Briefings in Functional Genomics, 17(1), 2018, 34–41

doi: 10.1093/bfgp/elx015
Advance Access Publication Date: 17 August 2017
Review paper

A systems-level analysis of drug–target–disease associations for drug repositioning

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Abstract

Drug repositioning is the process of finding new therapeutic uses for existing, approved drugs—a process thathas value when considering the exorbitant costs of novel drug development. Several computational strategies exist as a way to predict these alternative applications. In this study, we used datasets on: (1) human biological drug targets and (2) disease-associated genes and, based on a direct functional interaction between them, searched for potential opportunities for drug repositioning. From the set of 1125 unique drug targets and their 88 490 interactions with disease-associated genes, 30 drug targets were analyzed and (3) discussed in detail for the purpose of this article. The current indications of the drugs thattarget them were validated through the interactions, and new opportunities for repositioning were predicted. Among the set of drugs for potential repositioning werebenzodiazepines for the treatment of autism spectrum disorders; nortriptyline for the treatment of melanoma, glioma and other cancers; and *vitamin B6* in prevention of spontaneous abortions and cleft palate birth defects. Special emphasis was also placed on those new potential indications that pertained to orphan diseases—these are diseases whose rarity means that development of novel treatment is not financially viable. This computational drug repositioning approach uses existing information on drugs and drug targets, and insights into the genetic basis of disease, as a means to systematically generate the most probable new uses for the drugs on offer, and in this way harness their true therapeutic power.

Key words: drug targets; orphan disease; drug discovery; drug repositioning; molecular interactions

Introduction

Pharmaceuticals are the cornerstone of modern medicine, but the pharmaceutical industry has, in recent years, reached an 'innovation crisis' [1] The FDA defines a new molecular entity as an active ingredient that has never before been marketed in any form [2], and by this definition, according to FDA records, drug companies 'have delivered innovation at a constant rate for almost 60 years'[3]. However, the true crisis lies in the lack of therapeutic gains that these drugs offer, in that most of them are only minor chemical variations on existing drugs—altered to reduce deleterious side effects and optimize efficacy [1]. A review by Gupta *et al.*[4] notes that these slightly altered formulations may be a patent protection strategy, allowing pharmaceutical companies to renew the 20 year patent period on what is essentially the same drug, and in this way defend against the threatening competition of generics. These artful tactics find reason when considering the exorbitant costs of truly novel drug development.

The discovery and development of a novel drug areestimated to cost $US 2.87 billion and the time to market can exceed 10 years [5]. The key contributor to drug development costs is the resources that go into drugs which fail. The 1962 Amendments to the Food and Drug Cosmetics Act of 1938 requires proof of drug safety and efficacy before market approval, and the rigorous testing in respect of this is what makes the drug development process slow, expensive and often resulting in failure. Even successful drugs accrue preclinical and clinical costs [6]. A potential strategy to address the challenges of novel drug development and increase the therapeutic power of the drugs at hand is that of drug repositioning—this is the identification of new uses for

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existing drugs [7–11]. Repositioning approved drugs whose safety and efficacy arealready known reduces the risk of failure in the development process, and speeds up the time to market. These drugs also have known pharmacology and formulation, defined manufacturing routes and bioavailability [12].

The most well-known drug repositioning success is sildenafil (Viagra), which was previously indicated for angina (reduced oxygen supply to the heart) and is now indicated for erectile dysfunction. Sales of sildenafil reached $1.88 billion in 2003 alone [13]. Other successful examples include buprenorphine, previously prescribed as a painkiller but currently prescribed at higher dosages to interrupt heroin and other opioid addictions [14], and pregabalin, previously used as an antiepileptic drug but proved more useful in the treatment of anxiety disorders [15]. It is worth noting that these new indications were discovered serendipitously, but with vast datasets now available, which provide information on the molecular basis of disease, and the technology to compute meaningful associations from this, bioinformatics offers a far more systematic approach to discovery.

