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ORIGINAL ARTICLE

Drug contraindications in comorbid diseases: a protein interactome perspective

Kalyani B. Karunakaran[1,2] · Madhavi K. Ganapathiraju[3,4] · Sanjeev Jain[5] · Samir K. Brahmachari[6] · N. Balakrishnan[1]

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Abstract

Adverse drug reactions (ADRs) are leading causes of death and drug withdrawals and frequently co-occur with comorbidities. However, systematic studies on the effects of drugs on comorbidities are lacking. Drug interactions with the cellular protein–protein interaction (PPI) network give rise to ADRs. We selected 6 comorbid disease pairs, identified the drugs used in the treatment of the individual diseases 'A' and 'B'– 44 drugs in anxiety and depression, 128 in asthma and hypertension, 48 in chronic obstructive pulmonary disease and heart failure, 58 in type 2 diabetes and obesity, 58 in Parkinson's disease and schizophrenia, and 84 in rheumatoid arthritis and osteoporosis—and categorized them based on whether they aggravate the comorbid condition. We constructed drug target networks (DTNs) and examined their enrichment among genes in disease A/B PPI networks, expressed across 53 tissues and involved in ~ 1000 pathways. To characterize the biological features of the DTNs, we performed principal component analysis and computed the Euclidean distance between DTN component scores and feature loading values. DTNs of disease A drugs not contraindicated in B were affiliated with proteins common to A/B networks or uniquely found in the B network, similarly regulated common pathways, and disease-B specific pathways and tissues. DTNs of disease A drugs contraindicated in B were affiliated with common proteins or those uniquely found in the A network, differentially regulated common pathways, and disease A-specific pathways and tissues. Hence, DTN enrichment in pathways, tissues, and PPI networks of comorbid diseases will help identify drug contraindications in comorbidities.

Keywords Comorbidities · Interactomes · Adverse drug reactions · Drug contraindications · Drug target networks · Protein–protein interactions

1 Introduction

Comorbidity is the phenomenon in which one or more diseases co-exist with a primary disease in patients. Comorbidities pose a significant threat to patient well-being and are the norm, rather than exception, among chronic conditions (Gadermann et al. 2012). The number of comorbidities increases with age and leads to elevated mortality risk. Mortality risk increased by 25% in patients with 3–4 chronic comorbidities, and by 80% in those with 5 or more comorbidities, when compared with individuals having no chronic conditions, over a period of 14 years from 1992–2006 (Caughey et al. 2010). The prevalence of comorbidities increases from 10% in 0–19-year-olds to 78% in individuals aged 80 or more (Van den Akker et al. 1998). The primary disease in 73.8–98.2% of the respondents of the US National Comorbidity Survey (NCS) survey was accompanied by at least one comorbid condition (Gadermann et al. 2012). Strikingly, the estimates of individual disease morbidity

✉ Kalyani B. Karunakaran
kalyanithepebble@gmail.com

✉ Madhavi K. Ganapathiraju
madhavi@pitt.edu

✉ N. Balakrishnan
balki@iisc.ac.in

1 Supercomputer Education and Research Centre, Indian Institute of Science, Bangalore, India

2 Institute for the Advanced Study of Human Biology, Kyoto University, Kyoto, Japan

3 Carnegie Mellon University, Doha, Qatar

4 Department of Biomedical Informatics, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

5 National Institute of Mental Health and Neuro-Sciences (NIMHANS), Bangalore, India

6 Academy of Scientific and Innovative Research, CSIR-4PI Bangalore, India

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(based on the respondents' perception of their health condition) decreased substantially when adjusted for comorbidity, particularly for disorders that contributed immensely to global disease prevalence, such as neurological disorders, chronic pain, anxiety disorders, major depressive disorder, and diabetes (Gadermann et al. 2012).

The likelihood of experiencing adverse drug reactions (ADRs) may increase with disease comorbidity (Morales et al. 2017; Mittmann et al. 2012; Bassi et al. 2017). Drugs that are beneficial in the treatment of one disease may cause adverse events and aggravate or even cause comorbid conditions, e.g. beta-blockers that treat hypertension and heart disease may aggravate asthma (Morales et al. 2017). ADRs are the fourth leading cause of death in the U.S., with 100,000 deaths and ~ 2 million patients experiencing such reactions per year. Nineteen drugs were withdrawn from the U.S. market during 1998–2007 due to patient fatalities (Giacomini et al. 2007). These statistics highlight the importance of re-examining drug design and development, in the light of mechanisms governing comorbidities.

Network medicine is an integrative framework for examining the mechanistic effects of disease-associated genes in the human protein–protein interaction (PPI) network (or the 'interactome') (Barabási et al. 2011). This emerging systems biology paradigm has prompted a systematic data-driven investigation of drug effects on diseases. This framework combines theory and computation to facilitate the translation of biological data into biologically insightful and clinically actionable results. Its primary applications include identifying (or repurposing) drug targets and pathways for therapeutic intervention and biomarkers for improved disease screening and patient stratification (Brahmachari 2012).

Drugs that target proteins may perturb the PPI network to elicit the intended therapeutic responses or contribute to unintended adverse events (Chan and Loscalzo 2012). Perturbations at the genomic or proteomic level may affect specific PPIs and other proteins in the neighborhood network due to the extensive interconnectivity of the components in the PPI network. This, in turn, may disrupt cellular functions and have deeper implications for disease comorbidity and phenotypic responses to drugs (Barabási et al. 2011).

Adverse events precipitated by drugs in individual diseases have been investigated within the framework of the PPI network (Mizutani et al. 2012; Fliri et al. 2005; Wang et al. 2013; Campillos et al. 2008; Brouwers et al. 2011; Hase et al. 2009). However, the effects of multiple drugs and their contraindications on comorbid conditions remain largely unexplored. Nevertheless, several studies have described the influence of three critical biological factors on drug action, namely, disease-associated PPI networks, biological pathways, and tissue-specificity. Pairs of drugs used for the same disease induce adverse events when the network modules of their protein targets overlap with each other and with the network of disease-associated genes ('overlapping exposure') (Cheng et al. 2019). A 2.6-fold greater risk of side effects was seen with drugs that target genes having 5 specific genetic features, including tissue-specific gene expression (Duffy et al. 2020). The findings from this study also suggested that side effects arise from drug delivery to multiple tissues (including those unrelated to the disease) (Duffy et al. 2020).

In this study, we attempt to elucidate the mechanisms underlying drug contraindications in pairs of comorbid diseases by examining drug target networks (DTNs). The overlaps of the DTNs with the PPI networks of disease-associated proteins, biological pathways and tissue-specific genes were identified as critical factors influencing ADRs in comorbidities.

2 Methods

2.1 Selection of comorbid and non-comorbid disease pairs

For our analysis, we selected six pairs of comorbid diseases and three pairs of non-comorbid diseases as negative controls based on literature evidence.

The following were the six comorbid disease pairs:

(I) Anxiety—Depression: 75% of the individuals primarily diagnosed with depression in the Netherlands Study of Depression and Anxiety (NESDA) also had a (lifetime) history of comorbid anxiety disorder (Lamers et al. 2011). Conversely, 81% of the individuals with a primary diagnosis of an anxiety disorder had a (lifetime) history of comorbid depressive disorder (Lamers et al. 2011).

(II) Asthma—Hypertension: Logistic regression models have shown that asthmatics were 36% more likely to have hypertension compared to non-asthmatics in a study conducted among Canadian adults (Dogra et al. 2007). Moreover, asthmatics with comorbid hypertension exhibited morbid asthma symptoms as evidenced by increased usage of short-acting β-agonists and corticosteroids, and increased hospitalization and visits to the emergency department (Christiansen et al. 2016).

(III) Chronic obstructive pulmonary disorder (COPD)—Heart failure: Retrospective analysis has revealed that 20.5% of the patients diagnosed with COPD had undiagnosed heart failure (Rutten et al. 2005). Conversely, 35% of the patients admitted with heart failure showed comorbid COPD (Iversen et al. 2008).

(IV) Type 2 diabetes—Obesity: Obesity has been noted in 78.2% of individuals with type 2 diabetes (Iglay et al. 2016). Conversely, a higher prevalence (30.9% versus 4.5%) of type 2 diabetes has been noted in cohorts with individuals showing a higher body mass index (BMI ≥ 40 versus BMI > 25) (Pantalone et al. 2017).

(V) Rheumatoid arthritis—Osteoporosis: 32.6% of the patients with rheumatoid arthritis (RA) had osteoporosis (Llorente et al. 2020). This percentage for osteoporosis comorbidity increased to 50% in menopausal women with RA (Llorente et al. 2020). Moreover, RA patients seemed to exhibit a higher risk of fractures of bones with higher mineral density compared with healthy controls (Llorente et al. 2020).

(VI) Parkinson's disease—Schizophrenia: Comorbidity between Parkinson's disease and schizophrenia has been noted despite their links to seemingly converse states of the dopaminergic signalling pathway. A hypodopaminergic state in the nigrostriatal pathway characterized by reduced levels of dopamine (in comparison with baseline levels) has been historically cited as a causative factor for Parkinson's disease, whereas a hyperdopaminergic state characterized by enhanced levels of dopamine in the mesolimbic pathway has been historically linked to schizophrenia (Kuusimäki et al. 2020). A retrospective study showed that 1.5% of Parkinson's disease patients had a schizophrenia diagnosis earlier in life and that schizophrenia increases the risk (odds ratio = 1.17) for Parkinson's disease later in life (Kuusimäki et al. 2020). This comorbidity may result from the use of dopamine antagonists in schizophrenia treatment, which can induce Parkinsonian symptoms or alter Parkinson's disease risk via undiscovered mechanisms, as well as from schizophrenia-induced phase-dependent dopamine dysregulation that increases vulnerability to Parkinson's disease (Kuusimäki et al. 2020). Additionally, psychiatric symptoms are common in drug-naïve patients with early-onset Parkinson's disease, and behavioral symptoms overlapping with those seen in schizophrenia are often observed in drug-naïve patients with late-onset PD (Pachi et al. 2021; Waddington 2020).

The following were the three non-comorbid disease pairs:

(I) Multiple sclerosis—Peroxisomal disorders: Multiple sclerosis and peroxisomal disorders were used in an earlier study (Menche et al. 2015) to show that diseases lacking overlapping network modules do not exhibit a comorbid association.

(II) Schizophrenia—Rheumatoid arthritis: Schizophrenia and rheumatoid arthritis have been known to show an inverse prevalence, i.e., low incidence of rheumatoid arthritis among schizophrenia patients and vice versa (Vinogradov et al. 1991; Oken and Schulzer 1999; Benros et al. 2014).

(III) Asthma—Schizophrenia: Schizophrenia has been less commonly associated with asthma, although other psychiatric morbidities such as anxiety disorders and depression have been known to occur among asthma patients (Boulet 2009).

