

Toxicology knowledge graph for structural birth defects

John Erol Evangelista^{1,6}, Daniel J. B. Clarke^{1,6}, Zhuorui Xie¹, Giacomo B. Marino¹, Vivian Utti¹, Sherry L. Jenkins¹, Taha Mohseni Ahooyi², Cristian G. Bologa³, Jeremy J. Yang³, Jessica L. Binder³, Praveen Kumar³, Christophe G. Lambert³, Jeffrey S. Grethe⁴, Eric Wenger², Deanne Taylor², Tudor I. Oprea³, Bernard de Bono⁵ & Avi Ma'ayan¹✉

Abstract

Background Birth defects are functional and structural abnormalities that impact about 1 in 33 births in the United States. They have been attributed to genetic and other factors such as drugs, cosmetics, food, and environmental pollutants during pregnancy, but for most birth defects there are no known causes.

Methods To further characterize associations between small molecule compounds and their potential to induce specific birth abnormalities, we gathered knowledge from multiple sources to construct a reproductive toxicity Knowledge Graph (ReproTox-KG) with a focus on associations between birth defects, drugs, and genes. Specifically, we gathered data from drug/birth-defect associations from co-mentions in published abstracts, gene/birth-defect associations from genetic studies, drug- and preclinical-compound-induced gene expression changes in cell lines, known drug targets, genetic burden scores for human genes, and placental crossing scores for small molecules.

Results Using ReproTox-KG and semi-supervised learning (SSL), we scored >30,000 pre-clinical small molecules for their potential to cross the placenta and induce birth defects, and identified >500 birth-defect/gene/drug cliques that can be used to explain molecular mechanisms for drug-induced birth defects. The ReproTox-KG can be accessed via a web-based user interface available at <https://maayanlab.cloud/reprottox-kg>. This site enables users to explore the associations between birth defects, approved and preclinical drugs, and all human genes.

Conclusions ReproTox-KG provides a resource for exploring knowledge about the molecular mechanisms of birth defects with the potential of predicting the likelihood of genes and preclinical small molecules to induce birth defects.

Plain language summary

While birth defects are common, for most birth defects there are no known causes. During pregnancy, developing babies are exposed to drugs, cosmetics, food, and environmental pollutants that may cause birth defects. However, exactly how these environmental factors are involved in producing birth defects is difficult to discern. Also, birth defects can be a consequence of the genes inherited from the parents. We combined general data about human genes and drugs with specific data previously implicating genes and drugs in inducing birth defects to create a knowledge graph representation that connects genes, drugs, and birth defects. This knowledge graph can be used to explore new links that may explain why birth defects occur, particularly those that result from a combination of inherited and environmental influences.

¹Department of Pharmacological Sciences, Mount Sinai Center for Bioinformatics, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. ²The Children's Hospital of Philadelphia, Department of Biomedical and Health Informatics; Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA. ³Department of Internal Medicine, Division of Translational Informatics, University of New Mexico, Albuquerque, NM 87131, USA. ⁴Department of Medicine, University of California San Diego, La Jolla, CA 92093, USA. ⁵Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand. ⁶These authors contributed equally: John Erol Evangelista, Daniel J. B. Clarke. ✉email: avi.maayan@mssm.edu

The United States Department of Labor's Occupational Safety and Health Administration¹ defines reproductive toxicity as a characteristic of substances or agents that may affect the reproductive health of women or men or the ability of couples to have healthy children. These hazards may cause problems such as infertility, miscarriage, and birth defects. The prevention and clinical management of reproductive toxicity caused by chemical agents² requires the combined expertise from several medical fields, including public health and occupational health to protect against environmental/occupational toxins that lead to miscarriage³, food and drug regulatory medicine to avoid drug teratogenicity or toxins in food that impact fertility, as well as clinical genetics, obstetrics, gynecology, and pediatrics to screen, prevent, monitor, and manage birth defects. This multidisciplinary nature of reproductive health is challenging. For instance, prescribing drugs in pregnancy remains a complex and controversial issue for both pregnant persons and physicians⁴. Prescriptions given to pregnant persons of drugs known to potentially cause birth defects based on evidence from animal models, human studies, and based on post-marketing evidence is a frequent event. A cohort study of over a million pregnant persons found that 42% had at least one prescription for such medications⁵. A key challenge to prescribing for the gravid patient is that recommendations are based on limited human pharmacological data and conflicting cases of adverse outcomes, given that pregnant persons are routinely excluded from randomized controlled trials⁶. Combinatorial complexity challenges and data availability limitations are also key considerations in the prediction of drug–drug interactions^{7,8} that may impact reproductive health^{9,10}. It is likely that some birth defects may be caused by a combination of factors that may include interactions between genetics, drugs, and viral or bacterial infections, making it difficult to identify a specific cause. As a result, the causes of birth defects, which in the US account for an estimated 3% of births¹ and 20% of infant deaths^{11,12}, are still mostly poorly understood.

In recent years, knowledge graphs have gained popularity as a productive approach to integrate data from multiple sources to organize information and glean new knowledge¹³. Knowledge graph databases store information about the semantic relationships between objects and represent events as triples: subject->predicate->object, for example, chicken->lays->eggs. Once these assertions are combined, they form a network made of nodes and edges and this establishes the knowledge graph. Once data from multiple sources are organized in a knowledge graph, it can be queried to extract subgraphs that can illuminate unexpected associations between entities. Integrated data organized into knowledge graphs can also be used as input into graph embedding algorithms^{14,15} that aim to predict missing associations not present in the original knowledge graph. Such an approach is increasingly applied in the domain of drug discovery¹⁶. For example, there are efforts that combine drug reaction knowledge encoded in a knowledge graph with side effects information from databases such as the FDA Adverse Event Reporting System (FAERS) to predict adverse events^{17,18} or encoding pharmacogenomics data that connect drugs, variants, and adverse events to explain variant-adverse event associations¹⁹. In many cases, these types of efforts are derivatives of comprehensive projects that aim to abstract biomedical knowledge-bases into triples or gene-attribute associations, and make such integrated data available for search and knowledge imputation²⁰. There are currently several options for knowledge graph databases, including Neo4j²¹, Stardog²², ArangoDB²³, Amazon Neptune²⁴, and OrientDB²⁵. In this study, we endeavored to combine knowledge about birth defects with knowledge about genes and drugs to identify potential molecular mechanisms for known birth defects and

predict birth defects for preclinical drugs and other small molecules. We ranked genes based on their association with pathogenicity; predicted the likelihood of small molecules to cross the placental barrier and induce birth defects using semi-supervised learning; assembled knowledge about known drug targets for marked drugs²⁶ and abstracted knowledge about the effects of drugs and preclinical small molecules on gene expression²⁷. All these data are serialized into a knowledge graph representation, stored in a Neo4j database, and provided for access via an original user-friendly web-based user interface. By combining general information about genes, drugs, and preclinical small molecules with knowledge about the association of genes and drugs with birth defects, we were able to predict the likelihood that pre-clinical compounds will induce birth defects, and whether these compounds are likely to cross the placental barrier. In addition, by analyzing the knowledge graph topological structure, we were able to pinpoint previously unknown associations between drugs and genes based on the birth defects these drugs and genes are known to induce.

Methods

Curating phenotypic terms relevant to birth defects. The manual curation of birth defects terms started from a list of observed birth-defect cases from the Gabriella Miller Kids First Pediatric Research Program (Kids First) cohort. This list of real birth defects and their frequencies is provided as supporting materials (Supplementary Data 1) and is available for download from the ReproTox-KG website at the following URL: https://s3.amazonaws.com/maayan-kg/reprotox/HPO_Freq.tsv. The list of observed birth defects had to be pruned to exclude phenotypes that are not specific to birth defects, for example, glioma. Specifically, we focused on abnormal morphologies of the great vessels, heart, and central nervous system (CNS) phenotypes. Using the EMBL-EBI Ontology Lookup Service (OLS) human phenotype ontology (HPO) v2021-10-10²⁸ we mapped terms from the table of observed cases to HPO identifiers. To this end we considered the parent terms HP:0030962 (Abnormal morphology of the great vessels), HP:0001627 (Abnormal heart morphology) and HP:0012639 (Abnormal nervous system morphology) and extracted all the child nodes. In all, 166, 193, and 177 phenotype terms were retained for great vessels, heart, and CNS, respectively (Supplemental Data 2). The phenotype terms identified as relevant to the heart, large vessels, and CNS were filtered for terms that were not immediately relevant for structural birth defects. Specifically, clinical experts filtered for including phenotypes that could only have developed in utero. So, for example, a term under Conotruncal defect (HP:0001710) would qualify for our list, but not terms related to Cardiomyopathy (HP:0001638), Palpitations (HP:0001962) or Congestive Heart Failure (HP:0001635), even though the latter three phenotypes might also be a secondary consequence of a structural birth defect. In addition, 36 major birth-defect terms were separately extracted from the Centers for Disease Control and Prevention (CDC) website²⁹ on January 6, 2022, and manually mapped to HPO identifiers (Supplemental Data 3).

To enhance the consistent representation of the above phenotypic terms, and to link these birth defects with knowledge about the appropriate anatomical entities involved with these pathologies, we manually curated the HPO terms onto an anatomy connectivity knowledge graph. The schema adopted by this graph is based on the ApiNATOMY knowledge representation^{30,31}, which was developed as part of the Stimulating Peripheral Activity to Relieve Conditions (SPARC)³² connectivity mapping effort. The ApiNATOMY subgraph within the ReproTox-KG provides links to knowledge about constituent

anatomical structures such as cell types that may be involved in the birth-defect mechanisms, as well as representations of abnormal anatomical organizations that typify these pathological phenotypes.

