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Identification of novel immune-relevant drug target genes for Alzheimer's Disease by combining ontology inference with network analysis

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Abstract

Aims: Alzheimer's disease (AD) is one of the leading causes of death in elderly people. Its pathogenesis is greatly associated with the abnormality of immune system. However, only a few immune-relevant AD drug target genes have been discovered up to now, and it is speculated that there are still many potential drug target genes of AD (at least immune-relevant genes) to be discovered. Thus, this study was designed to identify novel AD drug target genes and explore their biological properties.

Methods: A combinatorial approach was adopted for the first time to discover AD drug targets by collectively considering ontology inference and network analysis. Moreover, a novel strategy limiting the distance of reasoning and in turn reducing noise interference was further proposed to improve inference performance. Potential AD drug target genes were discovered by integrating information of multiple popular databases (TTD, DrugBank, PharmGKB, AlzGene, and BioGRID). Then, the enrichment analyses of the identified drug targets genes based on nine well-known pathway-related databases were conducted to explore the function of the identified potential drug target genes.

Results: Eighteen potential drug target genes were finally identified, and 13 of them had been reported to be closely associated with AD. Enrichment analyses of these identified drug target genes, based on nine pathway-related databases, revealed that the enriched terms were primarily focus on immune-relevant biological processes. Four of those identified drug target genes are involved in the classical complement pathway and process of antigen presenting.

Conclusion: The well-reproducible results showed the good performance of the combinatorial approach, and the remaining five new targets could be a good starting point for our understanding of the pathogenesis and drug discovery of AD. Moreover, this study supported validity of the combinatorial approach integrating ontology inference with network analysis in the discovery of novel drug target for neurological diseases.

KEYWORDS

Alzheimer's disease, drug target, immune, network analysis, ontology inference

1 | INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia and one of the leading causes of death in elderly people, which is characterized by neurofibrillary tangles and amyloid β-peptide (Aβ) deposits in brain.[1,2] Based on two independent studies, the number of people newly diagnosed with AD is expected to reach 135 million around the world and 16 million in USA by 2050, respectively.[3,4]

So far, the deficiency of innate immune ability to clear Aβ deposits (rather than overproduction of them) has been widely accepted as one of the most important causes of AD pathogenesis, and current research was gradually moved from blocking the production of Aβ deposits to rebalancing the immune system of AD patients.[5–7] For example, interleukin (IL)-10, one of the best known inflammatory cytokines, was a major regulator of macrophages.[8] Recent study showed that the inhibition of the IL-10 pathway could rebalance the innate immunity and mitigate Alzheimer-like pathology.[9] Another member of interleukin family, IL-33, was found to play a potential therapeutic role for AD. A previous study reported that IL-33 could modulate the innate immune responses to reduce the accumulation of Aβ deposits and reverse the impairment of memory and synaptic plasticity in AD mouse.[10] The genome-wide association study found that a cluster of genes implicated in innate immune pathways was upregulated together with the downregulation of synaptic plasticity genes in AD patients.[11,12] Moreover, two lymphatic systems involved in the clearance of Aβ deposits in AD were reported. In particular, glymphatic (glial+lymphatic) system was a critical contributor to the clearance of interstitial solutes (including Aβ deposits) from the brain,[13] and the function of glymphatic transport was suppressed in a mouse AD model.[14] The malfunction of meningeal lymphatic vessel (the other cleaner of Aβ deposits closely related to lymphatic system) was found to be greatly associated with AD pathogenesis.[15–17] In the meantime, some immunotherapies and drugs for Aβ clearance have attempted to control progress of AD.[18–20] For example, a monoclonal antibody-based immunotherapeutic drug was reported under development in recent year, and it selectively targeted and cleared the aggregated Aβ in brain and effectively relieved the symptom of AD.[21–23]

However, only a few of drug target genes of AD implicated in immunity had been discovered up to now. Specifically, among the known target genes of therapeutic drugs for AD, only four of them (about 10.5%) belonged to the family of innate immune genes (Table S1, based on the information of InnateDB with over 1500 innate immune genes collected by literature review).[24] Moreover, the studies showed that the total number of the known AD drug targets had appeared to be also not complete enough, considering the wide range of pathologic features.[25] In fact, only five drugs have got FDA approval for the treatment of AD, and they target primarily on two therapeutic targets: *Acetylcholinesterase* and *NMDA receptor*.[26] Since 2003, no new target has been approved for treating AD. All the facts implied that there were still potential target genes of AD (especially the innate immune genes) remaining to be discovered.

