

RESEARCH ARTICLE

# Network Analysis of a Comprehensive Knowledge Repository Reveals a Dual Role for Ceramide in Alzheimer's Disease

Satoshi Mizuno<sup>1,2\*</sup>, Soichi Ogishima<sup>2\*</sup>, Kazuyuki Kitatani<sup>3</sup>, Masataka Kikuchi<sup>4</sup>, Hiroshi Tanaka<sup>2</sup>, Nobuo Yaegashi<sup>3</sup>, Jun Nakaya<sup>1</sup>

**1** Department of Clinical Informatics, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan, **2** Department of Clinical Record Informatics, Tohoku Medical Megabank Organization, Tohoku University, Sendai, Miyagi, Japan, **3** Department of Gynecology and Obstetrics, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan, **4** Department of Genome Informatics, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan

\* [samizuno@med.tohoku.ac.jp](mailto:samizuno@med.tohoku.ac.jp) (SM); [ogishima@sysmedbio.org](mailto:ogishima@sysmedbio.org) (SO)



OPEN ACCESS

**Citation:** Mizuno S, Ogishima S, Kitatani K, Kikuchi M, Tanaka H, Yaegashi N, et al. (2016) Network Analysis of a Comprehensive Knowledge Repository Reveals a Dual Role for Ceramide in Alzheimer's Disease. PLoS ONE 11(2): e0148431. doi:10.1371/journal.pone.0148431

**Editor:** Mária A. Deli, Hungarian Academy of Sciences, HUNGARY

**Received:** October 7, 2015

**Accepted:** January 18, 2016

**Published:** February 5, 2016

**Copyright:** © 2016 Mizuno et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, Grant number : 25330333, URL : <https://kaken.nii.ac.jp/d/p/25330333.en.html>. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

## Abstract

Alzheimer's disease (AD) is the most common cause of senile dementia. Many inflammatory factors such as amyloid- $\beta$  and pro-inflammatory cytokines are known to contribute to the inflammatory response in the AD brain. Sphingolipids are widely known to have roles in the pathogenesis of inflammatory diseases, where the precise roles for sphingolipids in inflammation-associated pathogenesis of AD are not well understood. Here we performed a network analysis to clarify the importance of sphingolipids and to model relationships among inflammatory factors and sphingolipids in AD. In this study, we have updated sphingolipid signaling and metabolic cascades in a map of AD signaling networks that we named "AlzPathway," a comprehensive knowledge repository of signaling pathways in AD. Our network analysis of the updated AlzPathway indicates that the pathways related to ceramide are one of the primary pathways and that ceramide is one of the important players in the pathogenesis of AD. The results of our analysis suggest the following two prospects about inflammation in AD: (1) ceramide could play important roles in both inflammatory and anti-inflammatory pathways of AD, and (2) several factors such as Sphingomyelinase and Siglec-11 may be associated with ceramide related inflammation and anti-inflammation pathways in AD. In this study, network analysis of comprehensive knowledge repository reveals a dual role for ceramide in AD. This result provides a clue to clarify sphingolipids related inflammatory and anti-inflammatory pathways in AD.

## Background

Alzheimer's disease is the most common cause of senile dementia. Nearly 36 million people were affected by dementia worldwide as of 2010, and this figure is estimated to increase to 65.7 million by 2030 [1]. The societal costs of dementia are already huge and could continue to

increase rapidly. Alzheimer's disease (AD) thus represents a major public health concern and has been identified as a research priority. To address this global social issue, clarification of the pathology and the identification of effective therapies for AD are urgently needed.

The principal pathological feature of AD is brain atrophy and neural cell death. Previous studies demonstrated the occurrence of inflammation in pathologically vulnerable regions of AD brain [2]. Inflammation contributes to pathogenic processes in the degenerating brain tissue [3]. Currently, many inflammatory factors such as amyloid- $\beta$  and inflammatory cytokines are known to contribute to the inflammatory response in the AD brain [2, 4, 5], and among the inflammatory factors, sphingolipids in particular have been implicated in AD. For instance, altered distributions of the gangliosides GM1 and GM2 [6], elevated levels of ceramide [7] and the up regulation of ceramide generating enzyme [7] have been reported in the AD brain. In addition, sphingolipids such as ceramide and gangliosides are widely known to have roles in the pathogenesis of inflammatory diseases. In cystic fibrosis, an accumulation of ceramide in lung epithelial cells was reported to mediate inflammation and cell death [8]. Ceramide metabolic pathways play vital roles in the pathogenesis of other inflammatory diseases as well, such as inflammatory bowel disease and rheumatoid arthritis [9]. However, the precise roles for sphingolipids in inflammation and neurodegeneration are not well understood in the pathogenesis of Alzheimer's disease [10].

