Systems biology

Screening novel drug candidates for Alzheimer's disease by an integrated network and transcriptome analysis

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Abstract

Motivation: Alzheimer's disease (AD) is a serious degenerative brain disease and the most common cause of dementia. The current available drugs for AD provide symptomatic benefit, but there is no effective drug to cure the disease. The emergence of large-scale genomic, pharmacological data provides new opportunities for drug discovery and drug repositioning as a promising strategy in searching novel drug for AD.

Results: In this study, we took advantage of our increasing understanding based on systems biology approaches on the pathway and network levels and perturbation data sets from the Library of Integrated Network-Based Cellular Signatures (LINCS) to introduce a systematic computational process to discover new drugs implicated in AD. Firstly, we collected 561 genes that have reported to be risk genes of AD, and applied functional enrichment analysis on these genes. Then, by quantifying proximity between 5595 molecule drugs and AD based on human interactome, we filtered out 1092 drugs that were proximal to the disease. We further performed an Inverted Gene Set Enrichment analysis on these drug candidates, which allowed us to estimate effect of perturbations on gene expression and identify 24 potential drug candidates for AD treatment. Results from this study also provided insights for understanding the molecular mechanisms underlying AD. As a useful systematic method, our approach can also be used to identify efficacious therapies for other complex diseases.

Supplementary information: Supplementary data are available at *Bioinformatics* online.

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1 Introduction

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative disorders and it accounts for the majority of people diagnosed with dementia (Ballard, et al., 2011). As a progressive and chronic neurological disease, AD has its origins decades before the initial symptoms of cognitive decline begin to appear. Currently, AD affects about 6% of people of 65 years and older (Burns and Iliffe, 2009), and is responsible for about half million deaths per year around the world (Lozano, et al., 2012). The prevalence rate of AD is increasing rapidly as the world's population ages and more people enter the major risk period for this age-related disorder. It is estimated that the total number of affected individuals will increase from the current about 30 million to a staggering 100 million by 2050 (Cummings, et al., 2017). In addition to affecting the life quality of those suffering from the disorder and their families, AD also causes severe burden to the society. In the USA alone, the health-care costs related to AD is about $172 billion per year (Reitz and Mayeux, 2014), and will grow to an unsupportable $1 trillion annually in 30 years (Cummings, et al., 2017).

Currently there is no meaningful pharmaceutical or other intervention that can successfully treat AD. Alzheimer's drugs are among the major strategies to help the patients to maintain mental function, manage behavioral symptoms, and slow down the development of the disease. There are two types of drugs approved by US Food and Drug Administration (FDA) to treat people diagnosed with AD, i.e., the cholinesterase inhibitors or the N-methyl D-aspartate (NMDA) antagonist. The cholinesterase inhibitors (i.e., donepezil, galantamine, and rivastigmine) can boost the amount of acetylcholine available to nerve cells and maintain cholinergic transmission by preventing its breakdown in brain (Birks and Grimley Evans, 2015; Howard, et al., 2015; Prvulovic, et al., 2010; Unzeta, et al., 2016). NMDA antagonist, on the other hand, is prescribed to treat moderate to severe AD by regulating glutamate, an excitatory neurotransmitter that may lead to brain cell death when produced in excessive amounts (Howard, et al., 2015; Savelieff, et al., 2019). However, these drugs don't work for everyone (Strohle, et al., 2015). Although they can improve symptoms such as mood swings or dyskinesia, they cannot halt AD progression nor improve memory performance in patients (Mangialasche, et al., 2010; Schneider, et al., 2014). At the same time, antibody therapies aiming to normalize the levels of amyloid beta and tau proteins in the brain have also been developed. But many clinical trials intended to reducing amyloid levels have not reached significant improvement in memory performance, or caused adverse effects (Huff and Kardon, 1989). Therefore, there is an urgent need to develop new treatments for AD (Cummings, et al., 2017; Loera-Valencia, et al., 2019). However, the

failure rate in AD drug development is very high, with 99% of trials showing no drug-placebo difference (Cummings, et al., 2019). Under such a situation, the repositioning of available drugs for other disorders as potential novel therapeutic agents for AD becomes an attractive strategy (Chand, et al., 2018; de Castro, et al., 2018; Jojo and Kuppusamy, 2019; Kabir, et al., 2019; Kabir, et al., 2019).

