Computational drug repositioning for ischemic stroke: neuroprotective drug discovery

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Background: A comprehensive approach to drug repositioning will be required to overcome translational hurdles and identify more neuroprotective drugs. Results & methods: Gene Set Enrichment Analysis was applied to identify related pathways and enriched genes. Candidate genes were optimized using ToppGene, ToppGenet and pBRIT. From the perspective of the local structures, gene–domain–substructure–drug relationships were constructed. Using the MCODE algorithm and K-means clustering, 31 functional subnetworks were obtained, and 252 drugs with proposed neuroprotective function were identified. Using computational analysis, 72 substructures with different scores were found to correspond to neuroprotective functions. The protective effects of benidipine and barnidipine were confirmed in vitro. Conclusion: The authors’ research has great potential to discover more neuroprotective drugs and obtain more information regarding mechanisms of action and functional substructures.

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Ischemic stroke (IS) is a leading cause of neurologic disability and mortality worldwide [1]. The disease itself has a significant economic burden on society that will increase with the aging population. For decades, the main approaches to therapy for IS have focused on reperfusion and neuroprotection [2]. In the acute stage of IS, recombinant tissue plasminogen activator remains the only US FDA-approved drug that can accelerate reperfusion via thrombolysis, but its use is unfortunately limited by a narrow time window, low recanalization rate and increased risk of hemorrhagic transformation [3]. Apart from reperfusion, neuroprotective therapy has been proposed since the 1980s, with the main aim of intervening in the events of the ischemic cascade, blocking the pathological process and avoiding the death of nerve cells [4]. Several key factors in ischemic cell death within the penumbra have been identified, including oxidative stress, excitotoxicity and inflammation. Targeting these mechanisms shows beneficial neuroprotective effect [5]. However, translating these mechanistic theories into clinically meaningful drugs for stroke is very challenging, and continuous efforts have been directed toward identifying new drugs [6].

Over the past few decades, de novo drug discovery has become increasingly expensive and time-consuming, and the number of novel compounds transferred to therapeutic drugs has stagnated [7]. To overcome this burden, drug repositioning (DR), which identifies new indications for existing drugs, has emerged as a significant strategy in new drug discovery [8]. However, because of the large number of diseases and known drugs, it is still very costly to completely screen new uses of known drugs via experiments. With the accumulation of omics and pharmaceutical informatics data, DR has entered the stage of combining rational design and experimental screening. Computational DR analysis strategies have become an important research direction in computational biology and systems biology. In addition, accurately identifying the interactions between drugs and targets is a key stage in accelerating drug development [9]. If we can accurately identify a significant correlation between the candidate drug studied and certain target proteins, we can avoid aimlessly screening candidate targets from the massive protein data and accelerate the entire drug development process [10]. At present, research on the prediction of drug–target interactions is mainly
based on the overall structure of the drug. However, the binding of drug and target protein is the interaction of the local structure of the drug (drug substructure) and the active pocket of the protein (protein domain). From the perspective of local structure, the relationship between structure and function can be more comprehensively expressed.

For these reasons, the authors’ study adopted a computational DR strategy based on drug–target interaction and identified drugs with strong neuroprotective effects. In addition, the correlations between substructures and drug functions were further explored. In contrast to previous studies, the strategy used in the authors’ study shows great promise in identifying more neuroprotective drugs, which can, to an extent, make up for the lack of treatment strategies.

**Methods**

**Preparing candidate gene set**

The authors searched the Gene Expression Omnibus database for gene expression profiling studies associated with IS. Genome-wide RNA sequencing data (GSE58294) with normalized values were downloaded from the Gene Expression Omnibus database [11]. In this dataset, blood samples were collected from 69 patients within 24 h of stroke onset and 23 controls with no history of symptomatic vascular disease. Microarray expression profile was determined by array using the [HG-U133 Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.