Curating small molecules associated with birth defects.

Manually curated teratogens and xenobiotics with potential to cause birth defects were extracted from various sources. We relied on existing resources such as those listed by DrugCentral³³ and Drugs.com³⁴ as FDA D and X category drugs, a report by the National Birth Defects Prevention Study (NBDPS)³⁵, a list provided by the National Birth Defects Registry³⁵, as well as drugs listed in several other publications^{36–40} (Supplementary Fig. S1 and Supplemental Data 4). In addition, we used DrugShot⁴¹ which is an automated way to obtain a ranked list of drugs for any search term using PubMed. We also extracted birth-defect/drug associations from the FAERS database via over-representation analysis (Supplemental Data 5). DrugCentral³³, an online drug information resource, was queried for FDA D and X category drugs and their associated Simplified Molecular Input Line Entry System (SMILES) with absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. FDA-approved drugs classified as X or D are drugs with evidence of inducing birth defects in humans and animal models. D category drugs are those that, despite potential risks shown in human studies and postmarketing, may be used in pregnant persons as the potential benefits outweigh the risks, while category X drugs are those that should not be used in pregnant persons because studies in animals or humans have shown fetal abnormalities, and these risks outweigh any potential benefits⁴². In addition, using DrugShot⁴¹, we queried each CDC birth-defect term through PubMed to extract Pubmed Identifiers (PMIDs) associated with each birth-defect term. Abstracts associated with these PMIDs were mined to extract drug PubChem IDs based on co-mentions of the birth defect with a drug. The DrugShot⁴¹ method first queries PubMed with drug names to collect PMIDs for each drug. Then we query PubMed with birth-defect terms and counted the overlapping PMIDs between the queries. To normalize for research focus biases, we also include the number of PMIDs returned for the drug and birth defect that do not overlap. The 30 most frequently co-occurring drugs for each birth defect were retained as the drug sets for each birth defect. The cutoff of 30 drugs was set to reasonably ensure that each drug that we retained for the birth defect has multiple publication co-mentions that serve as evidence for such associations. Only the CDC birth defects were used in this part of the analysis. Finally, the list of teratogens from FAERS birth-defect terms were mapped to drugs with a likelihood ratio (LLR) cutoff of $LLR > 2 * (\text{likelihood ratio test}) LLRT$. The formula assumes an over-representation of the presence of the birth-defect term and the drug without considering other factors that may induce the birth defect.

Evidence implicating genes with birth defects. Given the curated phenotype lists described above, human phenotype–gene associations were retrieved from multiple sources, including Pharos⁴³, Online Mendelian Inheritance in Man (OMIM)⁴⁴, Orphanet⁴⁵, ClinVar⁴⁶, DISEASES⁴⁷, Database of genomic variation and Phenotype in Humans using Ensembl Resources (DECIPHER)⁴⁸, the American Heart Association (AHA)⁴⁹, and Geneshot⁵⁰. From OMIM and Orphanet human phenotype–gene associations were obtained from the Jackson Laboratory HPO database (hpo.jax.org, October 2021 release), providing curated links between HPO terms and human genes²⁸. The OMIM and Orphanet-based HPO-term gene associations were retrieved for the human abnormal morphology of the great vessels, heart, and

CNS phenotypes. Gene-birth-defect associations were also obtained from ClinVar human genetic variants-phenotype submission summary dataset (v2021-11-03)⁴⁶. This dataset was utilized to extract relationships between human genes harboring a pathogenic variant and their associated phenotypes given the birth-defect phenotypes described above. Only genes with pathogenic variants and variants affecting a single gene were considered: That is, variants affecting multiple genes were excluded, due to the complexities in interpreting the relationships between their affected subset of genes and associated human diseases. The ClinVar-based HPO-gene associations were compiled for the human abnormal morphology of the great vessels, heart, and CNS phenotypes. Literature-based human disease–gene associations were obtained from the DISEASES portal⁴⁷. This dataset contains disease–gene associations text-mined from literature and genome-wide association studies. The disease ontology identifier (DOID) and ICD-10 codes listed in this database were converted to HPO terms using mappings directly taken from the Monarch initiative's Mondo disease ontology⁵¹. DECIPHER⁴⁸ provided this study with a curated list of genes reported to be associated with developmental disorders, processed by expert clinicians as a part of the Deciphering Developmental Disorders (DDD) study⁵² to facilitate clinical feedback of likely causal variants. The DECIPHER-based HPO-gene associations were compiled for the human abnormal morphology of the heart, and CNS phenotypes. We included a dataset of human congenital heart disease-associated genes associated with syndromic, non-syndromic, and ciliopathic cardiac disorders that was published by the AHA as general guidance for genetic testing by practitioners in 2018⁴⁹. Finally, using the Geneshot API⁵⁰, we queried each one of the 36 CDC birth-defect terms through PubMed to extract PMIDs associated with each term. These PMIDs were then converted into genes using the AutoRIF option of the Geneshot application. The top 50 most frequently occurring genes were retained as gene sets for each birth defect. A file containing all gene-birth-defect associations in JSON format can be retrieved from the ReproTox-KG download page.

Linking small molecule and drugs to their known targets.

Drugs and small molecules that have known targets were extracted from the TCRD database²⁶ and converted into KG assertions. Only compounds with a defined structure were included because other substances do not have PubChem⁵³ chemical IDs. In addition, only human targets were included, and only single gene/protein targets were included excluding some multi-component ion channels and transporters. Properties such as SMILES, binding affinity, original source, PubChem IDs, and common names are provided for each drug. These drug-target associations are available in JSON format from the ReproTox-KG download page.

Linking small molecules to genes based on changes in gene expression.

The ReproTox-KG holds knowledge about most FDA-approved drugs and over 30,000 preclinical small molecules profiled by the LINCS program for their effects on the transcriptome of selected human cell lines²⁷. To extract a set of genes that are up- or downregulated by each drug and small molecule profiled by the L1000 assay for LINCS, we computed the mean of the Characteristic Direction⁵⁴ gene expression vector for each drug in the LINCS L1000 chemical perturbation signature dataset downloaded from SigCom LINCS⁵⁵. We then retained the top 25 up- and downregulated genes for each drug. This allows us to take the top genes affected by the small molecule perturbation without overwhelming the database with differential gene expression

associations. All drug–gene associations can be downloaded from the ReproTox-KG download page.

Drug–drug similarity based on gene expression and chemical structure. To enable drug–drug similarity search across the ReproTox-KG, and to perform the semi-supervised machine learning predictions, we developed two drug–drug similarity matrices, one based on structure and one based on gene expression similarity. The drug–drug similarity matrix based on gene expression vector similarity was computed by transforming the consensus signatures described above using cosine similarity, comparing all pairs of consensus drug gene expression vectors to produce a square matrix where the value at (i, j) is the gene expression-based cosine similarity between the drugs at row i and column j . The matrices that contain the consensus signatures for all drugs and small molecules, and the drug–drug similarity matrix are available for download from SigCom LINCS⁵⁵ and the ReproTox-KG download page. To create drug–drug similarity based on chemical structure similarity, we first converted the SMILES strings of each compound to a binary feature vector using the Morgan fingerprint (2048 bits) method⁵⁶ with radius implemented in RDKit⁵⁷. We also used RDKit Chem module's QED and Crippen functions for physicochemical properties. Other chemical structure similarity methods such as MACCS, Avalon, Atom Pair, RDKit with maxPath 2 and 4, and Topological fingerprints using FingerprintMol were tested, confirming that the method chosen is justified as superior or comparable. Next, we computed the inverse document frequency (IDF) between all pairs of drug vectors as the distance measure between each pair of drugs. The resultant matrix of drug–drug similarity based on chemical structure is available from the ReproTox-KG download page. Chemical structure-based similarity search was also implemented using a workflow which queries the KG for compounds and generates fingerprints and similarity measures at runtime, for additional flexibility and interoperability.

Computing frequency of genetic variants. Gene intolerance scores were introduced to the knowledge graph from three main sources: haploinsufficiency, triplo-sensitivity, and general intolerance. Probability of being loss-of-function-intolerant (pLI) scores⁵⁸ for 18,225 human genes were obtained from a large-scale study conducted by the Exome Aggregation Consortium (ExAC)⁵⁹. Residual Variant Intolerance Score (RVIS)⁶⁰ values of 16,956 human genes were adopted from a large-scale analysis that processed 6503 human whole exome sequences made available by the NHLBI Exome Sequencing Project (ESP)⁶¹. The resulting scores were then compared with information on whether the gene causes any known Mendelian diseases. In this sense, genes with higher functional mutation to total variant site ratios are considered more tolerant⁶⁰. Dosage sensitivity scores⁶² such as haploinsufficiency and triplosensitivity for 17,263 human genes were presented by meta-analyzing 753,994 individuals with neurological disease phenotypes⁶². The provided scores are utilized in gene prioritization by their potential loss-of-function or gain-of-function through the introduction of de novo rare copy-number variants (rCNVs) as opposed to the point mutations.

Gene–gene similarity based on co-expression. Gene–gene similarity associations were obtained from the human gene–gene correlation matrix provided by the ARCHS4 resource⁶³. The matrix stores the Pearson correlation coefficient between genes across bulk RNA-seq expression samples uniformly processed by the ARCHS4 pipeline. Genes were filtered to include only protein-coding genes to keep the size of the graph manageable, and for each of the 17,966 genes, the top five most positively and

most negatively correlated genes based on the correlation coefficients were extracted for a total of 170,819 edges. Each edge was weighted by the correlation coefficient between the two connected genes. These gene–gene associations were then integrated into the ReproTox-KG. From these associations, it may be possible to identify genes that are potentially affected by known teratogens and discover the role that different groups of genes may play in inducing birth-defect phenotypes.

