Broad-Spectrum Profiling of Drug Safety via Learning Complex Network

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Drug safety is a severe clinical pharmacology and toxicology problem that has caused immense medical and social burdens every year. Regrettfully, a reproducible method to assess drug safety systematically and quantitatively is still missing. In this study, we developed an advanced machine learning model for de novo drug safety assessment by solving the multilayer drug-gene-adverse drug reaction (ADR) interaction network. For the first time, the drug safety was assessed in a broad landscape of 1,156 distinct ADRs. We also designed a parameter ToxicityScore to quantify the overall drug safety. Moreover, we determined association strength for every 3,807,631 gene-ADR interactions, which clues mechanistic exploration of ADRs. For convenience, we deployed the model as a web service ADRAlert-gene at http://www.bio-add.org/ADRAlert/. In summary, this study offers insights into prioritizing safe drug therapy. It helps reduce the attrition rate of new drug discovery by providing a reliable ADR profile in the early preclinical stage.

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ The adverse drug reaction (ADR) is a severe clinical pharmacology/toxicology problem that has caused immense medical and social burdens. It is also one of the two major causes leading to new drug discovery failure. Unfortunately, current mechanistic understanding and profiling of drug safety are still limited in both concept and methodology.

WHAT QUESTION DOES THIS STUDY ADDRESS?
☑ Most of the current experimental and computational methods primarily focus on drug toxicity in cell or tissue/organ instead of adverse consequences in the clinic. Furthermore, a general, repeatable, and systematic method to study the mechanism of ADR is still needed.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ This study implements a new method for broad-spectrum ADR profiling. It suggests a gene-based systematic exploration of the ADR mechanism, which is applicable to most ADRs.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ This study provides new insights to understand clinical pharmacology. This study may enhance the success rate of new drug discovery by reducing the drug safety problems in clinical trials. It also helps prioritize clinical pharmacogenetic tests for safe drug therapy.
large-scale postmarket surveillance, which is costly and time-consum-
ing. Sometimes, it has to compromise patients’ treatment and satisfac-
tion. For all this, the ADR profiles present in the drug labels can still
be incomplete or inaccurate due to the medical complexity.9

As a complementary solution to experimental assays, computa-
tional methods have been developed for drug toxicity evaluation.
For instance, a number of computer programs built upon the
quantitative structure-activity relationship for high-throughput
assessment of drug toxicity, such as hepatotoxicity, nephrotox-
icity, genotoxicity, oncocogenicity, and so on. Typical applications
include DEREK,10 TOPKAT,11 COMPACT,12 MULTICASE,13
HazardExpert,14 and OncoLogic. It is noteworthy that drug toxicity
and ADR are two linked but different concepts. Drug toxicity
is often determined under different medication dosing and timing
to assess damaging effects of definite chemical on cells or organs at
early drug discovery stage; whereas the ADRs are undesired clin-
cal consequences observed in drug therapy. However, the chemical
structure itself possesses some hidden features to cause cell/organ
toxicity and eventually induce ADRs. Hence, linking chemical
features to ADRs provide a feasible way to aid better investigation
of ADRs.19 Some machine learning algorithms, like decision tree,
have been applied to determine the chemical, physical, and struc-
tural properties of chemical drugs that predispose to ADRs.16–18