To assess gene expression signatures and activation status of pathways, Gene Set Enrichment Analysis (GSEA) was performed using Java GSEA implementation with 186 Kyoto Encyclopedia of Genes and Genomes gene sets of canonical pathways, a C2 subcollection in MSigDB [12]. Using a permutation test with 1000 repetitions, the cutoff for p-value significance level for the significant pathways related to IS was determined to be 0.05. Accordingly, GSEA gave the authors a subset of genes that better contributed to score enrichment and significant pathways by comparing the samples with IS and the controls. The subset genes were used as the candidate gene set in the subsequent process.

**Process of gene prioritization**

To further obtain the genes closely related to IS (or neuroprotection), three methods of gene prioritization – ToppGene, which prioritizes or ranks candidate genes based on functional similarity to the training gene list; ToppGenet, which identifies and prioritizes the neighboring genes of the seeds in the protein–protein interaction network based on functional similarity to the ‘seed’ list (ToppGene); and pBRIT, which prioritizes genes based on phenotypic concordance to the training genes – were used to select the most related genes among the gene candidate set. The three gene prioritization algorithms were used to measure the similarity of genes and to select the most related genes based on different complementary characteristics. The genes that had the greatest similarity with the genes the authors collected as the training set and had been documented to be associated with neuroprotection were selected [13]. Prioritized genes were screened (p < 0.05) and sorted using the three algorithms. The authors denoted score1(Gj) as the prioritization score of the j-th gene by integrating the scores of the j-th gene in the three tools using the formula

$$\text{Score}_1(G_j) = \frac{TG_j + TN_j + PB_j}{3T}$$

where T represents the total number of genes, j ∈ [1, T], and TGj, TNj and PBj are the score rankings of the j-th genes in ToppGene, ToppGenet and pBRIT, respectively. According to score1(Gj), the most promising neuroprotection-related genes with score1(Gj) greater than the mean were identified.

**Identification & quantification of gene–drug relationships**

To find drugs targeting these neuroprotection-related genes, a gene (protein)–domain–substructure–drug relationship network was established based on domain–substructure associations identified in the authors’ previous study [14]. Detailed information regarding the data used to establish the relationship network is shown in the identification and quantification of gene–domain–substructure–drug relationships in the Supplementary material. To evaluate the intensity of the interaction in each drug–gene pair, score2(Zpq), which was determined by the
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The intensity score of the interaction between the p-th drug and the q-th gene, was defined as

$$s_{\text{core}2}(Z_{pq}) = \frac{N_{pq}}{T_p} \times \frac{N_{pq}}{G_q}$$

where $N_{pq}$ represents the number of domain–substructure relationships shared between the p-th drug and the q-th gene, $p \in [1, 2436]$ and $q \in [1, 25]$; $T_p$ represents the number of all domain–substructure relationships corresponding to the p-th drug; and $G_q$ represents the number of all domain–substructure relationships corresponding to the q-th gene. The higher the score2($Z_{pq}$), the stronger the association between a drug and a gene. Finally, drug–gene relationships with a score2 above the average were obtained and used to optimize the gene–domain–substructure–drug network.

Obtaining drugs with strong similarity

To evaluate the similarity of drugs, the authors used a clustering method based on the assumption that drugs in the same class tend to be similar in function. Therefore, the MCODE algorithm [15] and K-means clustering [16] were used to cluster drugs based on the corresponding substructure–domain relationships in the authors’ neuroprotective network. The authors determined that the drugs that were clustered into one class by the two clustering algorithms simultaneously had strong similarity at the substructure level. A detailed description of this can be found in the supplementary material.