Placental crossing and D and X category predictions for small molecules. Using a semi-supervised learning approach, we generated placental crossing scores and D and X category scores for all FDA-approved and preclinical compounds profiled by LINCS that are included in the ReproTox-KG. To obtain true positives for placental crossing, we first extracted the list of 248 compounds assembled by Di Filippo et al.⁶⁴. Category D and X drugs were obtained from DrugCentral³³ and Drugs.com³⁴, and drugs were filtered by those which could be mapped to the LINCS L1000 compounds through the LINCS small molecule metadata, including drug names and synonyms. Out of the 248 placental crossing drugs, we were able to manually map 143 to the L1000 profiled compounds. Drugs associated with both categories were considered category X.

Predictions were made with the two drug–drug similarity matrices assembled based on gene–gene co-expression correlations, and chemical structure similarity as described above. Predictions were made using the same approach described in a publication describing DrugShot⁴¹. Specifically, we computed the average distance of each drug to the drugs labeled as positives, and then rank the drugs by this average distance. Importantly, the diagonal is removed to prevent contribution to the average distance from the drug to itself. This approach was used to score all LINCS compounds. The placental crossing scores and the category D and X scores for all drugs and small molecules in the ReproTox-KG are displayed as node properties for drugs and are depicted as the hue level of the drug nodes in the ReproTox-KG user interface. In addition, the predictions are provided as a supplementary table (Supplemental Data 6). To assess the reliability of the similarity scores, a Kolmogorov–Smirnov-like random walk statistic was applied. Half of the drugs were held-out when constructing the prediction scores for the compounds, and the other half of held-out set of drugs was used to construct a bridge plot and compute a normalized enrichment score (NES). The NES is the enrichment score (ES) divided by the average ES of 10,000 label permutations of those scores.

Two methods were developed to combine the predictions made by the gene expression and chemical structure similarity predictions. Given two scoring vectors produced by the two different similarity matrices, the Top Rank method takes the highest ranking of the drugs across all predictions to be the aggregated score. This score is then used for the bridge plot and normalized enrichment score. Alternatively, given two similarity score vectors, one based on expression and one based on structure, we aggregated these predictions by assigning a weight to each score coming from the two sources: expression and structure. These weights were optimized on a pre-task involving logistic classification of drugs into different mechanisms of action (MOA) using the same gene expression and chemical structure similarity prediction approach. More specifically, a dot product was performed between the two features and a weight matrix, the result is passed through a sigmoid function and the binary cross entropy loss is measured between the output and the true class where 1 means that the drug has the MOA, and 0 means that the drug does not. This is optimized for performance using the Adam optimizer⁶⁵ on 60 different MOAs, for which there are at

least 10 drugs, with 10 repeats shuffled randomly; class gradient contributions are weighted to counter the inherent class imbalance. The learned weights are then applied for combining the L1000 and structural features in the FDA drug categorization and placenta crossing sets into a singular score. This score is then used for the bridge plots and the normalized Enrichment Scores. This analysis is performed independently for predicting FDA drug categorization, and for the placenta crossing predictions.

UMAP visualization of L1000 perturbations. Uniform Manifold Approximation and Projection (UMAP)⁶⁶ was applied to the normalized L1000 count matrix of over 718,055 chemical perturbations performed with different drugs across different cell lines, time points, and concentrations. Perturbations with FDA drug categories D and X and drugs known to cross the placenta were colored by category. To identify the top MOAs in the L1000 perturbation space, we first clustered L1000 perturbations directly using Hierarchical Density-based Spatial Clustering of Applications with Noise (HDBSCAN)⁶⁷ with a minimum cluster size of 40 which struck a qualitative balance between the number of clusters and concordance with apparent clusters of FDA category D and X drugs in the UMAP. We then selected the top 25 clusters with highest concentration of drugs for each drug category, finally we identified the top 5 MOAs for drugs in those clusters. We colored the L1000 UMAP with those top MOAs.

ReproTox-KG backend KG database. The ReproTox-KG uses a graph-structured data model to integrate data. The KG is implemented using Neo4j²¹. The information in the ReproTox-KG represents a network of nodes representing birth defects, genes, and drugs, and edges representing their relationships. In addition, attributes/properties of the nodes and edges are provided. The ReproTox-KG is made up of datasets from the various sources listed above and listed in two tables (Tables 1 and 2) and illustrated in the associated schematic (Fig. 1). The ReproTox-KG uses a standardized JSON schema serialization to ingest data into the KG. Queries to the Neo4j platform are constructed using the Cypher query language⁶⁸.

Original graphical user interface to interact with the ReproTox-KG. Since Neo4j currently does not provide an open-source, free, and customizable standalone web-based user interface (UI) to visualize the results from Cypher queries, we developed an original UI with these features for this project. Leveraging the Cytoscape.js library⁶⁹, the UI renders Cypher query results in JSON format for network visualization. The UI provides the ability to perform queries for finding neighbors of single entities, finding shortest paths between pairs of entities, displaying the networks using various layouts, expanding, and shrinking the size of the displayed subnetwork, viewing properties of nodes and links, and downloading the displayed associations in tabular format.

Reporting summary. Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Results

Overall construction and composition of the ReproTox-KG. The ReproTox-KG contains semantic assertions that connect birth defects, genes, and drugs. In addition, drug–drug and gene–gene similarity assertions are included (Fig. 1 and Tables 1 and 2). Each entity in the ReproTox-KG has a set of attributes and properties. Some of these attributes are unique to the project. For example, we rank the likelihood of all included compounds

Table 1 ReproTox-KG node and edge sources and enumeration.

Assertion edge type	Name	Relationship	Reference	Nodes/nodes/edges
Birth defect-gene	HPO	Genes known to be associated with a birth defect	Human Phenotype Ontology	599/5093/127,023
Birth defect-drug	Geneshot	Genes co-mentioned with birth-defect terms in publications	Geneshot	32/1091/1565
Drug-gene	FDA Adverse Event Reporting System (Female/Male) Drugshot	Drugs with reported adverse events related to birth defects	FDA Adverse Event Reporting System	32/94/372
Drug-drug	IDG (Drug Target)	Drugs co-mentioned with birth-defect terms in publications	Drugshot	679/2207/13,000
Gene-gene	SigCom LINC5 Drug-to-Gene (upregulates/ downregulates) LINC5 Drugs Cosine Similarity	Drugs with known human gene targets	Target Central Resource Database	1363/1034/7326
	ARCHS4 (positively/ negatively correlated)	Drugs that up- or down-regulate genes across the LINC5 L1000 signatures	SigCom LINC5	4523/4419/225,509
		Drugs that induce similar gene expression patterns across LINC5 L1000 signatures based on cosine similarity	SigCom LINC5	4523/3449/20,785
		Genes positively or negatively correlated across ARCHS4 gene expression samples	ARCHS4	17,964/12,185/170,801

The ReproTox-KG is made of entities (nodes) representing birth defects, genes, and drugs that are connected based on semantic assertions (edges/relationships) extracted from different sources. The table lists the type of assertion, the nature of the relationship, the original source from where the assertion was extracted from, and the number of entities and relations for each entry in the table.