Drug repositioning, the application of established drug compounds to new therapeutic indications (Langedijk, et al., 2015), offers an alternative drug discovery route and has been successfully used to develop therapies in multiple areas (Nosengo, 2016; Rena and Lang, 2018; So, et al., 2017; Würth, et al., 2016). Compared with approaches focusing on developing novel therapeutic compounds, drug repositioning has some obvious advantages, e.g., the established safety of the candidates, substantial reduction in time and cost required to advance a candidate treatment into clinical trials (Pushpakom, et al., 2019). Especially, computational drug repositioning has become a promising and efficient tool for discovering new uses from existing drugs in the age of big data (Li, et al., 2016). Traditional drugs usually target the symptoms of a disease, and lack selectivity towards the genetic cause (Delavan, et al., 2018; Pushpakom, et al., 2018). The explosive growth and accumulation of data related to genomic, phenotypic and small molecular compounds, makes computational repositioning not only necessary, but also feasible.

In this study, in order to search novel candidate compounds for treating AD, we first collected the genes associated with the disease, then screened potential drugs by a network analysis on the AD-related genes and drug targets, and further filtered the drug candidates on the basis of their gene expression profiles. We eventually obtained a handful of hits that may have efficacy for AD treatment.

2 Methods

The procedure utilized in this study included the following steps: 1), collection of AD-related genes; 2), collection of available drugs; 3), screening drugs by their proximity to AD-related genes on the interactome network; 4), filtering the screened drugs based on their correlation with gene expression profiles (Fig. 1).

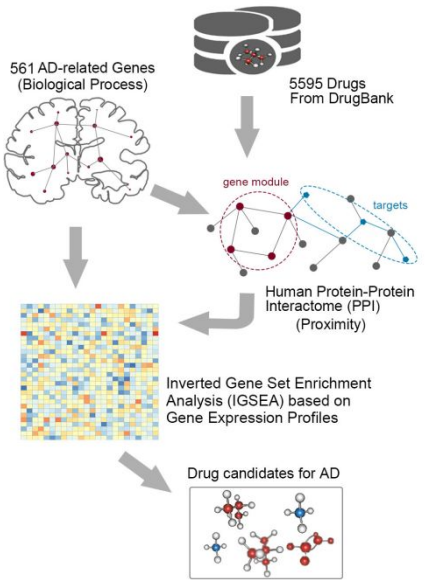

Fig. 1. The systematic framework of the approach used to identify potential therapies for AD.

2.1 Collection of AD-related genes, drug candidates and protein-protein interactome

Genes associated with AD (AD-related genes) were retrieved from Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/), Online Mendelian Inheritance in Man (OMIM) (https://www.ncbi.nlm.nih.gov/omim), and Phenotype-Genotype Integrator (PheGenI) (https://www.ncbi.nlm.nih.gov/gap/phegeni) (Amberger, et al., 2015; Kanehisa, et al., 2017; MacArthur, et al., 2017). Briefly, 171 genes were retrieved from the Alzheimer disease pathway (hsa05010) included in KEGG Pathway Database; 277 genes were retrieved from OMIM by querying the keyword "Alzheimer disease"; another list of 195 genes with genome wide significance (p-value $<5x10^{-8}$) were obtained from PheGenI by selecting the NHGRI genome-wide association study (GWAS) catalog as the source repository. Genes from the three lists were combined and were then mapped onto the official gene symbols of human reported in HUGO Gene Nomenclature Committee (HGNC) (https://www.genenames.org/), and 561 genes were kept as the AD-related genes (Supplemental Table S1).

The drug information was obtained from DrugBank (version 5.1.3, released on April 2, 2019), which contained 13,339 drug entries including 2,593 approved small molecule drugs, 1,289 approved biotech (protein/peptide) drugs, 130 nutraceuticals and over 6,304 experimental drugs (Wishart, et al., 2018; Wishart, et al., 2006). The target genes for each drug were converted into human gene symbols. Drugs without targets were exclude, resulting in a total of 5595 drugs (Supplemental Table S2).