Identification & quantification of the correlations between substructures & functions

The authors next identified drug substructures related to neuroprotective effect and quantified the correlations between substructures and neuroprotective effect. To this end, the authors attempted to find specified substructures of drugs in each cluster that were considered to have similar mechanisms related to neuroprotection. In this method, the 881-bit PubChem fingerprints were used as substructures to analyze the mechanism of drug neuroprotective action from the perspective of the local structure of the drug. The authors used the following formula to identify drug substructures that significantly correlated with neuroprotective function and elucidate the mechanism of drug action from the perspective of the local structure

$$\text{score}3(S_i) = \frac{D}{F_i}$$

$$\text{score}4(S_i) = \frac{M_i}{P}$$

$$\text{score}5(S_i) = \text{score}3(S_i) \times \text{score}4(S_i)$$

where score3($S_i$) is defined as the specificity score of the i-th substructure to highlight the rare substructures distributed in approved drugs; score4($S_i$) is defined as the commonality score of the i-th substructure in a specific drug group to highlight the common substructures of the drug functional mechanism in a cluster; score5($S_i$) is defined as the correlation intensity score of the i-th substructure and drug function, $i \in [1, 881]$; the number of all approved small-molecule drugs in the DrugBank database is represented by $D$; the number of drugs containing the i-th substructure in all approved small-molecule drugs is represented by $F_i$; the number of drugs in one cluster is represented by $P$; and the number of drugs containing the i-th substructure in the drugs of the cluster is represented by $M_i$.

In vitro experiments

Cell culture, reagents & antibodies

Mouse brain microvascular endothelial cells (bEnd.3) were obtained from the Bena Culture Collection (Beijing, China). Cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin in a humidified incubator with 5% CO2 at 37°C. Benidipine was purchased from Aladdin
Table 1. Antiapoptotic, anti-inflammatory and antioxidative genes related to ischemic stroke.

<table>
<thead>
<tr>
<th>Antiapoptotic gene</th>
<th>Antiapoptopic gene (cont.)</th>
<th>Anti-inflammatory gene</th>
<th>Antioxidative gene</th>
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<tr>
<td>ACOX1</td>
<td>IDH1</td>
<td>ACSL4</td>
<td>GSTM3</td>
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<td>GSTM3</td>
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<td>TXNDC12</td>
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(Shanghai, China). Barnidipine was purchased from Molbase (Shanghai, China). Antibodies used in this study were anti-GPX-1, anti-SOD-1, anti-Bcl-2, anti-Bax and anti-cleaved caspase-3 (Wanleibio Co. Ltd., Shenyang, China).

Oxygen−glucose deprivation & reoxygenation
The bEnd.3 cells were subjected to ischemia-like injury via oxygen−glucose deprivation (OGD) for 8 h by replacing the culture medium with serum- and glucose-free DMEM (Gibco, Shanghai, China) and placing cultures in a hypoxic incubator containing 5% CO₂ and 95% N₂, as described previously [17]. After 8 h of OGD, reoxygenation was induced by placing all cultures in normoxic conditions for a further 24 h and replacing the OGD medium with the normal medium supplemented with 10% fetal bovine serum. Control cultures (no injury) were incubated with DMEM containing glucose in a normoxic incubator.

Western blot analysis
Western blot analysis was used to determine levels of GPX-1, SOD-1, Bcl-2, Bax and cleaved caspase-3 in the bEnd.3 cells given that they are associated with apoptosis and oxidation. Cell lysates were obtained from the bEnd.3 cells at reoxygenation 24 h following OGD. Protein samples (30 μg) were resolved on polyacrylamide sodium gels and electrophoretically transferred to polyvinylidene difluoride membranes. The primary antibodies against GPX-1, SOD-1, Bcl-2, Bax and cleaved caspase-3 were used, with GAPDH as an internal control. The western blot bands were quantified using Odyssey (LI-COR Biosciences, NE, USA).

Statistical analysis
The in vitro experiment results were analyzed using Prism 7.0 (GraphPad Software, CA, USA). Statistical comparisons between experimental groups were assessed with one-way analysis of variance, and the level of statistical significance was set at p < 0.05.