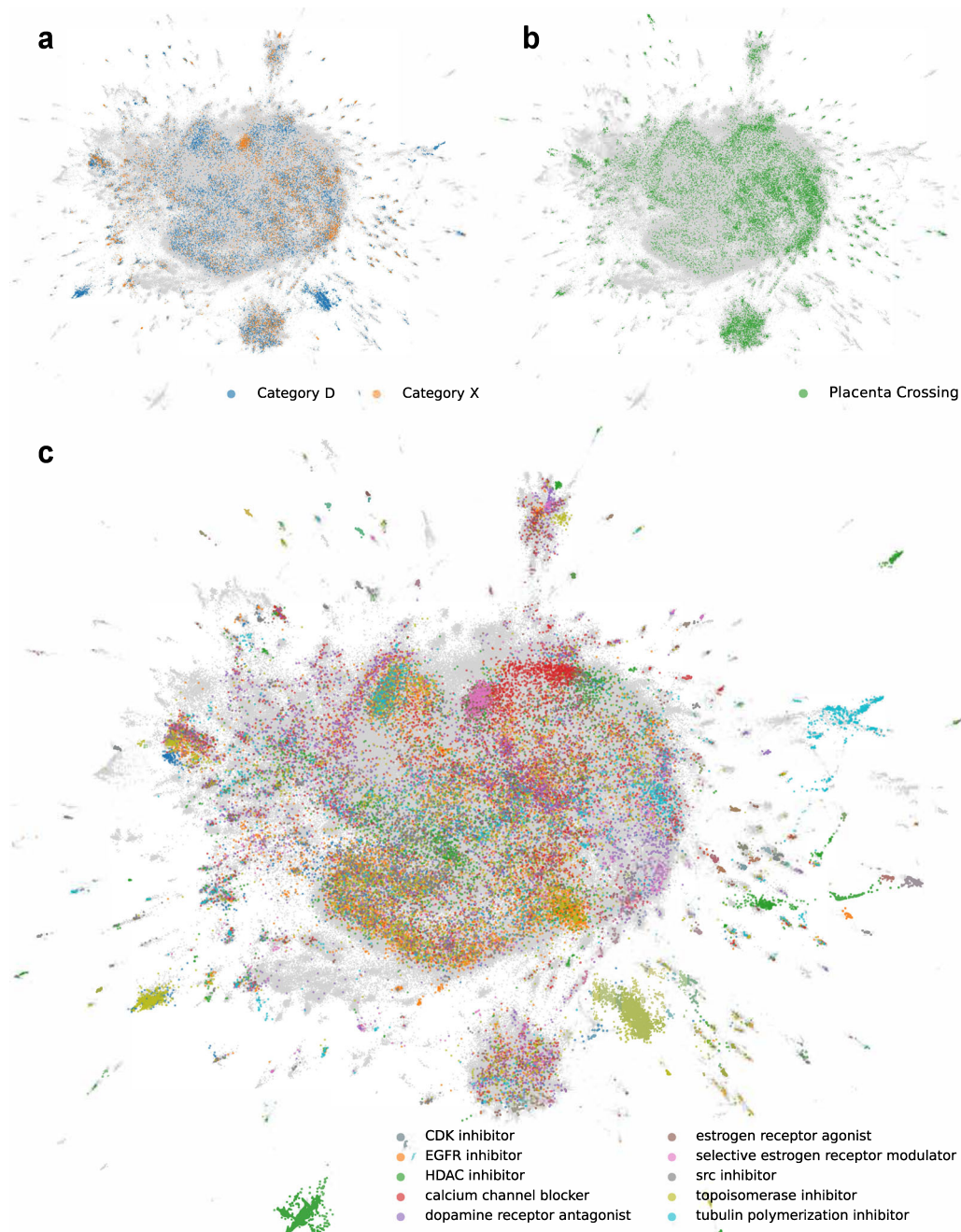


Fig. 3 Global visualization of gene expression signature similarity for LINCS drugs. UMAP of 718,055 L1000 perturbations, colored by **a** FDA D and X category; **b** known placental crossing; **c** top MOAs across clusters. Clusters computed using HDBSCAN with a minimum cluster size of 40, top 25 clusters for each category, and top five MOAs of those clusters are included.

data consists of a list of 248 drugs that are known to cross the placenta⁶⁴, and lists of FDA-approved drugs classified in the X ($n = 60$) and D categories ($n = 112$). We then manually mapped these drug names to small molecules profiled by the LINCS L1000 assay using terms, synonyms, and IDs derived from PubChem⁵³ resulting in 143 mapped placenta crossing drugs, as well as 60 X and 112 D category drugs (Fig. 2). Next, we constructed two drug–drug similarity matrices, one based on drug structural similarity, and one based on gene expression induced signature similarity. These matrices are used to perform semi-supervised machine learning to prioritize all drugs for the likelihood to cross the placenta or to be categorized as D and/or X. Before performing such predictions with these two matrices, we projected the category D and X drugs (Fig. 3a) and the known placental

crossing drugs (Fig. 3b) onto the LINCS L1000 gene expression space of 718,055 gene expression signatures induced by >30,000 small molecules using UMAP⁶⁶. We observe that these drugs fall into distinct regions within the L1000 gene expression space. By comparing the UMAP visualization of the known placental crossing drugs and the category D and X drugs to the same layout with highlighted known mechanisms of actions (Fig. 3c), we observe that dense clusters of D and X drugs involve estrogen disruptors and topoisomerase inhibitors. Other clusters colored by their unique MOAs also have many placental crossing drugs and the category D and X drugs within them. The observed punctate distribution strongly suggests that we can make predictions about the likelihood of preclinical drugs to induce birth defects and cross the placenta. The clinical relevance of these

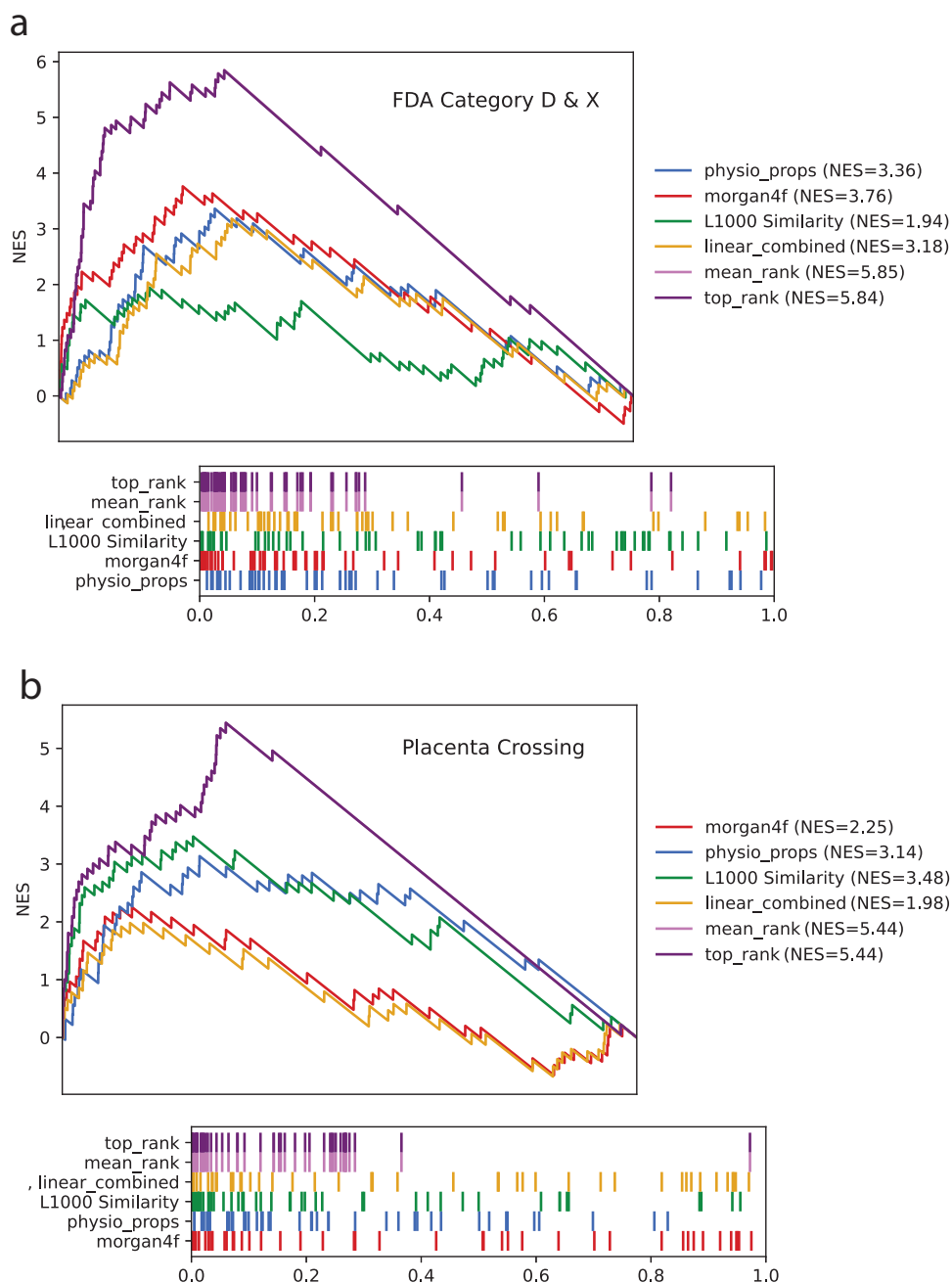


Fig. 4 Drug category and placental crossing prediction performance. Bridge plots colored by prediction method for **(a)** predicting FDA D and X categories; and **(b)** placenta crossing. The NES are shown in the legend. Leading edges of the same bridge plots are shown on the right of each complete bridge plot.

predictions needs to be qualified by considering additional factors, such as the impact of influx (solute carrier) proteins, efflux (e.g., ABC) transporters, as well as in situ metabolism mediated by, e.g., cytochrome P450 enzymes.

Next, we apply the semi-supervised learning approach to rank all mapped approved drugs and preclinical compounds to estimate their likelihood to cross the placental barrier, and to induce a birth defect. We use the category D or X drugs and the drugs known to cross the placenta as the true positives. The predictions are based on how close the other drugs are to these drugs in feature space. Such feature space is defined based on chemical similarity, gene expression similarity, or a combination of them. During benchmarking, we hold out a third of the drugs for testing. We observe that with the L1000 signature similarity

matrix alone we achieve an NES of 1.94 for predicting D and X category membership and 3.48 for placental crossing (Fig. 4). The predictions that are based on chemical structural similarity alone achieve NES of 3.76 and 3.14 for D and X category membership and for placental crossing, respectively (Fig. 4). Combining the predictions made by the gene expression data together with the chemical structure data with the Top Rank or Mean Rank methods improves such predictions to 5.85 for D and X category membership, and 5.44 for the placental crossing predictions. Overall, these are high-quality predictions for a semi-supervised approach. Importantly, these predictions perform well at the leading edge (Fig. 4a, b). It should be noted that predictions made with structural similarity only perform well when we define the similarity between compounds using IDF instead of Tanimoto.

Table 3 Top predicted X and D category drugs and preclinical compounds.