A comprehensive knowledge repository was proposed to consider global connections among disease factors such as the relationships among signaling molecules, exposures, phenotypes and well-known but not well-understood factors including sphingolipids [11–15]. Such a knowledge repository can provide a comprehensive map of pathogenic signaling pathways based on the ever-increasing data that have accumulated in specific fields such as AD. Each comprehensive knowledge repository includes various types of factors for a disease such as genomic variants, signaling molecules, exposures and phenotypes.

We have previously constructed (AlzPathway 1) and updated a comprehensive map of AD signaling pathways (AlzPathway 2) [16]. It is the first comprehensive knowledge repository, and it was constructed with manual curation from 123 review articles. AlzPathway has been used to simplify the signal transduction pathways of multiple risk factors [17], collect associated factors and pathways of biological interest [18], extract enriched modules among several datasets [19], and provide a training data set in a supervised text mining tool, which warrants future investigation [20]. AlzPathway would also be informative for pathway-based drug discovery efforts [21].

In the present study, 18 review articles related to both sphingolipids and AD were collected and manually curated to update AlzPathway as 'AlzPathway 3,' using Cell Designer [22], a modeling editor for biochemical pathways. We also performed a network analysis on AlzPathway 3 to clarify the importance of sphingolipids in AlzPathway and to model relationships among inflammatory factors and sphingolipids in Alzheimer's disease.

## Materials and Methods

### Update of AlzPathway

We collected all review articles based on these three criteria, 1) related to both sphingolipids and Alzheimer's disease 2) published after 2000 3) be accessible from PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>). We obtained 18 review articles [10, 23–39] fulfilling these criteria.

We manually curated these review articles, and then we updated AlzPathway by using Cell Designer. In the process of manual curation, we collect all molecular names and reactions from figures of review articles. We named the updated AlzPathway 'AlzPathway 3.' Molecules are distinguished by the following types: proteins, complexes, simple molecules, genes, RNAs, ions,

degraded products, and phenotypes. The detailed protocol of the construct relations, file format and gene symbols conformed to the construction method used for AlzPathway [16].

## Construction of binary-relation AlzPathway 3 and extraction of key molecules

We converted AlzPathway 3 from the Systems Biology Graphical Notation (SBGN) process description notation to binary-relation notation. In the SBGN process description notation, a reaction consists of reactants, modifiers, and products. We converted this notation to the binary-relation notation by decomposing reactions into the reactions between (1) reactants and products and those between (2) modifiers and products. For simplification, the molecules were limited to proteins, complexes, genes, RNAs, simple molecules and phenotypes. We calculated the betweenness centralities on the binary-relation AlzPathway 3 to evaluate the topological properties of nodes and edges.

In previous studies, the betweenness centrality [40] was shown to have important meaning in biological networks such as a protein-protein interaction networks [41] and gene regulatory networks [42]. Betweenness centrality is an indicator of how many shortest paths among all nodes in network pass through a node or edge. In AlzPathway, nodes correspond to molecules, and edges correspond to path between two molecules. It was also shown that in AlzPathway, primary factors of AD studies such as amyloid- $\beta$  and Tau protein have high betweenness centrality [16]. In the present study, we calculated the edge betweenness centrality [43, 44] to extract the high edge betweenness centrality network (primary pathway). We calculated the node betweenness centrality [41] of the binary-relation AlzPathway 3 to extract high centrality nodes and compare the node betweenness centrality between primary factors of AD.