Results
Candidate gene selection & validation
Based on GSEA of the dataset, which was achieved by comparing IS and control samples, the authors identified 45 significantly upregulated and enriched gene sets (pathways) (p < 0.05) associated with IS. These included the antiapoptosis/antioxidative-related (glutathione metabolism) and antiapoptosis/inflammation (PPAR signaling) [18] pathways as well as the proinflammatory-related (cytosolic DNA-sensing) and apoptosis-related (apoptosis) pathways (Figure 1). The authors obtained the gene sets that better contributed to score enrichment in the glutathione metabolism and PPAR signaling pathways (Figure 2).

To select antiapoptotic, anti-inflammatory and antioxidative genes, the authors used core-enriched genes from GSEA as the test set and genes that have known antiapoptotic, antioxidative and anti-inflammatory effects as the training set. Prioritized genes were identified with three algorithms (TopGene, TopGenet and pBRIT) (p < 0.05) and sorted by score1. The authors identified 18 antiapoptotic, nine anti-inflammatory and ten antioxidative genes that might serve as the targets of therapy and a total of 25 neuroprotective genes (Table 1).

The neuroprotective functions of 25 genes were confirmed by literature search. Searching in the PubMed database using ‘neuroprotective’, ‘antiapoptotic’, ‘anti-inflammatory’, ‘antioxidative’ and ‘gene symbol’ as search terms for
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**Figure 1.** Forty-five significantly upregulated and enriched gene sets associated with ischemic stroke. This is a set to set graph, showing the overlap between gene sets, using green color to indicate the number of genes shared between sets. The deeper the color, the more the number of shared genes.

The 25 genes, it was confirmed that ten genes were related to neuroprotective function and 13 genes were related to protective function in other tissues. The overall verification rate was 92%. The PubMed identifiers of the related literature can be found in Supplementary Table 1.

**Network construction, similarity analysis & verification of drug function**

The authors established the neuroprotective gene–domain–substructure–drug network (Supplementary Figure 1), which contained 22 genes, 24 domains, 480 substructures and 2297 drugs with score2(Zpq) greater than the mean (Supplementary Table 2). Using MCODE, the authors identified 37 modules and obtained scores for each module. The authors then conducted further analysis of ten modules whose scores were greater than the average score of 4.6 (Supplementary Table 3). Using the elbow method, the authors determined that the number of clusters in K-means clustering was 8 (Supplementary Figure 2). Finally, the authors screened out the drugs classified into the same class by MCODE and K-means clustering and formed 31 functional subnetworks, with a total of 252 drugs...
The authors predicted that these drugs had neuroprotective effects after cerebral ischemia and confirmed the brain protection effects of some drugs via literature search.

(Supplementary Table 4). The drugs in each network were considered to have strong neuroprotective function and functional similarities between drugs.

To confirm the authors’ prediction, literature verification of 252 drugs was conducted. Searching in the PubMed database using ‘neuroprotective,’ ‘antioxidative,’ ‘anti-inflammatory,’ ‘antiapoptotic’ and ‘drug name’ as search terms, it was confirmed that 85 drugs were related to neuroprotective function. In each subnetwork, there was at least one drug that was documented to be neuroprotective. Because of space limitations, the authors selected compounds from only two subnetworks for detailed analysis.

The 18 drugs in subnetwork 1 were shared by module 1 (MCODE) and cluster 3 (K-means clustering) (Figure 3). Using the method detailed in the current study, the authors predicted that these drugs had neuroprotective effects after cerebral ischemia and confirmed the brain protection effects of some drugs via literature search.
Digoxin is a drug used to control ventricular rate in atrial fibrillation and to aid in the management of congestive heart failure with atrial fibrillation. Peng et al. showed that preconditioning treatment using digoxin contributed to improved functional recovery and exerted a prominent neuroprotective effect, including reduction of apoptosis and promotion of cell proliferation [19]. Ouabain is used to treat congestive heart failure and to aid in the treatment of chronic atrial fibrillation. At present, studies have confirmed that ouabain has anti-inflammatory and antiapoptotic effects and can protect human renal cells [20]. However, there is no literature confirming its protective effect on the brain after ischemia. In the authors’ network, both drugs acted on the same target: RRM2, RRM2B, NR1H3, RXRB and SORBS1 (Figure 3A). In the initial gene prioritization, the authors identified the protective effects of these target genes. The authors also confirmed via the literature that these genes had antiapoptotic, antioxidative or anti-inflammatory effects [21]. In addition, ouabain has a large number of substructures that are identical to those found in digoxin. Among the 111 substructures of ouabain, 110 are shared with digoxin. Since the biological functions of a drug depend on its structure, the authors believe that ouabain also has neuroprotective effects. Furthermore, results of the MCODE algorithm and K-means clustering suggested that ouabain and digoxin play a role in brain protection through a similar mechanism of action. A study by Winnicka et al. indicated that 30 nM of digoxin and ouabain stimulated an antiapoptotic effect via promotion of the level of phosphorylated extracellular signal-regulated kinases [22].

