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Identifying patterns to uncover the importance of biological pathways on known drug repurposing scenarios

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Abstract

Background Drug repurposing plays a significant role in providing effective treatments for certain diseases faster and more cost-effectively. Successful repurposing cases are mostly supported by a classical paradigm that stems from de novo drug development. This paradigm is based on the “one-drug-one-target-one-disease” idea. It consists of designing drugs specifically for a single disease and its drug’s gene target. In this article, we investigated the use of biological pathways as potential elements to achieve effective drug repurposing.

Methods Considering a total of 4214 successful cases of drug repurposing, we identified cases in which biological pathways serve as the underlying basis for successful repurposing, referred to as DREBIOP. Once the repurposing cases based on pathways were identified, we studied their inherent patterns by considering the different biological elements associated with this dataset, as well as the pathways involved in these cases. Furthermore, we obtained gene-disease association values to demonstrate the diminished significance of the drug’s gene target in these repurposing cases. To achieve this, we compared the values obtained for the DREBIOP set with the overall association values found in DISNET, as well as with the drug’s target gene (DREG) based repurposing cases using the Mann-Whitney U Test.

Results A collection of drug repurposing cases, known as DREBIOP, was identified as a result. DREBIOP cases exhibit distinct characteristics compared with DREG cases. Notably, DREBIOP cases are associated with a higher number of biological pathways, with Vitamin D Metabolism and ACE inhibitors being the most prominent pathways. Additionally, it was observed that the association values of GDAs in DREBIOP cases were significantly lower than those in DREG cases (p -value < 0.05).

Conclusions Biological pathways assume a pivotal role in drug repurposing cases. This investigation successfully revealed patterns that distinguish drug repurposing instances associated with biological pathways. These identified patterns can be applied to any known repurposing case, enabling the detection of pathway-based repurposing scenarios or the classical paradigm.

Keywords Drug repurposing, Pathway-based, DISNET knowledge, Computational biology, Data-driven methodology

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Background

Biological pathways are described as a sequence of interactions between different biological entities [1]. These actions lead to a certain product or a change in the cell itself. They can trigger the assembly of a new molecule, stimulate cell movement, or even activate or deactivate



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gene expression [2]. Cells constantly receive chemical signals to react, and biological pathways are used to send and receive these signals [3]. These biological pathways are involved in all the actions that occur in the human body, giving rise to mechanisms and chains that perform important roles in the functioning of the whole organism [4]. For example, some pathways are involved in how the human body reacts to drugs. However, biological pathways do not always perform perfectly; when a pathway fails, it can trigger a certain problem in the organism, even a specific disease such as cancer [5] or Alzheimer's [6, 7]. Within biological pathways, we can highlight those involved in metabolic pathways, as well as in the regulation of gene expression [8].

Systems biology approaches, such as the analysis of drug actions at the pathway level, may reveal information about the underlying drug-induced mechanisms [9]. Therefore, deepening the knowledge of biological pathways may help to understand how diseases arise, which would accelerate the discovery of drugs to treat them [2]. Historically, drug development was based on designing a selective drug for a specific target [10]. However, this approach does not consider the physiological and cellular contexts involved in the mechanism of drug action [11]. The use of systems biology approaches, which move away from the one-drug-one-target concept, has consistently increased in recent years [12].

Several studies have shown that drugs can perform their functions because they act on the pathway itself rather than targeting a single target [13]. This, coupled with the fact that most complex diseases (such as neurological disorders or heart disease) are caused by the triggering of a set of dysfunctions in multiple biological pathways as opposed to a limited number of individual genes [14], opens the door to a new strategy for drug discovery. This innovative approach involves discovering new drugs based on biological pathways rather than targets [15].

However, the research and development of new drugs using experimental methods requires high economic cost and is time consuming [13]. For this reason, the purpose of this work was to investigate the key role of biological pathways in drug repurposing. The aim of this study was to identify Drug REpurposing based on BIOlogical Pathways cases (DREBIOP) and to discover the distinctive patterns of these drug repurposing cases.

Pathway-based drug repurposing approach is quite novel and has been developed in recent years. An important study developed a new computational method for predicting possible drug-pathway relationships through network-based approaches, where seven potential models were obtained to identify such associations in cancer [16]. Another pathway-based study, also focused

on cancer, performed an exhaustive review of the importance of pathways in drug repurposing and concluded that it is a field yet to be discovered for which a great development of new mechanisms that give rise to novel cases of pathway-based drug repurposing is forecasted [17]. Moreover, a further specific study based on breast cancer achieved a more personalized medicine for patients with this disease using pathway-based drug repurposing. The results of that study showed that different molecular subtypes of breast cancer were associated with novel molecular pathways and potential therapeutic targets [18]. Another study was based on exploring the pharmacological mechanisms of Enzastaurin to inhibit the progression of colorectal cancer as a new therapeutic approach based on biological pathways of colorectal cancer [19]. Also, a study has been conducted that created a metabolic pathway-targeted screening system that is able to identify compounds that control glyoxalase metabolism in living cells. This led to the discovery of compounds that can directly or indirectly inhibit the activities of this metabolic pathway in small cell lung carcinoma cells [20]. One study proposed *Gene2drug* as a computational tool for pathway-based drug repurposing. This approach combines transcriptomic data with previous understanding in the form of pathway databases and provides an efficient alternative to methods based on protein interaction networks [21]. A further important study performed modelling to identify systemic gene networks in specific drug pathways. The results showed five different pathways for five different drugs, suggesting that network-based detection of drug efficacy through a specific pathway may offer an opportunity to use a previous drug for a new disease with the same pattern in the selected pathway [22]. Following the network methodology, one study performed a multi-layer network representation to model the interaction between genes, drugs, diseases, and their associations. Through it, they visualized that the genes that form the communities extracted from the multilayer network regulate the activity of the protein metabolism pathway related to adverse or normal drug responses [23]. These studies open the door to focus our research on pathways since they indicate that choosing pathways as a key factor can lead us to discover additional cases of drug repurposing.

