

Alzheimer's Disease

Alzheimer's disease is a **brain** disease in which damaged and dying brain cells cause **mental deterioration over a period of time**. Its symptoms include increasingly poor memory, personality changes, and loss of concentration and judgment. The disease affects approximately 4.5 million persons in the United States. Most victims are over age 65, though Alzheimer's does sometimes occur in younger individuals. There is no effective treatment or cure. Its exact cause remains unknown.

Comment [1]: many researchers and doctors alike have been researching this... bc no cure, there is no real treatment for the disease

Words to Know

Autopsy

The **examination of a dead body** to find the cause of death or investigate the damage produced by disease.

Gene

A **sequence of DNA** that contains information directing an organism to function in a particular way.

Neuron

A nerve cell.

Protein

A large molecule built of amino acids that is essential to the structure and functioning of all living cells.

Synapse

A structure at the end of a neuron that allows the neuron to pass a signal to another cell.

History

Alzheimer's disease is named after German neurologist Alois Alzheimer (1864–1915), who was the first researcher to describe it. In 1906, he studied a 51-year-old woman whose personality and mental abilities were obviously deteriorating: She forgot things, became paranoid, and acted strangely. After the woman's death, Alzheimer examined her brain during an autopsy, the examination of a dead body to find the cause of death or investigate the damage produced by disease. He noted an unusual thickening and tangling of the organ's nerve fibers. He also found that the cell bodies and nuclei of nerve cells had disappeared. Alzheimer noted that these changes indicated some new, unidentified illness. More than seven decades would pass before researchers again turned their attention to this puzzling, destructive disease.

Comment [2]: cell bodies and nuclei direct nerve impulses... without it, connections stop short

A Progressive Disorder

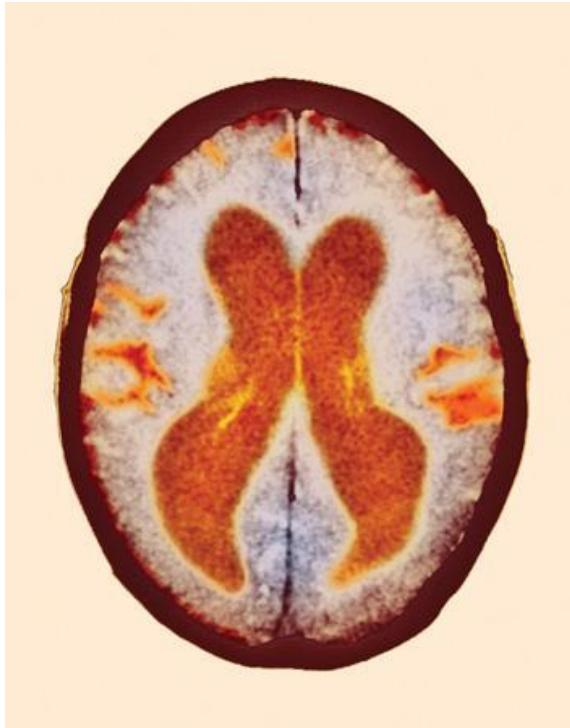
For individuals with this disease, Alzheimer's slowly destroys their brains, **robbing these individuals of the thoughts and memories that make them unique human beings**. Patients with Alzheimer's

typically progress through a series of stages that begin with relatively minor memory loss of recent events. Gradually, loss of memory is accompanied by forgetfulness, inattention to personal hygiene, impaired judgment, and loss of concentration. Later symptoms include confusion, restlessness, irritability, and disorientation. These conditions worsen until patients are no longer able to read, write, speak, recognize loved ones, or take care of themselves. **Survival after onset of symptoms is usually five to 10 years but can be as long as 20 years.** Persons with Alzheimer's are especially vulnerable to infection, particularly pneumonia, which is the usual cause of death.

Comment [3]: most people die of another disease and its symptoms are more deadly bc lack of attentiveness to personal hygiene due to disease

Changes in the Brain

A healthy brain is composed of billions of nerve cells, or neurons, each consisting of a cell body, dendrites, and an axon. Dendrites and axons together are called nerve fibers and are extensions of the cell body. Nerve messages enter a neuron by way of the dendrites and leave by way of the axon. Neurons are separated from one another by narrow gaps called synapses. Messages traveling from one neuron to another are carried across these narrow gaps by chemicals called neurotransmitters. **This highly organized system allows the brain to recognize stimuli and respond in an appropriate manner.**



In Alzheimer's patients, this orderly system becomes damaged to such a degree that it no longer works. The brains of Alzheimer's patients examined during autopsies show two hallmark features: **(1) a mass of fibrous structures called neurofibrillary tangles and (2) plaques consisting of a core of abnormal proteins embedded in a cluster of dying nerve endings and dendrites.** The brains display

Lowered levels of the neurotransmitter [acetylcholine](#) (pronounced uh-seh-tuhl-KOH-leen). The brains of Alzheimer's disease victims appear shrunken, particularly in large parts of the neocortex, the outer layer of gray matter responsible for higher brain functions such as thought and memory. Much of the shrinkage of the brain is due to loss of brain cells and decreased numbers of connections, or synapses, between them.