2.2 Compilation of disease-associated genes

The genes associated with 3 non-comorbid disease pairs and 6 comorbid pairs were compiled from the DisGeNET database (Piñero et al. 2016) (version 7) (Supplementary Data File 1). 100 top-ranking genes associated with each of the 14 diseases were curated based on their gene-disease association scores (GDA). The GDA score for a gene is computed based on multiple pieces of evidence, namely, the number of publications supporting its association with the disease, the number and types of database sources (based on curation method, i.e. expert-curated or computationally predicted) and the model organisms in which the association was validated. The range of the GDA scores varied across the 100 top-ranking genes for each of the 14 diseases. However, a minimum GDA of ≥ 0.01 was chosen to ensure that at least one publication reported the gene-disease association. Not all genome-wide association and targeted gene sequencing studies in the DisGeNET database involve drug-naïve populations, except for a few, such as studies conducted with untreated first-episode psychosis patients. Therefore, the influence of drug treatment on the disease-associated gene sets is unavoidable. Additionally, the patient population is naturally stratified into various groups responding differently to specific drugs based on their genetic predispositions (Loscalzo 2023). However, single-cell sequencing studies have begun to offer a clearer view of drug-induced perturbations at single-cell resolution (Srivatsan et al. 2020) and the impact of genetic variants on the multicellular underpinnings of disorders (Jin et al. 2020).

2.3 Construction of disease PPI networks

The PPI networks of the proteins encoded by the disease-associated genes were assembled by extracting their protein interactors from the Human Protein Reference Database (HPRD; version 9) (Keshava Prasad et al. 2008) and the Biological General Repository for Interaction Datasets (BioGRID; version 4.3.194) (Stark et al. 2006) using

the Cytoscape plugin, Bisogenet (Martin et al. 2010). The input nodes for disease network construction were the 100 top-ranking genes compiled from the DisGeNET database. The network building options were: organism—*Homo sapiens*, biorelation type—*protein–protein interaction*, data sources—*BioGRID and HPRD*, method—*input nodes and its neighbors upto a distance of 1*.

2.4 Compilation of drugs indicated for specific diseases

The Drug Bank database (Wishart et al. 2008) (version 5.1.8) was used to compile the lists of drugs indicated for each of the 14 diseases. Then, we used the TWOSIDES database (Tatonetti et al. 2012) (version 0.1)—a publicly available database of drugs and associated adverse events—to categorize these drugs with respect to their effects on the disease pairs. We separated the drugs into two groups, namely, disease A drugs that are (a) contraindicated and (b) not contraindicated in disease B, and disease B drugs that are (c) contraindicated and (d) not contraindicated in disease A. Drugs associated with specific adverse effects (belonging to (a) and (c)) were identified using their 'condition concept names' (which are descriptions of adverse events) (see Supplementary Data File 2 for the condition concept names and Supplementary Data File 3 for the drug lists). For example, to identify the anxiolytic drugs that may cause depression, the condition concept names, *depression*, *major depression*, *depressive symptom*, *depression suicidal*, *depression postoperative*, *postpartum depression*, *depressive delusion,* and *agitated depression*, were selected. The list of anxiolytic drugs was then compared with the list of drugs associated with these condition concept names. The matching drugs were compiled into groups 'a' and 'c', for example, "drugs effective in anxiety and contraindicated in depression". Similarly, groups 'b' and 'd' drugs were compiled.

2.5 Construction of drug-target networks

We compiled the proteins targeted by the drugs (Supplementary Data File 4) belonging to the 4 categories described above from the Drug Bank database (Wishart et al. 2008), by querying the DGIdb (drug-gene interaction database) web portal (Griffith et al. 2013). To construct the DTNs, we compiled the PPIs of the drug targets from HPRD (Keshava Prasad et al. 2008) and BioGRID (Stark et al. 2006) using Bisogenet (Martin et al. 2010). The network building options were identical to those described in the section for disease network construction.

2.6 Calculation of network similarity measures

Matching node ratio (N_M) was measured as the ratio of the total number of common nodes shared between the two disease PPI networks (in a comorbid pair) and the total number of unique nodes in these two networks (Brown et al. 2019).

$$N_M = \frac{A_n \cap B_n}{A_n \cup B_n} \tag{1}$$

A_n = Number of nodes in disease A PPI network, B_n = Number of nodes in disease B PPI network.

Matching link ratio (L_M) was measured as the ratio of the total number of common links (i.e. edges) shared between the two disease PPI networks and the total number of unique links in these two networks (Brown et al. 2019).

$$L_M = \frac{A_l \cap B_l}{A_l \cup B_l} \tag{2}$$

A_l = Number of links in disease A PPI network. B_l = Number of links in disease B PPI network.

The formula shown above was also used to calculate the matching link ratio for links of path lengths 2 and 3 in the two disease networks. Links of specific path lengths were retrieved using the Cytoscape application, NetworkAnalyzer (Assenov et al. 2008; Shannon et al. 2003).

2.7 Calculation of comorbid associations

Relative risk (RR_{AB}) measures comorbidity by comparing the observed prevalence of a pair of comorbid diseases (A and B) in the population with the expected number. The expected number is calculated based on the prevalence of the individual diseases A and B in the population.

$$RR_{AB} = \frac{N_{AB}N}{N_A N_B} \tag{3}$$

N_A = Total number of patients diagnosed with disease A. N_B = Total number of patients diagnosed with disease B.

N_{AB} = Total number of patients diagnosed with both disease A and disease B.

N = Total number of patients in the population.

For the calculation of relative risks of disease pairs, we downloaded the HuDiNe dataset (http://sbi.upf.edu/data/hudine/) containing processed hospital claims data of 13,039,018 U.S. individuals who had applied for support from the U.S. Medicare program during 1990–1993 (Hidalgo et al. 2009). Individual disease and comorbidity

data (i.e. N_A, N_B and N_{AB}) was available for 5 out of our 6 comorbid disease pairs (i.e. excluding anxiety—depression), and for 2 out of the 3 non-comorbid pairs (i.e. excluding multiple sclerosis—peroxisomal disorders). The diseases were specified in the form of their ICD-9 codes (at three-digits level). The population size N was considered to be 13,039,018, i.e. the total number of individuals represented in HuDiNe.

2.8 Pathway enrichment analysis

WebGestalt (Liao et al. 2019a) was used to compute the distribution of genes involved in specific signalling pathways in the DTNs and compare it with the background distribution of genes belonging to this pathway among all the genes associated with any pathway in the Reactome database (Liao et al. 2019b). Statistical significance of the enrichment was computed using Fisher's exact test and corrected for multiple hypotheses using the Benjamini–Hochberg method.

2.9 Gene expression enrichment analysis

The enrichment of the DTNs for genes expressed in specific tissues was computed using the GTEx database (Consortium 2015) (version 8). GTEx contains RNA-sequencing data from 53 postnatal human tissues. Genes that showed high or medium expression (transcripts per million (TPM) ≥ 9) in specific tissues were included in the analysis, provided that they were not housekeeping genes, i.e. those detected in all the tissues with transcripts per million ≥ 1, a criterion described in the Human Protein Atlas (Uhlén et al. 2015). TPM is a metric that measures the relative abundance of transcripts. Gene matrix transposed (GMT) files that contained the 52 tissues and the genes whose expression levels in these tissues matched the criteria described above were created. These GMT files served as inputs for gene over-representation analysis based on hypergeometric distribution. Tissues enriched with disease-specific single nucleotide polymorphisms (SNPs) were identified using TSEA-DB (Jia et al. 2020). TSEA-DB is a reference database for information on disease-associated tissues, specifically, the tissues in GTEx that show significant enrichment for genes harboring disease-associated variants compiled from the GWAS catalog (Jia et al. 2020).

BaseSpace Correlation Engine (https://covid-19.ce.basespace.illumina.com/c/nextbio.nb) was used to identify the correlations between the gene expression profile induced by maprotiline in PC3 cells (Broad Connectivity Map (CMAP 2.0) (Subramanian et al. 2017)), the profile associated with major depressive disorder and generalized anxiety disorder (GSE98793 (Leday et al. 2018)), and the tissue expression profile of the adrenal cortex. The software uses a non-parametric rank-based approach to compute the overlap of two gene sets (Kupershmidt et al. 2010).

2.10 Principal component analysis

Principal component analysis (PCA) was used to capture the relationships of the DTNs with disease networks, biological pathways and tissues. For each comorbid pair, negative log-transformed *P* values indicating the statistical enrichment of the disease networks/biological pathways/tissues in the 4 DTNs (constructed from drugs eliciting four types of reaction in the two diseases) were assembled into a data matrix. This data matrix contained disease protein sets/biological pathways/tissues as rows and DTNs as columns; each cell in the matrix contained a $-\log_{10}P$ value. Log transformation and unit variance scaling were performed to reduce the influence of extreme values on the obtained PCs (Love et al. 2015). PCA using singular value decomposition (SVD) with imputation was performed with a web-based tool called ClustVis (https://biit.cs.ut.ee/clustvis/) (Metsalu and Vilo 2015). The data matrix was pre-processed such that 70% of missing values were allowed across the rows and columns. PC scores are calculated as linear combinations of the original variables ($-\log_{10}P$) and the corresponding weights (otherwise known as component loadings). The importance of each disease protein set/pathway/tissue is reflected by the magnitude of their corresponding loading values on PC1 and PC2. Finally, for each of the comorbid pairs, the Euclidean distance between the PC scores of each of the DTNs was computed for all the component loading values pertaining to the particular biological modality. This resulted in a list of the specific disease protein sets/pathways/tissues that were potentially closely related to each of the different DTNs.

3 Results

To identify potential mechanisms of ADRs within comorbid diseases, we systematically studied pairs of comorbid diseases ('disease A' and 'disease B') and their FDA-approved drugs. We selected three pairs of non-comorbid diseases as negative controls and six pairs of comorbid diseases for our analysis. The non-comorbid pairs were: (I) multiple sclerosis—peroxisomal disorders (Menche et al. 2015), (II) schizophrenia—rheumatoid arthritis (Vinogradov et al. 1991; Oken and Schulzer 1999; Benros et al. 2014), (III) asthma—schizophrenia (Boulet 2009). The comorbid pairs were (IV) anxiety—depression (Lamers et al. 2011), (V) asthma—hypertension (Dogra et al. 2007; Christiansen et al. 2016), (VI) chronic obstructive pulmonary disorder (COPD)—heart failure (Rutten et al. 2005; Iversen et al. 2008), (VII) type 2 diabetes—obesity (Iglay et al. 2016; Pantalone et al. 2017), (VIII) rheumatoid

arthritis—osteoporosis (Llorente et al. 2020) and (IX) Parkinson's disease—schizophrenia (Kuusimäki et al. 2020). Four categories of drugs were compiled for each disease pair based on their clinical activity, namely, disease A drugs that are (a) contraindicated and (b) not contraindicated in disease B, and disease B drugs that are (c) contraindicated and (d) not contraindicated in disease A. Four corresponding DTNs were also constructed (see Methods for detailed descriptions and steps (a)-(c) in Fig. 1).