In a view of systems biology, the interactions between drug (or its metabo-
lites) and proteins or pathways are likely the driving force to
drug-induced adverse events. In recent years, different research groups
tried to depict ADRs by undesired drug-protein interactions, pertur-
bation of metabolic pathways, or organ malfunction.19 For instance,
the SePreSA built chemical-protein interactome to predict SADRs.20
LaBute et al. used logistic regression models on top of molecular
docking for ADR prediction.21 Huang et al.22 proposed a framework
for predicting ADR profiles by integrating protein-protein interac-
tion networks with drug structures. Liu et al.23 combined chemical
structures, biological properties, and phenotypic characteristics of
drugs for ADR prediction using the machine learning method. Cami
et al.24 developed the predictive pharmacosafety networks that com-
bined the safety, taxonomic, and biological information of specific
drugs and adverse events. Pan et al.25 used a high-throughput docking
program to create prostate-wide drug-off-target interaction profiles
for the network understanding of SADRs. It was proposed that tar-
get/pathway-based methods might outperform ligand-based meth-
ods in ADR assessment as they circumvented the hurdle of linking
the drug itself or its metabolites to specific ADRs.26 However, the
full drug-target interaction profiles are usually hard to obtain by
current experimental and computational technologies. Although
high-throughput screening systems can make use of robotics to test,
automatically and quickly, the interaction activities between drugs
and protein targets, the screening is usually limited to some well-
established protein panels, like kinases and receptors. As a result, the
target-based ADR studies often demonstrated on several ADRs of
interest.

Recent advances in systems pharmacology and toxicology in-
corporate microarray and next-generation sequencing to monitor
large-scale gene expression changes in response to outside chemical
stimulus simultaneously. By comparison of gene expression profiles
in different cell lines, drug efficacies to different tumor therapies
can be evaluated.27 This inspires us the opportunity to directly
link biological effects (i.e., gene expression changes) to clinical
outcomes (i.e., ADRs) upon drug treatment,19 leaving the interme-
diate drug-target/pathway interactions as a black box. Such a strat-
egy will take advantages of systems biology in network depiction
of ADRs and at the same time bypass the difficulty in acquiring
a full profile of the drug-target interactions underlying ADRs. In
this study, we will construct a multilayer complex drug-gene-ADR
interaction network by integrating heterogeneous, multiscale, and
historical data. Upon the network, we aim to develop an advanced
machine learning model for de novo ADR prediction via building
the full spectrum of gene-ADR associations statistically in a retro-
spective manner. Last, we will try to use the weighted gene-ADR
associations in unveiling ADR mechanisms systematically.

MATERIALS AND METHODS

Hypothesis

In this study, we built a model on top of a multilayer drug-gene-ADR
complex network (Figure 1) according to the assumption as followings:
As the clinical consequence of drug treatment, ADRs sometimes occur.
The occurrence of ADRs could attribute to any one or combination
of mechanisms, such as overdose, weak pharmacokinetic, drug-drug
interaction, off target, and so on. Underlining these mechanisms are
cascade molecular events led by drug treatment, including abnormal
protein activity, disturbance of biological pathways, and dysfunction of
organs. Regretfully, it is hard to determine the driving molecular events
and their subsequent ADR mechanisms in most cases. However, when
acted with drugs, the expressions of genes also change. It is the bio-
logical outcome of protein or pathway disturbance. In particular, the
gene expressions mostly change at the focus tissues where ADRs hap-
pen. Hence, associations exist between gene expression changes and the
occurrence of adverse reactions. Compared with the hard-to-acquire
drug-protein interactions, the drug-gene interactions are easy to deter-
dine, thanks to the wide application of transcriptome technologies.
Then, the problem is a shift to building the logic associations between
gene expression changes and ADRs. Taking advantage of machine
learning, we can determine the association strength for every gene-
ADR pair via statistically solving the known drug-gene-ADR relations
(Figure 1). By doing so, we leave the exact molecular mechanisms un-
derlying the ADRs as a black box. When the gene-ADR associations are
substantially represented and measured in the model, evaluation of an
ADR profile from the drug-regulated genes becomes feasible.

Data sources and data processing

To build the model, we incorporated a number of relations in the mul-
tilayer interaction network from various sources, including drug-ADR
relations, drug-gene regulations, gene-gene interactions, ADR concurre-
ence, and so on.