The current study is focused on the data contained within the DISNET¹ platform [24], which aims to provide a better understanding of diseases and to repurpose drugs by integrating biomedical data on a large scale [25–27]. Through this work, we provide several analytical approaches to demonstrate the importance of biological

¹ <https://disnet.ctb.upm.es/>

pathways in the generation of drug repurposing hypotheses. Our goal is to uncover an alternative paradigm to the original one based on target genes and focus on biological pathways instead, deciphering shared structures between known pathway-based repurposing scenarios. Relevant at this point is the definition of the concepts DREGE and DREBIOP, which refer to these two types of repurposing discussed above. DREGE (Drug REpurposing based on GENes) represents the original paradigm where drug repurposing is performed throughout the drug's target gene. DREBIOP (Drug REpurposing based on BIOlogical Pathways) represents the alternative proposed in this research, where drug repurposing is performed through the drug's biological pathway. For this purpose, several patterns will be provided throughout this study that will allow us to identify DREBIOP cases within any set of repurposing cases, as well as optimize the prioritization of potential new cases of pathway-based repurposing.

Materials

DISNET knowledge

DISNET is a biomedical knowledge platform that integrates data extracted from heterogeneous public sources. The data collected are related to diseases including characteristics such as genes, symptoms, or related drugs. This heterogeneous information is organized in three layers: (i) the phenotypical layer which mainly contains disease-symptoms associations; (ii) the biological layer which contains the associations of diseases with genes, proteins, and pathways among others; and (iii) the drugs layer which stores drug-related data, including their associations to diseases and the drugs targets.

The extraction and integration of the data that make up this platform were carried out through the web services and APIS of the different data sources consulted. In the biological layer, as discussed in Table 1, information on diseases and genes, as well as their relationship, was obtained from DisGeNET.² Information on biological pathways was obtained from WikiPathways.³ Also included are the proteins that encode the genes whose information is extracted from UniProt.⁴ For their extraction, SPARQL queries were used using Python through its SPARQL wrapper module. In the phenotypic layer, symptom data and their associations to diseases were obtained by combining text mining and natural language processing techniques applied to Wikipedia and PubMed. This process is developed in the paper by Lagunes-García et al. [24] in more depth. In the pharmacological

layer, DrugBank⁵ is used as a data source where the relevant XML files are mined for integration.

In addition, the data processing consisted of unifying the data through common identifiers that allowed us to relate all the information extracted from different data sources. Table 1 details each of these identifiers and their source.

DISNET was a crucial framework for conducting our research, as it provided us with all the biological insights needed to identify possible characteristic patterns of pathway-based repurposing cases. Since this platform provides all the diseases and drugs associated with a specific ID, it is particularly easy to relate different biological elements to each other and to obtain the whole network of information about a biological entity such as a disease. This represents highly valuable information for studies related to the repurposing of drugs based on biological similarities or, as in the present study, to be able to establish characteristic patterns of a particular dataset.

The data extracted from the DISNET platform date back to September 2022. Table 1 shows the data typology and the relationships between the different biological elements, and the information available on the platform at that date. The entire code developed during this research is available online at: https://medal.ctb.upm.es/internal/gitlab/b.otero/drebiop_dr_pathways-based.

Drug repurposing cases: RepoDB and scientific literature

The baseline data for this study are the successful cases of drug repurposing gathered from two data sources: RepoDB and the scientific literature. RepoDB [28] is a web-based platform that offers a dataset comprising both successful and unsuccessful drug repurposing cases. This resource can be leveraged to compare and evaluate computational drug repurposing methods. RepoDB data were extracted from DrugCentral⁶ and ClinicalTrials.gov.⁷ RepoDB enables users to search for drug repurposing cases on the bases of their specific interests, whether it is the drug or disease in question. Additionally, the data contained within the platform are readily available for download, providing convenient access to the dataset for further analysis and research. In this study, only approved drug repurposing cases were considered, with a total of 3757 drug-disease connections retrieved from this data source. When scientific literature is mentioned, references to successful cases of drug repurposing are collected in a set of papers. The selection and filtering of these papers were carried out in a previous study [27].

² <https://www.disgenet.org/home/>

³ <https://www.wikipathways.org/index.php/WikiPathways>

⁴ <https://www.uniprot.org/>

⁵ <https://go.drugbank.com/>

⁶ <https://drugcentral.org/>

⁷ <https://clinicaltrials.gov/>

Table 1 A description of the available data in the DISNET platform used in this study