To evaluate the correlation between drugs and AD, we adopted a network-based approach. We compiled a comprehensive human protein-protein interaction (PPI) network. The human interactome data sets were obtained from three databases, including the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (version 11.0: January 19, 2019) (Szklarczyk, et al., 2019), the Protein Interaction Network Analysis (PINA) (latest release version: May 21, 2014)(Cowley, et al., 2012) and the Human Reference Protein Interactome (HuRI) (downloaded on April 11, 2019)(Luck, et al., 2019; Rolland, et al., 2014). Particularly, STRING contained 648,304 edges (physical protein interactions) among 12,396 nodes (proteins/genes), the interactions with 'combined score' smaller than 900 were excluded; PINA contained 166,776 edges among 16,182 nodes; and HuRI involved 52,569 experimental validation interactions of 8,275 proteins. We then merged the three interactome data sets by excluding the self-interacting and redundant pairs. Meanwhile, the proteins in the list were mapped onto approved gene symbol from HGNC. We ultimately compiled a relatively comprehensive human physical interactome consisting of 493,232 interactions among 17,232 proteins/genes.

Moreover, we obtained drug-induced gene expression profiles from the Library of Integrated Network-based Cellular Signatures (LINCS; https://commonfund.nih.gov/LINCS/) L1000 dataset, the next generation Connectivity Map (CMap)(Lamb, et al., 2006), which aimed to create a network-based understanding of biology by cataloging changes in gene expression and other cellular processes that occurred when cells were exposed to a variety of perturbing agents (Subramanian, et al., 2017). In order to ensure the accuracy of the results, here we collected those perturbational data sets of 10,174 Best Inferred Genes (BING), including 978 landmark genes and 9,196 inferred genes.

2.2 Biological function enrichment analysis of AD-related genes

Functional enrichment analysis can help us gain insight about the mechanisms underlying the gene set of interest by identifying biological

processes, pathways or molecular functions enriched in the genes (Hu, et al., 2017). We performed functional enrichment analysis by using ClueGO (Bindea, et al., 2009), a Cytoscape (Shannon, et al., 2003) app that can improve biological interpretation of large list of genes. In this work, only the Gene Ontology (GO) biological process terms with false discovery rate (FDR) value smaller than 10^{-4} were kept as the significantly enriched terms. Network analyses were performed using Cytoscape version 3.7.1.

2.3 Network-based proximity between drugs and AD

A network-based approach could be used to analyze the relationship between drugs and diseases, in which the molecular interactions in biological systems were modular in the context of PPI network (Vidal, et al., 2011; Zhao and Zhao, 2013). Such a strategy could also be used in the repositioning of existing drugs for novel therapies, with the candidate drugs screened according to their characteristics similarity to known drugs used in a disease (Alaimo and Pulvirenti, 2019). In such an approach, correlation between drugs and the disease was measured by the network distance. Specifically, an unsupervised framework could be used to quantify the therapeutic effect of drugs by measuring proximity value between drugs and diseases, which had been proven to be unbiased and effective (Guney, et al., 2016). Here, we employed a similar method to evaluate distance between drugs and AD. Given S, the set of AD-related genes; D, the degree of AD-related genes in the PPI; T, the set of drug target genes and distance $d(s,t)$, the shortest path length between nodes s (AD-related gene in our case; $s \in S$) and t (drug target in our case; $t \in T$) in the network, as below:

$$d(S,T) = \frac{1}{|T|}\sum_{t \in T} \min_{s \in S}\left(d(s,t) + w\right) \qquad \text{(Eq.1)}$$

where w, the weight of a target gene, was defined as $w = -\ln(D+1)$ if the target was one of the AD-related genes; else, $w = 0$.