Computational drug repositioning can be categorized into two main approaches: the drug-based approach and the disease-based approach. The drug-based approach initiates from the chemical perspective, starting with the pharmacology of a particular drug, whereas the disease-based approach initiates from the pathological perspective, starting with the symptoms of a particular disease [13]. The approach used in this study is the drug-based approach. Based on the fact that a given drug can act on multiple targets and a given drug target can be involved in multiple disease pathways, there are two drug-based ways that one can search for repositioning opportunities: (1) using a known drug to find a new target or (2) using a known target to find a new indication [13]. Using the known target, new indication approach, this study looks at the human biological protein targets of current FDA-approved drugs and, to find new indications for these drugs, looks at the interactions that their targets have with disease-associated genes. Based on these interactions, it is possible that the drugs in question may additionally be used for the diseases with which these genes are associated. In this way, a list of potential opportunities for drug repositioning may be generated (Figure 1).

The decrease in the cost of drug development through drug repositioning means that drug development for orphan diseases is more financially viable. Orphan diseases are those defined as affecting <200 000 individuals in the United States, and there are currently 6000 of these that are known [8]. The cost, time and risk associated with traditional drug development make the development of drugs for rare diseases unappealing to the pharmaceutical industry—the cost of development will far outweigh the return on sales.

The FDA Orphan Drug Act (1983) [16] provides incentives to pharmaceutical companies manufacturing drugs for orphan diseases, and since its implementation, there has been an increase in new drugs available for rare diseases. However, the 325 drugs now available as treatment only cover 5% of the existing orphan diseases. There are 6000 known orphan diseases that cumulatively affect >25 million individuals in the United Statesalone—10% of the population [8]. Thus, there is a great potential for drug repositioning to bridge the gap in pharmaceutical production between common and orphan diseases. A further advantage of cheaper drug development is the broader economic spectrum of patients who will have access to treatment. Currently, the majority of the world's disease burden is borne by third world countries, while the most advanced medical technology (and its benefits) belongs to the first world. Pecoul *et al.* [17] report that of the 1233 drugs approved globally between 1975 and 1997, only 13 were for tropical diseases. Reducing the cost and risk of drug development increases the viability of making treatment affordable and available to the developing countriesthatneed it most.

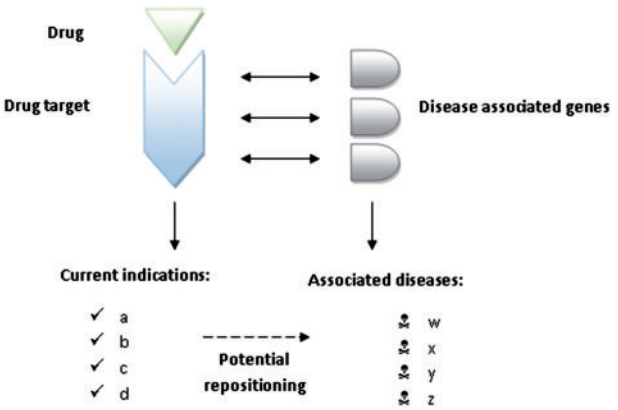


Figure 1. Every approved drug has a known biological target to which it binds, and a list of indications for which this drug is effective as a result of this. The drug target (most often a protein) has many interactions in the human protein–protein network, and some of these may be with genes, which are known to be associated with disease. Based on these interactions, it is possible that the drug in question may also be effective for the associated diseases, in addition to its current indications. (A colour version of this figure is available online at: https://academic.oup.com/bfg.)

Materials and methods

Retrieval of datasets

The full list of 1544 human biological drug targets (proteins) and the corresponding FDA-approved drugs thattarget them was downloaded from DrugBank (http://www.drugbank.ca/).

The full list of 24 755 one-to-one gene–disease associations was downloaded from DisGenNet (http://www.disgenet.org/web/DisGeNET/v2.1/downloads). The gene IDs in this file were Entrez IDs, which were converted to UniProt IDs using the UniProt ID mapping tool. The resulting file contained 5819 disease-associated UniProt proteins, each alongside its list of associated diseases. One central feature file for both drug targets and disease genes was created, containing the following data: gene ID, organism, type of gene (drug target or disease-associated gene), gene name, gene abbreviation, associated diseases (if disease-associated gene) or associated drugs (if drug target).