Due to the time-consuming and extremely high cost of modern drug discovery, computational methods have emerged as one of the most effective approaches for the discovery of new targets.[27–32] However, these computational methods focused mainly on single biologic perspective, such as pathway profile-based,[33] gene expression-based,[34–36] and similarity-based[37–40] methods. Important information might be neglected by these methods, as many essential relationships (such as gene-gene, gene-disease, and disease-drug) systematically contributed to the association between the disease and their corresponding drug targets.[41] As reported, target druggability was found to be collectively defined by target's disease relevance and its roles in human protein-protein interaction network,[42] and a novel strategy integrating ontology inference and network analysis was thus proposed to predict the candidate targets of colorectal cancer (CRC).[41] In this study, the inference performance of this published method[41] was substantially enhanced by limiting the distance of reasoning and in turn reducing noise interference (prediction accuracy of ontology-based inference was reported to be highly dependent on the distance from initial nodes).[43] Then, relationships among drug, gene, SNP, disease, and haplotype were integrated by combining ontology-based inference and biological network analysis to discover potential target of AD. Finally, the enrichment analysis to the in-depth investigation of the biological functions for AD was conducted. In conclusion, this was the first discovery of drug targets for neurological disease by improving the inference performance of the newly proposed combinatorial method, and the novel candidate target genes identified in this study did provide significantly added values to the discovery of drugs for treating AD.

2 | MATERIALS AND METHODS

2.1 | Collection of AD drugs and their known targets

The drugs approved by US FDA or in clinical trial for the treatment of AD were first collected from the Therapeutic Target Database (TTD)[44] which contained 31 614 drugs and 2589 targets covering over 125 diseases after its last update in 2018.[26] Then, the drugs that did not belong to PharmGKB database were removed, and full list of AD drugs was obtained for further analyses. The PharmGKB was a preeminent worldwide resource and web interactive tool for the knowledge of pharmacogenomics. It was funded by the National Institute of General Medical Sciences (NIGMS) and the National Institutes of Health (NIH), and the relationship data of PharmGKB could be used to research how the genetic variations affected the response of drugs.[45] For example, rs1800460 polymorphism was related to azathioprine, mercaptopurine, thioguanine based on the data of PharmGKB, and many studies reported this variant could cause adverse reactions to these drugs.[45–47] In this study, PharmGKB relationship data between drugs and drugs, drugs and haplotypes, drugs and SNPs, drugs and genes, diseases and genes, diseases and haplotypes, diseases and SNPs, diseases and diseases, genes and haplotype were collected for subsequent study. Finally, the known targets of the selected AD drugs were extracted from both TTD and DrugBank.[48,49]

2.2 | Discovery of the candidate AD target genes using ontology inference

Ontology was a hierarchically organized structure about existences, types of them and their relationships, according to their innate logic. Based on ontology, semantic web technology was developed to integrate and reason the heterogeneous data. As a machine-readable standard ontology language of semantic web technique, the web ontology language (OWL) effectively inherited triple model (subject-predicate-object) from resource description framework (RDF).[50–54] Thus, through integrating and reasoning relationships among drugs, genes, SNPs, diseases, and haplotypes, the OWL could find the genes directly or indirectly connected with an AD drug, and these genes were defined as the candidate AD target genes. According to the newly proposed strategy,[41] in OWL network, all of the drugs, genes, SNPs, diseases, haplotypes from PharmGKB were defined as nodes and the relationships of them were defined as links based on the Portégé (an editor and reasoner based on OWL). Then, the AD-related drug (ADDrug), gene (ADGene), SNP (ADSNP), disease (ADDisease), and haplotype (ADHaplotype) were defined according to the OWL description logic rule. Specifically, a gene belonged to the class of ADGene if it was associated with one of these defined concepts (ADDrug, ADGene, ADSNP, ADDisease, and ADHaplotype) according to the relationships in PharmGKB, where other concepts were defined similar as the ADGene. The distance of reasoning for an AD drug (ie the number of nodes away from the AD drug node) was limited according to the location containing the most number of their known target genes.