To assess the significance of association between a drug and AD, we generated a simulated reference distance distribution corresponding to the drug. Briefly, a group of proteins (denoted as R) matching the size of drug targets were randomly selected in the network, then the distance $d(S,R)$ (defined by Equation 1) between these simulated drug targets (representing a simulated drug) and AD-related genes was calculated. The reference distribution was generated by repeating the procedure for 10,000 times. The mean $\mu_{d(S,R)}$ and standard deviation $\sigma_{d(S,R)}$ of the reference distribution were utilized to convert the observed distance corresponding to the real drug into a normalized distance, i.e., proximity value:

$$z(S,T) = \frac{d(S,T) - \mu_{d(S,R)}}{\sigma_{d(S,R)}} \qquad \text{(Eq.2)}$$

3 Inverted Gene Set Enrichment Analysis of drug signatures

Genes associated with the same disease usually were more closely related to each other, and hence, also likely to have similar or correlated expression patterns. Consequently, when the disease status exists, these disease-related genes tended to collectively have an expression pattern divergent from the normal conditions. On the other hand, in the presence of drugs effective to the disease, the expression of these genes was driven back to normal levels, i.e., the expression of the overregulated genes was reduced and that of the under-expressed ones was induced by the treatment of the drugs. Thus, for a list of chemicals, their impacts on the expression of disease-related genes reflected their efficacy for treating the disease and could help to screen the potential drug candidates.

Suppose G was a gene set with L members that were known to be associated with a disease. X_1, X_2, …, and X_M were matrices of gene expression values, with each matrix containing N genes and their expression levels measured by microarrays or mRNA sequencing. Each matrix comprised samples from two classes. In our case, Class 1 corresponded to control samples and Class 2 corresponded the samples treated by a drug. If the drug was effective to the disease, then a larger fraction of genes in set G would be regulated compared to genes not in this gene set.

A similar problem was the common situation of significance analysis of gene sets, in which the gene sets like pathways significantly associated with a given experimental condition were screened from a list of pre-defined gene sets. A number of methods, e.g., Gene Set Enrichment Analysis (GSEA) (Subramanian, et al., 2005), Significance Analysis of Function and Expression (SAFE) (Barry, et al., 2005) and Gene Set Analysis (GSA) (Efron and Tibshirani, 2007), had been developed for such a task. Methods like GSEA was designed to determine whether a gene set was correlated with given phenotypic classes, but they are usually used to identify the gene sets significantly regulated in a single gene expression dataset. In our case, we needed to screen multiple gene expression datasets to identify the ones in which a single gene set of interest was significantly regulated. From these selected gene expression datasets, we could find potential drug candidates for a disease.

Since our problem could be simplified into a series of single gene set significance analysis problems, a procedure similar to GSEA could be adopted. To avoid confusion with the original GSEA that focused on gene sets screening, we named it Inverted Gene Set Enrichment Analysis (IGSEA) (Fig. 2), in which the AD-related genes were defined as the gene set G to be enriched. For an expression profile that measures the genes' responses to drug treatment vs. control, we adopted a procedure similar to GSEA to detect whether our gene set G was significantly enriched compared with a null distribution corresponding to a set of 1,000 permutated samples. Briefly, a number of gene expression datasets related to the effects of drug treatments were retrieved from LINCS; next, each dataset was analyzed by a customized GSEA with one gene set, and enrichment score (ES) and nominal p-value were used to measure the enrichment magnitude and statistical significance of the AD-related gene set, respectively. Then, multiple comparison correction was performed on the p-values of all the expression datasets, and the FDR was calculated via the method of Benjamini and Hochberg (Benjamini and Hochberg, 1995). The gene set was considered to be significantly enriched for gene expression datasets with FDR less than 0.25, and the corresponding drugs were identified as potential drug candidates for AD.

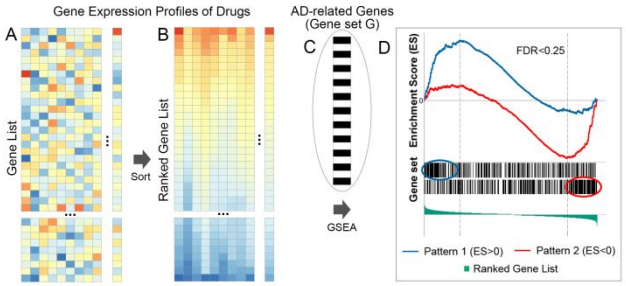

Fig. 2. Pipeline of filtering drugs for AD via Inverted Gene Set Enrichment Analysis (IGSEA). (A) Analyzing the genome-wide expression profile of cell line exposed to a drug screened from network analysis. (B) Ranking the genes in the dataset according to