3.1 Disease network similarity and comorbid associations

Relative risk is an experiential measure of comorbidity. It compares the observed prevalence of a comorbid disease pair in the population with the prevalence expected based on the number of patients with the individual diseases. We computed the relative risk of the disease pairs from the hospital claims data of 13,039,018 U.S. individuals in the HuDiNe dataset (Hidalgo et al. 2009) (see Methods).

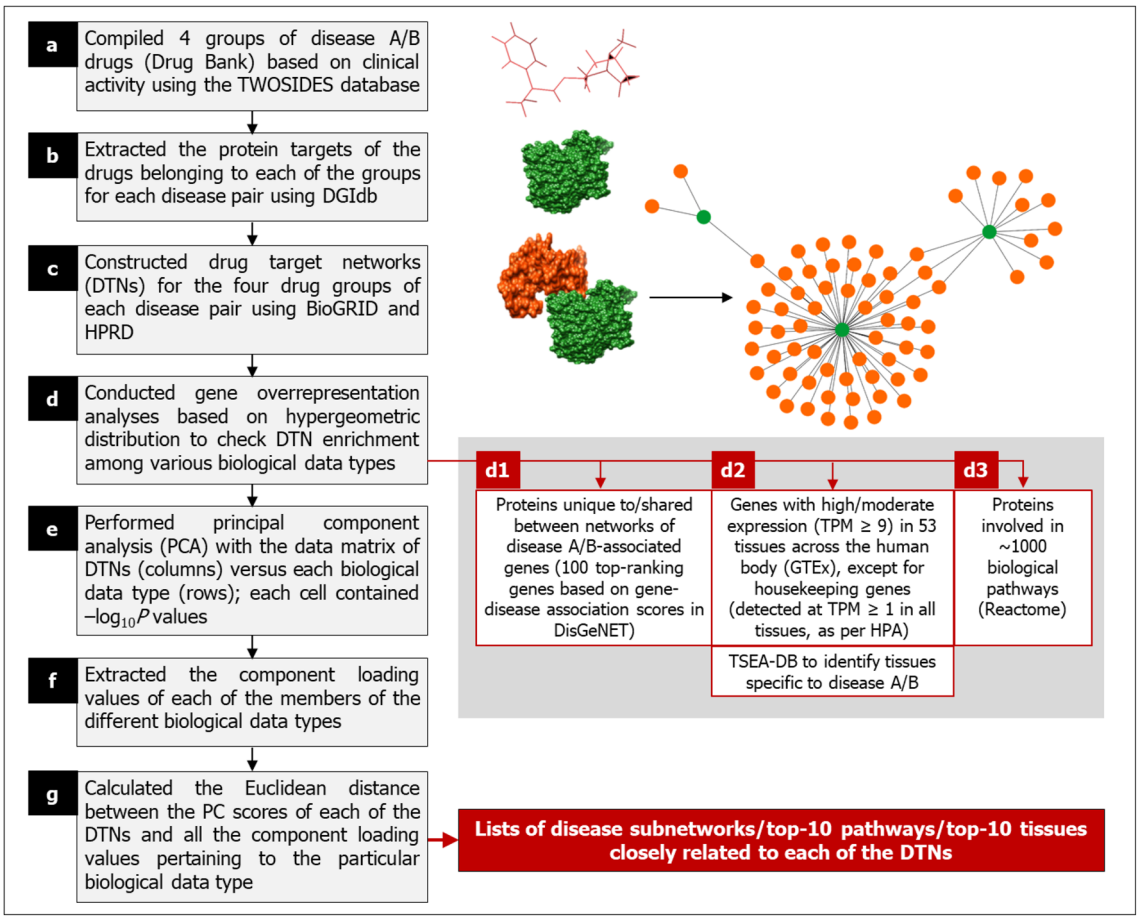

Fig. 1 Framework for characterizing the drugs that target comorbid disease pairs. Our methodology to characterize DTNs involved seven steps: **a** Retrieval of the drugs indicated for use against each of the diseases using Drug Bank and their categorization into four groups based on their clinical activity in the comorbid diseases. **b** Identification of the proteins collectively targeted by the drugs in each of the groups by querying Drug Bank through DGIdb. c Construction of DTNs using the protein targets as input nodes and assembling their immediate neighbors in the human PPI network using data from BioGRID and HPRD. **d** Performing gene enrichment analysis with the four DTNs (for each disease pair) in 3 biological data types: (d1) disease PPI networks, (d2) tissue gene expression and (d3) biological pathways. **e** Generation of a data matrix containing the enriched disease protein sets/tissues/pathways as rows, DTNs as columns and $-\log_{10}P$ values in the cells, and using the matrix as an input for PCA. **f** Extraction of component loadings of each of the enriched disease protein sets/tissues/pathways corresponding to each of the PCs. **g** Calculation of the Euclidean distance between the PC scores of each of the DTNs and the component loadings of the disease protein sets/tissues/pathways. These steps helped identify top disease protein sets, tissues and pathways that were closely associated with each of the DTNs. Databases: BioGRID (Biological General Repository for Interaction Datasets), DGIdb (Drug Gene Interaction database), DisGeNET (Disease Gene association NETwork), Drug Bank, GTEx (Genotype-Tissue Expression), HPRD (Human Protein Reference Database), Reactome, TSEA-DB (Tissue-Specific Enrichment Analysis DataBase) and TWOSIDES. Abbreviations: PPI—Protein–Protein Interaction, DTN—Drug Target Network, PCA—Principal Component Analysis and TPM—Transcripts Per Million

We then explored whether this relative risk of comorbidity would be reflected in the similarity of the disease networks. The disease networks were constructed using the 100 top-ranking genes associated with each of the 14 diseases (see Methods). For each of the disease pairs (including comorbid and non-comorbid negative control pairs), we systematically identified the proteins (a) shared between the two disease networks, (b) unique to disease A and (c) unique to disease B (Table 1). In Table 1, while we observed overlaps of higher statistical significance between the networks of comorbid disease pairs compared to non-comorbid pairs, the odds ratio of enrichments remained similar (see Supplementary Note 1 for factors contributing to this similarity).

Then, we computed four network similarity measures, namely, matching node ratio (N_M) for all the nodes shared between the two disease networks, and the matching link ratio (L_M) (Brown et al. 2019) for all the (i) shared links (i.e. edges), (ii) shared links of path length 2 (connecting two nodes via one intermediate node) and (iii) shared links of path length 3 (connecting two nodes via two intermediate nodes) between the two disease networks.

We found that the relative risk between diseases was proportional to the matching node and link ratios (Fig. 2). The negative control disease pairs showed low relative risks and smaller disease network overlaps, whereas 3 out of the 5 comorbid pairs showed high relative risks and larger network overlaps, namely, asthma—hypertension, COPD—heart failure and type 2 diabetes—obesity. However, this trend was not seen in 2 comorbid pairs, namely, rheumatoid arthritis—osteoporosis and Parkinson's disease—schizophrenia. Specifically, their higher relative risks (compared with other comorbid pairs), were not accompanied by a corresponding increase in the network overlap. This anomaly can, perhaps, be explained by multiple factors (see Discussion). As an overall trend, we noted that the relative risk of disease pairs varied in tandem with the similarity of their networks. Based on this, we speculated that drug action on the druggable proteins shared between the two disease networks may give rise to contraindications in comorbid pairs.

3.2 Druggability of disease networks

We examined the enrichment of the disease protein sets (common to both networks, unique to disease A and unique to disease B) for a group of 4,463 druggable proteins (Hopkins and Groom 2002), similar to the approach followed in a previous study (Sun et al. 2015). Drugs that follow Lipinski's 'rule-of-five' are bound by these proteins with high affinity at specific binding sites (Lipinski et al. 1997). We found that druggable targets were

Table 1 Overlap of the disease networks. The table shows the statistics of the overlaps shared between the two diseases in each of the nine disease pairs that were examined in our study

Disease pair	# Proteins in disease A network	# Proteins in disease B network	# Shared proteins	p-value of overlap	Odds ratio of overlap	% Shared proteins in disease A network	% Shared proteins in disease B network
Multiple sclerosis (A)—Peroxisomal disorders (B)	2418	727	284	5.97E-70	2.9	12	39
Schizophrenia (A)—rheumatoid arthritis (B)	2662	2424	918	6.86E-208	2.56	34.5	38
Asthma (A) – Schizophrenia (B)	3041	2662	1084	1.36E-228	2.41	36	41
Anxiety (A)—Depression (B)	3342	3054	1732	1.86E-628	3.06	52	57
Asthma (A)—Hypertension (B)	3041	2515	1371	1.85E-500	3.23	45	54.50
Chronic obstructive pulmonary disease (A) –heart failure (B)	3736	2922	1505	3.12E-371	2.48	40	51.50
Type 2 diabetes (A) – Obesity (B)	2471	2490	1232	3.66E-503	3.6	50	49
Rheumatoid arthritis (A)—osteoporosis (B)	2424	3681	1206	1.30E-270	2.43	50	33
Parkinson's disease (A)—schizophrenia (B)	3200	2662	1232	2.88E-310	2.6	38.50	46