Filtering datasets

The full set of human protein–protein interactions was downloaded from the STRING database [18]. This file was filtered to create dictionaries of both the proteins in the STRING file and the proteins in the feature file and find those STRING interactions where both proteins in the interacting pair exist in the feature file. The resulting interaction file, along with the feature file, was uploaded to the Protein Interaction Network Viewer (PINV) tool [19] for visualization. The interaction file was filtered further based on the STRING interaction score (a measure of experimental support for the interaction), using a cutoff score of 0.5. We identified those interactions where one protein in the pair is a drug target and the other a disease-associated gene. The resulting set of 88 490 interactions was grouped by drug target ID so that each drug target and its set of interactions with disease-associated genes could be analyzed individually.

Using the filtered interaction file, 30 unique drug targets in the set of 1325 were analyzed. There is the potential for an exhaustive analysis, but given the time constraints of this investigation and the manual nature of downstream analysis, the number was focused and a few cases exemplified. For each drug target, the IDs of the drugs thattarget them were used to extract the drug names and their current indications from DrugBank. For each disease-associated gene with which the drug targets interact, the associated diseases were extracted from the original feature file. The result was a 'repositioning file' in which the current indications and associated diseases could be directly compared withfind potential opportunities for drug repositioning.

Inferring disease–drug associations

The current indications of each drug in question were compared withthe diseases associated with its interacting genes to find (1) diseases in both sets (either the same or closely related based on disease phenotype), which validate the current indications; (2) diseases not currently indicated, but found among the associated diseases, which imply alternative diseases for which the drug can be used; and (3) orphan diseases for which the drug may be used. Orphan diseases were identified using Orphanet—a comprehensive database of rare/orphan diseases (http://www.orpha.net/consor/cgi-bin/index.php).

The information was tabulated and, in cases where the number of diseases for potential drug repositioning was too numerous to represent in table format, the online Wordle tool was used to convert the set of diseases (text) to a graphical representation. In the graphic, the most frequently occurring diseases appear as larger text, and those less frequently occurring as smaller. In this way, the most relevant repositioning opportunities can be visualized. For further validation and insight into potential repositioning opportunities, literature was mined, pathway analysis was done using the Kyoto Encyclopedia of Genes and Genomes (KEGG) [20], and the PINV tool [19] was used to visualize interactions of interest.

Results and discussion

The starting point of this study was the set of all 1544 targets of FDA-approved drugs, and all 5820 known human disease-associated genes. Using the computational methods described, 88 490 pairwise interactions were found where one protein in the pair was a drug target and the other a disease-associated gene. For the sake of this study, 30 drug targets were chosen, the current indications of the drugs thattarget them were found, and the disease-associated genes with which they interact were used to find new potential diseases to which these drugs may be repositioned.

The promiscuous nature of all drugs and drug targets was confirmed, in that some sets of drugs were found to bind to more than one target, and every drug target was seen to interact with more than one disease-associated gene. In addition, every disease-associated gene was associated with more than one disease. This alone suggests the potential of a given drug to have multiple indications.

For every drug target under consideration, at least one of its current indications was validated through its target's interaction with a disease-associated gene (i.e. if a drug is currently indicated for epilepsy, and its drug target directly interacts with a gene associated with epilepsy, the indication was said to be validated through the interaction). This implies that, if one interaction gave rise to a valid indication, the others are potentially valid indications too, and may be opportunities for drug repositioning.

We evaluated the computational strategy used and, from the graph (Figure 2), it appears it was most accurate in cases where a given drug had threeor fewer current indications. In this case, all indications were validated through the interaction. In cases where a drug's current indications exceeded three, some, but not all, indications were validated. This is most likely because of the fact that only direct interactions were analyzed in this study (i.e. where a drug target directly interacts with a disease-associated gene). There is further unexplored search space using indirect interactions (i.e. where a drug target interacts with a gene/protein, which in turn interacts with a disease-associated gene). In this way, branching one step out in the protein–protein interaction network could provide further possible repositioning opportunities and increased sensitivity of predictions.