		Description	DISNET layer	Count	Identifiers	Sources	Access date
Entities	Diseases	Data representing diseases	Phenotypical	15,415	DISNET own identifiers	Wikipedia Mayo Clinic PubMed	February 2018 – September 2022 (twice a month)
			Biological	24,314	UMLS CUIs	DisGeNET	May 2020
			Drugs	8366	UMLS CUIs	CTD	May 2020
	Symptoms	Data representing symptoms and phenotypical effects	Phenotypical	26,257	UMLS CUIs	Wikipedia Mayo Clinic PubMed	February 2018 – September 2022 (twice a month)
			Drugs	3944	UMLS CUIs	ChEMBL	May 2020
	Genes	Data representing genes	Biological	20,610	NCBI identifiers	DisGeNET	May 2020
	Pathways	Data representing pathways	Biological	1105	WikiPathways	WikiPathways	May 2022
	Proteins and Targets	Data representing proteins and drug targets	Biological	18,521	UniProt Accession Numbers	UniProt	May 2020
			Drugs	13,382	ChEMBL identifiers	ChEMBL	May 2020
	Drugs	Data representing drugs of different molecular types	Drugs	3944	ChEMBL identifiers	ChEMBL	May 2020
				2540	DrugBank identifiers	DrugBank	May 2020
Relationships	Disease - Gene	Associations between diseases and their related genes	Biological	358,209	–	DisGeNET	May 2020
	Disease - Symptom	Associations between diseases and their related symptoms	Phenotypical	201,541	–	Wikipedia Mayo Clinic PubMed	February 2018 – September 2022 (twice a month)
	Disease - Pathway	Associations between diseases and their related pathways	Biological	664	–	WikiPathways	Jun 2022
	Pathway - gene	Associations between pathways and their related genes	Biological	11,732	–	WikiPathways	May 2022
	Gene - Protein	Associations between genes and the proteins they encode	Biological	15,770	–	DisGeNET	May 2020
	Drug - Disease	Associations between diseases that are indications for drugs and drugs	Drugs	1,218,607	–	CTD	May 2020
	Drug - Target	Associations between targets to which drugs are directed and drugs	Drugs	7902	–	ChEMBL	May 2020
						DrugBank	May 2020

The data from each of the entities under consideration are described in detail, as well as the data on the relationships that exist between them.

A total of 457 successful drug repurposing cases were selected from Xue et al. [29], Jarada et al. [30], and Li et al. [31].

Being able to obtain successful cases of drug repurposing from these two data sources represents an important insight for this study. It was necessary to have this information to initialize the research and use these data as a

starting point. Through these repurposing cases, in combination with all the biological information available in DISNET, it has been possible to search for characteristic patterns of repurposing cases by pathways.

Before starting with the drug repurposing analysis, it is relevant to introduce the concepts of Drug REpurposing based on GENes (DREGE), and Drug REpurposing

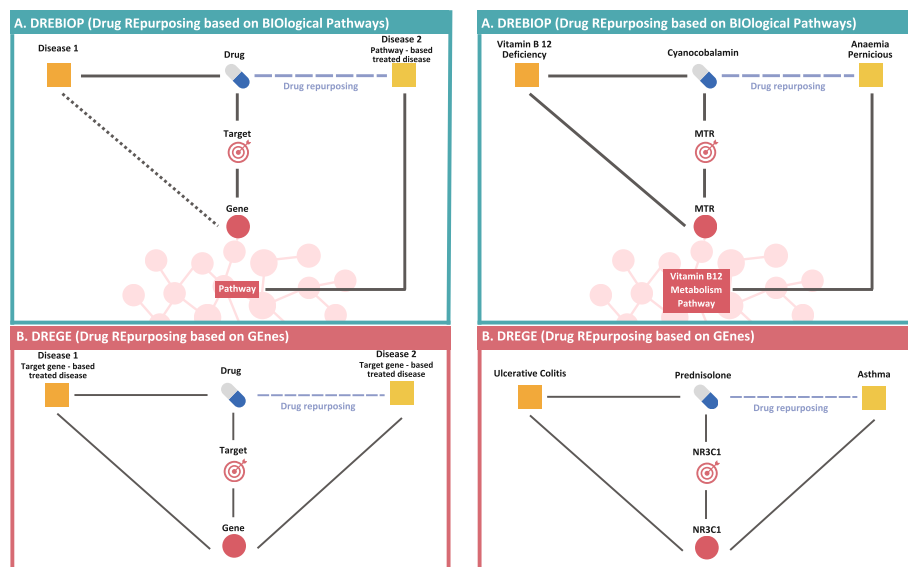


Fig. 1 Description of the two types of triples and a real case for each of them. In the left part, we can observe the description of the components that constitute the two different types of drug repurposing triples **A)** DREBIOP **B)** DREGE. On the right part, we can observe a real case of repositioning found in our results for both **A)** DREBIOP and **B)** DREGE

based on BIOlogical Pathways (DREBIOP). DREGE is the classical repurposing method, it focuses to use an existing drug for another disease through its drug's target gene. When a drug is designed to act on a certain gene product (for instance, a protein), we say that this gene is the drug's target gene. In other words, when we know the drug's target gene for a specific disease, we can explore other diseases where this gene plays a crucial role in their development. Consequently, the drug could be utilized to treat those diseases too. DREBIOP focuses on the biological pathway. In this method, the goal is to find an association with the pathway and both: 1) the drug's target gene and 2) the new disease to repurpose the drug for.

Upon understanding the two different methods of drug repositioning, DREGE, and DREBIOP, we will now elaborate on the procedure involved in creating both datasets.

DREGE cases are composed of "Disease 1 - Drug - Disease 2" triples (Fig. 1). In both the "Disease 1 - Drug" and "Drug - Disease 2" relationships, the diseases must contain the drug's target gene associated with them. This type of relationship between the disease and the drug will be referred to throughout the study as "Target gene-based treated disease". Triples registered in RepoDB and in the scientific literature that satisfy this criterion will be selected as part of the DREGE (970 triples) dataset.

DREBIOP cases also consist of "Disease 1 - Drug - Disease 2" triples (Fig. 1). In this case, it is of utmost importance and mandatory that Disease 2 satisfies the following requirements:

- 1) Disease 2 is directly associated with at least one pathway. The direct pathway-disease association was obtained from Wikipathway.
- 2) Disease 2 must not contain the drug's target gene. In this point, Disease 2 is not considered to contain the drug's target gene when the association of this disease with the gene has a GDA score of less than 0.9 according to DisGeNET.
- 3) The pathway associated with Disease 2 must have the drug's target gene.