Comment [4]: this neurotransmitter is used in parasympathetic division of autonomic nervous system (rest & digest) and in the somatic nervous system (voluntary control)

Diagnoses

There is no simple procedure, such as a blood test, to diagnose Alzheimer's. A definitive [diagnosis](#) can be made only by examining brain tissue after death. Diagnosis in a live patient is based on medical history, physical examination, laboratory tests to rule out other possible causes of symptoms, and neurological exams to test mental performance. Using these methods, physicians can accurately diagnose 90 percent or more of cases.

Comment [5]: potential for wrong diagnosis...

Risk Factors

The specific cause of Alzheimer's disease remains unknown, although risk factors include advanced age, trauma such as head injury, and gene mutations. When the disorder appears in a number of family members, it is called familial Alzheimer's disease and is thought to be caused by an altered gene, although only 1 to 5 percent of Alzheimer's disease cases are believed to be genetically based.

Comment [6]: gene mutations... the Human Genome Project has figured out different genes of the body... could help locate the genes and use CRISPR to change perhaps?

Treatment

There is no sure way to prevent Alzheimer's disease, though people who continue to engage in intellectually challenging activities as they age do seem less likely to get the disease. Although Alzheimer's had no cure and no treatments to halt the disease's progression as of 2013, five prescription medications had been approved for use in Alzheimer's patients. These medications are thought to help with cognitive functioning, though not all medical practitioners agree that these drugs are effective. Some behavioral and social therapies can help people in the early stages of Alzheimer's to adjust to their cognitive limitations. The main treatment for Alzheimer's disease is simple caretaking: as patients deteriorate, they need help with everyday tasks such as feeding themselves, bathing and brushing their teeth, and dressing. Nursing care can help prevent health problems caused by neglecting these areas.

Comment [7]: intellectual stimulation needed... in nursing homes and senior centers, have more games/ puzzles

Scientists continue to make strides in the early diagnosis and treatment of Alzheimer's disease. In January 2013, researchers from the U.S. National Institutes of Health published the results of a study in which they successfully reversed the symptoms of a disease similar to Alzheimer's disease in mice treated with TFP5, a molecule derived from the brain enzyme Cdk5. Researchers hoped that the technique would produce similar results in humans with Alzheimer's disease in future trials.

Comment [8]: look more into TFP5

A Growing Problem

The Alzheimer's Association, a nonprofit research and support organization based in Chicago, estimates that more than 100,000 Americans die of Alzheimer's each year, making it the fourth leading cause of death among adults in the United States after heart disease, cancer, and stroke. The cost of caring for Alzheimer's patients is projected to be more than \$100 billion per year. As

more and more citizens live into their eighties and nineties, the number of Alzheimer's disease cases could reach twelve to fourteen million or more before the middle of the twenty-first century.

"Alzheimer's Disease." *UXL Encyclopedia of Science*, edited by Amy Hackney Blackwell and Elizabeth Manar, 3rd ed., UXL, 2015. *Science In Context*, http://link.galegroup.com/apps/doc/CV2644300059/SCIC?u=j043905010&sid=SCIC&xi_d=a162dd8a. Accessed 28 Sept. 2018.

Shared Biological Pathways Between Alzheimer's Disease and Ischemic Stroke

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Alzheimer's disease (AD) and ischemic stroke (IS) are an immense socioeconomic burden worldwide. There is a possibility that shared genetic factors lead to their links at epidemiological and pathophysiological levels. Although recent genome-wide association studies (GWAS) have provided profound insights into the genetics of AD and IS, no shared genetic variants have been identified to date. This prompted us to initiate this study, which sought to identify shared pathways linking AD and IS. We took advantage of large-scale GWAS summary data of AD (17,008 AD cases and 37,154 controls) and IS (10,307 cases and 19,326 controls) to conduct pathway analyses using genetic pathways from multiple well-studied databases, including GO, KEGG, PANTHER, Reactome, and Wikipathways.