Similar drug-based methods to the one used show high sensitivity in predictions, such as that by Li *et al.*[21]who use drug pairwise similarity to predict new targets. However, it has been observed that more integrative methods are often better at

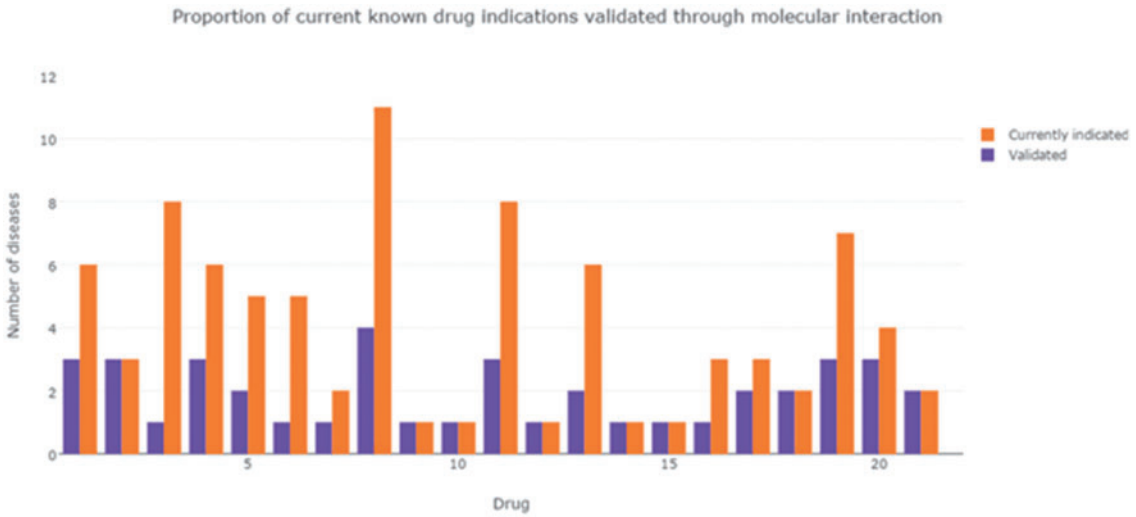


Figure 2. A bar graph showing prediction power of the computational strategy used, by comparing the number of current known indications for each drug under study, alongside the number of those indications that were confirmed through the molecular interaction between its drug target and human disease-associated genes. (A colour version of this figure is available online at: https://academic.oup.com/bfg.)

optimizing both sensitivity and specificity, such as Wang *et al.*[22], whose indication prediction approach uses a combination of chemical structures, target proteins and sideeffects. Thus, instead of trying to optimize prediction power by expanding the scope of the network analyzed, a worthwhile future direction may be to incorporate more features into the analysis, such as drug chemical structure, or target sequence similarities.

Three noteworthy repositioning opportunities, which were validated, are exemplified here for discussion, namely,benzodiazepines as treatment for symptoms of autism spectrum disorders, nortriptyline as treatment for cancer and vitamin B6 in preventing spontaneous abortions and cleft palate birth defects.

Benzodiazepines and autism

A large family of drugs known as benzodiazepines (Table 1, Row 1) was found to bind to three drug targets in the set: *GABRR3*, *GABRP* and *GABRD*—a group of gamma-aminobutyric acid (GAB) receptor subunits. Benzodiazepines are currently indicated for insomnia, anxiety, epilepsy, general anesthesia and alcohol withdrawal. Among the set of interactions with disease-associated genes, were genes associated with insomnia and epilepsy—validating current indications—but also 14 genes associated with autism.