It is necessary to obey these 3 conditions because the goal is to find pathway-based repurposing cases where the drug's target gene does not intervene in the repurposing process. All those cases collected in the two previously mentioned data sources that meet these criteria will be selected for the DREBIOP dataset (46 triples). We will refer to this new relationship between disease 2 and drug as a "Pathway-based treated disease". No conditions are imposed on the "Disease 1 - Drug" relationship.

Figure 1 shows a summary of the two different types of drugs repurposing we will consider in this research on the left side and a real case for each type of repurposing (DREBIOP and DREGE) on the right side. The DREGE cases are configured as "Disease 1 - Drug - Disease 2" triples. The triples are composed of two parts: in the left part, referred to as "Disease 1", we have the disease that presents a connection with the drug through the target gene (Target gene-based treated disease), and in the right part, "Disease 2", we find the same type of connection

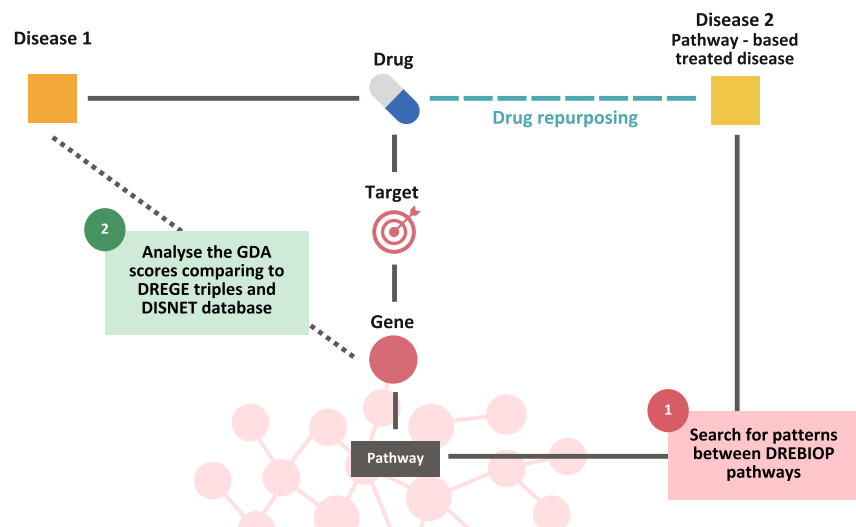


Fig. 2 Summary of the different analyses used in this research to determine the importance of biological pathways in drug repurposing

(Target gene-based treated disease). The DREBIOP cases are also formed by triplets containing two parts, but the connections with the diseases are different. On the left side of the triplet, we have “Disease 1” with no established connection to the drug, while on the right side, we have “Disease 2,” which is connected to the drug by pathway (Pathway-based treated disease). As for the right side of the figure, in the case of DREBIOP, we can see the triple “Vitamin B 12 Deficiency” - “Cyanocobalamin” - “Anaemia Pernicious”. The relationship between “Vitamin B 12 Deficiency” - “Cyanocobalamin” is established through the drug’s target gene “MTR”, while the relationship between “Cyanocobalamin” - “Anaemia Pernicious” is obtained through the “Vitamin B12 Metabolism pathway”. In this selected case, there is a relationship with the drug’s target gene although it is not required to appear. This example clearly represents what was sought to obtain in this paper. A successful case of repurposing through pathways where the drug’s target gene is not intervening in the relationship between “Cyanocobalamin” - “Anaemia Pernicious” and yet there is a successful repurposing through a pathway. In the case of DREGE, the triple “Ulcerative colitis” - “Prednisolone” - “Asthma” is observed. The relationship between both diseases and the drug is through the drug’s target gene “NR3C1”, present in all three entities.

Methods

To determine the role of pathways in drug repurposing, two distinct data analyses were conducted. The analyses are based on various biological characteristics or associations of the diseases and drugs under study. Figure 2 illustrates these approaches and the elements of the triples

that participate in each of them. The triples are composed of two distinct parts: on the left side is the “Disease 1” part and on the right side is the “Disease 2” (Pathway-based treated disease) part. In the following subsections, each of the procedures will be detailed.

Pattern search based on DREBIOP triples’ configuration

DREBIOP aims to provide higher consideration to pathways in drug repurposing processes. Focusing on this idea, we are looking for patterns that could contribute to the identification of cases of pathway-based drug repurposing.

The first step was to observe the biological elements that composed the triples based on pathway repurposing. Biological data related to each repurposing case were obtained through the DISNET platform. We took these triples and compared the DREBIOP left part, “Disease 1” with the DREBIOP right part, “Disease 2” (Pathway-based treated disease) as a function of:

- Number of genes: we counted the number of genes related to the diseases found on both parts of the triple.
- Number of gene-related pathways: we observed the number of pathways associated with each part of the triple through the genes.
- Number of disease-related pathways: in this case, only the pathways that had a direct association with the diseases in both parts were selected.
- Number of drugs: it was tested whether diseases considered as “Disease 1” had a higher number of drugs associated with them than “Disease 2” (Pathway-based treated disease).