Collectively, we discovered that AD and IS shared 179 GO categories (56 biological processes, 95 cellular components, and 28 molecular

Comment [1]: more older populations are affected, but because of this, more money spent on taking care of such patients since no cure

functions); and the following pathways: six KEGG pathways; two PANTHER pathways; four Reactome pathways; and one in Wikipathways pathway. The more fine-grained GO terms were mainly summarized into different functional categories: transcriptional and post-transcriptional regulation, synapse, endocytic membrane traffic through the endosomal system, signaling transduction, immune process, multi-organism process, protein catabolic metabolism, and cell adhesion. The shared pathways were roughly classified into three categories: immune system; cancer (NSCLC and glioma); and signal transduction pathways involving the cadherin signaling pathway, Wnt signaling pathway, G-protein signaling and downstream signaling mediated by phosphoinositides (PIPs). The majority of these common pathways linked to both AD and IS were supported by convincing evidence from the literature. In conclusion, our findings contribute to a better understanding of common biological mechanisms underlying AD and IS and serve as a guide to direct future research.

Comment [2]: perhaps a similar ligand/receptor molecule is used to activate channels that contribute to abnormal development

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. It is characterized pathologically by extracellular deposits of amyloid- β peptide (A β) and intracellular neurofibrillary tangles (NFTs) containing tau protein (Cuyvers and Sleegers, 2016). Ischemic stroke (IS) is a major health-threatening cerebrovascular disease with severe complications, such as post-stroke infection, physical disability, and cognitive deficits (Donnan et al.,

Comment [3]: both diseases lead to cognitive deficits

[2008](#)). These two neurological diseases have immense socioeconomic impact worldwide ([Norrving and Kissela, 2013](#); [Alzheimer's Association, 2016](#)).

Mounting evidence suggests that there is a potential correspondence between AD and IS. Firstly, epidemiological studies have revealed that AD is a contributing factor to the development of IS ([Chi et al., 2013](#); [Tolppanen et al., 2013](#)), and vice versa ([Gamaldo et al., 2006](#)). Secondly, AD and IS have common risk factors (e.g., hypertension, diabetes, obesity, and hyperlipidemia) ([de Brujin and Ikram, 2014](#); [Boehme et al., 2017](#)). Thirdly, recent evidence has indicated that brain ischemia can promote the development of AD by inducing β -/ γ -secretase-mediated A β accumulation and tau protein gene alterations ([Salminen et al., 2017](#); [Pluta et al., 2018](#)). Fourth, neuroinflammation elicited by the immune system is thought to play essential roles in the development and progression of both AD and IS ([Liu Y.H. et al., 2013](#); [Anrather and Iadecola, 2016](#)). Lastly, research shows that abnormal tau protein also plays crucial roles in IS ([Tuo et al., 2017](#)). Together, these findings support the hypothesis that shared genetic risk factors link AD and IS at the epidemiological and pathophysiological levels.

Recent genome-wide association studies (GWASs) have provided profound insights into the complex genetic architecture of AD ([Shen et al., 2015](#); [Li et al., 2016](#); [Liu et al., 2016](#); [Zhang et al., 2016](#); [Jun et al., 2017](#); [Sims et al., 2017](#)) and IS ([Traylor et al., 2012](#); [Williams et al., 2013](#); [Kilarski et al., 2014](#); [Malik et al., 2016](#); [Network et al., 2016](#); [Crawford et al., 2018](#)). Most recently, 29 risk loci for AD ([Jansen et al., 2018](#)) and 28 loci associated with IS and its subtypes [i.e., large vessel disease (LVD), cardioembolic stroke (CE), and small vessel disease (SVD)] ([Malik et al., 2018](#)) have been identified. Despite these findings, no significant genetic variants shared by AD and IS or its subtypes

Comment [4]: kill two birds with one stone if prevention methods/ national campaigns are used by the government

Comment [5]: immune system health is important because it can cause neuro problems

were discovered. From the perspective of pathways, however, four gene ontology (GO) categories jointly associated with AD and SVD were identified ([Traylor et al., 2016](#)). Moreover, single-disease pathway analysis demonstrates that AD and IS share common pathways that involve natural killer (NK) cells, i.e., NK cell mediated cytotoxicity in AD ([Lambert et al., 2010](#); [Liu et al., 2014](#); [Chen et al., 2016](#); [Jiang et al., 2017](#)) and NK cell signaling in IS ([Malik et al., 2016](#)). These findings imply that the link between AD and IS may have to do with **shared genetic signals at the pathway level**.