their response to the drug against control samples. (C) The list of AD-related genes, the gene set S to be checked for significance in the expression data. (D) The significance of the AD-related gene set was measured by the enrichment score, which was calculated based on the distribution of AD-related genes in the ranked gene list via procedure similar to GSEA. Pattern 1 (blue), the AD-related genes were centralized in the front of the list, indicating most genes were up-regulated by the drug in the cell line; Pattern 2 (red), the genes were centralized in the end of the list, indicating most of the genes were down-regulated by the drug in the cell line. For each drug, an empirical p-value was estimated by comparing the enrichment score with a null distribution generated from 1,000 permutations. Then, p-values corresponding to all drugs were pooled for multiple comparison correction and drugs with FDR<0.25 were selected as statistically significant.

4 Results

3.1 Biological process of AD-related genes

Identifying biological process enriched in the risk genes would provide meaningful information to give insight on the molecular mechanism underlying AD. In the current study, we collected 561 risk genes of AD (Supplemental Table S1), and 19 main GO biological processes were detected (Supplemental Table S3). Among the GO terms, amyloid-beta metabolic process, regulation of amyloid precursor protein catabolic process, regulation of protein oligomerization and regulation of nervous system development were included. Many proved risk genes of AD were identified to be related to GO terms mentioned above (e.g. APOE, APP, PSEN1, and PSEN2)(Verheijen and Sleegers, 2018). These results were consistent with previous studies suggesting that amyloid-β (Aβ) played important role in AD pathogenesis (Ittner and Götz, 2011). It worth noting that calcium-mediated signaling process was top ranking. Previous studies had signified that metal ions were involved in biologically vital processes, such as signal transmission, catalysis, stabilization of proteins' structures, and metabolism (Kepp, 2012). Therefore, we posited that the dyshomeostasis and miscompatmentalization of calcium ions might contribute to AD pathology. In addition, processes of proteolysis, second-messenger-mediated signaling, endopeptidase activity were also enriched. The diversity in the function of AD-related genes demonstrated the complexity of the disease. More importantly, the above results indicated the genes we collected here were representative and could be used to explore the molecular characteristics of AD.

3.2 Proximity between drugs and AD in the interactome network

The interactome would highlight non-obvious drug-disease associations in which the drug did not directly interact with known disease proteins. To investigate the relationship between drug targets and disease proteins (proteins encoded by genes associated with AD), we applied a relative proximity value to quantitatively measure the network-based relationship between drugs and the disease-related proteins. Proximal drugs were more likely to be therapeutically beneficial than distant drugs that usually correspond to palliative treatments. By the way, the weight value w was introduced for the risk genes of AD. Through this procedure, we excluded those irrelevant drugs with AD in the network, and constructed a rank list of the proximal drugs. We explored the distribution of the distance between drug targets and AD-related proteins (Fig. 3). The distribution of distance of drugs to AD-related genes was different from that for reference sets (simulated drugs) in the range of -4.0~4.0. The density curve of both real drugs and simulated drugs reached the summit at the point of 1.0, and followed by two smaller peaks, suggesting that the distances corresponding to most members were around this value. Drugs falling in the smaller peaks were less likely to be drug candidates for AD since much of the two distribution curves overlapped in this range. On the other hand, the density curves of drugs and reference sets dropped sharply near the point 0.8, but the density of the reference sets was much smaller after the point. In other words, drugs with distance smaller than 0.8 might be more likely to be effective for AD treatment. Thus, we selected this value as the threshold to screen the drug candidates for AD. When the distance was converted into proximity value, a drug was defined as proximal to the AD as long as the corresponding $z \leq -0.2$. In this way, we excluded those drugs that were distinctly irrelevant to AD and retained sufficient number of reliable drugs. Finally, 1092 drugs were considered to be close to AD (Supplemental Table S4). Currently, there are four FDA-approved treatments for AD, i.e., donepezil, rivastigmine, galantamine and memantine. Three of the drugs (donepezil, rivastigmine, and memantine) were included in the 1092 drug lists, indicating the method used here was effective in screening Alzheimer's drugs.