GAB is the chief inhibitory neurotransmitter in the central nervous system [21] and studies of the brain reveal that patients with autism have an increased ratio of excitatory to inhibitory neurotransmission (i.e. a decreased level of GAB receptors) [22]. Experimentation on mouse models showed that low (nonsedative) doses of benzodiazepines increased inhibitory neurotransmission by targeting these GAB receptors (restoring the distorted ratio) and improved deficits in social interaction, repetitive behavior and spatial learning, suggesting an alleviation of autism spectrum disorder symptoms [23].

Also, in the set of disease-associated genes, was a gene associated with the orphan disease, Angelman syndrome (AS). AS is a neurodevelopmental disorder characterized by intellectual and developmental disability, sleep disturbance, seizures, jerky movements and frequent laughter or smiling. The biological network connection of benzodiazepines to AS, as well as the similarity in phenotype of AS and autism, further implies the possible efficacy of benzodiazepines for AS.

Nortriptyline and cancer

Also of interest among the 30 drug targets analyzed was *PGRMC1*, which is targeted by the drugs dextromethorphan and nortriptyline (Table 1, Row 3). This drug target is itself a disease-associated gene, associated with prostate cancer, but also interacts directly with 18 other genes associated with disease. Among the set of 77 diseases associated with these genes, 35 of them are cancer related (Figure 3). Dextromethorphan is currently indicated for dry cough, and nortriptyline is a tricyclic antidepressant. A study done by Parker *et al.* [24] looked at the effect of three tricyclic antidepressants (amitriptyline, nortriptyline and clomipramine) on metastatic cutaneous melanoma cell cultures. All three drugs were active against the melanoma cells *in vitro*, but nortriptyline showed the highest level of activity.

A further study done by Levkovitz *et al.* [25] looked at the effect of tricyclic antidepressants in rat glioma and human neuroblastoma cell lines, and found that the drugs showed potent apoptotic activity against these cells, suggesting the efficacy of these drugs in the treatment of brain-derived tumors.

Pathway analysis was done, using the IDs of the drug target and the genes with which it interacts, and two genes in the set *EGFR* (epidermal growth factor receptor) and *AKT1* (a viral oncogene homologue) were found to be involved in the human glioma pathway (Figure 4).

Collectively, these drug–target–disease interaction data, pathway analysis and experimental observations mined from the literature strongly support the potential for nortriptyline to be repositioned as treatment for melanoma, glioma and possibly other human cancers. Also, notable is the connection of the nortiplyline drug target to genes associated with Charcot–Marie–Tooth disease (a neurological disorder) and Antley–Bixler syndrome (associated with skeletal malformation)—two phenotypically different orphan diseases, which collectively affect >150 000 Americans (Hereditary Neuropathy Foundation, http://www.hnf-cure.org/charcot-marie-tooth-disease/).

Vitamin B6, spontaneous abortion and cleft palate

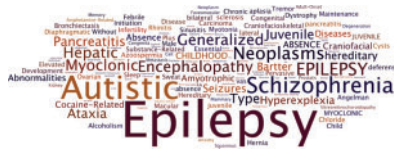
Another drug target of interest is *PDXK*, which is targeted by the drugs pryidoxal and pyridoxine—collectively referred to as vitamin B6 (Table 1, Row 4). This vitamin is currently indicated for nutritional supplementation and to treat a dietary shortage or imbalance. The *PDXK* drug target interacts with four genes associated with disease, and one of these genes, *CD8A*, is associated with spontaneous abortions. A study done by Ronnenberg *et al.* [26] looked at preconception folate, vitamin B6 status and clinical spontaneous abortions in Chinese women and found that the risk of spontaneous abortion was 4-fold higher among women with suboptimal concentrations of both folate and vitamin B6. This supports the potential of vitamin B6 to be used in preventing spontaneous abortions in women experiencing a vitamin B6 deficiency before and during gestation.

PDXK also interacts with a gene *AOX1*, associated with cleft palate birth defects. Vitamin deficiencies have been shown to induce oral clefts in animal experiments, and an additional human study was done by Munger *et al.* [27], which looked at maternal vitamin B6, folate status and the risk of oral cleft birth defects in the Philippines. The study found that the risk of cleft lip/palate was consistently associated with low maternal vitamin B6, further validating the interaction.