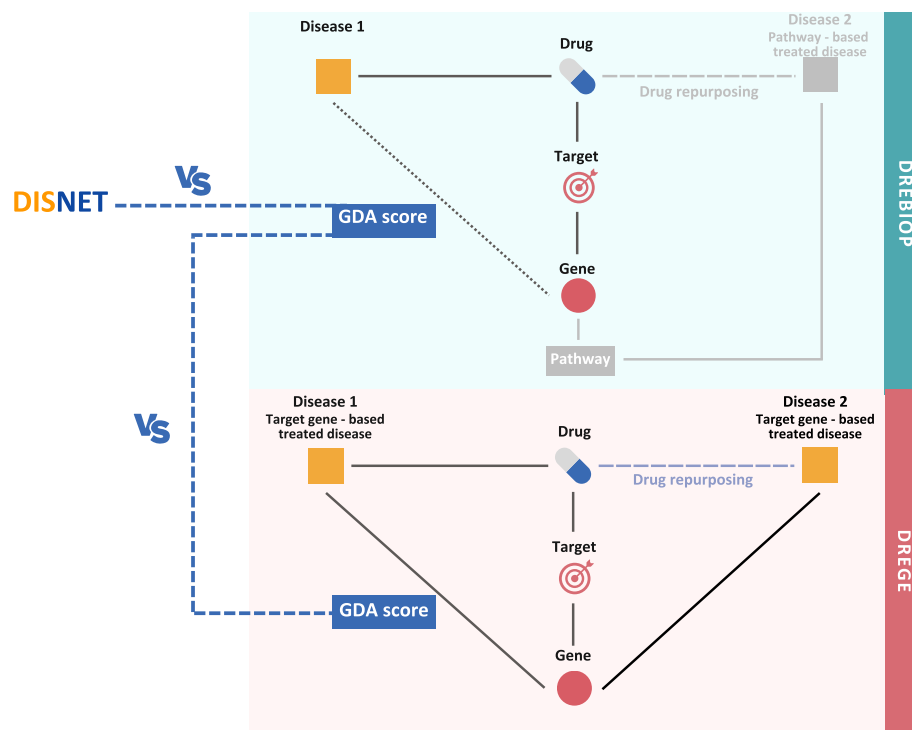


Fig. 3 Methodology applied to obtain patterns based on the GDAs values. In the first step, the GDA value of DREBIOP, DREGE and DISNET is calculated. Then, a statistical comparison is carried out by means of a Mann-Whitney U Test between the DREBIOP repositioning cases and the DREGE cases, and between DREBIOP and DISNET

- Number of symptoms: we quantify the number of symptoms assigned to the diseases found on both parts of the triplet.

In addition, we compared both sides of the triplet to determine whether there were statistically significant differences between “Disease 1” and “Disease 2” (Pathway-based treated disease). For this purpose, the Mann-Whitney U test was used since the data compared did not follow a normal distribution. Normality was tested by the Lilliefors test.

Furthermore, the different pathways of the DREBIOP triplets were classified according to their type. The objective of this study was to determine the type of biological pathways that were most abundant in DREBIOP cases and to establish a characteristic pattern. For this purpose, we first obtained the direct relationship between diseases and pathways from the WikiPathways [32] website. Once this relationship was acquired, we searched for the classification of the pathways in Pathway Ontology [33] since WikiPathways did not contain this information. This ontology collects information about the different types of biological pathways by capturing the relationships between them within the hierarchical structure of a controlled vocabulary.

Pattern search based on gene-disease associations (GDAs)

The measurement of gene-disease associations (GDAs) is derived from the scores present in DisGeNET scores [26]. The score indicates how well-established the association between the disease and the gene is. GDAs values range from 0 to 1, where higher values correspond to those associations supported by multiple databases, expert-curated resources, and scientific publications.

Through GDAs metrics, we have analyzed whether the association between disease and drug’s target gene is more robust in classical drug repurposing (DREGE) or in cases where repurposing has been performed via biological pathways (DREBIOP). In addition, this measure has been compared with the mean association between all gene-disease associations currently catalogued in the DISNET database. Figure 3 represents the methodology implemented and the part of the triplets (“Disease 1”) considered for the study. In the case of DREBIOP, all “Disease 1 - Drug” associations are considered even though they are not related to the drug’s target gene. This means that as in these types of repurposing no condition was imposed on “Disease 1”, and there will be cases where there is no GDA value for the drug’s “Disease 1 - Target Gene” relationship, and, in these cases, a value of 0 will be assigned. These cases are also considered because it is

relevant for the study to include those triplicates in which the repositioning occurs for another reason. DREGE is different, in these cases there must be a relationship between “Disease 1” and the drug’s target gene, so for all records, there is an associated GDA value. Furthermore, as this condition is also mandatory in the relationship with “Disease 2”, it was of great value to consider these associations as well. Thus, to calculate this section of the methodology, the DREGE triplets were considered in both directions. In addition, GDA values were extracted for the associations in DISNET.

After gathering the GDAs values, we checked if the differences between the GDA value in classical repurposing cases (DREGE), and the one in our pathway-based cases (DREBIOP) were significantly different. In essence, we examined if a stronger relationship existed between the genes and the diseases when the repurposing was based on the gene, as opposed to the pathway (DREGE Vs DREBIOP). For this purpose, the Mann–Whitney U Test [34] was used since the data examined did not conform to a Gaussian distribution and the number of samples within each dataset was noticeably dispersed. As previously noted, it was also of interest to further verify if this association value differs from the overall mean present in the DISNET database. Hence, the procedure mentioned in the previous paragraph was also applied here, but for comparing DREBIOP cases with the data in DISNET. Given the non-normal distribution of the data and the variability in the number of samples, the identical statistical test was used for this analysis.

Results and discussion

Biological pathways constitute a collection of processes that are essential for the proper functioning of the organism. Each of these pathways is involved in different key scenarios such as amino acid generation or gene transcription. The failure of these biological pathways triggers a series of events that may lead to a specific disease.

Bearing in mind the crucial role played by pathways in the development of diseases, this work has highlighted that biological pathways can also be employed to find potential treatments for diseases through drug repurposing. Drug repurposing allows us to use existing drugs to treat a new disease, thereby reducing the time and cost involved in developing drugs from scratch. On this basis, we selected Drug REpurposing based on BIOlogical Pathways (DREBIOP) cases to compare them with classic paradigm cases based on target genes (DREGE) to uncover patterns that prove the importance of pathways. Based on these premises, in the following subsection, we discuss the findings of our research and the implications for drug repurposing strategies based on biological pathways.