Drug-ADR relations. We derived the drug-ADR relations from the
Adverse Drug Reaction Classification System (ADReCS version 1.4;
http://bioinf.fxm.edu.cn/ADReCS),28 The ADReCS is a comprehen-
sive ADR ontology database that offers both standardization and hi-
erarchical classification of ADR terms via integrating the information
from multiple resources, such as MedDRA,29 WHO-ART,30 DailyMed,
and SIDER.31 The ADReCS version 1.4 covers total 6,778 standard
ADR terms, 1,378 marketed drugs, and 196,194 nonredundant drug-
ADR pairs from the drug labels. However, subject to the availability of
known drug-gene relations, the current model only included 365 drugs
and 1,156 ADR high level terms (HLTs). In MedDRA and ADReCS,
each HLT represents a group of related ADRs on the basis of anatomy,
pathology, physiology, etiology, or function.
Drug-gene relations. We derived 106,739 literature-documented human drug-gene relations from the Comparative Toxicogenomics Database (CTD; http://ctdbase.org). The direction and strength of drug-gene regulations were downloaded when available. As a complementary source, we also extracted the drug-gene relations from the Library of Integrated Network-based Cellular Signatures (LINCS; http://www.lincsproject.org).
The LINCS was elaborately designed to determine how perturbations like drug treatment affect gene expressions across multiple cells and perturbation types. In this study, we obtained normalized gene expression profiles (Z-score) from 14 cell line experiments treated with drugs at a concentration of 10 μM for 6 hours. The selection of experimental conditions was in consideration of acquiring as much as possible drug-gene relations under the constraints of data availability. For each drug treatment, differentially expressed genes, comparing with the untreated control, with the moderated Z-scores ≥ 2 or Z-scores ≤ −2 over at least two experiments were taken as reliable signature genes for the drug (i.e., drug-regulated genes). For modeling, we only adopted the consensus drug-gene pairs that had the same regulation direction (upregulation or downregulation) in all experiments. To weight the regulation strength for a consensus drug-gene regulation pair, we selected either the maximum positive value of Z-scores for upregulation or the minimum negative value of Z-scores for downregulation in all experiments. Ultimately, we obtained 25,274 drug-gene relations from the LINCS. Compared with the CTD, the drug-gene relations mined from the LINCS are comparatively full-scale than those from scientific literature or individual experiments deposited in ArrayExpress37 and Gene Expression Omnibus (GEO). The inclusion of LINCS data may improve the integrity and reliability of the drug-gene-ADR trilateral network.

**Gene-gene relations.** We obtained the quantitative gene-gene relations from the GeneMANIA Cytoscape plugin. GeneMANIA measures gene-gene relations using a guilt-by-association approach over publicly available biological big data. The big data include multiple molecule interaction networks of protein-protein, protein-DNA, genetic interaction, pathway, co-expression, colocalization, and protein domain similarity from multiple organisms.40,41 In this study, we measured the gene-gene relations quantitatively with all Homo sapiens interaction networks and incorporated those relations with weight ≥ 0.001 into the model.

**ADR-ADR concurrence.** The information ADR concurrence was determined on the basis of the AdrReCS data. We calculated the concurrence rate, denoted as , between a pair of ADR HLTS — (consisting of of preferred terms) and (consisting of of preferred terms) by:

\[
\begin{align*}
\omega_{PT_i,PT_j} &= \frac{D_{PT_i,PT_j}}{D_{PT_i} \times D_{PT_j}} \times \frac{D_{PT_i} \times D_{PT_j}}{D^2}, \\
\omega_{A_i,A_j} &= \frac{\sum_{i=1}^{x} w_{PT_i,PT_j} \times \left( \frac{D_{PT_i}}{D_{A_i}} + \frac{D_{PT_j}}{D_{A_j}} \right)}{\sum_{i=1}^{y} \left( \frac{D_{PT_i}}{D_{A_i}} + \frac{D_{PT_j}}{D_{A_j}} \right)},
\end{align*}
\]

where stands for the number of drugs inducing of the number of drugs inducing , stands for the number of drugs inducing preferred term , stands for the number of drugs inducing , and stands for the total number of drugs in the model. Only ADR-ADR pairs with concurrence ≥ 0.01 were used in the model.