Population genetics and pharmacogenetics

The use of drugs to effectively treat communicable or noncommunicable diseases is still challenging because of variable responses among a significant number of individuals. Approximately 50% of patients do not respond to a given drug [28]. Several studies [29–31] have indicated that single-nucleotide polymorphisms (SNPs) found in different populations are associated with significant changes in drug efficacy; therefore, efficacy may vary depending on the population to which they are administered [32]. Thus, it is important to understand to what degree the results are relevant in terms of different populations. A given SNP associated with a disease or contributing to drug response may be more prevalent in one population than another because of differences in frequency between populations. In this way, both population genetics and pharmacogenetics should come into play in further studies. We used ANNOVAR [33] to independently perform gene-based annotation using high-confidence haplotypes from 1000 Genome Phase 3 [34] to catalog the closest gene to each variant (60 kb downstream/upstream) and extract gene–SNP mapping. SNPs were mapped to their corresponding biological drug targets (proteins) based on the SNP–gene physical distance. We looked at the average number of SNPs in the set of these human biological drug targets compared withthose in the rest of the human proteome and the set of

Table 1. The current indications of the drugs under study, their potential alternative uses and those potential uses that pertain to orphan diseases

Drug names	Current indications	Validation through associations	Potential new indications	Potential new indications for orphan diseases
Temazepam, adinazolam, midazolam, flurazepam, halazepam, diazepam, oxazepam, triazolam, estazolam, bromazepam, clotiazepam, cinolazepam and nitrazepam	Insomnia, anxiety, epilepsy, general anesthesia, alcohol withdrawal and used an anticonvulsant	Sleep initiation and maintenance disorders, epilepsy, essential tremor and ataxia		AS
Dextromethorphan and nortriptyline	Dry cough, depression, chronic pain, IBS, diabetic neuropathy, agitation, insomnia, and migraine prophylaxis.	Pain		Antley–Bixler syndrome andCharcot–Marie–Tooth disease
Pregabalin, verapamil, loperamide, bepridil and spironolactone	Neuropathic pain, prostherpetic neuralgia, seizures, fibromalgyia, hypertension, angina, cluster headache prophylaxis, diarrhea, hypertension, hypokalemia and Conn's syndrome	Pain, seizures, hypertension and hypokalemia		Liddle syndrome, Bartter syndrome andSturge–Weber syndrome
Pyridoxal and pyridoxine	Nutritional supplementation and dietary shortage/imbalances	Pyridoxamine 5-prime-phosphate oxidase deficiency	Status epilepticus, CD8 deficiency, pulmonary disease, spontaneous abortion, neurodegenerative diseases, peripheral nervous system diseases, polycystic ovary syndrome, multiple sclerosis, rheumatoid arthritis, hepatitis C, retinal diseases, immunodeficiency, acromegaloid features, overgrowth, cleft palate and hernia	None

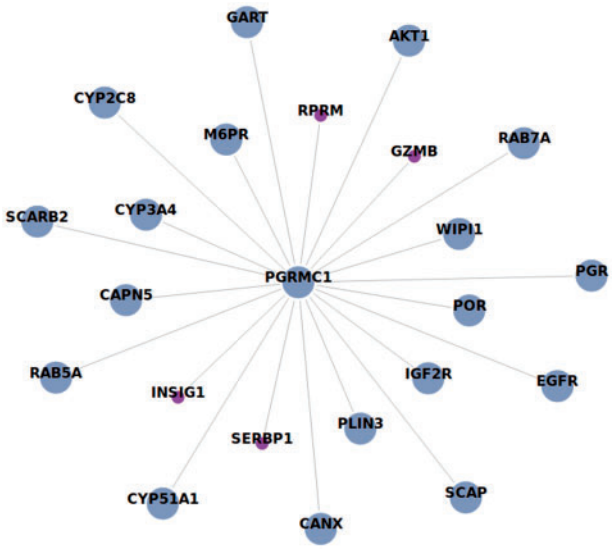


Figure 3. A PINV visualization of the *PGRMC1* drug target, and its network of interactions with disease-associated genes. Those genes with big circles are associated with cancer-related diseases. (A colour version of this figure is available online at: https://academic.oup.com/bfg.)