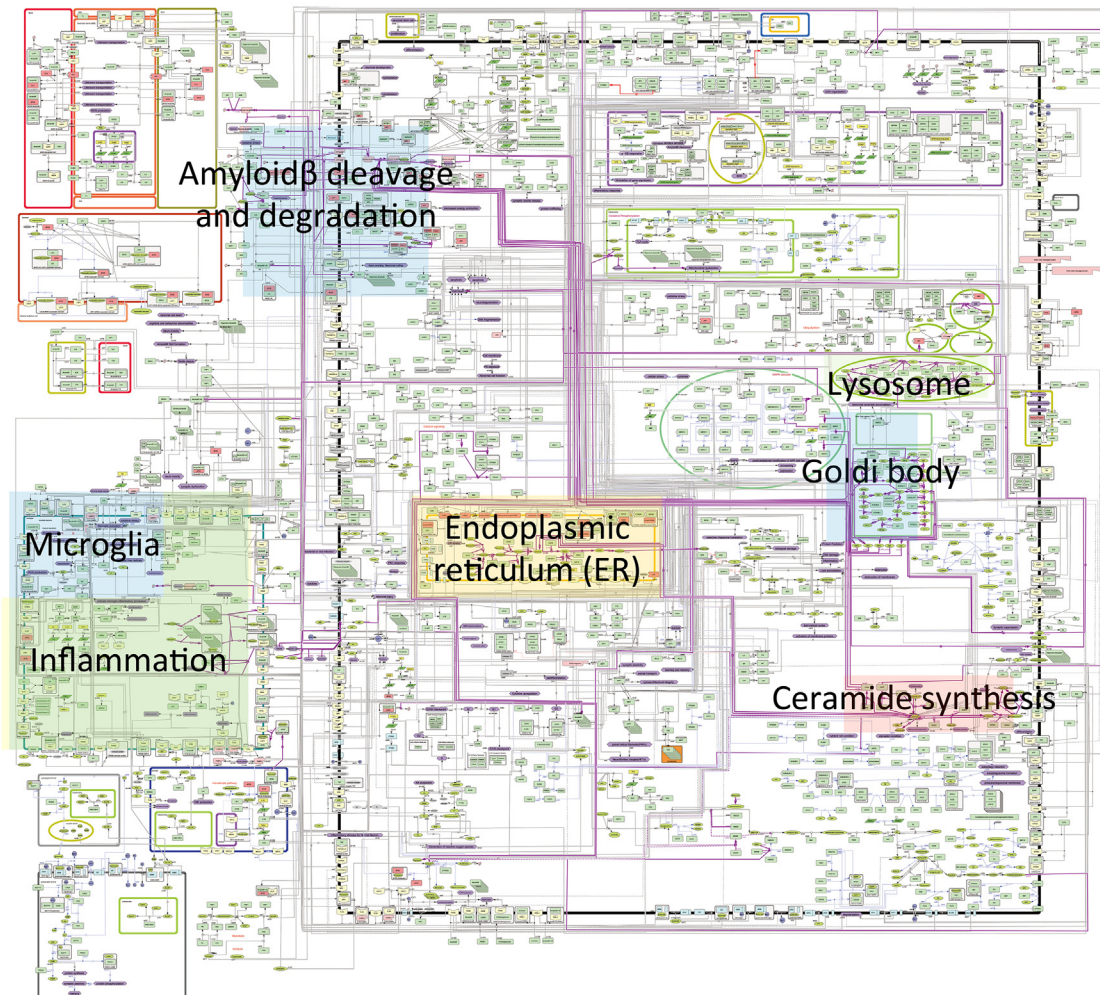
## Evaluation of relationships among sphingolipids and inflammatory factors

A sub-network, namely two hops from “Ceramide” and “Inflammation” was extracted from the binary-relation AlzPathway 3 to model the comprehensive relationships among them. The reason for extracting the sub-network between “Ceramide” and “Inflammation” is that ceramide has high node betweenness centrality next to amyloid- $\beta$  in AlzPathway 3. Two (hops) is the minimum number to make a connection between “Ceramide” and “Inflammation.” Top 50 high betweenness centrality relations were future extracted from this sub-network to simplify and evaluate the relationships between “Ceramide” and “Inflammation.”

## Results and Discussion

### Overview of AlzPathway 3

Here, we present an updated map of AD signaling networks we established (Fig 1). We collected 18 review articles related to both sphingolipids and AD, manually curated these review articles, and updated AlzPathway using Cell Designer ver. 4.2. The AlzPathway 3 map consists of 1,538 species, 1,127 reactions and 138 phenotypes. The molecules shown on AlzPathway 3 can be categorized as follows: 721 proteins, 246 complexes, 300 simple molecules, 33 genes, 37 RNAs, 24 ions, and 23 degraded products. The breakdown of reactions is as follows: 472 state transitions, 22 transcriptions, 30 translations, 184 heterodimer associations, 56 dissociations, 106 transports, 22 unknown transitions, six unknown negative influences and 226 omitted transitions. In AlzPathway 3, sphingolipid-involved relations can be found in the following canonical pathways: “amyloid- $\beta$  cleavage and degradation”, “inflammation”, “ganglioside synthesis in the endoplasmic reticulum”, “sphingolipids metabolism in lysosomes”, and



**Fig 1. Overview of AlzPathway 3 overlaid with spingolipid-related canonical pathway annotations.** AlzPathway 3 consists of 1,384 molecules, 1,127 reactions, and 138 phenotypes. Purple lines are newly added relations involving spingolipids.