Table 2 Statistical and descriptive analysis of the biological components building both parts of the DREBIOP triples

	Disease 1	Disease 2 Pathway-based treated disease	P-value
Number of genes	105.13	423.66	< 0.05 *
Number of gene-related pathways	391.04	1568.00	< 0.05 *
Number of disease-related pathways	0.00	25.83	< 0.05 ***
Number of drugs	384.6	657.33	< 0.05 *
Number of symptoms	68.60	106.66	< 0.05 *

Data reported in the table correspond to the means obtained for each biological component and part of the triples. The data highlighted in bold refer to the most relevant data in this section since they are the differences in the number of mean pathways between the two sides of the triplet. P-value annotation legend: *: $1.00 \times 10^{-2} < p \leq 5.00 \times 10^{-2}$, ***: $1.00 \times 10^{-4} < p \leq 1.00 \times 10^{-3}$

Table 3 Classification and quantification of the different pathways involved in DREBIOP cases

Pathway_id	Pathway_name	Pathway_type	Count
WP1531	Vitamin D Metabolism	Classic metabolic pathway	18
WP554	ACE Inhibitor Pathway	Drug pathway	18
WP4756	Renin Angiotensin Aldosterone System (RAAS)	Classic metabolic pathway	9
WP2371	Parkinsons Disease Pathway	Disease pathway	6
WP3303	RAC1/PAK1/p38/MMP2	Signaling pathway	6
WP1533	Vitamin B12 Metabolism	Classic metabolic pathway	3

Pattern search based on DREBIOP triples’ configuration

DREBIOP cases have several patterns. Table 2 shows a comparative analysis of the target-based and pathway-based parts of these repurposing cases. The data in this table shows the mean of each of these biological elements calculated from the selected dataset for DREBIOP. In these results, we can observe how the number of associated pathways is higher in the pathway-based part of the triple, both for the associations through the genes and the direct disease-pathway associations (we highlight the results in bold in the table). It was also observed that the number of genes involved in the DREBIOP right part “Disease 2” (pathway-based) was substantially higher than in the DREBIOP left part “Disease 1”. This is an important revelation that could enhance the identification of future potential cases to be repurposed by pathways. Furthermore, we checked whether these differences, which can be seen visually, are also statistically significant. For this purpose, in the

“*P*-value” column of Table 2 shows the results obtained by applying the Mann-Whitney U test.

In addition, we identified the most characteristic pathway categories among the considered DREBIOP cases. Table 3 shows the names of the pathways involved, the type of pathway to which they belong, and the number of times they were present in these instances. It is noteworthy that the most abundant pathways, in number and type, belong to pathways related to metabolism. Moreover, despite the occurrence of more than 60 different cases, only 6 different pathways were identified in DREBIOP cases. The different pathways found in these cases were classified into the following four groups: metabolic, drug-related, signaling, and disease-related pathways. Some biological pathways play crucial roles that suggest that they are implicated in several diseases. A clear example of this can be the ACE Inhibitor drug pathway which is found in about 30% of repurposing cases. ACE inhibitors prevent an enzyme in the body from producing angiotensin II, a substance that narrows blood vessels and can cause hypertension. There is a multitude of drugs based on these ACE inhibitors that are used to treat or palliate symptoms of various diseases such as scleroderma, heart failure, or diabetes [35]. In addition, new studies are indicating that these inhibitors also play an important role in certain autoimmune diseases [36].

To conclude the discussion of this work, we wanted to prove through the scientific literature the biological relationship between the diseases and pathways involved in DREBIOP cases. For this purpose, 6 different cases were selected based on each of the pathways that have been implicated in the DREBIOP cases studied. These cases are shown below, divided by the type of pathway involved (classical metabolic pathway, drug pathway, disease pathway, signaling pathway).

Within the classic metabolic pathways, the selected triple is the one formed by the disease’s anaemia pernicious and vitamin b12 deficiency, in which anaemia pernicious is related to the drug Cyanocobalamin through the pathway-based part of this triple. The pathway involved in this case is vitamin b12 metabolism. Several studies have found that anaemia pernicious can be caused by vitamin b12 deficiency due to a deficient nutritional diet [37, 38] or inadequate enteric vitamin B12 absorption [39]. Therefore, the metabolic pathway of vitamin b12 development is strongly linked to the possible development of anaemia pernicious. Another selected example related to metabolic pathways is the relationship between rickets and hypoparathyroidism through the drug Ergocalciferol. In this case, the vitamin D metabolism pathway plays an important role. Rickets is the disease established in the pathway-based part of the triplet. Rickets is usually caused by a prolonged and severe vitamin D deficiency

because vitamin D aids the absorption of calcium and phosphorus by the bones [40]. Thus, multiple scientific articles have linked rickets and vitamin D deficiency through this pathway [41, 42]. This example is also representative of the type of metabolic drug used. Finally, to illustrate the Renin Angiotensin Aldosterone System (RAAS) pathway, the triplet linking hypertension and diabetic nephropathy disease through the drug Irbesartan was selected. Hypertension is on the pathway-based part of the triplet. The relationship of this disease with the pathway is powerful since blockers of RAAS are a cornerstone for treating hypertension [43].

In the case of drug pathways, a triple of Hypertensive disease and Congestive heart failure related through the drug Enalapril was selected. Enalapril is a cardiovascular agent and both diseases are related through the ACE Inhibitor Pathway. This example is also valid to explain the case of the relationship between circulatory category diseases. Hypertensive disease is part of the pathway-based process. The relationship between hypertension with ACE inhibitors is widely known and there are several articles link the two concepts. ACE inhibitors are a class of drugs used to control hypertension, which is a major risk factor for stroke and heart failure [44, 45].

