent on sample size. The learning curves show that for most drugs, ≈ 400 genotype-phenotype examples were required for optimal predictive accuracy and that the MSE then saturates at 0.15–0.20, suggesting that factors other than TSMs influence susceptibility. For example, polymorphic sites influence susceptibility either directly or indirectly by affecting virus replication capacity (16). In addition, protease cleavage site mutations, primarily those in the *gag* gene, also influence viral replication capacity and drug susceptibility (17).

Although it is likely that the effect of individual mutations on drug susceptibility depends on the presence of other mutations, we did not demonstrate an improvement in predictive accuracy using a LARS regression model that included all possible two-way interactions or using a polynomial kernel for SVR (data not shown). This may be because the process of exploring interactions without model selection to choose sets of interacting mutations often leads

Drug Susceptibility Results. For consistency, only susceptibility results generated by the PhenoSense method (Monogram Biosciences, South San Francisco, CA) were analyzed (7, 8). Drug susceptibility results were expressed as fold change in susceptibility defined as the ratio of the IC₅₀ of an isolate and a standard wild-type control isolate. Results were classified into three categories: susceptible, low/intermediate resistance, and high-level resistance. The cut-off between susceptible and low/intermediate resistance was based on the distribution of results in HIV-1 isolates from untreated persons lacking drug-resistance mutations (18). The cut-off between low/intermediate and high-level resistance was based partly on the drug's dynamic susceptibility range (fold difference of the most highly resistance isolates) and partly based on levels of resistance associated with markedly reduced clinical activity.

For the PIs, <3.0-fold resistance was considered susceptible, 3.0- to 20-fold was considered low/intermediate resistant, and >20-fold was considered highly resistant. For the NNRTIs and the NRTIs AZT and lamivudine, <3.0-fold resistance was considered susceptible, 3.0- to 25-fold was considered low/intermediate, and >25-fold was considered highly resistant. For the NRTIs ddI, d4T, and TDF, a fold resistance of <1.5 was considered susceptible, 1.5–3.0 was considered low/intermediate, and >3.0 was considered highly resistant. For the NRTI ABC, <2.0-fold resistance was considered susceptible, 2.0- to 6.0-fold was considered low/intermediate, and >6.0-fold was considered highly resistant. Susceptibility results were log-transformed and standardized before analysis.

Prediction Algorithms. Decision trees. The C4.5 algorithm (19) was used to construct decision trees for the 16 drugs in the study. Mutations were the attributes, and phenotypic classifications were the decision variables.

Neural network. Feed-forward neural network models with a single hidden layer were trained to predict susceptibility by using the R package AMORE (<http://cran.r-project.org>). The input layer contained a node for each of the mutation attributes. The hidden layer was assigned 12 nodes for all drugs. The output layer consisted of three nodes, one for each of the susceptibility classes. The weights were learned by using the error back propagation algorithm.

Support vector regression. SVR was used to learn a regression function of the form: $f(\mathbf{x}) = \sum \alpha_i K(\mathbf{x}, \mathbf{x}_i) + b$, where $f(\mathbf{x})$ is the logarithm of the fold value for the training sample and \mathbf{x} is the binary vector of mutations (20). K is the kernel function, the nonzero α_i correspond to the support vectors, and b is the bias term. Both linear and polynomial kernels were used. The latter was used to model interactions between mutations. SVR was performed with the PyML package (available at <http://pyml.sourceforge.net>).

Linear regression. LSR and LARS (20, 21) were used to predict the logarithm of the fold decrease in susceptibility. For each of the regression methods, the coefficients were learned after scaling the log-fold values to zero mean and unit variance to analyze the relative impact of a mutation on different drugs. LARS is a constrained model building procedure similar to the LASSO (23) that constructs a model by first finding the mutation most correlated with susceptibility and then incrementally builds the model by following the “equi-angular vector” until another variable is equally correlated with the residual. The process would eventually terminate at a least-squares solution. However, a validation set (20% of the data) was used to decide when to stop adding variables to the model. With LARS, second-order polynomials were also used to model interactions among each of the input mutations.

Cross-Validation. Five-fold cross-validation was used to determine the mean generalization accuracy of each learning method on test data. Five-fold cross-validation was run 10 times on different subdivisions of the data set to estimate variability of the mean generalization accuracy. For decision trees, LSR, and SVR, 80% of the data were used for training and 20% for testing. For neural networks and LARS, 60% of the data were used for training, 20% for validating the selected model, and 20% for testing.

Performance Criteria. Accuracy was the proportion of correctly predicted samples. The regression methods were also evaluated by using the MSE and r^2 between actual and predicted standardized log-fold values. Both measurements indicate how much of the variability in the response variable (the standardized log-transformed reduction susceptibility) was explained by the regression model.

All comparisons between drug classes, drugs, mutation sets, or learning methods were done in a pairwise fashion, using paired differences of the averaged accuracy or MSE for each repeated run of cross-validation. For instance, the accuracy for mutations sets was averaged over all drugs and learning methods, resulting in a single observation per run of cross-validation. A one-sample permutation test on the 10 paired differences was used to ensure the correct null distribution was used for computing P values.

To assess the effect of the number of training examples (genotype–phenotype correlations) on prediction accuracy, we created sets of randomly selected training examples that were multiples of 50 and ranging in size from 50 to 600 for testing and training using 5-fold cross-validation.

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