2.3 | Collecting the AD disease genes

To further identify the potential AD target genes from AD target genes, the levels of association between known AD disease genes and each of the candidate AD target genes were compared and ranked. The AD disease genes were searched in the AlzGene, a publicly available database collecting AD genetic variants from the publications of genetic association study about AD. To the best of our knowledge, the AlzGene is so far the only database specialized in the selection and analysis of AD risk genes, and its latest version (updated in 2011) contained 695 disease genes, 2973 polymorphisms, and 320 systematic meta-analyses performed for genotype data (including at least three case-control samples).[55] To ensure the credibility of this study, the significantly associated genes with AD (95% confidence interval of OR values should not include 1)[56] were selected for the subsequent analyses based on the results of the meta-analyses.

2.4 | Discovering potential AD target genes via association level test and aggregation rank

Based on the hypothesis that the drugs will more efficiently work on some disease genes if they show a tighter connection,[41] the levels of association between each of the candidate AD target genes and the AD disease genes were assessed to identify potential AD target genes. The human protein-protein interaction (PPI) network was thus collected for conducting such calculation. The BioGRID (Mount Sinai Hospital, Toronto, ON, Canada) was a monthly updated comprehensive PPI network database.[57] It captured 836 212 nonredundant biological interactions from 57 058 published biomedical articles involving all major organisms and human beings by September 2016. Moreover, the different reliability of each interaction was provided in the BioGRID based on available evidences that how many independent studies consistently support the result. First, human PPI data were download from BioGRID (version 3.4.140), and the high reliability data supported by at least two independent studies were selected. Second, after removing self-interaction data, the human PPI networks which centered on each of the candidate target genes were built. Within these networks, the nodes directly linked with the candidate target genes were defined as the first-degree neighbor. Similarly, the nodes linked with the first-degree neighbors (except the candidate AD target genes) were defined as the second-degree neighbors, and the nodes linked with the second-degree neighbors (except first-degree neighbors) were defined as the third-degree neighbors, etc. Thirdly, the previous studies showed that the disease genes were mainly enriched in the first three degree neighbors of the drug target genes.[58,59] Thus, the AD disease genes to the first-, second-, third-degree neighbors of the PPI networks were mapped, and their percentages in each neighbor to assess the levels of association between each of the candidate AD target genes and AD disease genes were calculated. Specifically, for a given AD candidate gene, there were N genes in its first-degree neighbor, and of which n genes belong to AD disease genes. Then, the closer that this ratio (n/N) was to 1, the more significant association was built between this given AD candidate gene and AD disease genes. Thus, this given candidate gene more likely becomes a potential drug target. Similarly, the ratio in other degree neighbors was calculated by this approach. Finally, based on the ratios in the three degree neighbors, the candidate AD target genes were ranked in three lists.

To identify the potential AD drug targets, robust rank aggregation (RRA) method was used to integrate the three lists of rank order. RRA was a computationally efficient and statistically stable order algorithm, and it assigned the *P*-value to measure how well a candidate gene was positioned in the ranked lists than expected by chance.[60] The R package of RRA (RobustRankAggreg) was available at the Comprehensive R Archive Network (https://www.icesi. edu.co/CRAN/web/packages/RobustRankAggreg/).

2.5 | Enrichment analysis of the potential AD drug target genes

AD was reported to be closely related to immune system,[5,61] but very little of immune-relevant AD drug target genes had been discovered comparing with other target genes. Therefore, it was

TABLE 1 Summary of the final list of AD drugs and their known target genes

Drug Name	TTD PharmGKB	Drug Status	Known drug target genes					Mode Value of Degrees	Average Value of Degrees
			Degree 1	Degree 2	Degree 3	Degree 4	Degree 5		
Donepezil	DAP000560 PA449394	Approved	ACHE	NaN	HTR2A	NaN	NaN	1, 3	2
Rivastigmine	DAP000149 PA451262	Approved	ACHE, BCHE	NaN	NaN	NaN	NaN	1	1
Memantine	DAP000493 PA10364	Approved	NaN	NaN	GRIN3A, GRIN2A, GRIN2B, HTR3A, CHRNA7, DRD2, GRIN1	NaN	NaN	3	3
Galantamine	DAP000559 PA449726	Approved	CHRNA4, CHRNA7, CHRNB2, ACHE, BCHE	NaN	CHRNA1, CHRNB1, CHRNA2, CHRNA3, CHRNA5, CHRNA6, CHRNB3, CHRNB4	NaN	CHRND, CHRNG	3	3
Tacrine	DAP000558 PA451576	Approved	NaN	NaN	ACHE, BCHE	NaN	NaN	3	3
Rosiglitazone	DCL000633 PA451283	Phase III	PPARG	NaN	NaN	NaN	NaN	1	1