Complex diseases like AD and IS are mostly driven by the joint interactions of associated genes affected by large proportions of SNPs well below genome-wide significance, and the crosstalk of regulatory pathways ([Furlong, 2013](#)). Based on this concept, pathway-based analysis has been an effective strategy to investigate the potential mechanisms of complex disease ([Luo et al., 2010](#); [Jin et al., 2014](#)), thus is widely used to unravel either single disease etiology or pleiotropism of clinically distinct diseases ([Lesnick et al., 2007](#); [Liu G. et al., 2012](#); [Liu Y. et al., 2013](#); [Liu et al., 2014](#); [Bao et al., 2015](#); [Xiang et al., 2015](#)). Taken together, these considerations prompted us to perform pathway analyses using AD and IS GWAS data to further dissect their common molecular mechanisms.

Comment [6]: on the genetic levels, there are not many connections

Materials and Methods

Samples

We obtained AD GWAS summary data from the International Genomics of Alzheimer's Project (IGAP). IGAP performed a large, two-stage joint

association analysis of AD on individuals of European descent ([Lambert et al., 2013](#)). In stage 1 (discovery stage), 17,008 AD cases and 37,154 controls from four previously published GWAS samples were included. These were the European Alzheimer's disease Initiative (EADI), the Alzheimer Disease Genetics Consortium (ADGC), the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (CHARGE), and the Genetic and Environmental Risk in AD Consortium (GERAD). After quality control procedures, 7,055,881 SNPs remained for further analysis. In stage 2 (replication stage), the top SNPs with a *P*-value less than 1E-3 in stage 1 were selected for replication in an independent sample (8,572 cases and 11,312 controls). Finally, the results of the two stages were combined in a meta-analysis in order to identify loci reaching genome-wide significance ($P < 5.00E-08$). In the present study, we used the GWAS summary data from stage 1.

We selected the IS GWAS summary results from the METASTROKE collaboration published by Malik et al ([Malik et al., 2016](#)). In the discovery stage, METASTROKE conducted a meta-analysis of 12 case-control GWAS comprising 10,307 Caucasian IS cases and 19,326 Caucasian controls, producing quality-controlled 8.3 million SNPs for further analysis. In the replication stage, the top SNPs ($P < 1.00E-05$) in the discovery meta-analysis were selected for replication in three independent samples consisting of Caucasian (13,435 cases and 29,269 controls) and South Asian (2,385 cases and 5,193 controls) samples. Ultimately, a transethnic meta-analysis combining the two stages was conducted. Here, we used the IS GWAS summary statistics from the discovery phase.

Comment [7]: combining data from many different studies to achieve the best conclusion

Statistical Analyses

Firstly, we computed gene-level association statistics through gene-based association tests. Next, gene-based pathway analysis was performed to identify pathways shared between AD and IS.

Gene-Based Testing for AD and IS GWAS Datasets

We performed gene-level analysis implemented in the versatile gene-based association study software (VEGAS). By incorporating the effects of a full set of markers within a gene and correcting for linkage disequilibrium (LD) between SNPs, we were able to calculate gene-based *P*-values. Compared with other gene-based testing approaches like PLINK, a set-based test based on permutations (Purcell et al., 2007), VEGAS is much more computationally efficient by using simulations from multivariate normal distributions (Liu et al., 2010). In addition, because VEGAS only requires SNP rs IDs and *P*-values, it is particularly appropriate for GWAS summary results, in which individual genotyped information is not available. We used VEGAS2, a recently updated version of VEGAS that uses LD estimates from the 1000 Genomes Project phase I panel (Mishra and Macgregor, 2015). We chose the following defaults to define the gene boundaries for locating SNPs in VEGAS2: “SNPs within a gene plus SNPs outside of the gene with $r^2 > 0.8$ with SNPs in the gene” (Mishra and Macgregor, 2015). By using this default setting, we were able to capture the effects of regulatory SNPs and minimize non-specificity caused by large boundaries (i.e., ± 50 kb) (Mishra and Macgregor, 2015).

Recently, Boyle et al. proposed the hypothesis that most complex traits are largely driven by the vast majority of peripheral genes with smaller effects by

Comment [8]: basic bell curve with 99.7% of the data within 3 standard deviations

propagating through regulatory networks to core genes ([Boyle et al., 2017](#)). As such, genes in AD and IS with $P < 0.05$, rather than with stringent threshold like gene-wide statistical significance, were selected for subsequent pathway analysis.