Drug	D & X L1000	D & X structure	Weighted	Top rank	D known	X known
Enalaprilat		1	1	1	0	0
TAK-715	1			1	0	0
Pitavastatin		2	11	2	0	0
BRD-K08703257	2			2	0	0
Ramipril		3	2	3	1	0
Pentoxifylline	3			3	0	0
Trandolapril		3	6	4	1	0
Troglitazone	4			4	0	0
Perindopril		4	3	5	1	0
FTI-276	5			5	0	0
BRD-K76846644		5	5	6	0	0
Gossypetin	7			6	0	0
Pravastatin		6	4	7	0	1
Phenothiazine	9			7	0	0
Evodiamine		7	7	8	0	0
Lorazepam	10			8	1	0
BRD-K40167599		8		9	0	0
Losartan	11			9	1	0
Lovastatin		9		10	0	1
Zebularine	13			10	0	0
CTPB		10	15	11	0	0
Risperidone				11	0	0
VX-765		11	8	12	0	0
BMS-191011				12	0	0
Methiopril		12	10	13	0	0
EX-527				13	0	0
Canrenone		13	12	14	0	0
BRD-K55591206				14	0	0
BRD-K80672993				15	0	0
Oxytetracycline		14		15	0	0
BRD-K63784565			9		0	0
Zofenopril-calcium			13		0	0
BRD-K93367411			14		0	0
BRD-K08533345	14				0	0
BRD-K27194553	12				0	0
BRD-K27889081	8				0	0
BRD-K37848780	15				0	0
BRD-K76551930	6				0	0
Imidapril		15			0	0

The top-15 ranked compounds predicted using semi-supervised learning with L1000 gene expression similarity or chemical structure similarity, or two by two methods that combine the predictions from the two sources, namely, weighted, and top rank, are listed together with whether these were previously known to belong to the X or D categories.

This is likely because there is a bias with the Tanimoto method which emphasizes similarity between complex larger compounds that share common features. The predictions made by the semi-supervised method highly rank compounds that are known as ACE inhibitors, antibiotics, and statins (Tables 3 and 4). This is not surprising because such compounds are already common among known category X & D drugs⁷⁰ and drugs that are known to cross the placenta. For example, the top ranked drug by structural similarity to be categorized as X and D is enalaprilat. Enalaprilat, an ACE inhibitor, is the active metabolite of the oral drug, enalapril. Both are used to treat high blood pressure⁷¹. They are listed as category C for the first trimester and as category D for the second and third. ACE inhibitors also cause fetal renal failure resulting in oligohydramnios. These birth defects are deformations caused by uterine pressure on the fetus in the second and third trimesters. Overall, such predictions can be used to warn about the potential of newly approved drugs to cross the placenta and induce birth defects, but it should be noted that these predictions cannot be taken as a substitute for experimental confirmation.

In addition, for each gene included in the KG, we computed likelihood for deleterious mutations using three established

methods: (pLI) scores⁵⁸, RVIS⁶⁰, and dosage sensitivity scores [20]. Each entity in the ReproTox-KG also includes links out to databases based on entity ID resolution. In particular, 694 birth defects are mapped to HPO identifiers²⁸, 18,233 genes and proteins are mapped to HGNC IDs, and 5403 drugs are mapped to their PubChem identifiers⁵³. Lists of birth-defect terms were extracted from HPO²⁸ and the CDC website²⁹. The 127,023 associations between birth-defect terms and genes were extracted from OMIM⁴⁴, Orphanet⁴⁵, ClinVar⁴⁶, DISEASES³³, DECIPHER⁴⁸, American Heart Association (AHA)⁴⁹ and Geneshot⁵⁰. The 13,561 assertions between birth defects and drugs were extracted from DrugCentral⁵⁰, and DrugShot⁴¹. Two types of assertions connect genes and drugs within the ReproTox-KG: 1) genes that are differentially expressed after drug treatment based on transcriptomics, and 2) known drug targets for the drugs. Overall, 225,509 drug-gene associations were extracted from the LINCS L1000 data⁵⁵, and 7326 drug-target assertions were extracted from Pharos²⁶. Similarly, 9546 drug-drug similarity assertions are identified based on chemical similarity and 33,608 based on gene expression signature similarity. Finally, gene-gene similarity included in the KG is based on gene-gene co-expression⁶³.

Table 4 Top predicted placental crossing drugs and preclinical compounds.

Drug	L1000	Structure	Weighted	Top rank	Known
Nafcillin		1	1	1	0
FTI-276	1			1	0
Piperacillin		2	4	2	0
Gossypetin	2			2	0
Cefotaxime		3	3	3	0
TAK-715	3			3	0
Ciclacillin		4	2	4	0
BRD-K08703257	4			4	0
7-aminocephalosporanic-acid		5	6	5	0
Temozolomide	5			5	0
Penicillin		6	5	6	0
CGS-21680	6			6	0
Ceforanide		7	9	7	0
Y-27632	7			7	0
Lorazepam	8			8	1
BRD-K43966364		8	8	9	0
Benzathine		8		9	0
Estradiol-cypionate	9			10	0
BRD-K50776152	10			11	0
Isoetharine		9		11	0
Enalaprilat		10	12	12	0
Rolipram	11			12	0
BRD-A66025870		11		13	0
PT-630	12			13	0
EMF-csc-9	13			14	0
Practolol		12		14	0
Cephalothin		13	15	15	1
DL-TBOA	14			15	0
Pravastatin		14	7		0
Cefoperazone			10		1
Orciprenaline			11		0
Ampicillin			13		1
Dicloxacillin			14		0
BRD-K55591206	15				0
Micropenin		15			0

The top-15 ranked compounds predicted using semi-supervised learning with L1000 gene expression similarity or chemical structure similarity, or two by two methods that combine the predictions from the two sources, namely, weighted, and top rank, are listed together with whether these were previously known to cross the placenta.

The processed data from these resources was created by customized extract, transform, and load (ETL) scripts and stored as a JSON schema data model. This processed data was ingested into a Neo4J database, and it is made available for download on the ReproTox-KG website at: <https://maayanlab.cloud/reprotox-kg/downloads>. The ETL scripts are open-source and available from: <https://github.com/nih-cfde/ReproToxTables/>. To provide access to the processed data in a user-friendly manner, we developed an original graphical user interface (Fig. 5). The example shown provides possible links that connect valproic acid and Spina Bifida, a known association⁷². The interface can be accessed from <https://maayanlab.cloud/reprotox-kg>.

Extraction of birth-defect gene-small molecule cliques to explain potential MOAs. To demonstrate the utility of the ReproTox-KG to illuminate knowledge, we queried the graph to identify all three-node cliques. That is, we extracted from the ReproTox-KG all instances where a birth defect was connected to a gene and a drug that are also connected. In total, 533 such cliques are identified (Supplemental Data 7). From this collection of cliques, there are cliques for six drugs and small molecules that were not previously listed as crossing the placenta and have a placental scoring rank of less than 3000 (out of 30,000) (Fig. 6). This sub-network demonstrates how the ReproTox-KG can be used to suggest MOA for how these drugs may induce specific birth defects by

affecting the gene expression of genes known to be associated with the birth defect. For example, LINCS L1000 transcriptomics data show that the approved drug methotrexate, a chemotherapeutic and immunosuppressive drug, inhibits the expression of the mitotic checkpoint serine/threonine-protein kinase BUB1. *BUB1* is known to cause microcephaly when mutated⁷³, and methotrexate is known to cause microcephaly and atrial defects⁷⁴. Hence, this adverse effect of methotrexate can be attributed to its direct influence on the expression levels of *BUB1*. It should be noted that *BUB1* is a critical component of the cell cycle pathway⁷⁵. Hence, it is likely that methotrexate interferes with a specific stage of development that requires cell proliferation via indirect downregulation of *BUB1*. Similarly, the experimental drug LY-294002 which is a morpholine-containing chemical compound that is a strong inhibitor of PI3K, was previously shown to influence cell proliferation of epithelial cells isolated from human fetal palatal shelves (hFPECs)⁷⁶. Besides inhibiting the activity of PI3K, LY-294002 increases the expression of *DUSP6*, a dual specificity phosphatase that dephosphorylates members of the PI3K pathway. There is evidence that PI3K phosphorylates *DUSP6*, and this phosphorylation induces *DUSP6* degradation⁷⁷. Such observation is consistent with the ReproTox cliques' subgraph.

The approved antidepressant drug sertraline is reported to induce cardiac and vascular birth defects based on analysis of FAERS⁷⁸. The ReproTox-KG subnetwork of cliques suggests that such adverse birth defects could be mediated via the activation of the



Toxicology Screening Knowledge Graph for Structural Birth Defects

Queries Submitted: 489



Start with: label: Example: [Anencephaly](#) / [Trisomy 21](#)

End with: label: [Remove End Filter](#)

Select relation: Size:

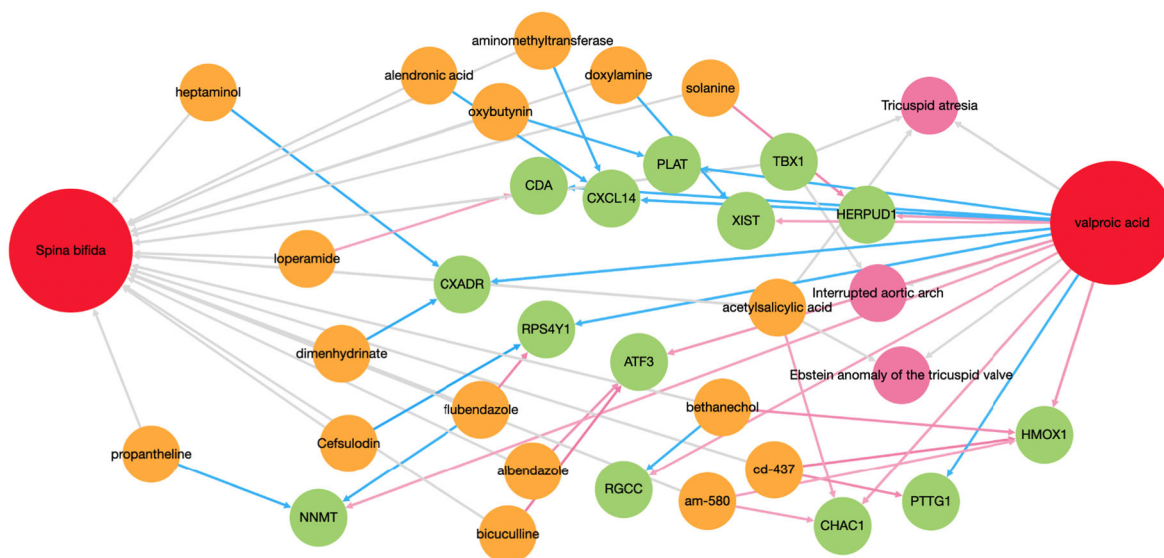


Fig. 5 Screenshot from the ReproTox-KG user interface. A query to identify connections between the birth defect Spina Bifida and the drug valproic acid with a limit of 25 nodes is provided as an example.

dehydrocholesterol reductase *DHCR7* and *DHCR24*⁷⁹. These two enzymes are critical components of the cholesterol biosynthesis pathway. Mutations in *DHCR7* are known to cause Smith–Lemli–Opitz syndrome, a disease of multiple congenital abnormalities⁸⁰, while mutations in *DHCR24* can cause desmosterolosis⁸¹. Hence, it is plausible that sertraline mediates induction of cardiac and vascular birth defects via up-regulatory effects on *DHCR7* and *DHCR24*. Unlike methotrexate, sertraline is currently not contraindicated in pregnancy and it is classified as a category C drug. Taken together, ReproTox-KG evidence suggests that pregnancy should be listed as a contraindication for sertraline. Overall, these are just a few examples of how the ReproTox-KG can illuminate knowledge about potential mechanisms of how drugs and preclinical small molecules may induce birth defects.