Ginseng is promoted as an adaptogen, a position that is, to a certain extent, supported with reference to its anticarcinogenic and antioxidant properties. A study conducted by Liu et al. suggested that apoptosis induced by cerebral ischemia/reperfusion was attenuated by ginseng via downregulation of the levels of cleaved caspase-3 and...
caspase-9 [23]. Sood et al. showed that ginseng ameliorated middle cerebral artery occlusion-induced oxidative stress, apoptosis, mitochondrial dysfunction and cognitive impairment [24]. Steviolbioside, ivermectin and ginseng act on the same protective targets — RRM2, RRM2B, NR1H3 and RXRB — and the substructures are similar (Figure 3B). Therefore, the authors concluded that steviolbioside and ivermectin have neuroprotective effects, and ivermectin has been shown to have antioxidant and anti-inflammatory effects. By the same token, the rest of the drugs in subnetwork 1 all have neuroprotective effects.

The seven drugs in subnetwork 21 are shared by module 8 (MCODE) and cluster 2 (K-means clustering), and six of these drugs are dihydropyridines, which are widely used in the treatment of hypertension and cerebrovascular disease (Figure 4A). Azelepidine demonstrates a neuroprotective effect in the ischemic brain [25]. Lercanidipine possesses anti-inflammatory, antioxidant and antiapoptotic properties, which are cardinal mechanisms involved in acute IS [26]. Nicardipine has anti-neuroinflammatory effects on microglial cells and exerts a protective effect against rotenone-induced apoptosis [27]. The combination of manidipine and idebenone significantly ameliorates neurological deficits and histological changes in the rat brain following stroke [28]. In addition, benidipine has been shown to prevent inflammatory changes and oxidative stress in a rat model of myocardial infarction [29]. Barnidipine reduces the plasma levels of inflammatory and oxidative biomarkers in hypertensive rats [30]. Although the targets and substructures of these drugs are similar, the neuroprotective effects of benidipine and barnidipine against cerebral ischemia have not been confirmed. Therefore, the authors' study verified the neuroprotective effects of these two drugs, which are widely used in clinical practice in vitro.

The protein expression of GPX-1, SOD-1, Bax, Bcl-2 and cleaved caspase-3 was determined using western blot assay. The authors' results showed that OGD and reoxygenation (OGD/R)-induced apoptosis and oxidative stress were inhibited by benidipine and barnidipine. As shown in Figures 4B & 5, the level of protein expression of Bax and cleaved caspase-3 was upregulated by OGD/R compared with the control group. Benidipine and barnidipine suppressed the expression of Bax and cleaved caspase-3 compared with the OGD/R group. The level of Bcl-2, SOD-1 and GPX-1 was downregulated by OGD/R compared with the control group. Benidipine and barnidipine promoted an increase in the level of these proteins compared with the OGD/R group.