Pathway-Based Analysis for Shared Disease Pathways

It is likely that genes of AD and IS are involved in one pathway, but in different proportions, such as in the upstream and downstream of the pathway, respectively. Hence, we performed pathway analysis for AD and IS separately by integrating genes for each disease from VEGAS2 ($P < 0.05$) into known functional annotations using WebGestalt ([Wang et al., 2017](#)). Next, their respective enrichment results were overlapped to identify shared pathways jointly associated with AD and IS.

The latest version of WebGestalt 2017 included genetic pathways from GO annotations ([Ashburner et al., 2000](#)), KEGG ([Kanehisa et al., 2004](#)), PANTHER ([Mi et al., 2016](#)), Reactome ([Fabregat et al., 2016](#)), and WikiPathways ([Pico et al., 2008](#)). We chose over-representation analysis (ORA), a hypergeometric test, to identify the enrichment of the disease-related genes among all the genes in a given pathway. The P -value for observing at least **m** disease-related genes in a given pathway can be calculated as follows:

$$P = 1 - \sum_{i=0}^m \frac{(n_i)(N-n)(M-i)(NM)}{\sum_{i=0}^m (ni)(N-n)(M-i)(NM)}$$

where M is the total number of genes of interest that are associated with a given disease, N is the number of reference genes, and n is the number of genes in the pathway. The smaller the P -value is, the more likely the list of disease-related genes of interest will be overrepresented in this pathway. To avoid testing overly narrow or broad pathways, which could result in some

Comment [9]: formula used to figure out similarities in disease pathways

false positives, we chose pathways comprising at least 20 and at most 300 genes for analysis ([Jiang et al., 2017](#)). The threshold of statistical significance for a pathway associated with AD and IS was $P < 0.05$ in both diseases.

Meta-Analysis Using Fisher's Method

Here, Fisher's method was used to combine the P -values of each shared pathway in AD and IS into one test statistic. For a given pathway, the Fisher equation for the statistic is:

$$x_2 = -2 \sum_{i=1}^k \ln(P_i)$$

where P_i is the P -value of the pathway in the i th study and k is the number of total studies. x_2 follows chi-square distribution with $2k$ degrees of freedom ([Begum et al., 2012](#)).

Results

Gene-Based Testing for AD and IS GWAS Datasets and Validation of VEGAS2

We identified 1915 AD genes and 1288 IS genes with $P < 0.05$. More detailed results are provided in Supplementary Table [S1](#). To verify the reliability of the VEGAS2 gene-based testing approach, we compared our gene-based association results of AD with previously established AD risk loci identified through conventional GWAS approaches. We replicated 15 genes (*APOE*, *TOMM40*, *APOC1*, *EXOC3L2*, *PVRL2*, *CR1*, *HLA-DRB6*, *EPHA1*, *CLU*, *PICALM*, *ABCA7*, *CD33*, *MS4A2*, *MS4A6A*, *BIN1*) with gene-wide significance ($P < 2.35E-6$, Bonferroni corrected for 21,244 genes). These genes belong to 12

established risk loci for AD (i.e., *APOE/TOMM40/APOC1* locus; *EXOC3L2/BLOC1S3/MARK4* locus; *PVRL2; CR1; HLA-DRB5-DRB1* region; *EPHA1; CLU; PICALM; ABCA7; CD33; MS4A* locus; *BIN1*). In addition, we replicated 11 genes (*SORL1, CD2AP, MS4A6E, MS4A4A, MS4A3, HLA-DQB1, HLA-DRB1, HLA-DQA1, PTK2B, CELF1, and SLC24A4*) with suggestive association ($P < 1.00\text{E-}04$); these belong to seven known significant loci (*SORL1, CD2AP, MS4A* locus, *HLA-DRB5-DRB1* region, *PTK2B, CELF1, and SLC24A4*). Thus, the replication of these established findings shows that VEGAS2 can be trusted as a validated tool for our subsequent pathway analysis.

Pathway-Based Analysis for Shared Pathways Between AD and IS

Using the 1915 AD genes and 1288 IS genes having a $P < 0.05$, pathway analysis for AD and IS were separately conducted followed by overlapping their respective significant enrichment results.