Eventually, we built the model using 38,761 drug-ADR relations, 20,867 drug-gene relations, 19,229 gene-gene interactions, 6,195 ADR-ADR pairs, 365 drugs, 8,571 genes, and 1,156 distinct ADR HLTS.

**Construction of the machine learning model**

**The weighted Bayesian model.** We denoted the three partners of drug-gene-ADR complex network as the drug set , the gene set , and the ADR set . The weighted Bayesian model incorporates those relations with weight ≥ 0.001 into the model. Furthermore, we assumed that a drug in may induce multiple ADRs in . And vice versa, an ADR in may be induced by multiple drugs in . In the meantime, a drug in may regulate multiple genes in ; and vice versa, a gene in may be regulated by multiple drugs in .

In previous work, we constructed an unweighted naive Bayesian model prototype for ADR prediction, assuming that the drug-gene-ADR trilateral relations were all independent, unweighted, and directionless. Such an assumption is too idealized. In the real world, all of the elements in the molecular network can actually interact with each other. Therefore, we significantly improved the model as following in this study.

In the case of ADR induced by the expression change of a single gene , the drug set that regulates , (i.e., the drug-gene pairs) were denoted as . The set that regulates and, thus, leads to ADR is denoted as . Accordingly, the posterior probability of induced by the expression change of a single gene , (despite of gene-gene regulation and ADR concurrence), denoted as , can be calculated by:

\[
P(A_m | G_n) = \frac{\sum_{i=1}^{p} w_{D_i G_n} \times w_{D_i A_m}}{\sum_{i=1}^{q} w_{D_i G_n} \times w_{D_i A}}
\]

where stands for the regulation strength of drug on gene ; stands for the frequency of drug in drug treatment, stands for the regulation strength of drug on gene ; stands for the frequency of any ADR in drug treatment.

In the real world, ADR can be induced by the expression changes of different genes, denoted as , where . As the result, the probability of triggered by , can be calculated by:

\[
P(A_m | G_{tgt}) = 1 - \prod_{i=1}^{r} \left( 1 - P(A_m | G_{n_i}) \right),
\]

where stands for the probability of triggered by .

**Incorporation of gene-gene regulation and ADR concurrence.** In the real world, genes have interactions between each other, and ADRs often happen together. We denoted the genes that interact with , as , where . As well, we denoted the ADRs that occur concurrently with , as . When incorporating the gene-gene regulation and ADR concurrence into the model, the probability of triggered by gene set , denoted as , can be calculated by:

\[
P(A_m | G_{tgt}) = 1 - \prod_{i=1}^{r} \left( 1 - \omega_{G_i G_{tgt}} \times P(A_m | G_{i}) \times \omega_{A_m A_i} \right),
\]

where , stands for the weight of -gene interaction , and stands for the frequency of ADR concurrence . The probability can also be taken as the estimated frequency or occurrence of ADR .

**Normalization of ADR probability by adjusting the bias caused by drug-gene regulations.** As our observation, the estimated frequency was affected by the number of effective input genes. Hence, we demonstrated a retrospective ADR prediction for 324 drugs, analyzing the change of detection rates (DRs) and false-positive rates (FPRs) by the number of effective input genes. Hence, we demonstrated that the average DR, as well as higher FPR (Figure 2). Therefore, to eliminate the bias caused by...
the number of input genes and at the same time normalize the estimated ADR frequency into the same scale of the observed ADR occurrence rate, we made an empirical correction of estimated frequency \( P(A_m | G_{k|t}) \) to a normalized value, denoted as \( P(A_m | G_{k|t, norm}) \), which can be determined by:

\[
P(A_m | G_{k|t, norm}) = 1 - \prod_{k=1}^{t} \left( 1 - \frac{3 + [t/10]}{t} \times P(A_m | G_k) \right)
\]

if \( (3 \leq t < 100) \),

\[
P(A_m | G_{k|t, norm}) = 1 - \prod_{k=1}^{t} \left( 1 - \frac{12 + [t/100]}{t} \times P(A_m | G_k) \right)
\]

if \( (100 \leq t < 1,000) \),

\[
P(A_m | G_{k|t, norm}) = 1 - \prod_{k=1}^{t} \left( 1 - \frac{21 + [t/1,000]}{t} \times P(A_m | G_k) \right)
\]

if \( (t \geq 1,000) \),

where \( t \) stands for the number of effective input genes for ADR assessment.