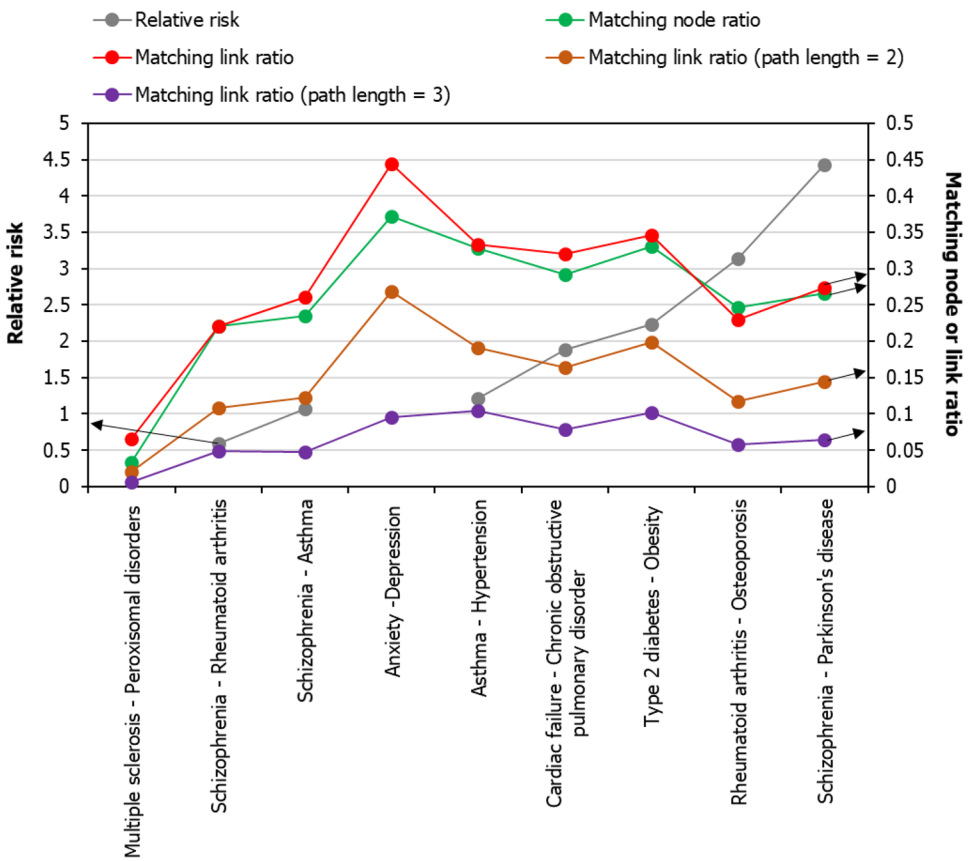

Fig. 2 Comparison of disease network similarity measures and comorbid associations. The graph shows the relationship between relative risk (black data points) and four measures of network similarity, namely, matching node ratio (green data points), matching link ratio of all shared edges (red data points), matching link ratio of all shared edges of path length 2 (brown data points) and matching link ratio of all shared edges of path length 3 (purple data points). Note that the values for relative risk have been plotted with respect to the Y-axis on the left. The matching node and link ratios have been plotted on the Y-axis on the right

most significantly enriched among the proteins shared between the two disease networks (Table 2). This trend was observed in 5 out of the 6 comorbid pairs (Table 2). In anxiety–depression, druggable targets were most enriched among proteins exclusively found in the depression network (Supplementary Note 2 and Supplementary Table 1).

Based on this result, we hypothesized that (i) the DTNs of the group 'a' and 'c' drugs (effective in disease A and contraindicated in disease B or vice versa) may show the highest enrichment for the proteins/pathways/tissues shared between the two disease networks and (ii) the DTNs of the groups 'b' and 'd' drugs (effective in disease A and *not* contraindicated in disease B or vice versa) may

Table 2 Overlaps of the disease protein sets with druggable targets. $-\log_{10}P$ values computed for each of the nine tested disease pairs using a hypergeometric test. The $-\log_{10}P$ values indicate the statistical significance of the overlaps shared by each of the disease protein sets (top column headings) with a group of 4463 druggable proteins. *, ** and *** indicate low, medium and high levels of statistical significance. †, †† and ††† indicate non-significant overrepresentation, non-significant underrepresentation and significant underrepresentation respectively

Disease pairs	Common to both the networks	Unique to disease A network	Unique to disease B network
Multiple sclerosis **(A)**—peroxisomal disorders **(B)**	7.38**	19.52***	2.09*
Schizophrenia (A)—Rheumatoid arthritis	13.26**	14.36***	2.4*
Asthma **(A)**—schizophrenia **(B)**	19.18***	9.41**	0.89†
Anxiety **(A)**—Depression **(B)**	5.57**	0.001†††	12.05***
Asthma **(A)**—Hypertension **(B)**	31.34***	3.19*	9.59**
Chronic obstructive pulmonary disease **(A)**—heart failure **(B)**	34.73***	1.06††	9.16**
Type 2 diabetes **(A)**—Obesity **(B)**	18.65***	1.47*	7.05**
Rheumatoid arthritis **(A)**—Osteoporosis **(B)**	21.96***	7.17**	1.97*
Parkinson's disease **(A)**—Schizophrenia **(B)**	19.93***	0.27†	0.3†

show the highest enrichment for proteins/pathways/tissues unique to disease A (or B respectively).

3.3 Framework to characterize the drug target networks

The methodology of our study is illustrated in Fig. 1. To characterize the 4 DTNs associated with each of the comorbid pairs, we examined 3 types of data that may reflect their biological profiles, namely (i) disease PPI networks, (ii) biological pathways and (iii) tissue gene expression. Specifically, we conducted gene overrepresentation analyses based on hypergeometric distribution to check the enrichment of the DTNs among proteins that are unique to/shared between networks of disease A and disease B, genes showing high/moderate expression in 53 tissues across the human body, and proteins involved in ~ 1000 biological pathways. Overlaps computed in this manner were considered to be statistically significant at $P < 0.05$ after correction for multiple hypotheses using the Benjamini–Hochberg method.

We sought to identify the specific disease protein sets, pathways and tissues that were more closely related to each of the 4 DTNs for each comorbid pair in terms of Euclidean distance. For this, we performed principal component analysis (PCA) with a data matrix containing DTNs (columns) versus the specific disease protein sets, pathways or tissues (rows). For example, for the data modality 'disease protein set', the rows would be 'common to both the networks', 'unique to disease A network' and 'unique to disease B network' and for the data modality 'tissue', the members would be 'amygdala', 'aorta', 'lungs', etc. Each cell contained $-\log_{10}$ transformed P values, which have been used as inputs for PCA in previous studies (Chang and Keinan 2014; McGuirl et al. 2020). PCA has been applied to matrices containing gene-level association scores in several studies (McGuirl et al. 2020). All the PCs generated after this analysis were considered for our study, and the PC scores of the DTNs were used to identify their grouping patterns. Following this, we extracted the component loading values denoting the weights of each of the biological modalities on the PCs. Component loadings depict the correlation of the original variables ($-\log_{10}P$ values) in our data matrix with each of the extracted PCs. Their magnitudes can be used to assess the influence of the different biological modalities on the 4 DTNs separated along the PCs. Lastly, we calculated the Euclidean distance between the PC scores of each of the DTNs and the corresponding component loadings of the biological modalities. This yielded a list of the specific disease protein sets/pathways/tissues that may be closely related to each of the 4 DTNs of the comorbid pair.

3.4 Disease networks and drug target networks

For each disease pair, we systematically computed the overlaps of the 4 DTNs with proteins that are (a) common to disease A and disease B networks, (b) unique to disease A network and (c) unique to disease B network (Supplementary Table 2). Previous studies have examined the overlaps between DTNs and disease networks (Cheng et al. 2019; Han et al. 2021). A data matrix of DTNs (columns) versus disease protein sets (rows), which contains $-\log_{10}P$ values indicating the statistical significance of their overlaps was used as the input for PCA. We computed the Euclidean distance between the PC scores of each of the DTNs across all the extracted axes and the corresponding component loadings of all the disease protein sets across these axes.

In 10 out of the 12 cases, the DTNs of drugs used for a specific disease and not contraindicated in a comorbid condition were found to be closest/second closest to the proteins uniquely found in the network of the comorbid condition. Additionally, in 9 out of the 12 cases, they were closest/second closest to the proteins shared between the networks of both diseases. Hence, disease A drugs that are not contraindicated in disease B may target proteins unique to the disease B subnetwork. Based on this, we speculated that these proteins unique to disease B network may be involved in mechanisms that are not critical or beneficial for disease B, but whose modulation is certainly beneficial for the treatment of disease A. Note that the absence of contraindications in disease B cannot be solely attributed to the favourable modulation of disease B by the drug. It could also result from the drug not interfering with the mechanisms of disease B. However, in cases where a beneficial modulation by the drug is suspected, it can be the underlying reason for the absence of contraindications. Alternatively, this same category of drugs (disease A drugs that are not contraindicated in disease B) may target common mechanisms that are dysregulated in a similar manner in both diseases and pharmacologically modulate them in a similar direction.

In contrast, in 8 out of the 12 cases, the DTNs of drugs used for a specific disease and contraindicated in a comorbid condition were found to be closest/second closest to the proteins uniquely found in the network of the disease for which these drugs were primarily used. Additionally, in 9 out of the 12 cases, they were closest/second closest to the proteins shared between the networks of both diseases. These results led us to speculate two scenarios for disease A drugs that are contraindicated in disease B. They may either target (a) common mechanisms that are pharmacologically oppositely modulated in a manner that benefits disease A but aggravates disease B or (b) mechanisms unique to disease A that aggravate disease B.

Altogether, this led us to suspect that biological processes functioning at a higher level than disease subnetworks could be regulating drug action under comorbid conditions.

3.5 Biological pathways and drug target networks

For each disease pair, we identified the pathway associations of the DTNs using the gene set analysis toolkit called WebGestalt (Liao et al. 2019a). For each of the 6 disease pairs, a data matrix of DTNs (columns) versus Reactome pathways (rows) containing corresponding $-\log_{10}P$ values of enrichments was used as inputs for PCA. The Euclidean distance between the PC scores of each of the DTNs across all the extracted axes and the corresponding component loading values of all the pathways across these axes were computed. For each of the disease pairs, we retrieved the top-10 pathways closest to each of the DTNs out of all the pathways enriched in the DTNs (Supplementary Fig. 1–6). Confirming our earlier premise, we noted that disease A DTNs without contraindications in disease B were nearest to pathways possibly underlying both the diseases or uniquely associated with B, which are similarly regulated, i.e. upregulated or downregulated together, in the two comorbid diseases. On the other hand, disease A DTNs with contraindications in disease B were nearest to pathways underlying both the diseases or unique to disease A that are differentially regulated, i.e. upregulated in one disease and downregulated in the other or vice versa.

The drug maprotiline was among our list of anxiety drugs without contraindications for depression. Corroborating this, clinical data suggested that the drug is effective in alleviating anxiety symptoms co-occurring with depression (Lacy 2006). 'G α(12/13) signalling events' and 'muscarinic acetylcholine receptors' were identified among the top-10 pathways that were close to anxiety drugs not contraindicated in depression (Supplementary Fig. 7). G α(12/13) regulates adrenergic receptor signaling (Maruyama et al. 2002), and acts as an antagonist on adrenergic and cholinergic receptors (Supplementary Note 3). It targets a higher number of proteins associated uniquely with depression (ADRA2A, HTR2C, SLC6A2 and CHRM2) in the adrenergic, serotonergic and cholinergic systems (Fig. 3). It targets only one receptor associated with both anxiety and depression (HTR2A), and no gene uniquely associated with anxiety (Fig. 3). These observations are in line with our findings in the previous section. DTNs of disease A drugs that are not contraindicated in disease B (e.g. maprotiline) are closely associated with proteins uniquely found in the disease B network (which is depression in this example). Maprotiline, however, is being cited here only as a demonstrative example, since its usage has been discontinued since 2020 in U.S. (Data 2017).