Correlation analysis between substructure & drug function
With regard to the 252 drugs with strong neuroprotective function, the authors further explored the relationships between drug substructures and drug functions to elucidate the structural basis of the neuroprotective effects of these drugs. Based on score5(Zpq), the authors obtained correlation scores between drug substructures and neuroprotective function, with a total of 72 substructures (Supplementary Table 5). The higher the score, the stronger the correlation intensity. Since the drugs in each subnetwork had a common brain protection effect and a similar mechanism of action, the authors concluded that these shared and specific substructures were likely to be an important structural basis for the neuroprotective role played by these drugs. For example, in subnetwork 21 (Figure 4A), the six dihydropyridine drugs all had highly correlated substructures: SUB402(N∼O)(∼O)) and SUB456(N∼O)(∼O)). These substructures may allow the six dihydropyridines to exert brain protection effects through the interaction of similar mechanisms with the domains (PF00268, PF00104 and PF00105).

Discussion
The basic aim of neuroprotective therapies for IS is to interfere with the events of the ischemic cascade by focusing on one or more of the mechanisms of damage, including excitotoxicity, oxidative stress and inflammation, blocking the pathological processes and preventing the death of vulnerable nerve cells in the ischemic penumbra [4]. However, clinically effective neuroprotectants have thus far remained elusive. Therefore, the authors’ study aimed to identify new treatment targets and look for neuroprotective drugs through a screening strategy that targeted multiple mechanisms of ischemic injury.

Since the interaction of drug and protein is essentially the interaction of the drug substructures and protein domains, the relationship between structure and function can be better reflected from the local perspective. For this reason, the authors used substructure–domain relationships as the basis for predicting drug–target protein interaction relationships. The strategy used in the authors’ study can identify not only drugs with similar overall structures and targets (e.g., nicardipine, benidipine, barnidipine and lercanidipine) but also drugs with different overall structures but similar targets (e.g., clenbuterol, entacapone, verapamil and oxymetazoline).

The occurrence and development of complex diseases rarely stem from mutations of a single gene, but rather involve comprehensive changes in the intracellular molecular network. From the perspective of a complex network,
Figure 4. Sub-network 21, and benidipine inhibited OGD/R-induced apoptosis and oxidative stress in bEnd.3 cells. (A) Sub-network 21. The red node represents the gene, the blue node represents the drug, and the green node represents the domain–substructure. (B) The level of protein expression of Bax and Cleaved caspase-3 were upregulated by OGD/R compared with the control group. Benidipine suppressed the expression of Bax and Cleaved caspase-3 compared with the OGD/R group. The expression of Bcl-2, SOD-1 and GPX-1 were downregulated by OGD/R compared with the control group. Benidipine promoted the increase in these proteins level compared with the OGD/R group (n = 3).

*p < 0.05; **p < 0.01 compared with the OGD/R group; ##p < 0.01 compared with the control group.
with the concept of a multilevel and multiangle interaction network (‘gene–domain–substructure–drug’), the authors used the idea of network pharmacology to establish a large-scale association analysis of genes and drugs and discover new combinations of drugs and targets. In addition, the authors' study used two methods – MCODE and K-means clustering – to identify drugs with the strongest neuroprotective effects according to the modularity of the gene–drug network. Drugs clustered into the same subnetwork were determined to protect the ischemic brain via the same mechanism. For example, in subnetwork 1, the authors predicted that ouabain and digoxin had similar targets and substructures and may exert neuroprotective effects through similar mechanisms of action. A study by Winnicka et al. confirmed this inference [22]. Similarly, drugs in different modules were determined to
have different mechanisms of action. Therefore, researchers could try to combine the drugs in different modules
to exert the neuroprotective effects through a variety of mechanisms, a process that is expected to increase the
neuroprotective effects of drugs in future research.

It is well known that structure determines function, but previous research has mainly focused on the overall
structure of drugs. This study, for the first time, explored the mechanisms of action of drugs from the perspective
of the local structure and used computational analysis to quantify the correlations between drug functions and
substructures. Seventy-two substructures related to neuroprotective function were identified, and the score of each
substructure was obtained. The higher the score of the substructure, the stronger the correlation intensity between
the substructure and the neuroprotective effect of the drug. This theory has strong practical significance. First,
it allows us to better focus on the similarities of these specific substructures rather than the drugs themselves to
discover drugs that contain these substructures and have neuroprotective effects, further expanding the space for
drug discovery. Second, in future rational drug design, the specificity of drug substructures can be used to retain
the therapeutic effects, delete the substructures that produce side effects and optimize druggability.