In GO, we identified 179 common GO categories associated with both AD and IS: 56 biological processes, 95 cellular components, and 28 molecular functions (Supplementary Table [S2](#)). Here, we paid more attention to the more fine-grained GO terms and weeded out their ancestors to solve the problem of redundancy. Taken as a whole, the remaining GO terms of biological process, cellular component and molecular function mainly fall into categories listed below: transcriptional and post-transcriptional regulation, synapse, endocytic membrane traffic through the endosomal system, signaling transduction, immune process, multi-organism process, protein catabolic metabolism, cell adhesion, and others (Figure 1 and Supplementary Table [S2](#)).

Comment [10]: different proponents of cells were compared and contrasted

The detailed information about complete GO annotation results and summarized GO annotation results as well as their relevant GO terms is displayed in Supplementary Table [S2](#).

FIGURE 1



FIGURE 1. Summarized GO annotation results shared by AD and IS. The complete GO annotation results shared by AD and IS are summarized and reorganized here, with only more fine-grained terms labeled and classified into different functional categories: transcriptional and post-transcriptional regulation, synapse, endocytic membrane traffic, signaling transduction, immune process, multi-organism process, protein catabolic metabolism, cell adhesion, and others, which are shown in nodes. The relevant GO terms of biological process, cellular component, and molecular function are shown in targets. The threshold for statistical significance for a GO term is $P < 0.05$ in both AD and IS. The edges connecting the nodes and targets are attributed by the value of $-\log_{10} P$.

In KEGG, we identified six significant pathways shared by AD and IS: NK cell mediated cytotoxicity [hsao4650], Toll-like receptor signaling pathway [hsao4620], non-small cell lung cancer [hsao5223], glioma [hsao5214], phospholipase D signaling pathway [hsao4072], and hepatitis B [hsao5161]. Besides these, we also identified 2 shared significant PANTHER pathways between AD and IS: cadherin signaling pathway [P00012] and Wnt signaling pathway [P00057]. Four significant Reactome pathways shared by AD and IS

were immunoregulatory interactions between a lymphoid and a non-lymphoid cell [R-HSA-198933], synthesis of PIPs at the plasma membrane [R-HSA-1660499], PI metabolism [R-HSA-1483255] and cooperation of PDCL (PhLP1) and TRiC/CCT in G-protein beta folding [R-HSA-6814122]. In the WikiPathways dataset, one significant pathway was identified: signaling pathways in glioblastoma [WP2261]. The detailed results are described in Table 1.

TABLE 1

TABLE 1. Shared biological pathways between AD and IS.

Discussion

Large-scale GWAS of AD and IS have identified a set of risk loci with genome-wide significance and provided deep insights into the genetics of AD and IS ([Cuyvers and Sleegers, 2016](#); [Boehme et al., 2017](#)). Despite these discoveries, a large proportion of heritability remains underappreciated ([Zhang et al., 2013](#); [Markus and Bevan, 2014](#)). To date, no shared genetic determinants jointly associated with AD and IS have been revealed. However, AD and IS have common biological pathways that may account for their similarities at the epidemiological, neuropathological, and molecular levels. Indeed, our pathway-based association tests using large-scale GWAS summary datasets

Comment [11]: confirm the association

for AD and IS prove the genetic links between these two diseases at the pathway level.

In order to fully understand the biological features shared by AD and IS, we included in our analyses different functional annotation databases (i.e., GO annotation, KEGG, PANHTER, Reactome, and Wikipathways). GO annotation sets represent the most comprehensive spectrum of functional categories but are mainly computationally inferred, with less than 1% being experimentally confirmed ([Ashburner et al., 2000](#)). In contrast, KEGG, PANTHER, and Reactome pathway databases are manually curated from the literature based on experimentally validated evidence ([Kanehisa et al., 2004](#); [Fabregat et al., 2016](#); [Mi et al., 2016](#)). Compared to the centrally curated databases above, open community-based Wikipathways allows for broader exploration into the entire biological spectrum ([Pico et al., 2008](#)). Altogether, we uncovered 179 GO annotation terms, 6 shared KEGG pathways, 2 PANTHER pathways, 4 Reactome pathways, and one pathway in Wikipathways jointly associated with AD and IS.

Shared KEGG Pathways

Together with one Reactome pathway [R-HSA-198933 immunoregulatory interactions between a lymphoid and a non-lymphoid cell], two KEGG pathways are related to the immune system (i.e., Toll-like receptor signaling pathway [hsao4620] and NK cell mediated cytotoxicity [hsao4650]). Toll-like receptor signaling pathway has also been reported to be associated with AD ([Liu et al., 2014](#)). It is now widely accepted that Toll-like receptors (TLRs) play essential roles in A β -induced microglial inflammatory activation in AD ([Liu S. et al., 2012](#)). The persistent inflammatory stimuli fueled by activated microglia

in turn leads to a disturbance in microglial clearance of A β , eventually causing neuronal degeneration ([Heneka et al., 2015](#)). Following IS, damage-associated molecular patterns (DAMPs) released from the intracellular compartment of dying and dead cells activate microglia and perivascular macrophages by pattern recognition receptors (PRRs), especially TLRs. The triggering cascades of innate immunity further exacerbate stroke lesions in the acute phase of IS ([Iadecola and Anrather, 2011](#)).