disease-associated proteins. Results obtained indicate that the average number of identified SNPs in the set of these human drug targets is significantly greater than those in the background human proteome and the set of disease-associated proteins, with Wilcoxon rank sum test *P*-values < 2e-16 and equal to 2.572e-07, respectively. These genetic variants are potentially influential, as they may directly impact drug metabolizing enzymes, drug targets and drug receptors [35].

Communicable diseases

Asthis investigation was based on the knowledge of disease-associated genes or putative genes causing disease, the scope of repositioning is limited to genetically well-characterized, hereditary diseases, and does not directly address possible alternative treatment for communicable diseases, such as malaria or tuberculosis.

Although communicable diseases are not genetically rooted, there are genetic components, which may contribute to their effect, such as genes for 'susceptibility to malaria' or genes for 'progression of respiratory tract infections'. Genes for related diseases may also be of interest, such as the HBB gene associated with sickle cell anemia. This gene, if heterozygous, confers immunity to malaria [36]. In this way, drug repositioning through disease-associated genes could still be applied to communicable diseases.

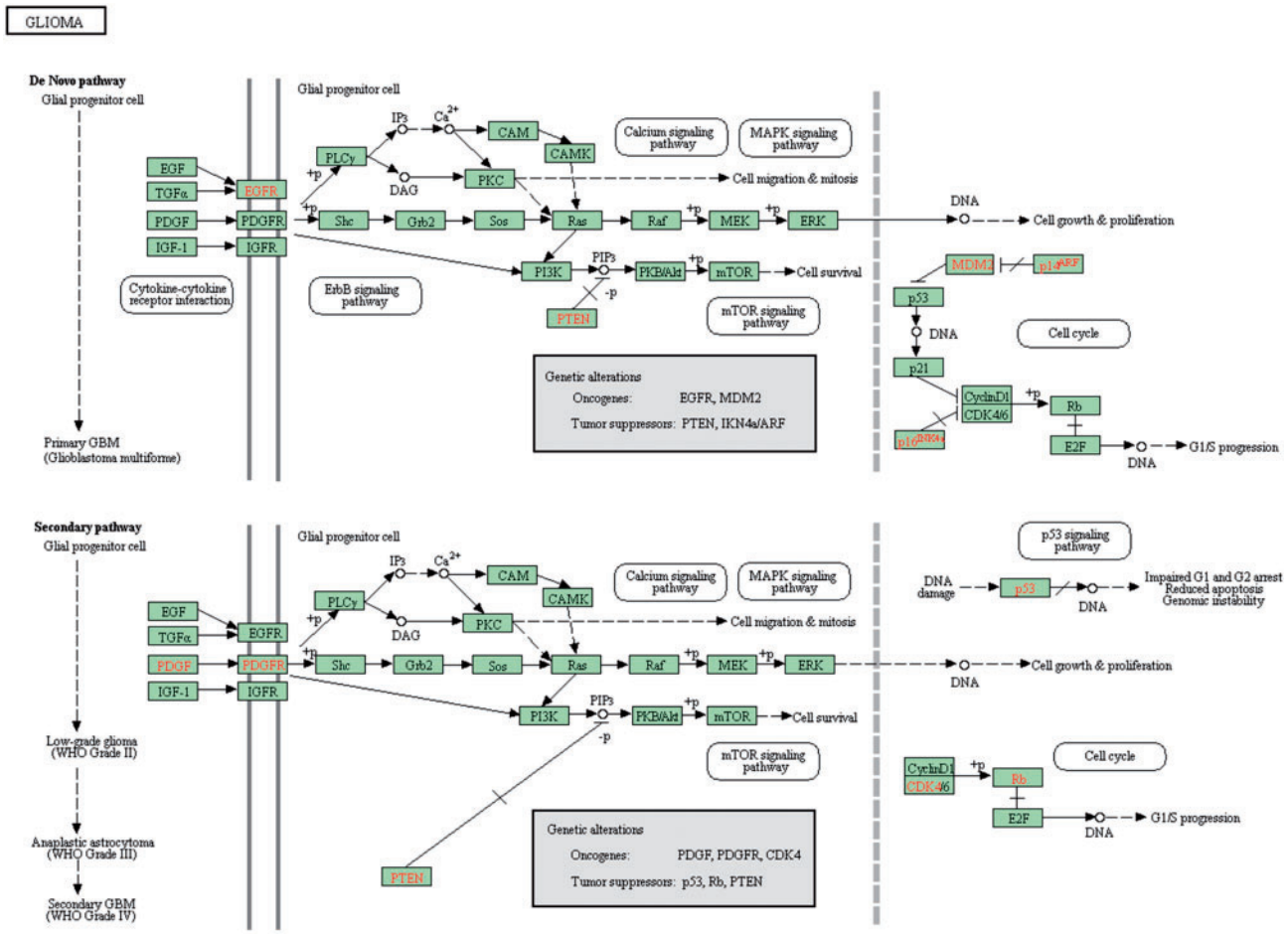


Figure 4. KEGG human glioma pathway (http://www.kegg.jp/pathway/hsa05214), showing EGFR and AKT1 in both the *de novo* and secondary pathways, causing cell migration, mitosis, growth, proliferation and cell survival of glioma cells. Gliomas are the most common brain tumors, and one of the most severe human cancers. *De novo*/primary glioblastomas develop in older patients and secondary glioblastomas in younger patients.

Conclusions

This study put into practice a computational framework for drug repositioning, by harvesting data on current approved drugs, their targets and their indications, and human genes found to be associated with disease. In this way, current information was filtered and analyzed on a large scale to find new possible uses for existing drugs, without the cost and resources required for conventional drug discovery. Although this process is theoretical and does not discount the need for clinical validation and drug safety testing, it is an effective way to systematically generate a list of most probable therapeutic