However, the authors’ approach has several limitations with regard to predicting drugs with neuroprotective
effects in IS. The approach is limited by the number of protein domains and drug substructures and is not
applicable to predicting drugs without substructures or proteins without domains. These problems will be solved
with further developments in structural chemistry and pharmacology.

Conclusion
Our study proposed a strategy to discover more neuroprotective drug candidates and confirmed that benidipine
and barnidipine exerted neuroprotective effects in ischemic injury by inhibiting apoptosis and oxidative stress. In
addition, our study obtained more information regarding mechanisms of action and functional substructures.

Future perspective
Neuroprotection is important for reducing cerebral injury as much as possible in the context of stroke. It is very
urgent that we find more drugs that can be translated to clinical practice. Drug repositioning, a strategy for finding
new indications for old drugs, is an important strategy for drug research and development in both the present
and future because of its advantages of low risk of failure and short development time (e.g., the discovery of anti-
coronavirus drugs). Moreover, from the perspective of the local active structures of drugs and proteins, this study
demonstrates new indications for old drugs. This strategy not only can quantify the effects of drugs by measuring
the connection strength of the local structures between drugs and proteins but can also find more drugs candidates
with neuroprotective effects, which is consistent with the development trend in innovative drugs. It is also the trend
to extract structural features based on the relationships between local active structures and functions to combine
effective structures and generate new compounds. At the same time, based on the method used in this study,
a drug–substructure–domain–adverse drug reaction correlation network will be established in a future study to
discover new drug candidates with neuroprotective effects and less toxicity and improve the success rate of new drug
research and development. In addition, dihydropyridine calcium antagonists are widely used as antihypertensive
drugs in patients with IS. From the perspective of clinical treatment, the neuroprotective effect of these drugs is of
great significance and needs to be further confirmed in animal models and clinical trials.

Supplementary data
To view the supplementary data that accompany this paper, please visit the journal website at: www.futuremedicine.com/doi/sup pl/10.2217/fmc-2021-0022

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Summary points

- Using the bioinformatics tools Gene Set Enrichment Analysis, ToppGene, ToppGenet and pBRIT, the authors’ study identified 25 neuroprotective target genes.
- The relationships between protein domains and drug substructures were predicted, and gene–domain–substructure–drug relationships were constructed.
- Using computational analysis, the authors’ study quantified gene–drug relationships and constructed a neuroprotective gene–domain–substructure–drug network.
- Using the MCODE algorithm and K-means clustering, the similarity of drug functions was analyzed, and promising drugs were identified.
- The modularity of the network can better aggregate drugs with the same mechanism. Since the drugs and corresponding target proteins are different between the modules, it can be inferred that the mechanisms of action of the drugs are also different. In subsequent studies, the drugs between the various modules can be used in combination to study their potential therapeutic effects.
- Using computational analysis, the relationships between substructures and neuroprotective effect were identified and quantified. Seventy-two substructures with different scores were identified as corresponding to neuroprotective functions.
- In future rational drug design, the specificity of drug substructures can be used to retain the therapeutic effects, delete the substructures that produce side effects and optimize druggability.
- In in vitro experiments, the authors’ study demonstrated that benidipine and barnidipine exerted neuroprotective effects in ischemic injury by inhibiting apoptosis and oxidative stress.

References

Papers of special note have been highlighted as: ● of interest; ●● of considerable interest


Establishes a gene (protein)–domain–substructure–drug relationship network based on the association of domain and substructure.


- Confirms that the glutathione metabolism pathway is related to antiapoptotic and antioxidative functions.