Discussion

Currently, in the clinical genomic diagnostic research setting, methods for prioritizing variants and genes for association with birth defects are done by utilizing databases such as ClinVar⁸², ClinGen⁸³, as well as gene lists related to birth defects from other annotated resources. In contrast, the ReproTox knowledge graph links evidence about associations between chemicals, phenotypes, and genes. This data integration effort offers hypotheses about drugs and compounds that potentially could be involved in the induction of birth defects. There are many cases where there are no causative genetic variants that have been attributed to birth defects. It is possible that exposure to certain teratogens could

either alone or via interaction with gene variants potentially cause birth-defect phenotypes. Such interactions may be illuminated by the ReproTox knowledge graph. In addition, the ReproTox knowledge graph has the potential to make an impact on the field of regulatory toxicology by identifying teratogenic potential for preclinical compounds, including their potential MOA to induce such birth defects. Currently, agencies such as the FDA and EPA are faced with the pressures of an increase in applications and a greater demand for making decisions without animal testing.

To characterize associations between small molecule compounds and their potential to induce reproductive toxicity, we gathered knowledge from multiple sources to construct a reproductive toxicity knowledge graph with an initial focus on associations between birth defects, drugs, and genes. The idea of abstracting genes, drugs, and diseases into networks is not new. We and others have pioneered the construction of networks to represent functional and physical associations between genes/proteins^{84–86}, drugs and their targets^{87,88}, and diseases based on their gene set similarity⁸⁹. The unique feature of the ReproTox-KG is that it provides a flexible framework not only to connect entities such as gene–drug, gene–gene, gene–birth defect, drug–drug, and drug–birth defects, but also to query this network, extend it, visualize it, and add attributes to different node types.

Similar efforts to ReproTox-KG have recently been published, including studies that attempted to use graph embedding Deep Learning algorithms to predict missing associations between drugs and diseases⁹⁰, drug repurposing opportunities^{91,92}, predicting drug targets^{93,94}, adverse events⁹⁵, and drug–drug

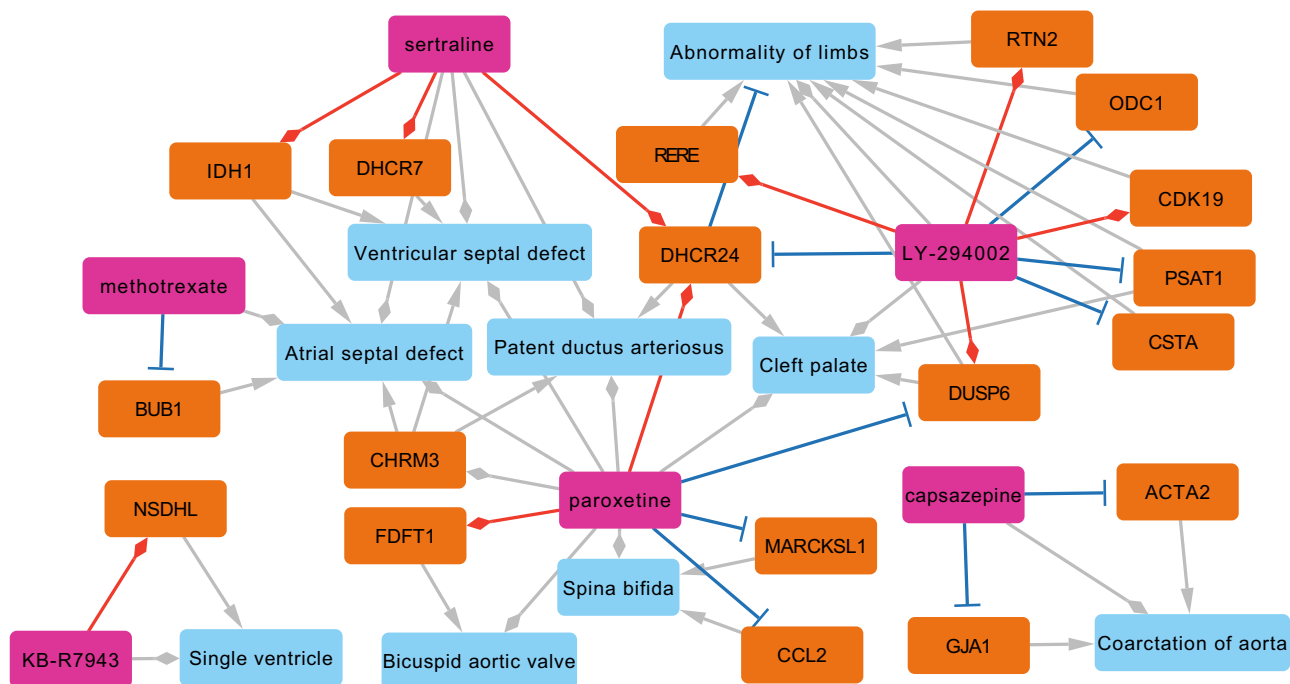


Fig. 6 Network of cliques connecting drugs, birth defects and genes. Cliques are made of drugs that have a placenta crossing predicted rank of less than 3000 and are known to induce a birth defect based on literature evidence. These drugs are connected to the genes that their expression is affected by the drugs based on LINCS L1000 data. Finally, associations between genes and birth defects are established based on known mutations that are known to cause the same birth defect. Light blue nodes represent birth-defect terms, orange nodes represent genes, and pink nodes represent drugs and preclinical small molecules. Red lines with diamond-heads indicate an L1000 consensus drug signatures that upregulates the gene, and plungers indicate an L1000 consensus drug signature that downregulates the target gene. Gray arrowheads indicate genes that their mutations induce a birth defect, and gray diamond-heads connect drugs to the birth defects they are known to induce.

interactions⁹⁶. These are just a few studies in this domain. Here, we did not attempt to make such graph-based predictions but provided the needed building blocks to enable such future applications. Hence, the ReproTox-KG was developed as a resource for the community to explore and expand.

One of the limitations of knowledge graphs is their inability to cover many associations between many entities. For example, we decided to only consider the top 25 up and downregulated genes for each drug. This leaves out many genes that may be affected by drugs. These genes will be missed from queries and post-hoc analyses. We also created consensus signatures for each drug from the LINCS L1000 data. Computing such consensus masks the effect of drugs in specific cellular contexts. To make the ReproTox-KG project focused and manageable, we kept its scope limited. However, tissue and cell type distribution of the affected genes, and how drugs and small molecules induce such differential effects, are critical information for associating genes and drugs with birth defects. Such information is partially available and could be included in future releases of ReproTox-KG. The L1000 transcriptomics dataset is not a mainstream assay, but the data produced by this assay provides a glimpse to the effect of many drugs and preclinical small molecules on human cells. Before such data became available, it was mostly unknown whether drugs that are labeled with the same known MOAs, or having the same targets, will induce similar gene expression patterns when applied to human cell lines. Our observation, that in general, we can see that gene expression signature clusters by the same known MOAs is profound. Another excellent resource for gene expression during development is DESCARTES, a human cell atlas for fetal tissues⁹⁷. Such a dataset should be considered in future studies because it provides gene expression across tissues at different stages of development. Understanding

the time window of the expression of a gene in a specific tissue during development is critical for better elucidating the molecular mechanisms of many birth defects. It also should be noted that the ReproTox-KG is preliminary and should not be used for clinical applications and clinical decision support.

Data availability

All files needed to reconstruct the knowledge graph with instructions are available from <https://maayanlab.cloud/reprotox-kg/downloads>. All additional processed data files used for the analysis are available from <https://maayanlab.cloud/reprotox-kg/downloads>. Initial versions of those files, scripts used to process the data, and links to the original sources are available from <https://github.com/nih-cfde/ReproToxTables>. The interactive web interface to access the knowledge graph is available at <https://maayanlab.cloud/reprotox-kg>. All the data used in the study is processed from other primary sources. All other data are available from the corresponding author on request.

Code availability

The scripts used to process the data are available from <https://github.com/nih-cfde/ReproToxTables>. Code needed to reconstruct the knowledge graph locally is available from <https://maayanlab.cloud/reprotox-kg/downloads>. Code needed to recreate the figures can be found at <https://github.com/MaayanLab/ReproToxPaper>. An alternative method to compute chemical similarity: <https://github.com/unmtransinfo/CFChemDb>. Snapshot of the code and processed datasets are available from <https://doi.org/10.5281/zenodo.8049261>⁹⁸.