Natural killer cell mediated cytotoxicity has been reported by previous GWAS and brain expression datasets of AD ([Lambert et al., 2010](#); [Liu et al., 2014](#); [Chen et al., 2016](#); [Jiang et al., 2017](#)). The association for IS and NK cell signaling was also observed ([Malik et al., 2016](#)), and supported by evidence that infiltrating NK cells exacerbate ischemia lesions and increase neuronal death either in experimental IS models or in human IS ([Gan et al., 2014](#)). The inflammatory responses in both AD and IS are initiated locally within the central nervous system (CNS) under aberrant local conditions, and presumably elicited by the innate immune system. Therefore, the similarity of their pathophysiological processes may provide a reasonable explanation for our findings that AD and IS share common biological pathways involved in the immune system.

Comment [12]: can direct future research here

Another two KEGG pathways are related to cancer (i.e., glioma [hsao5214] and non-small cell lung cancer [hsao5223]). Convincing evidence suggests an inverse relationship and significant genetic overlap between AD and cancer ([Roe et al., 2005](#); [Driver et al., 2012](#); [Musicco et al., 2013](#); [Ou et al., 2013](#)). For instance, the cellular behavior of proliferative cells in cancer is essentially the opposite of the processes leading to degenerating neurons in AD ([Behrens et al., 2009](#); [Driver and Lu, 2010](#); [Driver, 2014](#)). Bioinformatics analyses has

unraveled shared genes and signaling pathways connecting AD and glioblastoma ([Liu T. et al., 2013](#)). The one significant pathway we found in the Wikipathways dataset (signaling pathways in glioblastoma [WP2261]) also supports this linkage. Though some illustrative cases suggested that glioblastoma should be considered in the etiology of acute IS ([Pina et al., 2014](#); [Lasocki and Gaillard, 2016](#)), little is known about the relationship between IS and glioma or other prevalent cancers.

The biological significance of the phospholipase D (PLD) signaling pathway [hsao4072] has been suggested for both AD and IS ([Oliveira and Di Paolo, 2010](#); [Stegner et al., 2013](#); [Frohman, 2015](#)). Hepatitis B pathway [hsao5161] has already been revealed in hippocampal CA1 region of AD patients ([Mastroeni et al., 2018](#)). Epidemiology studies suggested that hepatitis B virus (HBV) infection was associated with decreased risk of IS, although the underlying mechanism was unclear ([Sung et al., 2007](#); [Kuo et al., 2017](#)). Part of the GO terms we deciphered, i.e., positive regulation of viral transcription [GO:0050434], positive regulation of viral life cycle [GO:1903902] and defense response to virus [GO:0051607] appeared to support this linkage.

Shared PANTHER Pathways

In the present study, we uncovered 2 significant PANTHER pathways shared by AD and IS (cadherin signaling pathway [Pooo12] and Wnt signaling pathway [Pooo57]). Similar to cadherin signaling pathway, cadherin binding involved in cell-cell adhesion [GO:0098641] was implied in GO. Both enriched PANTHER pathways primarily arose from the *Pcdha* gene cluster (*Pcdha*) shared by AD and IS in our VEGAS2 gene-based analysis. Protocadherins (Pcdhs) constitute the largest group of the cadherin superfamily of cell-

adhesion molecules. Pcdhs are predominantly expressed in the CNS (i.e., neurons, astrocytes, pericytes, choroid plexus epithelial cells, and brain microvascular endothelial cells), and are crucial for neuronal survival, neural circuit assembly, and maintenance of the blood-brain barrier (BBB) ([Takeichi, 2007](#); [Dilling et al., 2017](#)). Moreover, the interactions between Pcdhs and Wnt signaling pathway have recently been uncovered ([Mah and Weiner, 2017](#)). Wnt signaling pathway plays critical roles in neurogenesis and synaptic transmission and plasticity, whereby its deregulation shows significant linkage to the development of AD ([Caruso et al., 2006](#); [De Ferrari et al., 2007](#); [Inestrosa and Varela-Nallar, 2014](#)). For IS, experimental evidence has demonstrated that Wnt signaling activation may exert effective neuroprotection by providing an appropriate compensatory microenvironment for neurogenesis or neuronal survival in focal ischemic injury ([Shruster et al., 2012](#); [Wu et al., 2015](#)).