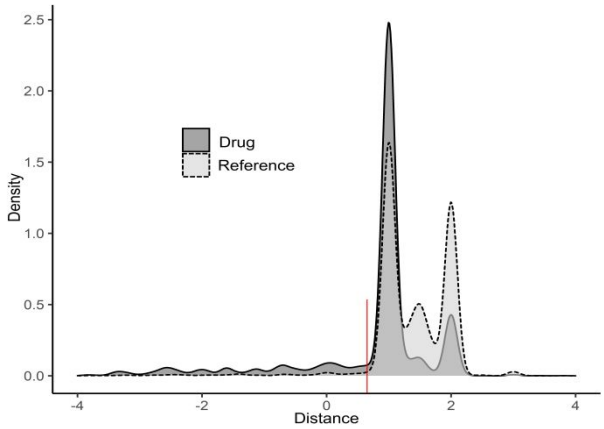

Fig. 3. Proximity between drugs and AD. Distance distribution of all 5595 drugs compared with that of the null distribution. The distribution of distance of drugs to AD-related genes was overlapped with but different from that for the reference sets. The density curves of drugs and the reference sets dropped suddenly near the point 0.8, but the density for the reference was much smaller than the drug set in the left part of the curves, which meant drugs with distances smaller than 0.8 might to be more likely to be Alzheimer's drugs. This value (corresponding proximity value z was about -0.2) was selected as the threshold to screen the drug candidates for AD.

3.3 Interpreting in drugs signatures

The network analysis on the relationship between drugs and AD helped us identified novel drug-AD associations; we then filtered the drug candidates by checking their effect of the transcriptome of neural cell lines. From the LINCS database, we retrieved the gene expression datasets on the effects of the screened drugs on various concentrations and cell lines. Given the fact that AD was a chronic neurodegenerative disease, we further narrowed our selection to neural cell lines. Of 1092 drugs, 148 were tested on two neural cell lines (i.e., NEU, NPC), with a total of 295 gene expression datasets extracted (Supplemental Table S5). Via IGSEA, we identified 24 drugs with FDR<0.25 (Table 1), which meant they had higher impacts on the expression of AD-related genes in the tested neural cell lines. All these drugs had target genes overlapped with the AD-related genes or targets of known Alzheimer's drugs. For example, tubocurarine targeted on genes including ACHE, HTR3A, CHRNA2, CHRNA7, which were also targets of donepezil and memantine. Besides, targets of drugs like fluphenazine, lisuride, nefazodone and cisapride also overlapped with tubocurarine.

5 Discussion

While there was still no therapy to cure AD due to its complex pathology, some medicines could be used to manage the symptoms and

slowed down the development of the disease. In recent years, the repositioning of drugs for other disease as potential therapeutic agents for AD had drawn much attention (Chand, et al., 2018; de Castro, et al., 2018; Jojo and Kuppusamy, 2019; Kabir, et al., 2019; Kabir, et al., 2019). In the current study, we adopted a computational framework to reposition potential drugs for AD based on by integrating interactome network and transcriptome analysis.

Since drugs usually selectively acted on specific targets to exert their biological function, we collected the genes associated with AD as the representation of its molecular features. Biological function enrichment analysis on these genes revealed that GO terms like amyloid-beta metabolic process and several pathways related to signaling transduction were involved in progression of AD, which was consistent with our understanding on the molecular mechanisms of AD and provided a foundation for explore novel drugs for the disease.