The nodes directly linked with a certain drug according to the relationships of PharmGKB are defined as degree 1. The nodes linked with this drug through degree 1 are defined as degree 2. Degree 3, degree 4, and degree 5 are defined by similar way. NaN means that the known drug target gene is not in this degree. "Mode value of degrees" means that most of the known drug target genes are found in this degree.

possible that there were more potential immune-relevant genes than others among the undiscovered AD drug target genes. However, it was improper to simply compare the numbers of them due to the different backgrounds (the various sizes of gene sets). So far, these backgrounds (such as human disease, organism system, cellular processes and signaling pathways) were provided in Kyoto Encyclopedia of Genes and Genomes (KEGG) for achieving this comparison.[62] Apart from KEGG, there were several other data sources providing such signaling pathway information, including MetaCyc[63] (database of the metabolic pathways and enzymes), NetPath[64] (resource of curated signal transduction pathways), PathWhiz[65] (database of biology pathways and web server for creating biologically accurate pathway diagram), Pathway Interaction Database PID[66] (collection of curated and peer-reviewed pathways composed of human molecular signaling, regulatory event and cellular process), and WikiPathways[67] (multifaceted pathway database bridging metabolomics to other omics research). Moreover, there were three ontology-based databases offering pathway-related data, including Gene Ontology GO[68] (gene ontology organized by biological process, molecular function and cellular component), PANTHER[69] (gene products organized by biological function), and Reactome[70] (molecular detail of signal transduction, transport, metabolism, and other cellular processes). Apart from PID, the comprehensive data of the remaining eight databases were fully downloadable, and the enrichment analyses of the potential AD drug target genes based on data from these eight databases were thus conducted using the R package clusterProfiler[71] to validate the presumption of this study.

3 | RESULTS AND DISCUSSION

3.1 | Collecting AD drugs and limiting search criteria using their known targets

First, by selecting AD drugs from TTD database[72] and removing the drugs not belonging to PharmGKB database,[73] five approved and one phase III clinical trial drugs were obtained. Second, 23 known targets of these drugs were collected from TTD and DrugBank.[48,49] Third, to limit the distance of reasoning, the number of the known AD target genes was counted in each degree of OWL network. As demonstrated in Table 1, for the selected drugs, their known target genes were mainly concentrated in a specific degree. These results implied that for a given drug, its target genes were mainly distributed in a specific scope of OWL network. In this study, to reduce the effect of noise, the reasoning distance of each drug was thus limited to the degree where most of its known target genes can be found.

3.2 | Discovering the candidate AD target genes using OWL ontology method

An OWL ontology of AD drug, gene, SNP, disease, and haplotype was built based on their relationship in PharmGKB, and the reasoning was performed using Portégé in a specific scope limited by the previous step. The process of reasoning and the result is displayed