'Serotonin receptors' was identified among the top 10 pathways that were close to depression drugs not contraindicated in anxiety (Supplementary Fig. 7). This is in line with the observed efficacy of drugs acting on serotonin receptors both in short-term and long-term treatment of major depressive disorder and anxiety disorders (Goodwin 2015). Two such drugs in our study display antagonistic activity on the serotonin receptors HTR2A and HTR2C—flupentixol (Pöldinger and Sieberns 1983) and mirtazapine (Alam et al. 2013)—and have been used to treat depression accompanied by anxiety symptoms (Fig. 3). Note that flupentixol is sparingly used to treat depression and is cited here only as a demonstrative example.

The pathway 'dopamine receptors' was found to be close to Parkinson's disease (PD) drugs contraindicated in schizophrenia (SCZ) (Supplementary Fig. 8). This is in line with the observation that enhanced dopamine levels induced by PD drugs may in fact induce SCZ-like symptoms, which has been linked to a hyperdopaminergic state (Kuusimäki et al. 2020). These dopamine agonist PD drugs have been shown to induce psychosis, namely, levodopa (acting on DRD1, DRD2, DRD3, DRD4 and DRD5) and ropinirole (DRD2, DRD3 and DRD4) (Fig. 4) (Zahodne and Fernandez 2008; Stoner et al. 2009). Similar symptoms were observed with the increased dopamine levels resulting from allografts containing dopaminergic stem cells from the ventral mesencephalon (Barker et al. 2013). It is notable that levodopa and ropinirole target a higher number of dopamine receptors associated with PD (DRD1 and DRD2) (Fig. 4). They target only one dopamine receptor (DRD3) associated with SCZ (Fig. 4). These observations are in line with our finding that the DTNs of drugs (used for disease A) that are contraindicated in disease B (e.g. levodopa and ropinirole) are closely associated with proteins found in the disease A network (i.e. PD in this specific example).

3.6 Tissues and drug target networks

Using RNA-sequencing data of 53 postnatal human tissues obtained from GTEx (Consortium 2015) (version 8), we attempted to identify whether the four DTNs (of each disease pair) showed enrichment for tissue-specific genes. We generated a data matrix of DTNs (columns) versus tissues (rows) containing the $-\log_{10}P$ values of enrichment and performed PCA with this matrix as the input. We calculated the Euclidean distance between the PC scores of each of the DTNs and the component loading values of all the tissues. For each of the disease pairs, we retrieved the top 10 tissues that were nearest to the four DTNs (Supplementary Fig. 9–15). For each of the diseases, we used TSEA-DB (Jia et al. 2020) to retrieve the top 3 tissues that showed significant enrichment for disease-associated variants (see Methods). We then checked whether the top 3 tissues associated

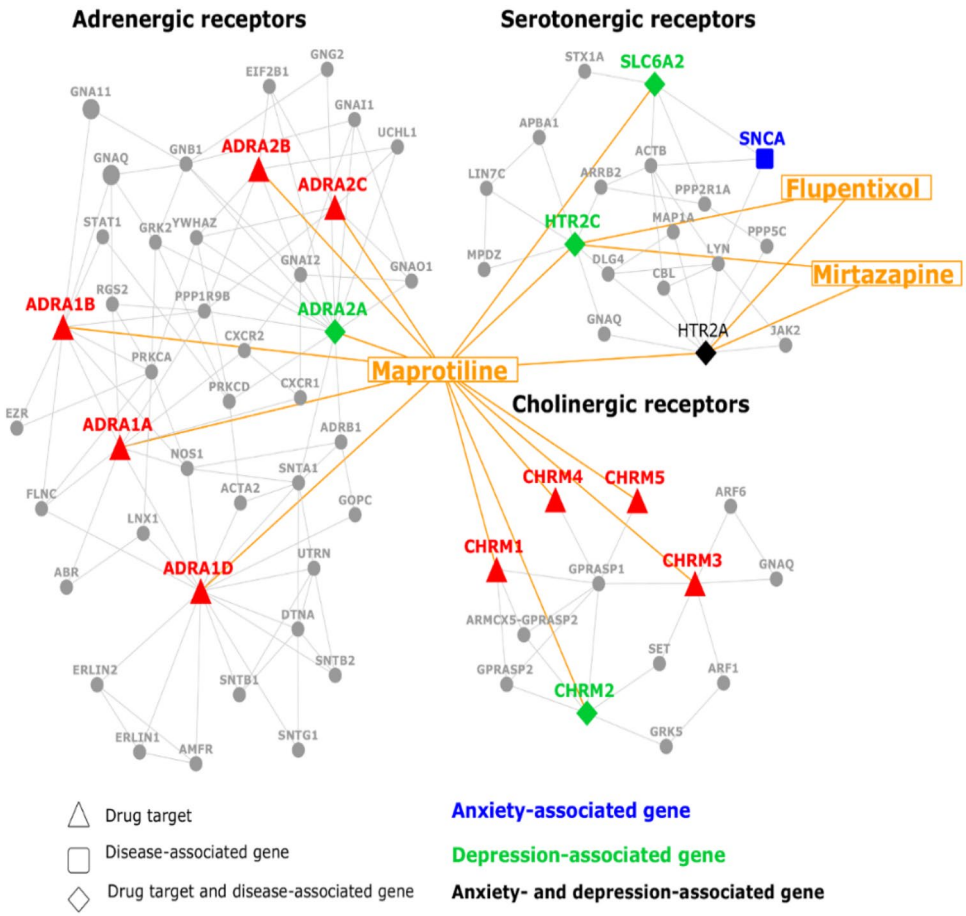

Fig. 3 Network diagram showing the relationship between the targets of maprotiline, flupentixol and mirtazapine, and genes associated with anxiety and depression. The different families of receptors and transporter proteins targeted by maprotiline, flupentixol and mirtazapine and their interactions with the proteins encoded by anxiety (disease A) and/or depression (disease B) associated genes have been shown. Note that maprotiline (an anti-anxiety (disease A) drug not contraindicated in depression (disease B)) targets a higher number of proteins associated uniquely with depression in the adrenergic, serotonergic and cholinergic systems, which is in line with our observation that disease A drugs that are not contraindicated in disease B are closely associated with proteins uniquely found in the disease B network (i.e. depression in this specific example). Serotonin receptors were found to be associated in our analysis with depression drugs not contraindicated in anxiety; antagonistic activity on serotonin receptors is exhibited by two such drugs shown in the diagram (flupentixol and mirtazapine)

with individual diseases appeared among the list of tissues identified to be closely related to the 4 DTNs (associated with each disease pair). Out of the 11 tissues identified to be closer to the DTNs of drugs used for a primary disease and not contraindicated in a comorbid condition, 6 were found to be associated with the comorbid condition (as per TSEA-DB); 3 were associated with the primary disease for which the drugs were used and 2 were associated with both the primary disease and the comorbid condition. Conversely, out of the 9 tissues identified to be closer to the DTNs of drugs used for primary disease and contraindicated in a comorbid condition, 5 were found to be associated with the primary disease, whereas 3 were associated with the comorbid condition in which the drugs were contraindicated and one was associated with both the disease and the comorbid condition.

These percentages should be cautiously interpreted as they were obtained with a small number of tissues. Nevertheless, the results seem to corroborate our previous findings with disease subnetworks and biological pathways. Specifically, the DTNs of disease A drugs that are not contraindicated in disease B seemed to be nearest to tissues preferentially affiliated with disease B. This indicated that these tissues could be important in the pathophysiology and therapeutic alleviation of both disease A and disease B, despite showing high enrichment of disease B-associated variants.

Adrenal gland was detected as a tissue highly specific to depression by TSEA-DB. In our analysis, this tissue appeared to be nearest to the DTN of anxiety drugs that were not contraindicated in depression (Supplementary Fig. 16). This suggested that this class of anti-anxiety drugs

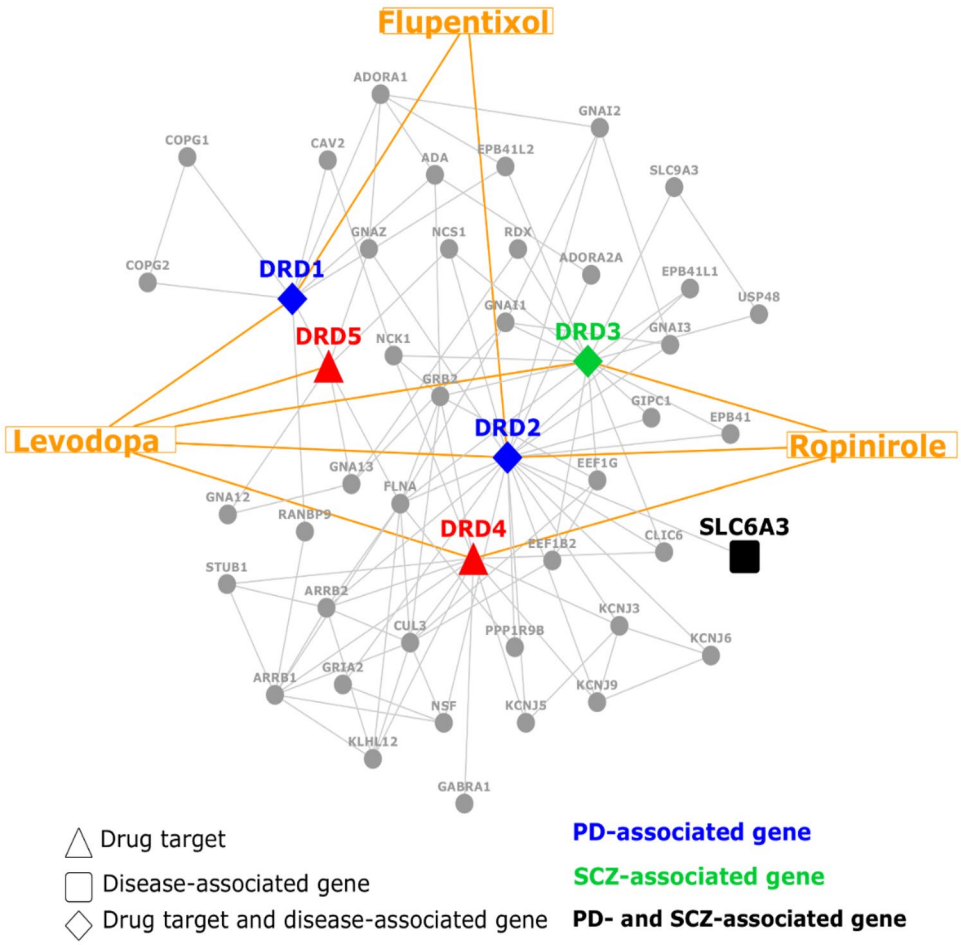

Fig. 4 Network diagram showing the relationship between the targets of levodopa and ropinirole and genes associated with Parkinson's disease and schizophrenia. The specific dopamine receptors targeted by levodopa, ropinirole and flupentixol and their interactions with the proteins encoded by Parkinson's disease and/or schizophrenia associated genes have been shown. Note that levodopa and ropinirole are used in the treatment of Parkinson's disease (disease A), but contraindicated in schizophrenia (disease B), and flupentixol is used in the treatment of schizophrenia, but contraindicated in Parkinson's disease. Note that levodopa and ropinirole target a higher number of dopamine receptors associated uniquely with Parkinson's disease, which supports our finding that disease A drugs that are contraindicated in disease B are closely associated with proteins uniquely found in the disease A network (i.e. Parkinson's disease in this specific example)