Model evaluation

In this study, we adopted the conventional 10-fold cross-validation strategy to evaluate model performance on the basis of 365 marketed drugs. All of the drugs were randomly divided into 10 folds (subsamples) of nearly equal size, each of which had similar data distribution in 14 Anatomical Therapeutic Chemical (ATC) categories (Supplementary Figure S1). For the 10 folds, 9 were used for model training and 1 was retained as the validation dataset for model testing. The cross-validation process was repeated 10 times, at which each of the 10 folds was chosen as the validation data once. We also derived 18 external trial drugs extracted from the Clinical Trials database (http://clinicaltrials.gov) by the criteria of: (i) the trial drugs are not recorded in both ADReCS database and DrugBank database and (ii) the drugs have information of at least three drug-gene regulations. Unlike most deterministic methods that make the true or false classification, the Bayesian model of this study outputs the probability for every ADR in a broad spectrum of 1,156 distinct ADR HLTs. Therefore, we adopted the DR to measure the ratio of known ADRs predicted by the model:

\[
DR = \frac{PKA}{KA} \times 100% ,
\]

where PKA stands for the number of known ADRs predicted by the model and KA stands for the number of known ADRs. In addition, we also adopted metrics, like accuracy, sensitivity, specificity, the receiver operating characteristic curve, and the area under the curve of ROC (AUC) for model evaluation.

The ToxicityScore for summarizing drug safety

In this study, we introduced a new parameter, the ToxicityScore, to summarize the toxicity effects of a drug. This parameter evaluates the overall drug safety by integrating the information of both ADR occurrence rate (here, the estimated ADR frequency) and ADR severity. The ToxicityScore overcomes the shortages of current methods that relied on several preset toxicity features, like hepatic toxicity and kidney toxicity, for drug safety assessment in early drug discovery. In advance, we pre-assigned ADRs into five severity categories of mild, moderate, severe, life-threatening, or death according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.43,44 The CTCAE is a predominant system for describing the severity of adverse events commonly encountered in clinical trials. Here, we determined the severity grade for each ADR HLT used in the model manually according to the guideline of CTCAE. As each HLT may consist of multiple ADR preferred terms of different severity grades, we adopted the lowest grade of the most frequent preferred term induced by most drugs as the grade for HLT. Examples of ADR severity assignment were given in Table S1. To quantitatively differentiate the grades, we further assigned ADR a severity score \( S_i \) of 1, 10, 100, 1000, and 10,000 for grades 1 to 5, respectively.

The ToxicityScore can be determined by:

\[
\text{ToxicityScore} = \sqrt{\sum_{i=1}^{n} (F_i S_i)^2} = \sqrt{F_1 S_1}^2 + (F_2 S_2)^2 + \ldots + (F_n S_n)^2, \quad (8)
\]

where \( F_i \) stands for the estimated/observed occurrence rate of ADR \( A_i \), \( S_i \) stands for the severity score of ADR \( A_i \), and \( A_i \) belongs to ADR list \( \{ A_{i1}, A_{i2}, \ldots, A_{in} \} \) of the drug. The estimated frequency \( F_i \) comes from the model prediction.