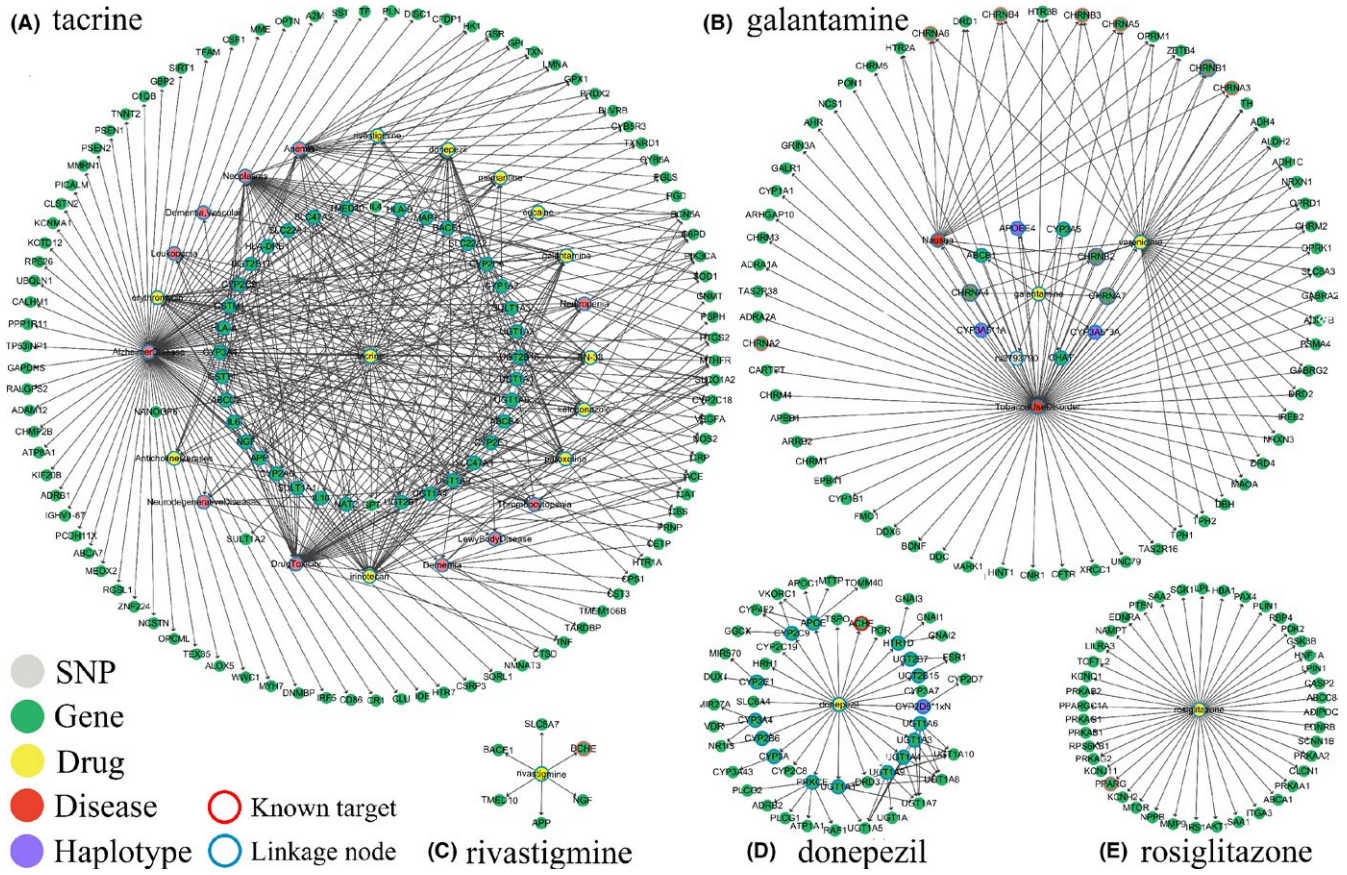

FIGURE 1 Process of discovering the candidate AD target genes from each final selected AD drug. Each part of this figure shows the process of discovery from one of the drugs, respectively. The process of discovery from memantine is shown in Figure S1 because it is relatively complex. Different colors of the nodes mean different data types. In particular, green represents gene, yellow represents drug, red represents disease, purple represents haplotype, and gray represents SNP. The nodes with red and blue circles represent known targets and linkage nodes, respectively. The annulus areas constituted of these nodes represent different discovery degrees. Only the linkage nodes and the discovered candidate target genes are retained in this figure. The duplicate data have been eliminated. This figure can be viewed more clearly by enlarging in the electronic version

in Figures 1 and S1. After eliminating duplicate data, 623 candidate AD target genes directly or indirectly connected with one of the AD drugs were discovered, and 109 of them had been reported to be closely related to AD.

3.3 | Identifying the potential AD target genes by AD disease genes and RRA method

First, 41 AD disease genes confirmed by meta-analysis (including at least three case-control experiments) were collected from AlzGene database (Table S2). Second, 623 human PPI networks of candidate AD target genes were built by BioGRID data. Third, the 41 AD disease genes were mapped to the first-, second-, and third-degree neighbors of the 623 PPI networks, and the percentages of AD disease genes in each degree neighbor were calculated to rank these candidate target genes at 3 different levels. Finally, these three levels of rank were integrated using RRA method. As shown in Table 2, 18 potential AD target genes with significant *P*-values (<0.05) were identified, and of which 13 genes were reported to be closely associated with AD by previous studies.