For the disease pathway, the Parkinson’s disease pathway itself has been considered. The triple is formed by the relationship between Parkinson’s and Parkinsonism through the drug Carbidopa. In the treatment of Parkinson’s disease, Carbidopa is used to block the conversion of levodopa to dopamine in peripheral tissues outside of the central nervous system. This helps to prevent unwanted side effects of levodopa on organs located outside of this system. Carbidopa’s mechanism of action is particularly important in the management of Parkinson’s disease, as it allows for a more targeted and effective approach to treating the disease [46].

Among the signaling pathway, the pathway RAC1/PAK1/p38 MAPK/ MMP2 is related to ovarian neoplasm and Acne vulgaris through the drug Minocycline. The ovarian neoplasm is the disease of the part pathway-based in the triple. A study suggests that the RAC1/PAK1/p38 MAPK/ MMP2 axis that conforms to this pathway is involved in angiogenesis, tumor growth, and cell proliferation in ovarian cancer. When RAC1 is inactivated, these activities are reduced in this tumor [47, 48].

Pattern search based on GDAs

The GDA was obtained for the two types of repurposing (DREGE and DREBIOP), as well as for the DISNET database. This association was obtained on the left part of the triple corresponding to “Disease 1” in both repurposing cases. In DISNET, the data obtained corresponded to all the associations reported in the platform that relate

Table 4 Descriptive analysis of the several data sets considered for the study of GDAs

Metrics	DREBIOP	DREGE	DISNET
Count	53	1369	353,628
Mean	0.1816	0.2445	0.1374
Standard Deviation	0.1998	0.1951	0.1295
Minimum	0.0000	0.0100	0.0100
Maximum	0.7000	1.0000	1.0000

and the disease for which we are repurposing the drug is of great importance. Therefore, the GDA value may be a metric to consider in determining the success of a repurposing case. However, in DREBIOP cases, GDA scores are lower. This fact leads us to think that they do not have the same relevance. Instead, the pathways would seem to influence more in determining the success or non-success of the repurposing.

Finally, through Fig. 2 of [Methods](#) section. Methods, we would like to provide a brief illustrative summary (Fig. 5) of the results obtained for a real DREBIOP case

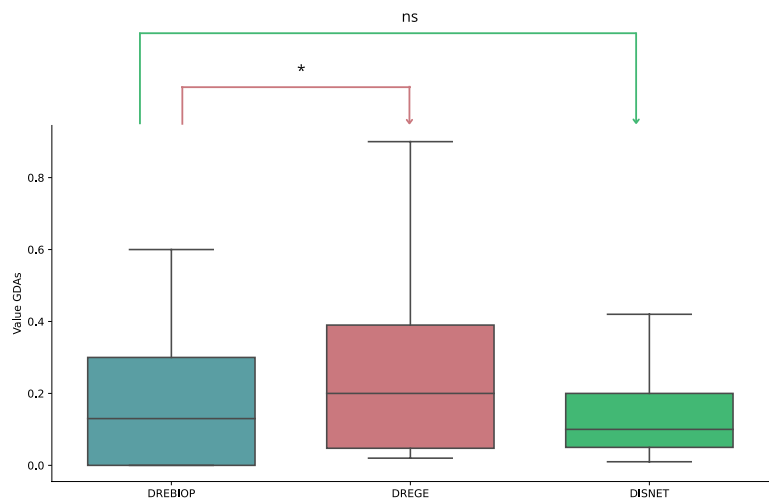


Fig. 4 Summary of DREBIOP, DREGE and DISNET datasets GDA score distributions. GDA score distributions in the DREBIOP, DREGE and DISNET datasets as well as the statistical results obtained by performing the Mann-Whitney U-test to compare the GDA values between the cases represented. P-value annotation legend: ns: $5.00 \times 10^{-2} < p \leq 1$, *: $1.00 \times 10^{-2} < p \leq 5.00 \times 10^{-2}$

genes with diseases, so we could have a reference of the overall mean of this association.

Table 4 shows the descriptive analysis performed on each dataset. In this table, the count of the total number of cases considered for each dataset (DEBRIOP, DREGE, and DISNET) is shown. In addition, the mean of the GDAs obtained for each dataset was calculated, as this metric is used to determine if there were statistically significant differences between the data studied. Related to these mean values, a related standard deviation was obtained. Finally, the maximum and minimum values of the GDA metric in each set are shown. Note that the value of GDA, as discussed throughout the manuscript, ranges from 0 to 1.

Performing the Mann-Whitney U-test (Fig. 4), it was found that there are statistically significant differences between the DREBIOP and DREGE cases. It was observed that DREGE cases had a much stronger association with genes than DREBIOP cases. This result indicates that in what we call “classical repurposing”, the strength of the association between the drug’s target gene

in each of the different strategies carried out. Figure 5 shows a DREBIOP repositioning triple formed by “Osteoporosis” - “Ergocalciferol” - “Rickets”. In point 1, concerning the characteristic biological elements of these triples and the type of pathway related to “Disease 2”, it has been obtained that Rickets has a moderate number of associated genes and a high number of associated pathways. In addition, the pathway classification indicates that it is a “classic metabolic pathway”, specifically the Vitamin D pathway. In point 2, where we were looking for the GDAs value between “Disease 1” and the drug’s target gene, whether this relationship existed, we obtained that Osteoporosis has an associated value of 0.70 (on a scale of 0–1) with the drug’s target gene VDR.