targeted the adrenal gland, perhaps because it produces cortisol, which may be regulated in a similar manner in depression and anxiety (Supplementary Note 4). Upon comparative transcriptome analysis, we found that the differential gene expression profile induced by maprotiline in PC3 cells was negatively correlated with the blood sample profiles of patients with major depressive disorder (MDD) and generalized anxiety disorder (GAD), and positively correlated with the expression profile of the adrenal cortex (Fig. 5a and Supplementary Note 5). This could indicate that maprotiline-mediated MDD/GAD alleviation may be dependent on the adrenal gland, i.e. the reversal of MDD/GAD-associated expression profile induced by maprotiline could occur in the adrenal cortex. We also found that the genes differentially expressed in the drug, disease and tissue profiles converged on protein folding and cell cycle processes (Fig. 5b, c and Supplementary Note 6). However, note that this is only anecdotal evidence of the role played by the adrenal gland in mediating stress and anxiety. A detailed discussion on the same would require strong results from multiple sources.

On the other hand, DTNs of disease A that are contraindicated for disease B were closely related to tissues preferentially affiliated with disease A. This indicated that these disease A-specific tissues mediate beneficial effects in disease A while mediating deleterious effects in disease B (e.g. spleen, see Supplementary Note 7 and Supplementary Fig. 17).

4 Discussion

Despite the increased prevalence of ADRs in comorbidities, knowledge of the mechanistic basis of drug contraindications in such conditions is limited. In our study, we attempted to characterize the biological profiles of the DTNs of drugs used in specific diseases that are either contraindicated or not contraindicated in comorbid diseases. We sought to provide an integrated interactome, pathway and tissue-level view of the DTNs.

The first key finding in our study was that the relative risk of comorbidity between diseases was proportional to their network similarity measures (Fig. 2), a trend that was seen with all the 3 negative control pairs and 3 out of the 5 comorbid pairs. The higher relative risk of rheumatoid arthritis—osteoporosis and Parkinson's disease—schizophrenia (compared with the other comorbid pairs) was not accompanied

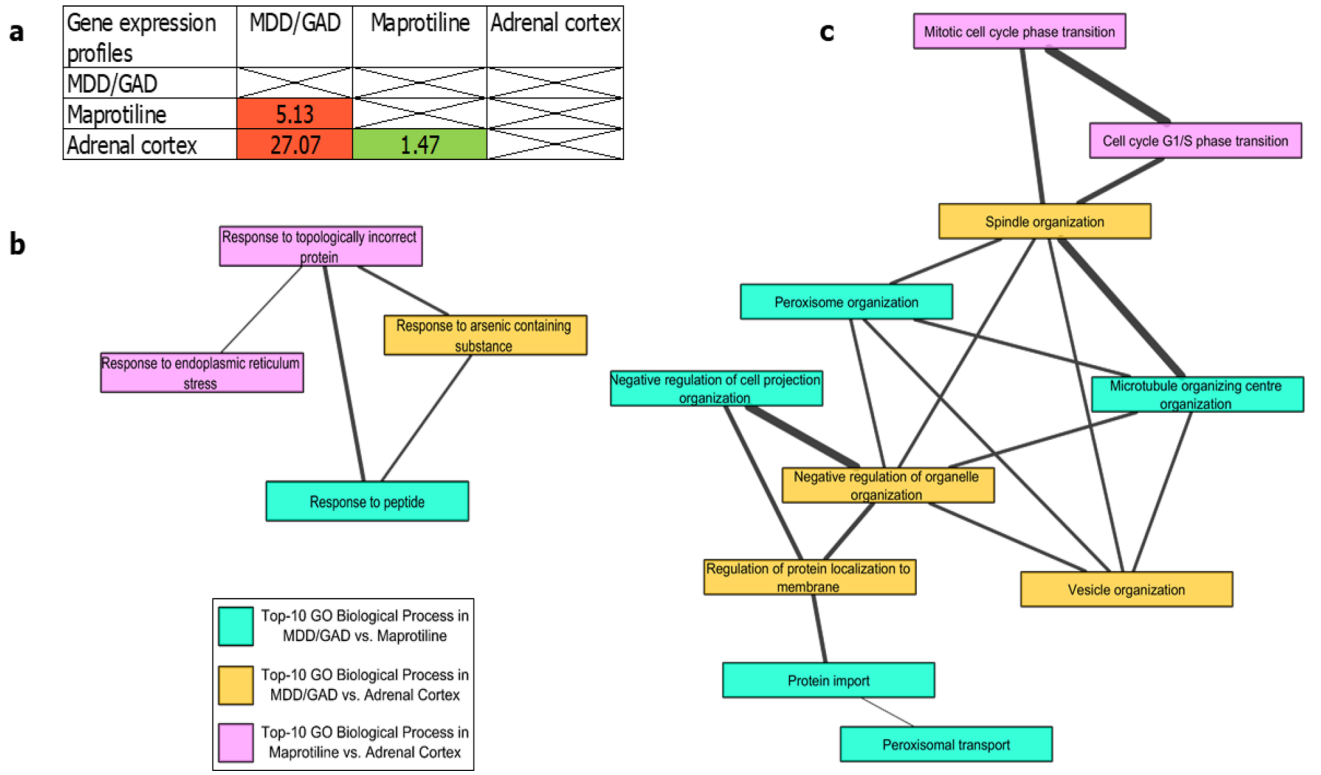

Fig. 5 Relationship between MDD/GAD, maprotiline and adrenal cortex at transcriptomic and biological process levels. **a** Correlation of differential gene expression profiles associated with a comorbid condition (major depressive disorder and generalized anxiety disorder), a drug (maprotiline) and a tissue (adrenal cortex). $-\log_{10}P$ indicating the overlap of the expression profiles have been shown; red and green colors indicate negative and positive correlations between the profiles respectively. Significant overlap was found among the genes that are upregulated in patients with both major depressive disorder (MDD) and generalized anxiety disorder (GAD) and downregulated on treating PC3 cells with maprotiline ($P=7.4E-06$), among the genes that are upregulated in MDD/GAD patients and downregulated in adrenal cortex ($P=8.4E-28$), and among the genes that are downregulated on treating PC3 cells with maprotiline and downregulated in adrenal cortex ($P=0.034$). **b**, **c** The functional networks of the Gene Ontology (GO) biological processes related to (**b**) protein folding and (**c**) cell cycle events that were enriched in the three expression profiles. The GO terms associated with each of the expression profiles have been shown using different node colors. The thickness of the edges corresponds to the Resnik semantic similarity score for GO terms (the greater the thickness of the edges, the greater is the similarity between the linked GO terms)

by a corresponding increase in the network similarity measures. Several factors may explain this anomaly. First, ~ 85% of the human interactome awaits experimental discovery (Hakes et al. 2008). Hence, network overlaps may have been underestimated due to the inherent incompleteness of these disease networks, the tendency of incomplete networks to exhibit small overlaps and sampling biases introduced as a result of selective PPI discovery (Hakes et al. 2008). Secondly, rheumatoid arthritis may progress to osteoporosis; and schizophrenia to Parkinson's disease over time due to biological and pharmacological mechanisms (Kuusimäki et al. 2021; Smeland et al. 2021). We expect to see higher concordance between relative risks and network overlaps for these disease pairs when the networks are partitioned based on developmental stages (e.g. upon integration with temporal transcriptomic data). Thirdly, it has been shown that relative risk overestimates the comorbid associations between rare diseases and underestimates the associations between highly prevalent diseases (Hidalgo et al. 2009). The number of cases in the HuDiNe database for rheumatoid arthritis—osteoporosis and Parkinson's disease—schizophrenia are 24,629 and 5439, respectively, which can be classified as rare occurrences when compared with the other comorbid pairs. Supplementary Fig. 18 shows the relationship of the relative risks of 9 disease pairs with individual and comorbid disease prevalence.

Our second key finding was that druggable proteins were highly enriched among proteins shared between the networks of two comorbid diseases (Table 2). Based on this, we speculated that drug action on shared targets may give rise to contraindications in comorbidities. This was based on the assumption that adverse events stem from drugs inducing opposing pharmacological effects in comorbid diseases, by targeting effectors shared between the diseases. However, our findings indicate that mechanisms underlying the pathology of disease A may contribute to contraindications

in the comorbid disease B. Specifically, the DTNs of disease A drugs that are contraindicated in a comorbid disease B were preferentially affiliated with disease A-associated tissues, and disease sub-networks and pathways that are either shared between the two diseases or uniquely associated with disease A (Table 3). Although further studies are required to examine the basis of this finding, it suggests that contraindications may arise when drugs used in disease A are highly specific to the said disease A, in terms of the targeted PPI network, pathway and tissue. Therefore, the causative and correlational influences of comorbid conditions (i.e. disease B) should be taken into account for rational drug development.

The DTNs of disease A drugs that are not contraindicated in a comorbid disease B were preferentially affiliated with disease B-associated tissues, and disease sub-networks and pathways that are either shared between the two diseases or uniquely associated with disease B (Table 3). This was contrary to our expectation that these DTNs would be preferentially affiliated with biological modalities pertaining to disease A. This was based on the assumption that for a drug to be specifically active against disease A without aggravating a comorbid disease B, it had to reverse the phenotypes specifically associated with disease A. In this model, phenotypes of disease B were considered as 'off-targets' in line with the principles of conventional pharmacology, in which unintended effects of the drugs were attributed to interaction with pathways inconsequential to disease A pathology (i.e. pathways relevant to disease B) (Chan and Loscalzo 2012). Our findings on the contrary indicate that the mechanisms underlying the pathology of the comorbid disease B may contribute to the therapeutic alleviation of disease A. It is possible for the emergence and development of the two diseases to be interdependent based on etiological associations. Future studies should concentrate on etiological models of comorbidity (Valderas et al. 2009). The risk factors of disease B will influence the development of disease A directly, or through correlation with the risk factors of disease A, according to the 'heterogeneity' and 'associated risk factors' models respectively. This could explain why our study connected disease B-associated PPI networks, pathways and tissues with disease A drugs not contraindicated in disease B. For example, the alterations in disease B-associated genes may lead to pathway perturbations in specific tissues, which if counteracted by disease A drugs, may lead to disease A alleviation.