Taking RELN gene (coding reelin, an extracellular matrix glycoprotein) as an example, it participated in the regulation of neuronal migration, position, growth, and synaptic plasticity linking memory/learning formation,[74] and it could prevent synaptic dysfunction induced by accumulation of Aβ deposits in AD.[75] Some studies reported a significant association between RELN and AD among different populations.[76–78] Another example could be DNMBP, which was a scaffold protein to bring dynamin and actin regulatory proteins together[79] and participated in synaptic vesicle trafficking.[80–82] This process could be disturbed by accumulation of Aβ deposits in AD.[83] Previous studies have reported significant association between DNMBP and AD in Japanese population.[84] The following studies also found a significant association between DNMBP and AD in Belgian and Chinese populations.[85,86]

The distribution of 13 reported genes in the 18 identified potential AD target genes were calculated. The 18 potential target genes identified in this study were first arranged in the descending order of *P*-values of robust rank aggregation (RRA) analysis. Second, the cumulative number N was defined as the first N potential target genes, and the percentage of reported genes in each cumulative number

TABLE 2 Summary of the 18 potential AD drug target genes identified in this study

Gene symbol	Protein name	Gene ID	*P*-value	Reported or NOT
RELN	Reelin	5649	0.000546	Reported
DNMBP	Dynamin binding protein	23268	0.003415	Reported
APOD	Apolipoprotein D	347	0.003759	Reported
LEP	Leptin	3952	0.008264	Reported
HLA-B	Major histocompatibility complex, class I, B	3106	0.011278	Reported
C1QB	Complement C1q B chain	713	0.015038	Reported
HSPG2	Heparan sulfate proteoglycan 2	3339	0.018797	Reported
MTTP	Microsomal triglyceride transfer protein	4547	0.022556	NOT
IL–10	Interleukin 10	3586	0.024793	Reported
TF	Transferrin	7018	0.026316	Reported
NOS1AP	Nitric oxide synthase 1 adaptor protein	9722	0.027164	NOT
A2M	Alpha–2-macroglobulin	2	0.030075	Reported
NEK4	NIMA related kinase 4	6787	0.033058	NOT
CD86	CD86 molecule	942	0.033835	Reported
PIK3C2A	Phosphatidylinositol–4-phosphate 3-kinase catalytic subunit type 2 alpha	5286	0.041353	NOT
ADAM12	ADAM metallopeptidase domain 12	8038	0.044259	Reported
TARBP1	TAR (HIV–1) RNA binding protein 1	6894	0.045113	NOT
GABRG2	Gamma-aminobutyric acid type A receptor gamma2 subunit	2566	0.048872	Reported

was calculated. As shown in Figure 2, the slope of the fitted curve is continually decreasing. This phenomenon reflected a good reliability of the applied prediction method because it was consistent between the significance of potential AD target genes and the possibility of it being an AD associated gene. Specifically, nearly half of the genes reported to be closely associated with AD were gathered in the first 30% identified potential target genes. Then, another phenomenon was observed by comparing candidate and potential AD target genes. Percentage of reported genes were significantly higher in potential AD target genes (about 72.2%) than it in candidate AD target genes (about 17.5%), which further reflect the reliability of the prediction.

3.4 | Enrichment analysis of the potential target genes based on pathway-related databases

To explore the function of those 18 identified potential AD target genes, the KEGG enrichment analysis was conducted. As shown in Table 3, two KEGG pathways were enriched in this analysis by setting the *P*-value cutoff as 0.01, and both belonged to the human immune disease. If the *P*-value cutoff was further set as 0.05, six KEGG pathways could be enriched, and they all belonged to immune disease or immune system. The remaining KEGG pathway (Type I diabetes mellitus) was also an autoimmune disease.[87,88] Altogether, these pathways contained four immune genes: C1QB, HLA-B, IL-10, and CD86. Among these genes, C1QB coded the B chain of complement C1q which was an activator in the classical complement pathway, and the remaining were involved in the process of antigen presenting. Major histocompatibility complex (MHC) is a cell surface protein to bind and present antigen peptide fragments to T cell. Studies demonstrated that the expression of both MHC class I and class II was markedly increased in AD.[89,90] HLA-B is a member of the human leukocyte antigen gene family, and coded a part of heavy chain of the MHC class I.[91] CD86 expresses on most antigen-presenting cells and is a critical co-stimulatory factors for antigen presenting from MHC to T cell.[92] IL-10 is an inhibitory factor of excessive immune response, and strongly downregulates the expression of both MHC class II and CD86.[93–95] A recent study showed that inhibiting the IL-10 pathway could relieve the symptom of AD.[9]