RESULTS

The performance of the advanced machine learning model

According to conventional practice, we evaluated the model performance using both the internal dataset and the external dataset. The internal 10-fold cross-validation yielded an average DR of 91.70% if all predicted ADRs were included (frequency threshold > 0; Table 1). When setting the ADR frequency threshold to 0.1%, a
frequency threshold of “less common ADRs” defined by Wooten, the average DR dropped to 75.10%. The drop of DR was the result of excluding the rare known ADRs with occurrence rate < 0.1%. At the same time, the threshold also helped eliminate about 65.26% of potential FPRs (newly predicted ADRs). The independent test was undertaken on 18 external real-world trial drugs extracted from the Clinical Trials database, which yielded an average DR of 98.22% or 84.38% (estimated occurrence rate ≥ 0.1%). In addition, we also evaluated the model performance using several metrics that are often adopted in evaluating deterministic models. In general, the model achieved an accuracy of 81.11% and 75.59% for internal and external datasets at the frequency threshold of 0.1%, respectively; accordingly, the AUC value was 78.91% and 79.85%, respectively (Table 1). It should be noted that these metrics were underestimated due to excluding the rare ADRs (estimated frequency < 0.1%), which are usually counted in reality. Hence, both the internal and external evaluations consolidated the model robust in ADR prediction.

The web service of ADRAAlert-gene for rapid drug safety profiling

For user convenience, we deployed the well-established model as a web service ADRAAlert-gene at http://www.bio-add.org/ADRAAlert/ or its mirror site at http://bioinf.xmu.edu.cn/ADRAAlert/gene. We constructed the server upon a Linux + Tomcat architecture and developed interactive user interfaces using JavaScript. The ADRAAlert-gene could provide not only de novo assessment of drug safety but also the quantitative measure of gene-ADR relations. We described the details of ADRAAlert-gene access in the Supplementary Information.

ToxicityScore is a suitable parameter for evaluating overall drug safety quantitatively

In this study, we introduced a novel parameter, the ToxicityScore, to summarize the overall drug safety in a broad spectrum of 1,156 distinct ADR HLTs. To evaluate the reliability of ToxicityScore in representing the overall drug safety, we made statistical analyses on the ToxicityScores of 432 selected drugs, covering 20 over-the-counter drugs, 412 prescription drugs, and 1,058 distinct known ADRs. The average ToxicityScore for the known ADRs of over-the-counter drugs, determined by either estimated or observed ADR frequency well correlated with the observed ADR occurrence (R = 0.94, P < 10⁻⁶; Figure 3a,c). This result partially proved the feasibility and reliability of ToxicityScore in the evaluation of drug safety. Therefore, the ToxicityScore of current marketed drugs can serve as a suitable reference for selecting “safe” drug candidates according to their indications in the early drug discovery stage.

Example applications

Example 1: Identification of novel ADRs for known drugs. Lansoprazole is usually used to inhibit the acid production of stomach. Searching “lansoprazole” via the “From Drug” view responds 8 signature genes from the LINCS project and 32 lansoprazole-interacting genes from the CTD. Based on these regulated genes, the server predicted 368 ADRs for lansoprazole by the frequency threshold of 0.1%. The 368 predicted ADRs fully cover all 329 documented ADRs in the drug label in the ADReCS database; the DR is 100%. Of note, the majority of 329 known ADRs have generally higher estimated frequency than those of the remaining 39 novel ADRs. Of the 39 novel ADRs, we found 15 ADRs, including heart failures, mental disorders, and non-site-specific injuries, in a recent ADR collection of lansoprazole treatment in the Side Effect Resource (SIDER 4.1), DailyMed (updated by February 2017), and ClinicalTrials.gov databases (by January 2019).