AD was generally considered to be a complex disease, which was caused by defects or aberrant activity in multiple genes. Earlier studies shown that the disease-related genes were not randomly distributed in the interactome; instead, they agglomerated into modules that correspond to neighborhoods of the interactome (Barabási, et al., 2011). By measuring the relationship between drug targets and AD-related genes on the PPI network, we could exclude drugs with low proximity to AD-related genes from the drug list extracted from Drug Bank, and kept the potential drug candidates. Among the four FDA-approved medicines for treating AD (i.e., donepezil, rivastigmine, galantamine and memantine), three (donepezil, rivastigmine, and memantine) were found to be closely related to AD by the network analysis. Some drugs used for neural disorders were included the identified candidates; for instance, milnacipran (Yokoyama, et al., 2014), a selective serotonin and norepinephrine reuptake inhibitor (SNRI) for depression, and tacrine (Horak, et al., 2017), a cholinesterase inhibitor used in the treatment of mild to moderate dementia of the Alzheimer's type and other central nervous system disorders. In addition, many drugs that were investigated for treating AD showed high proximity to the AD gene module, including acetylcysteine, huperzine A, methylene blue, milnacipran, NADH, choline, aripiprazole, edonerpic, Mito-4509, CAD106, EVT-101, huperzine B, ganstigmine, E-2012, phenserine, CTS-21166, arundic acid, GTS-21, VP025, tesofensine, encenicline, xaliproden, zanapezil, tideglusib, verubecestat, CX717. Notably, here we choose $d(S,T)<0.8$ as the threshold to measure whether a drug was proximal to AD based on distribution of distance. Although this was an empirical selection, it could effectively reduce the number of drugs to be screened in the following steps.

Previous studies show that the cardinal features of AD pathology are amyloid plaques and neurofibrillary tangles. In addition, neuropil threads, dystrophic neurites, associated astrogliosis and microglial activation are also common symptoms of AD. Hence, we selected perturbational signatures of neural cell lines to perform IGSEA analysis. This would help us to identify the potential repositioning drug candidates. Of the drugs screened by network approach, 148 drugs had gene expression data in two neural cell lines (NEU and NPC) in LINCS, which were further filtered by the IGSEA according to their impacts on the expression of AD-related genes. According to the concept of pharmacogenomics, a drug effective for a disease should be able to affect the expression of the disease genes (Ma and Lu, 2011). Via such a process, we identified a number of candidates for which there were encouraging evidence to support their potential as treatments for AD. As a result, 24 drugs were kept after the filtration. Some of these drugs were originally approved drugs for neurological disorders (e.g. depression, Parkinson's disease), such as fluphenazine, loxapine, nefazodone, tolcapone. While some had been tested to treat mental illness. For example, formoterol was usually used for asthma, reversible obstructive airways disease, and bronchospasm. Recently, it was found to be able to reverse streptozotocin-induced alteration in acetylcholine and glutamate levels and provided protection to brain by combating neuro-inflammation and retarding the development of the main pathological hallmarks in mouse model of sporadic AD (Abdel Rasheed, et al., 2018). Nimodipine was originally developed for the treatment of high blood pressure, but now it was mainly used for preventing a major complication of subarachnoid hemorrhage termed vasospasm. As a calcium channel blocker, its potential role in AD treatment drawn much attention (Ishii, et al., 2019; Nimmrich and Eckert, 2013; Pourbadie, et al., 2017). Xaliproden, a 5HT1-A receptor antagonist that might enhance cognition by stimulating release of acetylcholine and glutamate, is studied for treating amyotrophic lateral sclerosis (ALS) and AD (Zarin, et al., 2019). Thus, our work provided further evidence to their potential efficacy for AD treatment. Moreover, the current available Alzheimer's drugs usually did not address the underlying disease process or their molecular mechanisms; instead, they mainly focused on addressing some symptoms. In this line, a complementary strategy based on repositioning drugs which were approved for the treatment of other disorders could provide new clues on drug exploring for AD. Especially, some clinical trials or animal studies indicate that the drugs screened by our approach might have potent and beneficial effects on the AD treatment.

There are some limitations with our method. First, our approach relies on known previously identified genes associated with AD, known drug targets and drug-disease annotations, all of which are still far from complete. Identification of the genes and targets, as well as the accumulation of data related to them, is still an ongoing task and could be a long-term challenge in the development of computational drug repositioning. Second, the human PPI network is extremely complicated and huge; the currently available information might be biased and only covers a relatively small portion of the proteome due to the limitation of experimental techniques and algorithms. Moreover, for the drugs screened by network analysis, only a fraction of them have gene expression data in LINCS, some promising drug candidates might be missed due to the lack of such data. In addition, the process also reflects some discrepancies with the current evidence, particularly where there is no supporting clinical evidence. Although some of the drugs discussed here appear to be good candidates for further investigation, it is still far from certain whether any of them would emerge as successful therapies for AD, and more systematic investigations at molecular and clinical levels are needed. Especially, their efficacy on AD-treatment should be verified by experiment in future.