Extensive enrichment analyses on these potential drug target genes based on eight databased providing the pathway information (MetaCyc, NetPath, PathWhiz, PID, and WikiPathways) and

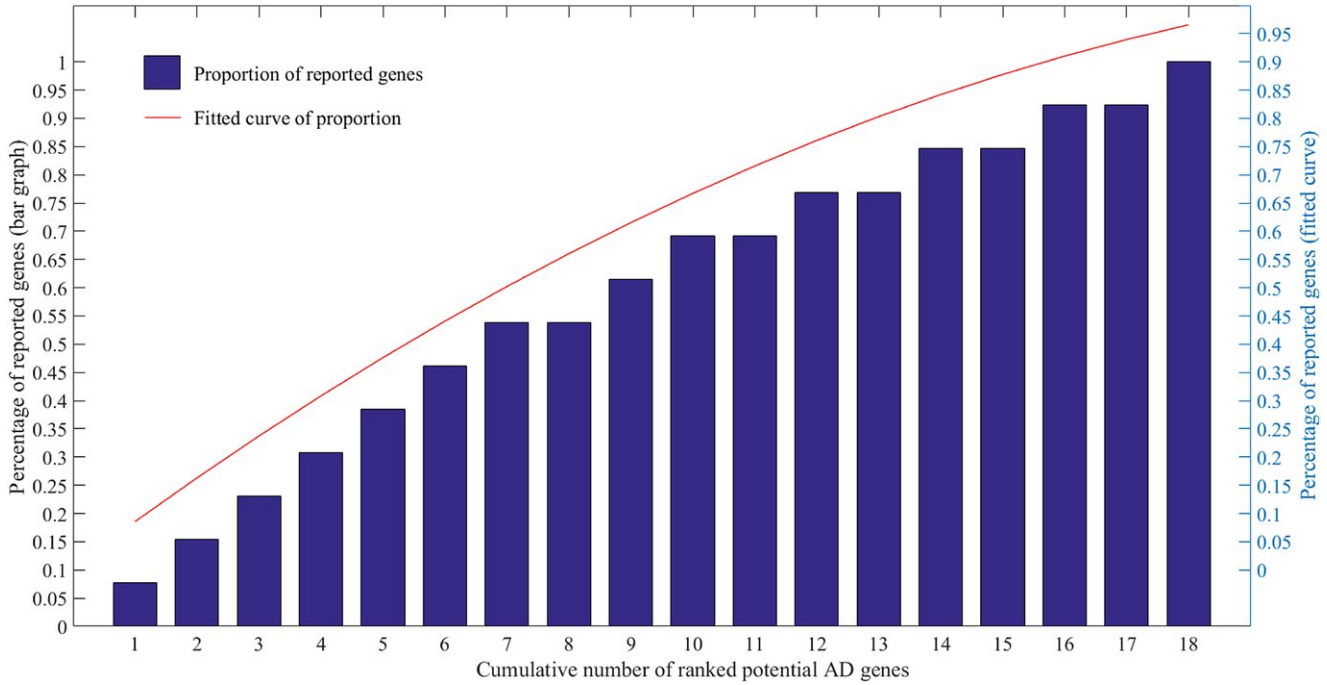

FIGURE 2 The distribution of reported genes among the ranked potential AD target genes. The bar graph shows percentage of reported genes in the first N identified potential AD genes which are ranked by RRA method. For example, there are 53.8% reported genes in first seven identified potential AD target genes. The red line is a fitted curve of the percentage values. The left and right ordinates show the percentage values of bar graph and fitted curve, respectively