Conclusions

The current work aims demonstrates that biological pathways play an essential role in drug repurposing. The main conclusion of this study is that pathways may be of out-most importance in certain drug repurposing processes

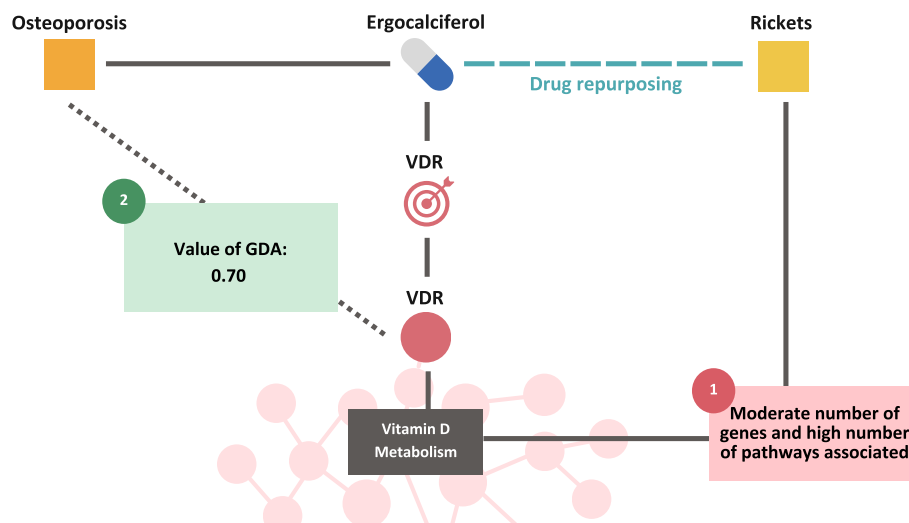


Fig. 5 Representation of a real DREBIOP repurposing triple through all the results obtained for the different proposed strategies

and should be thoroughly considered when generating future hypotheses. Using the DISNET knowledge platform, we found significant patterns characteristic of the DREBIOP cases. Considering the information gathered around these pathway-based repositioning cases, we can suggest DISNET as a useful tool for the generation of essential patterns for potential future drug repositioning cases. This opens the door to a possible new way of thinking about drug repositioning, not only focused on their targets, but also on the biological pathways involved in the development of diseases. We reached other conclusions based on the results of the analyses we developed. Considering different features, we uncovered patterns that will allow us to detect pathway-based drug repurposing scenarios. (1) The configuration of DREBIOP triples showed differentiable characteristics in terms of the number of genes, symptoms, drugs, and pathways involved in the process. (2) DREBIOP and DEGRE GDA values differed statistically significantly, indicating the minor contribution of drug's target genes in pathway-based drug repurposing processes. To summarize, we can conclude that the patterns obtained by this methodology can be applied to any known repositioning case and determine if this case has been produced through pathways, making it a DREBIOP case. Moreover, it could facilitate the prioritization of potential new instances of pathway-based repurposing by identifying the resulting patterns.

Nevertheless, we have identified some limitations in this study. First, the scarcity of drug repurposing based on biological pathway information. Also, the complexity behind the pathways that involves intricate biological mechanisms. Moreover, the categorization of the

pathways was also an arduous task because it was difficult to find an ontology that contained information about all the pathways in our experiment. To conclude, it is important to point out that, as in any *in silico* experiment, the results obtained could not be evaluated in a laboratory but were tested by the scientific literature, as shown in the [Results and Discussion](#) section.

Regarding future lines of this work, we will explore new pathway-based repositioning cases to broaden our analysis and search for more patterns that define them. Eventually, we would like to implement the uncovered patterns and generate novel drug repurposing hypotheses using the DISNET platform, exhaustively considering the biological pathways.

Abbreviations

DISNET	Drug repurposing and DISEase understanding through complex NETWORKs creation and analysis
DREBIOP	Drug REPurposing based on BIOlogical Pathways
DREGE	Drug REPurposing based on GENes
GDAs	Gene-Disease Associations
ACE inhibitors	Angiotensin-Converting Enzyme inhibitors
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase
MAPK	Mitogen-Activated Protein Kinase
MMP2	Matrix MetalloProteinase-2

Acknowledgements

Not applicable.

Authors' contributions

BO-C: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization
 EUC: Conceptualization, Methodology, Validation, Investigation, Writing – Review & Editing, Visualization
 LPS: Conceptualization, Methodology, Validation, Investigation, Writing – Review & Editing
 MDU: Methodology, Validation
 JPC-VH: Writing – Review & Editing, Supervision
 AR-G: Conceptualization, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

Funding

This research was funded by the projects "Data-driven drug repositioning applying graph neural networks (3DR-GNN)" (PID2021-122659OB-I00) from the Spanish Ministerio de Ciencia e Innovación, "Drug repurposing hypotheses through a data-driven approach (GRENADA)" (PDC2022-133173-I00) from the Spanish Ministerio de Ciencia e Innovación and MadridDataSpace4Pandemics, funded by Comunidad de Madrid (Consejería de Educación, Universidades, Ciencia y Portavocía) with FEDER funds as part of the response from the European Union to COVID-19 pandemia. Belen Otero Carrasco's work is supported by "Formación de Personal Investigador" grant (FPI PRE2019-090912) as part of the project "DISNET (Creation and analysis of disease networks for drug repurposing from heterogeneous data sources applied to rare diseases)" (RTI2018-094576-A-I00) from the Spanish Ministerio de Ciencia, Innovación y Universidades.

Availability of data and materials

All data and methods developed for this study are available in open access at the following link:

https://medal.ctb.upm.es/internal/gitlab/b.otero/drebiop_dr_pathways-based.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 19 July 2023 Accepted: 15 December 2023

Published online: 09 January 2024

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