In summary, by the mean of an integration of molecular network-based approach and pharmacoepidemiologic method, we identified several drug candidates that could potentially be used for treating AD. Although the result was still preliminary, it could provide clues for further investigation. The computational framework presented here might provide new ideas for exploring potential drugs of other complex disease.

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Table 1. Predicted drugs for AD

Drug	Indication	Target	Proximity	ES	FDR
cilomilast	chronic obstructive pulmonary disease (COPD)	PDE4D, PDE4B	-0.461	1.427	<0.001
exemestane	advanced breast cancer	CYP19A1	-0.449	1.482	<0.001
imatinib	leukaemia, malignant gastrointestinal stromal tumors (GIST)	KIT, RET, NTRK1, CSF1R, PDGFRA, DDR1, ABL1, PDGFRB	-0.237	1.481	<0.001
loxapine	psychotic disorders, such as schizophrenia	HTR, ADR, CHRM, DRD, HRH, SLC6A	-0.304	1.517	<0.001
nimodipine	neurologic outcome following subarachnoid hemorrhage (SAH)	CACN, NR3C2, AHR	-0.427	-1.535	<0.001
phenoxybenzamine	phaeochromocytoma, prostatic hypertrophy and essential hypertension	ADR, CALM1	-0.481	1.375	<0.001
staurosporine	-	LCK, PIM1, ITK, SYK, MAPKAPK2, GSK3B, CSK, CDK2, PIK3CG, PDPK1, PRKCQ, ZAP70, CHRM1	-0.248	-1.498	<0.001
tamoxifen	metastatic breast cancer and ductal carcinoma in Situ	ESR1, ESR2, EBP, PRKCA, AR, KCNH2, NR1I2, ESRRG, SHBG	-0.220	1.599	<0.001
tubocurarine	myasthenia gravis	CHRNA2, HTR3A, ACHE, CHRNA7	-0.472	1.516	<0.001
hexachlorophene	skin cleanser, gram-positive infection	SDHD, GLUD1, ESR1	-0.519	-1.371	0.202
norepinephrine	vasodilatory shock states, critical hypotension	ADR, PAH, SLC18A2, SLC18A1	-0.235	1.360	0.176
xaliproden	amyotrophic lateral sclerosis (ALS) and Alzheimer's disease	HTR1A	-1.189	-1.381	0.163
formoterol	reversible obstructive airways disease, bronchospasm	ADRB2, ADRB1, ADRB3	-0.585	1.390	0.168
metoclopramide	gastroesophageal reflux disease (GERD), nausea and vomiting	DRD2, CHRM1, HTR4, HTR3A, ACHE	-0.307	-1.370	0.153
mocetinostat	-	HDAC1, HDAC3, HDAC2	-0.505	-1.297	0.163
rescinnamine	hypertension	ACE	-0.655	1.306	0.169
glucosamine	osteoarthritis	MMP9, NFKB2, TNF, IFNG	-0.465	-1.345	0.168
tolcapone	Parkinson's Disease	COMT	-0.776	1.353	0.193
alprenolol	hypertension, angina, and arrhythmia	ADRB1, ADRB2, HTR1A, ADRB3	-0.736	-1.309	0.192
raloxifene	osteoporosis	ESR1, ESR2, SERPINB9, TFF1	-0.381	1.272	0.193
fluphenazine	psychotic disorders	DRD2, DRD1, CALM1, AR, HTR2A, HTR2C	-0.736	1.248	0.228
nadolol	arrhythmias, angina pectoris, and hypertension	ADRB1, ADRB2	-0.801	1.309	0.222
nefazodone	depression	HTR, SLC, ADR, KCNH2	-0.555	1.257	0.236
dextromethorphan	dry cough	SIGMAR1, SIGMAR1, GRIN3A, CHRN, SLC6A, PGRMC1, OPR, CYBB	-0.298	1.290	0.238