TABLE 3 KEGG enrichment results of potential AD drug target genes

KEGG ID	KEGG pathway	Class	Nominal *P*-value	Adjusted *P*-value	Enriched genes
Cutoff *P*-value = 0.01					
hsa05330	Allograft rejection	Immune disease[D]	3.87×10^{-5}	2.13×10^{-3}	HLA-B, IL–10, CD86
hsa05320	Autoimmune thyroid disease	Immune disease[D]	1.06×10^{-4}	2.91×10^{-3}	HLA-B, IL–10, CD86
Cutoff *P*-value = 0.05					
hsa05322	Systemic lupus erythematosus	Immune disease[D]	1.66×10^{-3}	2.93×10^{-2}	C1QB, IL–10, CD86
hsa05332	Graft-versus-host disease	Immune disease[D]	2.42×10^{-3}	2.93×10^{-2}	HLA-B, CD86
hsa04940	Type I diabetes mellitus	Endocrine and metabolic disease[D]	2.66×10^{-3}	2.93×10^{-2}	HLA-B, CD86
hsa04672	Intestinal immune network for IgA production	Immune system[O]	3.45×10^{-3}	3.16×10^{-2}	HLA-B, CD86

The capital letters beside the class name represent KEGG categories. Among them, D means human disease; O means organismal systems. The adjusted *P*-values are obtained through multiple hypothesis testing to correct the nominal *P*-values.

the ontology-based data (Gene Ontology, PANTHER and Reactome) were further conducted to reveal their functions. Apart from PID, the data of the remaining seven databases were fully downloadable, and the enrichment analyses of the potential AD target genes based on the data from the seven databases were thus conducted using R package clusterProfiler to validate the presumption of this study. As a result, no term was enriched based on the data of PANTHER, PathWhiz, MetaCyc, and NetPath, and the enriched terms based on GO, Reactome, and WikiPathways databases are provided in Tables 4, S3 and S4, respectively. As shown, the enriched terms based on GO and Reactome are focused on the biological process of lipoproteins (eg phosphatidylinositol) with *P*-value <0.05, that is significantly associated with the immunity.[96] Particularly, the activity of phosphatidylinositol 3-kinase affects the expression of MHC class

TABLE 4 GO enrichment results of the potential AD drug target genes identified in this study

GO ID	GO pathway	Subontology	Nominal *P*-value	Adjusted *P*-value	Enriched Genes
GO:0042157	Lipoprotein metabolic process	Biological Process	1.26E−05	1.39E−02	APOD, LEP, HSPG2, MTTP
GO:0048015	Phosphatidylinositol mediated signaling	Biological Process	5.29E−05	2.06E−02	RELN, LEP, CD86, PIK3C2A
GO:0048017	Inositol lipid-mediated signaling	Biological Process	5.60E−05	2.06E−02	RELN, LEP, CD86, PIK3C2A
GO:0050746	Regulation of lipoprotein metabolic process	Biological Process	1.15E−04	2.28E−02	APOD, LEP
GO:0032682	Negative regulation of chemokine production	Biological Process	1.48E−04	2.28E−02	APOD, IL10
GO:0030258	Lipid modification	Biological Process	1.53E−04	2.28E−02	APOD, LEP, CD86, PIK3C2A
GO:0043235	Receptor complex	Cellular Component	2.49E−04	1.93E−02	MTTP, NOS1AP, TF, GABRG2
GO:0072562	Blood microparticle	Cellular Component	3.71E−04	1.93E−02	C1QB, TF, A2M

The adjusted *P*-values are obtained through multiple hypothesis testing to correct the nominal *P*-values.

Il gene.[97] Moreover, one enriched terms based on WikiPathways is related to lipid metabolism, and the remaining enriched terms are immunity related pathways (*Allograft Rejection* & *SIDS Susceptibility Pathways*). SIDS neuroimmune disorder is reported as a neuroimmune disorder in brain, which is involved in the T-cell deficiency in immune inflammatory response.[98] In sum, these in-depth analyses further supported the discovery of KEGG enrichment and further associated AD pathogenesis with the immunity.

4 | CONCLUSIONS

A total of 18 potential AD target genes were identified by the combinatorial method of ontology-based inference and biological network analysis. Further, the results of enrichment analysis showed that these 18 potential AD target genes were significantly enriched in the immune-related pathways, and of which C1QB, HLA-B, IL-10, and CD86 were involved in the process of antigen presenting from MHC to T cell. These results implied that the pathogenic mechanism of AD may be relevant to the abnormal process of antigen presenting and may be an effective point of further drug development. In summary, our findings showed the importance of immune-related drug target genes to the therapy of AD and would benefit to the AD research in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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