Drug design is historically based on findings from studies that describe genetic and pharmacological modulation of specific targets and pathways, which elicit measurable changes in pathophenotypes (Chan and Loscalzo 2012). This framework suggests that side effects arise from unintended manipulation of 'off-targets' in other pathways. However, both beneficial and adverse outcomes of drug treatment in complex disorders (and their distinct pathophenotypes) may arise from shared effectors and pathways, albeit active in distinct combinations in specific cells and tissues (Chan and Loscalzo 2012). In line with this, we found that both categories of drugs used to treat primary conditions (whether contraindicated or not in a comorbid condition) were affiliated with proteins shared between the two diseases (Table 3) and that it may be difficult to delineate the separate mechanisms underlying the two outcomes. Future analysis should focus on biological variables that differentially affect the functions of such shared proteins, e.g. their cellular, pathway and tissue landscapes.

Our current approach has some limitations. First, our study is based on 9 disease pairs that were selected based on a literature survey. Future studies should include all the known pairs of comorbid and non-comorbid disorders. Second, our analysis did not take the overlaps among the DTNs into account. This would have allowed us to identify disease network and DTN configurations. Third, although we were able to support our findings by citing evidence based on the known clinical activity of specific drugs, further investigations with the six comorbid pairs are essential to confirm their validity. These should focus on large-scale analysis of patient treatment data collected from observational studies and functional assays in animal models of human comorbidities. Fourth, in our comparative analysis of drug-induced, disease-associated and tissue-associated transcriptomes, we utilized the differential gene expression profile induced by maprotiline in the PC3 prostate cancer cell line available

Table 3 Disease network, pathway and tissue-level characterization of drugs that are contraindicated/not contraindicated in comorbid conditions. A ✓ has been used to indicate the close affiliation of a specific category of drug-target network with specific disease protein sets, disease-associated pathways and tissues. Disease PPI protein sets, pathways and tissues that are common to disease A and disease B have been marked in columns 'a', those unique to disease A in columns 'b' and unique to disease B in columns 'c'

Drug target networks	Disease PPI protein sets			Pathways			Tissues		
	a	b	c	a	b	c	a	b	c
Disease A drugs not contraindicated in Disease B	✓		✓	✓		✓	✓		✓
Disease A drugs contraindicated in Disease B	✓	✓		✓	✓		✓	✓	

through the CMAP database. Maprotiline-induced profiles in neuronal cell lines, more aligned with the anxiety phenotype, or adrenocortical cell lines, which would have been relevant due to the comparison with adrenal cortex profiles, were unavailable in the CMAP database or in the L1000 CMAP database hosted by the NIH LINCS Consortium. Therefore, to strengthen the biological significance of our findings, it is important to confirm the observed correlations of maprotiline with disease- and tissue-associated profiles in more appropriate cell lines, when they become available. Fifth, while we considered adverse effects associated with the drugs for each pair of diseases during data collection from the TWOSIDES database, our current analysis did not offer interpretations of specific adverse effects. Future studies will focus on deriving conclusions on specific ADRs associated with comorbid conditions. Nevertheless, we offer specific examples, such as those related to PD and SCZ. Our aim was to examine whether the effects of drugs within their target networks were related to the mechanisms of the primary disease that they were designed to treat or to possibly secondary, albeit potentially significant effects on comorbid diseases. We found that contraindications stemmed from specificity to the primary condition and insufficient consideration of correlated conditions. Hence, our findings from a collective analysis of DTNs suggest that preventing contraindications necessitates a more rational drug selection process informed by etiological associations. Lastly, additional parameters including the effects of aging on PPIs and family history on comorbidities should be taken into account in future studies. Bayesian probabilistic models can be used to integrate the current set of evidence with other factors, e.g., pharmacological characteristics of the drugs, gene- and protein-level features of the drug targets, cell-type specificity of disease-associated genes and drug targets, etc.

In the current work, our aim was to provide a conceptual model for understanding drug contraindications in comorbid disease pairs. Therefore, we focused on specific pairs of comorbid diseases identified in the literature. We used relative risk ratios from the HuDiNe database to analyze their relationship with network similarity measures of comorbid disease interactomes. However, given that the likelihood of ADRs increases with disease comorbidity (Morales et al. 2017; Mittmann et al. 2012; Bassi et al. 2017), our future works will incorporate all comorbid diseases with a relative risk greater than 1 in the HuDiNe database co-occurring with a specific disorder. This approach will provide a clearer view of how disease networks, pathways, and tissues interact with multiple comorbidities and comedication.

In summary, our findings suggest that the pathway membership and the tissue-specificity of the DTNs and their overlap with disease PPI networks will influence contraindications in comorbidities. These biological modalities need to be examined for rational drug development and minimization of adverse events. The results from our study have therapeutic applications, and may directly benefit future assessments of drug contraindications in individuals with comorbidities.

5 Conclusions

We observed that disease B-associated PPI networks, pathways and tissues were affiliated with the DTNs of disease A drugs that were not contraindicated in disease B. On the other hand, disease A-associated PPI networks, pathways and tissues were affiliated with the DTNs of disease A drugs that were contraindicated in disease B. This suggested that etiological associations between the two diseases play a role in their therapeutic alleviation. In summary, our findings suggest that the enrichment patterns of DTNs in pathways, tissues and the PPI networks of comorbid diseases will help identify drugs with/without contraindications in comorbidities.

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Author contributions NBK conceived and designed the research. KBK designed and performed the analyses. NBK and MKG supervised the interactome-based analyses, and SKB and SJ provided scientific inputs on the biological aspects of the study. KBK prepared the manuscript and NBK, SKB, MKG and SJ edited the manuscript through extensive mutual consultations.

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Data availability The genes associated with the comorbid disease pairs, the condition concept names from the TWOSIDES database that were used to categorize the drugs associated with each of the comorbid disease pairs, the drug lists and the proteins targeted by the drugs, have been made available as Supplementary Data Files 1–4.

Declarations

Conflict of interest None.

References

Alam A, Voronovich Z, Carley JA (2013) A review of therapeutic uses of mirtazapine in psychiatric and medical conditions. The primary care companion for CNS disorders 15 (5)

Assenov Y, Ramírez F, Schelhorn S-E, Lengauer T, Albrecht M (2008) Computing topological parameters of biological networks. Bioinformatics 24(2):282–284

Barabási A-L, Gulbahce N, Loscalzo J (2011) Network medicine: a network-based approach to human disease. Nat Rev Genet 12(1):56–68

Barker RA, Barrett J, Mason SL, Björklund A (2013) Fetal dopaminergic transplantation trials and the future of neural grafting in Parkinson's disease. The Lancet Neurology 12(1):84–91

Bassi PU, Osakwe AI, Ogar CK, Elagbaje C, Nwankwo BB, Balogun ST, Ntadom GN, Isah AO (2017) Impact of comorbidity on adverse drug reaction profile in a cohort of patients treated with artemisinin combination therapies for uncomplicated malaria in Nigeria. Pharmacol Res Perspect 5(2):e00302

Benros ME, Pedersen MG, Rasmussen H, Eaton WW, Nordentoft M, Mortensen PB (2014) A nationwide study on the risk of autoimmune diseases in individuals with a personal or a family history of schizophrenia and related psychosis. Am J Psychiatry 171(2):218–226

Boulet L (2009) Influence of comorbid conditions on asthma. Eur Respir J 33(4):897–906

Brahmachari SK (2012) Introducing the medical bioinformatics in Journal of Translational Medicine. Springer,

Brouwers L, Iskar M, Zeller G, Van Noort V, Bork P (2011) Network neighbors of drug targets contribute to drug side-effect similarity. PLoS ONE 6(7):e22187

Brown NJ, Frazier CR, Cauthen KR, Nozick LK (2019) A Unique Graph Similarity Metric for Anomaly Detection. Sandia National Lab.(SNL-NM), Albuquerque, NM (United States),

Campillos M, Kuhn M, Gavin A-C, Jensen LJ, Bork P (2008) Drug target identification using side-effect similarity. Science 321(5886):263–266

Caughey GE, Ramsay EN, Vitry AI, Gilbert AL, Luszcz MA, Ryan P, Roughead EE (2010) Comorbid chronic diseases, discordant impact on mortality in older people: a 14-year longitudinal population study. J Epidemiol Community Health 64(12):1036–1042

Chan SY, Loscalzo J (2012) The emerging paradigm of network medicine in the study of human disease. Circ Res 111(3):359–374

Chang D, Keinan A (2014) Principal component analysis characterizes shared pathogenetics from genome-wide association studies. PLoS Comput Biol 10(9):e1003820

Cheng F, Kovács IA, Barabási A-L (2019) Network-based prediction of drug combinations. Nat Commun 10(1):1–11

Christiansen SC, Schatz M, Yang S-J, Ngor E, Chen W, Zuraw BL (2016) Hypertension and asthma: a comorbid relationship. The Journal of Allergy and Clinical Immunology: In Practice 4 (1):76–81

Consortium G (2015) The Genotype-Tissue Expression (GTEx) pilot analysis: Multitissue gene regulation in humans. Science 348(6235):648–660

Data E (2017) Orange book: Approved drug products with therapeutic equivalence evaluations.