Received: 20 September 2022; Accepted: 29 June 2023;

Published online: 17 July 2023

References

1. Update on Overall Prevalence of Major Birth Defects. CDC MMWR 57, 1–5 (2008).

2. Principles For Evaluating Health Risks To Reproduction Associated With Exposure To Chemicals. (2001) <https://inchem.org/documents/ehc/ehc/ehc225.htm>.
3. Kumar, S., Sharma, A. & Kshetrimayum, C. Environmental & occupational exposure & female reproductive dysfunction. *Indian J. Med. Res.* **150**, 532–545 (2019).
4. Leong, C. et al. Prescription medication use during pregnancies that resulted in births and abortions (2001–2013): a retrospective population-based study in a Canadian population. *PLoS ONE* **14**, e0211319 (2019).
5. Palmsten, K. et al. The most commonly dispensed prescription medications among pregnant women enrolled in the U.S. Medicaid program. *Obstet. Gynecology* **126**, 465–473 (2015).
6. Challa, A. P. et al. Machine learning on drug-specific data to predict small molecule teratogenicity. *Reprod. Toxicol.* **95**, 148–158 (2020).
7. Lindhout, D. Pharmacogenetics and drug interactions: role in antiepileptic-drug-induced teratogenesis. *Neurology* **42**, 43–47 (1992).
8. Ahn, M. R., Li, L., Shon, J., Bashaw, E. D. & Kim, M.-J. Teratogenic drugs and their drug interactions with hormonal contraceptives. *Clin. Pharmacol. Ther.* **100**, 217–219 (2016).
9. Lesko, L. J. et al. Establishing a multidisciplinary framework to study drug-drug interactions of hormonal contraceptives: an invitation to collaborate. *CPT Pharmacometrics Syst. Pharmacol.* **7**, 706–708 (2018).
10. Sudsakorn, S., Bahadduri, P., Fretland, J. & Lu, C. 2020 FDA drug-drug interaction guidance: a comparison analysis and action plan by pharmaceutical industrial scientists. *Curr. Drug Metab.* **21**, 403–426 (2020).
11. Matthews, T. J., MacDorman, M. F. & Thoma, M. E. Infant mortality statistics from the 2013 period linked birth/infant death data set. *Natl. Vital Stat. Rep.* **64**, 1–30 (2015).
12. Arth, A. C. et al. Inpatient hospitalization costs associated with birth defects among persons of all ages—United States, 2013. *MMWR Morb. Mortal. Wkly. Rep.* **66**, 41–46 (2017).
13. Hogan, A. et al. Knowledge graphs. *ACM Comput. Surv.* **54**, 1–37 (2021).
14. Ji, S., Pan, S., Cambria, E., Marttinen, P. & Yu, P. S. A survey on knowledge graphs: representation, acquisition, and applications. *IEEE Trans. Neural Netw. Learn. Syst.* **33**, 494–514 (2022).
15. Wang, Q., Mao, Z., Wang, B. & Guo, L. Knowledge graph embedding: a survey of approaches and applications. *IEEE Trans. Knowl. Data Eng.* **29**, 2724–2743 (2017).
16. MacLean, F. Knowledge graphs and their applications in drug discovery. *Expert Opin. Drug Discov.* **16**, 1057–1069 (2021).
17. Kamdar, M. R. & Musen, M. A. PhLeGrA: graph analytics in pharmacology over the web of life sciences linked open data. *Proc. Int. World Wide Web Conf.* **2017**, 321–329 (2017).
18. Myklebust, E. B., Jiménez-Ruiz, E., Chen, J., Wolf, R. & Tollefsen, K. E. Prediction of adverse biological effects of chemicals using knowledge graph embeddings. *Semantic Web* **13**, 299–338 (2022).
19. Monnin, P. et al. PGxO and PGxLOD: a reconciliation of pharmacogenomic knowledge of various provenances, enabling further comparison. *BMC Bioinforma.* **20**, 139 (2019).
20. Dumontier, M. et al. Bio2RDF release 3: a larger connected network of linked data for the life sciences. in *Proceedings of the 2014 International Conference on Posters & Demonstrations Track* Vol. 1272, 401–404 (Citeseer, 2014).
21. Miller, J. J. Graph database applications and concepts with Neo4j. in *Proceedings of the southern association for information systems conference*, Atlanta, GA, USA, 2324, 36, 141–147 (2013).
22. Cerans, K. et al. Graphical schema editing for stardog OWL/RDF databases using OWLGrEd/S. *OWLED* 849 (2012).
23. Dohmen, L., et al. Algorithms for large networks in the NoSQL database ArangoDB (Doctoral dissertation, Bachelors thesis, RWTH Aachen, Aachen) (2012).
24. Bebee, B. et al. Amazon Neptune: Graph data management in the cloud. <http://ceur-ws.org/Vol-2180/paper-79.pdf>.
25. Tesoriero, C. *Getting Started with OrientDB* (Packt Publishing Birmingham, 2013).
26. Sheils, T. K. et al. TCRD and Pharos 2021: mining the human proteome for disease biology. *Nucleic Acids Res.* **49**, D1334–D1346 (2021).
27. Keenan, A. B. et al. The library of integrated network-based cellular signatures NIH program: system-level cataloging of human cells response to perturbations. *Cell Syst.* **6**, 13–24 (2018).
28. Köhler, S. et al. The human phenotype ontology in 2021. *Nucleic Acids Res.* **49**, D1207–D1217 (2021).
29. Learn about Specific Birth Defects. CDC website. Last Reviewed: June 28, 2023 <https://www.cdc.gov/ncbddd/birthdefects/types.html>.
30. de Bono, B. et al. Representing normal and abnormal physiology as routes of flow in ApiNATOMY. *Front. Physiol.* **13**, 795303 (2022).
31. Kokash, N. & de Bono, B. Knowledge representation for multi-scale physiology route modeling. *Front. Neuroinform.* **15**, 560050 (2021).
32. Osanlouy, M. et al. The SPARC D. R. C.: building a resource for the autonomic nervous system community. *Front. Physiol.* **12**, 693735 (2021).
33. Avram, S. et al. DrugCentral 2021 supports drug discovery and repositioning. *Nucleic Acids Res.* **49**, D1160–D1169 (2021).
34. Abigail, L. P. Drugs.com: drug information online 2004. *Ref. Rev.* **18**, 41–41 (2004).
35. Dawson, A. L. et al. Maternal exposures in the National Birth Defects Prevention Study: time trends of selected exposures. *Birth Defects Res. A Clin. Mol. Teratol.* **103**, 703–712 (2015).
36. Schaefer, C. Drug safety in pregnancy: utopia or achievable prospect? Risk information, risk research and advocacy in Teratology Information Services. *Congenit. Anom* **51**, 6–11 (2011).
37. De Santis, M. et al. Risk of drug-induced congenital defects. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **117**, 10–19 (2004).
38. Dawson, A. L., Riehle-Colarusso, T., Reefhuis, J. & Arena, J. F., National Birth Defects Prevention Study. Maternal exposure to methotrexate and birth defects: a population-based study. *Am. J. Med. Genet. A* **164A**, 2212–2216 (2014).
39. Tinker, S. C. et al. Next steps for birth defects research and prevention: the birth defects study to evaluate pregnancy exposures (BD-STEPS). *Birth Defects Res. A Clin. Mol. Teratol.* **103**, 733–740 (2015).
40. Schaefer, C., Peters, P. W. J. & Miller, R. K. *Drugs During Pregnancy and Lactation: Treatment Options and Risk Assessment*. (Academic Press, 2014).
41. Kropiwnicki, E. et al. DrugShot: querying biomedical search terms to retrieve prioritized lists of small molecules. *BMC Bioinformatics* **23**, 76 (2022).
42. Millstein, L. G. FDA's pregnancy categories. *N. Engl. J. Med.* **303**, 706 (1980).
43. Nguyen, D.-T. et al. Pharos: Collating protein information to shed light on the druggable genome. *Nucleic Acids Res.* **45**, D995–D1002 (2017).
44. Hamosh, A., Scott, A. F., Amberger, J. S., Bocchini, C. A. & McKusick, V. A. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. *Nucleic Acids Res.* **33**, D514–D517 (2005).
45. Weinreich, S. S., Mangon, R., Sikkens, J. J., Teeuw, M. E. E. N. & Cornel, M. C. [Orphanet: a European database for rare diseases]. *Ned. Tijdschr. Geneesk.* **152**, 518–519 (2008).
46. Landrum, M. J. et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res.* **44**, D862–D868 (2016).
47. Grissa, D., Junge, A., Oprea, T. I. & Jensen, L. J. Diseases 2.0: a weekly updated database of disease-gene associations from text mining and data integration. *Database* **2022**, baac019 (2022).
48. Foreman, J. et al. DECIPHER: Supporting the interpretation and sharing of rare disease phenotype-linked variant data to advance diagnosis and research. *Hum. Mutat.* **43**, 682–697 (2022).
49. Pierpont, M. E. et al. Genetic basis for congenital heart disease: revisited: a scientific statement from the American Heart Association. *Circulation* **138**, e653–e711 (2018).
50. Lachmann, A. et al. Geneshot: search engine for ranking genes from arbitrary text queries. *Nucleic Acids Res.* **47**, W571–W577 (2019).
51. Vasilevsky, N. A. et al. Mondo: Unifying diseases for the world, by the world. Preprint at *bioRxiv* <https://doi.org/10.1101/2022.04.13.22273750> (2022).
52. Kaplanis, J. et al. Evidence for 28 genetic disorders discovered by combining healthcare and research data. *Nature* **586**, 757–762 (2020).
53. Kim, S. et al. PubChem 2019 update: improved access to chemical data. *Nucleic Acids Res.* **47**, D1102–D1109 (2019).
54. Clark, N. R. et al. The characteristic direction: a geometrical approach to identify differentially expressed genes. *BMC Bioinforma.* **15**, 79 (2014).
55. Evangelista, J. E. et al. SigCom LINCS: data and metadata search engine for a million gene expression signatures. *Nucleic Acids Res.* <https://doi.org/10.1093/nar/gkac328> (2022).
56. Morgan, H. L. The generation of a unique machine description for chemical structures—a technique developed at chemical abstracts service. *J. Chem. Doc.* **5**, 107–113 (1965).
57. Landrum, G. RDKit: Open-source cheminformatics (2006) <https://www.rdkit.org/>.
58. Lek, M. et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536**, 285–291 (2016).
59. Karczewski, K. J. et al. The ExAC browser: displaying reference data information from over 60 000 exomes. *Nucleic Acids Res.* **45**, D840–D845 (2017).
60. Petrovski, S., Wang, Q., Heinzen, E. L., Allen, A. S. & Goldstein, D. B. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet.* **9**, e1003709 (2013).
61. Auer, P. L. et al. Guidelines for large-scale sequence-based complex trait association studies: lessons learned from the NHLBI Exome Sequencing Project. *Am. J. Hum. Genet.* **99**, 791–801 (2016).
62. Collins, R. L. et al. A cross-disorder dosage sensitivity map of the human genome. *Cell* **185**, 3041–3055 (2022).
63. Lachmann, A. et al. Massive mining of publicly available RNA-seq data from human and mouse. *Nat. Commun.* **9**, 1366 (2018).