Example 2: De novo ADR assessment. Levosalbutamol is a short-acting β₂ adrenergic receptor agonist used in the treatment of asthma and chronic obstructive pulmonary disease. As of October 2019, levosalbutamol has been tested against asthma in phase
III and IV clinical trials. As documented in the Clinical Trials database, levosalbutamol might cause 71 different ADRs in 217 studies, covering a few hundred of selected patients. As well, mining of the US Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) identified 114 potential levosalbutamol-ADR associations in 306 reports. Using seven levosalbutamol-regulated genes (RFC2, LOXL1, PCSK1N, BID, HOXA10, LSR, and HSPA4L) extracted from the LINCS, ADRAAlert-gene predicted total 225 ADRs via the “From Gene” view at the frequency threshold of 0.1%. Of the 71 observed ADRs, 36 (about 50.70%) were predicted by the server with comparatively high frequency (3.84% average). For the 114 possible levosalbutamol-induced ADRs mined from the FAERS, 72 (about 63.16%) were predicted by the server. The remaining 138 predicted ADRs were either novel ADRs that have not been reported due to the limitation of trial data or potentially false positives.

Figure 3 Statistics of ToxicityScores by drug types. The ToxicityScores of different Anatomical Therapeutic Chemical (ATC) drug types, determined by the observed occurrence rate (a) and the estimated occurrence rate (c) of known adverse drug reactions (ADRs), respectively. The ToxicityScores of 20 over-the-counter drugs and 412 prescription drugs, determined by the observed occurrence rate (b) and the estimated occurrence rate (d) of known ADRs, respectively. The color box stands for 75% of data, the black line stands for the median value, and the asterisk stands for the mean value. [Colour figure can be viewed at wileyonlinelibrary.com]
In the real world, the clinical safety of a new molecular entity can be evaluated in a simple way of comparing the calculated ToxicityScore against that of the marketing drugs, especially the drugs of same indication (e.g., ATC category; Figure 3). If the new molecular entity has a substantially higher ToxicityScore and consists of severe ADRs with estimated frequency ≥ 0.1% particularly, further clinical trials should be prudentially conducted. In this example, the server evaluated levosalbutamol safety with a ToxicityScore of 60.870 at the frequency threshold of 0.1%. This score was much higher than that of the marketing drugs of the Respiratory System category, which is 18.78 on average. Two common (estimated frequency ≥ 10%) severe ADRs (“cardiac signs and symptoms” and “circulatory collapse and shock”) and a less common (estimated frequency of about 0.89%) life-threatening ADR “sepsis” may answer for the high ToxicityScore. These three severe ADRs have been reported in the Clinical Trials and FAERS. Therefore, more attention should be paid to levosalbutamol safety in future clinical trials.

Example 3: Mechanistic understanding of ADRs by network analysis of gene-ADR associations. Allergic conditions (ACs; ADReCS ID: 10.01.03) is in a group of ADRs, including allergic reactions, hypersensitivity, asthma, Stevens-Johnson syndrome, and so on. Up to date, the molecular mechanisms underlying ACs have not yet been fully explored. By searching “Allergic conditions” or “10.01.03” via the “Gene-ADR From ADR” view, we extracted 8,571 AC-associated genes (3,852 upregulated, 757 downregulated, and 3,962 both upregulated and downregulated). We selected 563 comparatively strong AC-associated genes (the association strength ≥ 0.025) for later mechanistic study. Of them, 424 were upregulated genes and 139 were downregulated genes. We mapped these 563 genes against the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, which identified 209 AC-associated pathways. Of them, we specified 34 major AC-associated pathways (covering at least six AC-associated genes), of which about one-third (12 pathways) belong to the signal transduction pathways and immune system pathways (Figure 4a). The 209 AC-associated pathways include a total of 225 AC-associated genes. Eight of them (ALOX5, DNMT1, HLA-B, IFNG, IL15, ITGB2, KNG1, and TBXA2R) have literature evidence from the CTD and the Online Mendelian Inheritance in Man (OMIM) to support their associations with the allergy symptoms. On the basis of the 225 KEGG-mapped genes, we constructed a gene-gene interaction network (Figure 4b), from which we identified 5 hub genes (CXCL10, HUWE1, ITGB2, RPS23, and SELL) by satisfying the criteria of: owning a connection degree ≥ 8 and neighboring to two or more known AC-related genes. These hub genes are potential major gene players in understanding drug-induced allergic reactions. Of them, ITGB2 was previously reported to be a biomarker in monitoring the dysregulated allergic response. CXCL10 exhibited significantly higher concentration in allergic patients than that of the healthy subjects. The protein of HUWE1 might function as an E3 ubiquitin ligase that played a role in modulating allergic responses.