Shared Reactome Pathways

Two shared Reactome pathways are PI (phosphatidylinositol) metabolism [R-HSA-1483255] and synthesis of PIPs (phosphoinositides) at the plasma membrane [R-HSA-1660499], which are connected by a parent–child relationship. Similarly, phosphatidylinositol binding [GO:0035091] was also identified in GO. PIPs consist of seven diverse phosphorylated forms of phosphatidylinositol. Among these, PI(4,5)P₂ and PI(3,4,5)P₃ occur in relatively high abundance at the plasma membrane ([Di Paolo and De Camilli, 2006](#); [Riehle et al., 2013](#)). PI(4,5)P₂ primarily serves as a pool for the formation of intracellular second messengers—inositol 1,4,5-trisphosphate (IP₃), diacylglycerol (DAG), and PI(3,4,5)P₃—to mediate downstream signal

cascades [e.g., Ca_{2+} response, PKC activation and $\text{PI}(3,4,5)\text{P}_3/\text{Akt}/\text{mTOR}$ pathway] from plasma membrane receptors like GPCRs ([Riehle et al., 2013](#)). PIPs play important roles in the nervous system by regulating lipid signaling, receptor signaling and membrane dynamics (e.g., endocytic membrane traffic). Thus, deregulation of these pathways has been implicated in neurological diseases including AD and IS ([Waugh, 2015](#)).

Another significant Reactome pathway shared by AD and IS is cooperation of PDCL (PhLP1) and TRiC/CCT in G-protein beta folding [R-HSA-6814122]. Recent studies reveal the interplay of the protein-folding chaperone CCT (also called TRiC) and its co-chaperone PhLP1 in G-protein $\beta\gamma$ ($\text{G}\beta\gamma$) dimer formation, which is a particularly critical step in G-protein signaling ([Plimpton et al., 2015](#)). Comparably, heterotrimeric G-protein complex [GO:0005834] was also identified in GO. In AD, G-protein signal transduction pathways mediated by G protein-coupled receptors (GPCRs) are strongly related to α -/ β -/ γ -secretase-mediated APP processing through the endosomal system ([Teng et al., 2010](#); [Thathiah and De Strooper, 2011](#); [Heese, 2013](#)).

In summary, we discovered several shared significant pathways jointly associated with AD and IS, which can roughly be classified into three categories: immune system, cancer (NSCLC and glioma), and signal transduction pathways. The latter includes cadherin signaling pathway, Wnt signaling pathway, G-protein signaling, and downstream signaling mediated by PIPs. Convincing evidence in the literature corroborates the majority of these shared pathways.

Despite these interesting and consequential findings, our study had some limitations. Firstly, we were able to analyze only the overall IS summary data. However, IS is genetically heterogeneous, as nearly all its risk loci are specific

Comment [13]: the shared pathways hint at the relationship between the two diseases

Comment [14]: too rigid of an experiment

to individual subtypes. Secondly, the input lists of significant genes for pathway analysis were selected by setting an arbitrary threshold ($P < 0.05$), which might lead to information loss of some marginally less significant genes (e.g., $P = 0.051$). Lastly, the statistical model we used for pathway analysis was ORA, a hypergeometric test, which treats each gene equally and does not take into account interdependencies between genes in a pathway. However, the significance of each gene (P -value) can be informative in assigning different weights to input genes in the pathway analysis. In the future, we aim to conduct more robust statistical analysis using multiple pathway analysis methods and multiple datasets to replicate and expand our findings.

Conclusion

Collectively, in our gene-based tests and comprehensive pathway analyses of AD and IS summary GWAS datasets, we discovered several shared novel functional pathways linking AD and IS. By providing an in-depth investigation here of the shared biological mechanism associated with these two neurological disorders, our findings may advance the current understanding of the biology of AD and IS and guide future research on AD or IS in new directions.

Cui, Pan, et al. "Shared Biological Pathways Between Alzheimer's Disease and Ischemic Stroke." *Frontiers in Neuroscience*, 2018. *Science In Context*, <http://link.galegroup.com/apps/doc/A553341998/SCIC?u=j043905010&sid=SCIC&xid=5e701c65>. Accessed 28 Sept. 2018.