Dogra S, Ardern CI, Baker J (2007) The relationship between age of asthma onset and cardiovascular disease in Canadians. J Asthma 44(10):849–854

Duffy Á, Verbanck M, Dobbyn A, Won H-H, Rein JL, Forrest IS, Nadkarni G, Rocheleau G, Do R (2020) Tissue-specific genetic features inform prediction of drug side effects in clinical trials. Science Advances 6 (37):eabb6242

Fliri AF, Loging WT, Thadeio PF, Volkmann RA (2005) Analysis of drug-induced effect patterns to link structure and side effects of medicines. Nat Chem Biol 1(7):389–397

Gadermann AM, Alonso J, Vilagut G, Zaslavsky AM, Kessler RC (2012) Comorbidity and disease burden in the National Comorbidity Survey Replication (NCS-R). Depress Anxiety 29(9):797–806

Giacomini KM, Krauss RM, Roden DM, Eichelbaum M, Hayden MR, Nakamura Y (2007) When good drugs go bad. Nature 446(7139):975–977

Goodwin GM (2015) The overlap between anxiety, depression, and obsessive-compulsive disorder. Dialogues Clin Neurosci 17(3):249

Griffith M, Griffith OL, Coffman AC, Weible JV, McMichael JF, Spies NC, Koval J, Das I, Callaway MB, Eldred JM (2013) DGIdb: mining the druggable genome. Nat Methods 10(12):1209–1210

Hakes L, Pinney JW, Robertson DL, Lovell SC (2008) Protein-protein interaction networks and biology—what's the connection? Nat Biotechnol 26(1):69–72

Han Y, Wang C, Klinger K, Rajpal DK, Zhu C (2021) An integrative network-based approach for drug target indication expansion. PLoS ONE 16(7):e0253614

Hase T, Tanaka H, Suzuki Y, Nakagawa S, Kitano H (2009) Structure of protein interaction networks and their implications on drug design. PLoS Comput Biol 5(10):e1000550

Hidalgo CA, Blumm N, Barabási A-L, Christakis NA (2009) A dynamic network approach for the study of human phenotypes. PLoS Comput Biol 5(4):e1000353

Hopkins AL, Groom CR (2002) The druggable genome. Nat Rev Drug Discovery 1(9):727–730

Iglay K, Hannachi H, Joseph Howie P, Xu J, Li X, Engel SS, Moore LM, Rajpathak S (2016) Prevalence and co-prevalence of comorbidities among patients with type 2 diabetes mellitus. Curr Med Res Opin 32(7):1243–1252

Iversen K, Kjaergaard J, Akkan D, Kober L, Torp-Pedersen C, Hassager C, Vestbo J, Kjoller E, Group ELFS (2008) Chronic obstructive pulmonary disease in patients admitted with heart failure. J Intern Med 264(4):361–369

Jia P, Dai Y, Hu R, Pei G, Manuel AM, Zhao Z (2020) TSEA-DB: a trait–tissue association map for human complex traits and diseases. Nucleic Acids Res 48(D1):D1022–D1030

Jin X, Simmons SK, Guo A, Shetty AS, Ko M, Nguyen L, Jokhi V, Robinson E, Oyler P, Curry N (2020) In vivo Perturb-Seq reveals neuronal and glial abnormalities associated with autism risk genes. Science 370 (6520):eaaz6063

Keshava Prasad T, Goel R, Kandasamy K, Keerthikumar S, Kumar S, Mathivanan S, Telikicherla D, Raju R, Shafreen B, Venugopal A (2008) Human protein reference database—2009 update. Nucleic acids research 37 (suppl_1):D767-D772

Kupershmidt I, Su QJ, Grewal A, Sundaresh S, Halperin I, Flynn J, Shekar M, Wang H, Park J, Cui W, Wall GD, Wisotzkey R, Alag S, Akhtari S, Ronaghi M (2010) Ontology-based meta-analysis of global collections of high-throughput public data. PloS one 5 (9). https://doi.org/10.1371/journal.pone.0013066

Kuusimäki T, Al-Abdulrasul H, Kurki S, Hietala J, Hartikainen S, Koponen M, Tolppanen AM, Kaasinen V (2021) Increased risk of Parkinson's disease in patients with schizophrenia spectrum disorders. Mov Disord 36(6):1353–1361

Kuusimäki T, Al-Abdulrasul H, Kurki S, Hietala J, Hartikainen S, Koponen M, Tolppanen AM, Kaasinen V (2020) Increased Risk of Parkinson's Disease in Patients With Schizophrenia Spectrum Disorders. Movement Disorders

Lacy C (2006) Drug information handbook: a comprehensive resource for all clinicians and healthcare professionals. Lexi-Comp Incorporated,

Lamers F, van Oppen P, Comijs HC, Smit JH, Spinhoven P, van Balkom AJ, Nolen WA, Zitman FG, Beekman AT, Penninx BW (2011) Comorbidity patterns of anxiety and depressive disorders in a large cohort study: the Netherlands Study of Depression and Anxiety (NESDA). J Clin Psychiatry 72(3):341–348

Leday GG, Vértes PE, Richardson S, Greene JR, Regan T, Khan S, Henderson R, Freeman TC, Pariante CM, Harrison NA (2018) Replicable and coupled changes in innate and adaptive immune gene expression in two case-control studies of blood microarrays in major depressive disorder. Biol Psychiat 83(1):70–80

Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B (2019b) WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. Nucleic Acids Res 47(W1):W199–W205

Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B (2019a) WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. Nucleic acids research

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 23(1–3):3–25

Llorente I, García-Castañeda N, Valero C, González-Álvaro I, Castañeda S (2020) Osteoporosis in Rheumatoid Arthritis: Dangerous Liaisons. Front Med 7:802

Loscalzo J (2023) Molecular interaction networks and drug development: Novel approach to drug target identification and drug repositioning. FASEB J 37(1):e22660

Love MI, Anders S, Kim V, Huber W (2015) RNA-Seq workflow: gene-level exploratory analysis and differential expression. F1000Research 4

Martin A, Ochagavia ME, Rabasa LC, Miranda J, Fernandez-de-Cossio J, Bringas R (2010) BisoGenet: a new tool for gene network building, visualization and analysis. BMC Bioinformatics 11(1):1–9

Maruyama Y, Nishida M, Sugimoto Y, Tanabe S, Turner JH, Kozasa T, Wada T, Nagao T, Kurose H (2002) Gα12/13 Mediates α1-Adrenergic Receptor-Induced Cardiac Hypertrophy. Circ Res 91(10):961–969

McGuirl MR, Smith SP, Sandstede B, Ramachandran S (2020) Detecting shared genetic architecture among multiple phenotypes by hierarchical clustering of gene-level association statistics. Genetics 215(2):511–529

Menche J, Sharma A, Kitsak M, Ghiassian SD, Vidal M, Loscalzo J, Barabási A-L (2015) Uncovering disease-disease relationships through the incomplete interactome. Science 347 (6224)

Metsalu T, Vilo J (2015) ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res 43(W1):W566–W570

Mittmann N, Knowles SR, Koo M, Shear NH, Rachlis A, Rourke SB (2012) Incidence of toxic epidermal necrolysis and Stevens-Johnson Syndrome in an HIV cohort. Am J Clin Dermatol 13(1):49–54

Mizutani S, Pauwels E, Stoven V, Goto S, Yamanishi Y (2012) Relating drug–protein interaction network with drug side effects. Bioinformatics 28(18):i522–i528

Morales DR, Lipworth BJ, Donnan PT, Jackson C, Guthrie B (2017) Respiratory effect of beta-blockers in people with asthma and cardiovascular disease: population-based nested case control study. BMC Med 15(1):1–9

Oken RJ, Schulzer M (1999) At issue: schizophrenia and rheumatoid arthritis: the negative association revisited. Schizophr Bull 25(4):625–638

Pachi I, Maraki MI, Giagkou N, Kosmidis MH, Yannakoulia M, Dardiotis E, Hadjigeorgiou G, Sakka P, Ntanasi E, Xiromerisiou G (2021) Late life psychotic features in prodromal Parkinson's disease. Parkinsonism Relat Disord 86:67–73

Pantalone KM, Hobbs TM, Chagin KM, Kong SX, Wells BJ, Kattan MW, Bouchard J, Sakurada B, Milinovich A, Weng W (2017) Prevalence and recognition of obesity and its associated comorbidities: cross-sectional analysis of electronic health record data from a large US integrated health system. BMJ open 7 (11)

Piñero J, Bravo À, Queralt-Rosinach N, Gutiérrez-Sacristán A, Deu-Pons J, Centeno E, García-García J, Sanz F, Furlong LI (2016) DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. Nucleic acids research:gkw943

Pöldinger W, Sieberns S (1983) Depression-inducing and antidepressive effects of neuroleptics. Neuropsychobiology 10(2–3):131–136

Rutten FH, Cramer M-JM, Grobbee DE, Sachs AP, Kirkels JH, Lammers J-WJ, Hoes AW (2005) Unrecognized heart failure in elderly patients with stable chronic obstructive pulmonary disease. Eur Heart J 26(18):1887–1894

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11):2498–2504

Smeland OB, Shadrin A, Bahrami S, Broce I, Tesli M, Frei O, Wirgenes KV, O'Connell KS, Krull F, Bettella F (2021) Genome-wide association analysis of Parkinson's disease and schizophrenia reveals shared genetic architecture and identifies novel risk loci. Biol Psychiat 89(3):227–235

Srivatsan SR, McFaline-Figueroa JL, Ramani V, Saunders L, Cao J, Packer J, Pliner HA, Jackson DL, Daza RM, Christiansen L (2020) Massively multiplex chemical transcriptomics at single-cell resolution. Science 367(6473):45–51

Stark C, Breitkreutz B-J, Reguly T, Boucher L, Breitkreutz A, Tyers M (2006) BioGRID: a general repository for interaction datasets. Nucleic acids research 34 (suppl_1):D535-D539

Stoner SC, Dahmen MM, Makos M, Lea JW, Carver LJ, Rasu RS (2009) An exploratory retrospective evaluation of ropinirole-associated psychotic symptoms in an outpatient population treated for restless legs syndrome or Parkinson's disease. Ann Pharmacother 43(9):1426–1432

Subramanian A, Narayan R, Corsello SM, Peck DD, Natoli TE, Lu X, Gould J, Davis JF, Tubelli AA, Asiedu JK (2017) A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. Cell 171 (6):1437–1452. e1417

Sun J, Zhu K, Zheng WJ, Xu H A comparative study of disease genes and drug targets in the human protein interactome. In: BMC bioinformatics, 2015. vol 5. BioMed Central, pp 1–9

Tatonetti NP, Patrick PY, Daneshjou R, Altman RB (2012) Data-driven prediction of drug effects and interactions. Science translational medicine 4 (125):125ra131–125ra131

Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A (2015) Tissue-based map of the human proteome. Science 347 (6220)

Valderas JM, Starfield B, Sibbald B, Salisbury C, Roland M (2009) Defining comorbidity: implications for understanding health and health services. The Annals of Family Medicine 7(4):357–363

Van den Akker M, Buntinx F, Metsemakers JF, Roos S, Knottnerus JA (1998) Multimorbidity in general practice: prevalence, incidence, and determinants of co-occurring chronic and recurrent diseases. J Clin Epidemiol 51(5):367–375

Vinogradov S, Gottesman II, Moises HW, Nicol S (1991) Negative association between schizophrenia and rheumatoid arthritis. Schizophr Bull 17(4):669–678

Waddington JL (2020) Psychosis in Parkinson's disease and parkinsonism in antipsychotic-naive schizophrenia spectrum psychosis: clinical, nosological and pathobiological challenges. Acta Pharmacol Sin 41(4):464–470

Wang X, Thijssen B, Yu H (2013) Target essentiality and centrality characterize drug side effects. PLoS Comput Biol 9(7):e1003119

Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, Gautam B, Hassanali M (2008) DrugBank: a knowledgebase for

drugs, drug actions and drug targets. Nucleic acids research 36 (Database issue):D901-D906. https://doi.org/10.1093/nar/gkm958
Zahodne LB, Fernandez HH (2008) Pathophysiology and treatment of psychosis in Parkinson's disease. Drugs Aging 25(8):665–682