DISCUSSION
This work introduces an advanced machine learning model and its web service ADRAlert-gene for rapid drug safety assessment via a learning drug-gene-ADR complex network. Compared with prior methods, it has several advantages: (i) To our limited knowledge, ADRAlert-gene is the only method that provides broad-spectrum ADR profiling. It allows reviewing the drug safety in a landscape of 1,156 distinct ADRs. (ii) Unlike many target-based models that heavily rely on complete chemical-protein interaction profile for reliable ADR prediction, ADRAlert-gene just requests representative drug-gene regulation profile (i.e., significantly differentiated genes of drug treatment), which is easier to obtain via state-of-the-art transcriptome sequencing.
technology in current practice. (iii) The ADRAlert-gene is up-to-date and the first tool that provides comprehensive information more than simple ADR prediction. The ToxicityScore integrates multiple information of the ADR number, ADR frequency, and ADR severity quantitatively to measure overall drug safety. As we know, the high-incidence or SADRs usually receive more attention in the clinic and new drug discovery. (iv) Last but not least, the ADRAlert-gene provides a new thought to reveal major gene players in ADR via genomewide quantifying gene-ADR associations. Such a quantitative gene-ADR association profile is hard to obtain through conventional molecular technologies.

We also acknowledge several limitations of the model. First, the current version of the ADRAlert-gene model was partially built upon drug-gene regulations derived from the LINCS project. Unfortunately, the experimental design and data quality of the LINCS project is arguable itself because it was not particularly designed for drug toxicity research. Especially, most cells used in the LINCS project are tumor cell lines instead of primary normal cells in which most ADRs may occur. Second, the drug-treated transcriptomes determined on various cell lines are likely to be different from those of individual patients, even though recent research suggested that the transcriptomes on human cell lines could honestly reflect human response.53 Third, the drug-gene relations mined from heterogeneous cell transcriptomes are not always consistent, pending improvement of data analysis and new technology. Last but not least, the model was developed on the basis of monotherapy assumption. In reality, most patients are typically on a polypharmacy regimen, which sometimes confronts the ADRs caused by drug-drug interactions. The drug-drug interactions are so complex that they are hard to measure quantitatively. Therefore, the model has not yet integrated the information of drug-drug interactions in drug safety evaluations in the current version. Therein, multiple factors may take part in the ADRs caused by the drug-drug interactions, including the combination of drug dosages, the order, the interval of polypharmacy treatment, the administration routes of drugs, and so on. More efforts are expected to improve the model in the future.

Nevertheless, ADRAlert-gene can serve as a powerful, practical, and economical tool for drug safety profiling. It helps to reduce the attrition rate of new drug discovery by offering reliable ADR profile in the early preclinical stage. It also provides a shortcut for network understanding of molecular mechanisms underlying ADRs. In particular, the identification of ADR-associated genes allows targeting the potential molecular causes of ADR directly. This could accelerate precision medicine because genetic testing would build the pharmacogenetic profile of different patients’ responses to the same drug quickly and undistractedly. Therefore, we believe that ADRAlert-gene will benefit the communities of both clinical pharmacology and toxicology. It will be especially useful for drug design, ADR mechanism study, and individual drug therapy.

SUPPORTING INFORMATION
Supplementary information accompanies this paper on the Clinical Pharmacology & Therapeutics website (www.cpt-journal.com).

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CONFLICTS OF INTEREST
All authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
K.L., L.X.Y., R.F.D., and Z.L.J. wrote the manuscript. Z.L.J. designed and supervised the experiment. K.L., H.X., Y.M.Q., Q.S.H., R.F.D., P.Y., F.D., Y.Z., and Y.P.X. performed the research. K.L. analyzed the data.

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