64. Di Filippo, J. I., Bollini, M. & Cavasotto, C. N. A machine learning model to predict drug transfer across the human placenta barrier. *Front. Chem.* **9**, 714678 (2021).
65. Kingma, D. P. & Ba, J. Adam: a method for stochastic optimization. Preprint at <https://arxiv.org/abs/1412.6980> (2014).
66. McInnes, L., Healy, J. & Melville, J. UMAP: uniform manifold approximation and projection for dimension reduction. Preprint at <https://arxiv.org/abs/1802.03426> (2018).
67. Malzer, C. & Baum, M. A hybrid approach to hierarchical density-based cluster selection. in *2020 IEEE International Conference on Multisensor Fusion and Integration for Intelligent Systems (MFI)* 223–228 (IEEE, 2020).
68. Francis, N. et al. Cypher: an evolving query language for property graphs. in *Proceedings of the 2018 International Conference on Management of Data* 1433–1445 (Association for Computing Machinery, 2018).
69. Franz, M. et al. Cytoscape.js: a graph theory library for visualisation and analysis. *Bioinformatics* **32**, 309–311 (2016).
70. Vahedian-Azimi, A., Makvandi, S., Banach, M., Reiner, Ž. & Sahebkar, A. Fetal toxicity associated with statins: a systematic review and meta-analysis. *Atherosclerosis* **327**, 59–67 (2021).
71. Reisenberger, K. et al. Placental passage of angiotensin-converting enzyme inhibitors. *Am. J. Obstet. Gynecol.* **174**, 1450–1455 (1996).
72. Nau, H. Valproic acid-induced neural tube defects. *Ciba Found. Symp.* **181**, 144–152 (1994).
73. Carvalho, S. et al. Biallelic BUB1 mutations cause microcephaly, developmental delay, and variable effects on cohesion and chromosome segregation. *Sci. Adv.* **8**, eabk0114 (2022).
74. Verberne, E. A., de Haan, E., van Tintelen, J. P., Lindhout, D. & van Haelst, M. Fetal methotrexate syndrome: a systematic review of case reports. *Reprod. Toxicol.* **87**, 125–139 (2019).
75. Roberts, B. T., Farr, K. A. & Hoyt, M. A. The *Saccharomyces cerevisiae* checkpoint gene BUB1 encodes a novel protein kinase. *Mol. Cell. Biol.* **14**, 8282–8291 (1994).
76. Gao, Z. et al. Effect of TCDD on the fate of epithelial cells isolated from human fetal palatal shelves (hFPECs). *Toxicol. Appl. Pharmacol.* **305**, 186–193 (2016).
77. Bermudez, O., Marchetti, S., Pagès, G. & Gimond, C. Post-translational regulation of the ERK phosphatase DUSP6/MKP3 by the mTOR pathway. *Oncogene* **27**, 3685–3691 (2008).
78. Hong, F., Qiu, J., Zhang, S. & Zhang, L. Fetal congenital cardiac and vascular disorders associated with sertraline treatment during pregnancy: analysis of FAERS data. *Biomed Res. Int.* **2022**, 9914931 (2022).
79. Luu, W., Hart-Smith, G., Sharpe, L. J. & Brown, A. J. The terminal enzymes of cholesterol synthesis, DHCR24 and DHCR7, interact physically and functionally. *J. Lipid Res.* **56**, 888–897 (2015).
80. Smith, D. W., Lemli, L. & Opitz, J. M. A newly recognized syndrome of multiple congenital anomalies. *J. Pediatr.* **64**, 210–217 (1964).
81. Rohanizadegan, M. & Sacharow, S. Desmosterolosis presenting with multiple congenital anomalies. *Eur. J. Med. Genet.* **61**, 152–156 (2018).
82. Landrum, M. J. et al. ClinVar: improvements to accessing data. *Nucleic Acids Res.* **48**, D835–D844 (2020).
83. Rehm, H. L. et al. ClinGen—the clinical genome resource. *N. Engl. J. Med.* **372**, 2235–2242 (2015).
84. Ma’ayan, A. et al. Formation of regulatory patterns during signal propagation in a mammalian cellular network. *Science* **309**, 1078–1083 (2005).
85. Bork, P. et al. Protein interaction networks from yeast to human. *Curr. Opin. Struct. Biol.* **14**, 292–299 (2004).
86. Dannenfels, R., Clark, N. R. & Ma’ayan, A. Genes2FANs: connecting genes through functional association networks. *BMC Bioinforma.* **13**, 156 (2012).
87. Ma’ayan, A., Jenkins, S. L., Goldfarb, J. & Iyengar, R. Network analysis of FDA approved drugs and their targets. *Mt. Sinai J. Med.* **74**, 27–32 (2007).
88. Yildirim, M. A., Goh, K.-I., Cusick, M. E., Barabási, A.-L. & Vidal, M. Drug-target network. *Nat. Biotechnol.* **25**, 1119–1126 (2007).
89. Goh, K.-I. et al. The human disease network. *Proc. Natl Acad. Sci. USA* **104**, 8685–8690 (2007).
90. Zhu, C. et al. Multimodal reasoning based on knowledge graph embedding for specific diseases. *Bioinformatics* <https://doi.org/10.1093/bioinformatics/btac085> (2022).
91. Sosa, D. N. et al. A literature-based knowledge graph embedding method for identifying drug repurposing opportunities in rare diseases. *Pac. Symp. Biocomput.* **25**, 463–474 (2020).
92. Gao, Z., Ding, P. & Xu, R. KG-Predict: a knowledge graph computational framework for drug repurposing. *J. Biomed. Inform.* **132**, 104133 (2022).
93. Alshahrani, M. et al. Combining biomedical knowledge graphs and text to improve predictions for drug-target interactions and drug-indications. *PeerJ* **10**, e13061 (2022).
94. Ye, C., Swiers, R., Bonner, S. & Barrett, I. A knowledge graph-enhanced tensor factorisation model for discovering drug targets. *IEEE/ACM Trans. Comput. Biol. Bioinform.* **19**, 3070–3080 (2022).
95. Joshi, P., V. M. & Mukherjee, A. A knowledge graph embedding based approach to predict the adverse drug reactions using a deep neural network. *J. Biomed. Inform.* **132**, 104122 (2022).
96. Karim, M. R. et al. Drug-drug interaction prediction based on knowledge graph embeddings and convolutional-LSTM network. in *Proceedings of the 10th ACM international conference on bioinformatics, computational biology and health informatics* 2019 Sep 4 (pp. 113–123).
97. Cao, J. et al. A human cell atlas of fetal gene expression. *Science* **370**, eaba7721 (2020).
98. Evangelista, J. E. et al. Supporting files for an article titled: “Toxicology Knowledge Graph for Structural Birth Defects” (1.0). *Zenodo* <https://doi.org/10.5281/zenodo.8049261> (2023).

Acknowledgements

This project was supported by NIH grants OT2OD030160, OT2OD030546, OT2OD032619, and OT2OD030162.

Author contributions

J.E., Z.X., D.C., V.U., T.M.A., C.B., J.Y., and J.B.: processed the datasets from the various sources to be included in the knowledge graph. J.E., D.C., and Z.X.: developed the ReproTox website. J.E., D.C., and J.G.: developed the ReproTox Neo4j database. D.C., G.M., A.M., and J.E.: generated the figures. D.C., J.E., Z.X., A.M., G.M., J.B., J.Y., and P.K.: performed the data analysis. B.D. and A.M.: conceived the project. A.M., B.D., S.J., J.B., E.W., D.T., T.O., and C.L.: managed the project. A.M., D.C., J.E., Z.X., G.M., T.M.A., J.Y., J.B., P.K., C.L., D.T., T.O., and B.D.: wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information


Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s43856-023-00329-2>.

Correspondence and requests for materials should be addressed to Avi Ma’ayan.

Peer review information *Communications Medicine* thanks Vit Novacek, Julia Gomes and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2023