successes from putative opportunities, and thereby reduce the risk of failure in the drug development process, and the expenses associated with this. With the great disease burden still borne by the developing world, the vast majority of orphan diseases that remain untreated and the pharmaceutical giants coming up dry in the search for new therapeutics, there is a great opportunity for a drug development process more effective than the one at play. Computational drug repositioning offers a systematic, efficient and reliable way to predict new uses for existing drugs, and in this way harness their full therapeutic power.

In among the 88 490 interactions between known drug targets and disease-associated genes found, there is vast potential for new indications of drugs to be further explored. The information age brought with it new insights into the molecular basis of disease and pharmacology, and this information should be harvested to draw meaningful conclusions for the advancement of molecular medicine. Given the highly interconnected nature of drug targets and disease genes, the 'one gene, one drug, one disease' paradigm that classical drug development follows can more effectively be replaced with that of 'polypharmacology'—a holistic, systems approach to drug development.

Key Points

- Integration of protein–protein functional interactions, disease-associated proteins and drug targets to enhance drug repositioning.
- Illustration of computational drug repositioning prediction power and prediction of drugs that can target orphan diseases.
- Incorporating population genetics and pharmacogenetics may improve disease–drug mapping prediction for communicable and noncommunicable diseases
- The number of identified genetic variants in drug targets or disease-associated proteins is significantly higher than in other human proteins.

Acknowledgements

The authors thank everyone involved with free software, from the core developers to those who contributed to the documentation. Many thanks to the authors of the freely available libraries.

Funding

The authors appreciate financial support received from the South Africa National Research Foundation (NRF). Some of the authors are funded in part by Government of Canada via the International Development Research Centre (IDRC) through the African Institute for Mathematical Sciences-Next Einstein Initiative (AIMS-NEI).

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