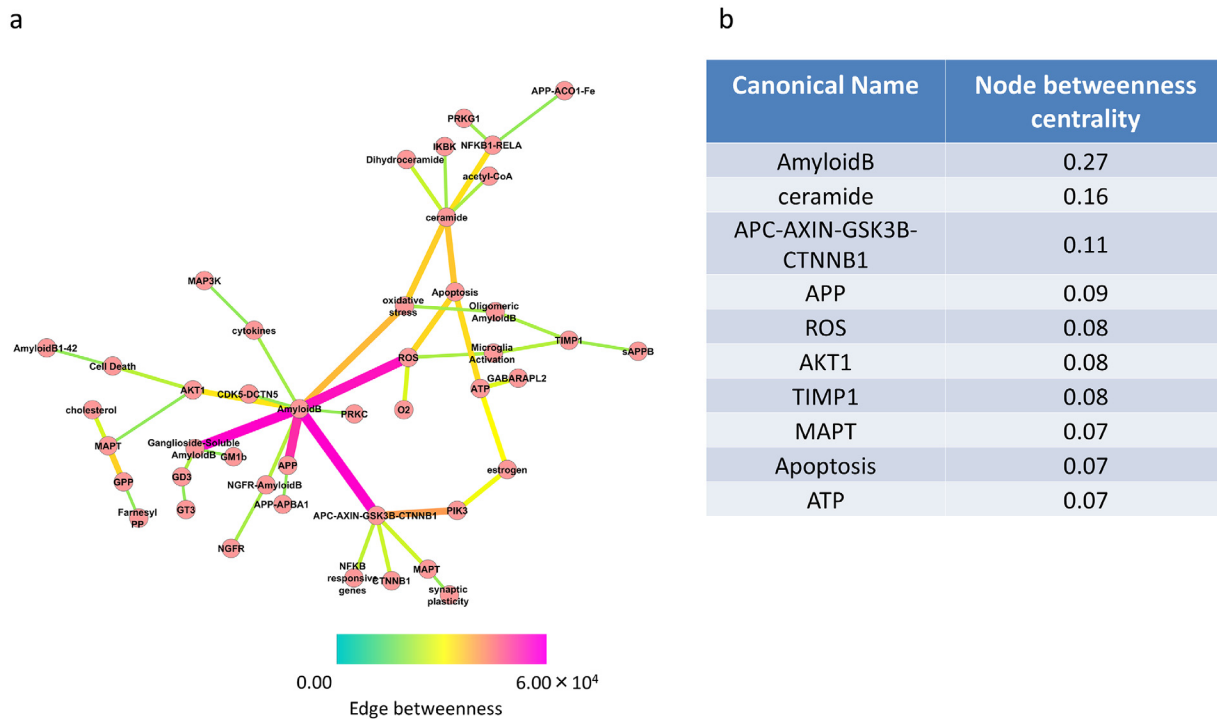
doi:10.1371/journal.pone.0148431.g001

“spingolipid synthesis in Goldi network” and “ceramide synthesis” (Fig 1). The AlzPathway 3 is available as the SBML map for CellDesigner (S2 File). The latest version of AlzPathway map is accessible at <http://www.alzpathway.org/>.

### Binary-relation notation and key molecules

To clarify the principal structure of AlzPathway 3, we constructed the binary-relation notation AlzPathway 3. Binary-relation notation AlzPathway 3 is available as the cys file (S3 File) which can be opened by Cytoscape 3. [45] High-centrality relations were highlighted as primary relations in accordance with the edge betweenness centrality of each reaction. The top 50 high-centrality relations are shown in Fig 2a. Among the several highlighted binary relations were AD hallmark pathways that are the same as in AlzPathway [16]: amyloid plaques (amyloid- $\beta$  accumulation), i.e., relations involving amyloid- $\beta$ , and neurofibrillary tangle accumulation (hyperphosphorylated tau accumulation), i.e., relations connecting amyloid- $\beta$  precursor protein (APP), APC-AXIN-GSK3 $\beta$ -CTNNB1 complex and microtubule-associated protein tau. Some less known relations were also found among the highlighted binary relations: those connecting





**Fig 2. Binary-relation notation AlzPathway 3 and the key molecules.** (a) The top 50 high-centrality relations as the highlighted primary pathway of AlzPathway. Circles are nodes in AlzPathway 3. Lines are edges between nodes. As represented, red lines have high edge betweenness centrality and blue lines have low. (b) The top 10 high-betweenness centrality nodes and their centrality.

doi:10.1371/journal.pone.0148431.g002

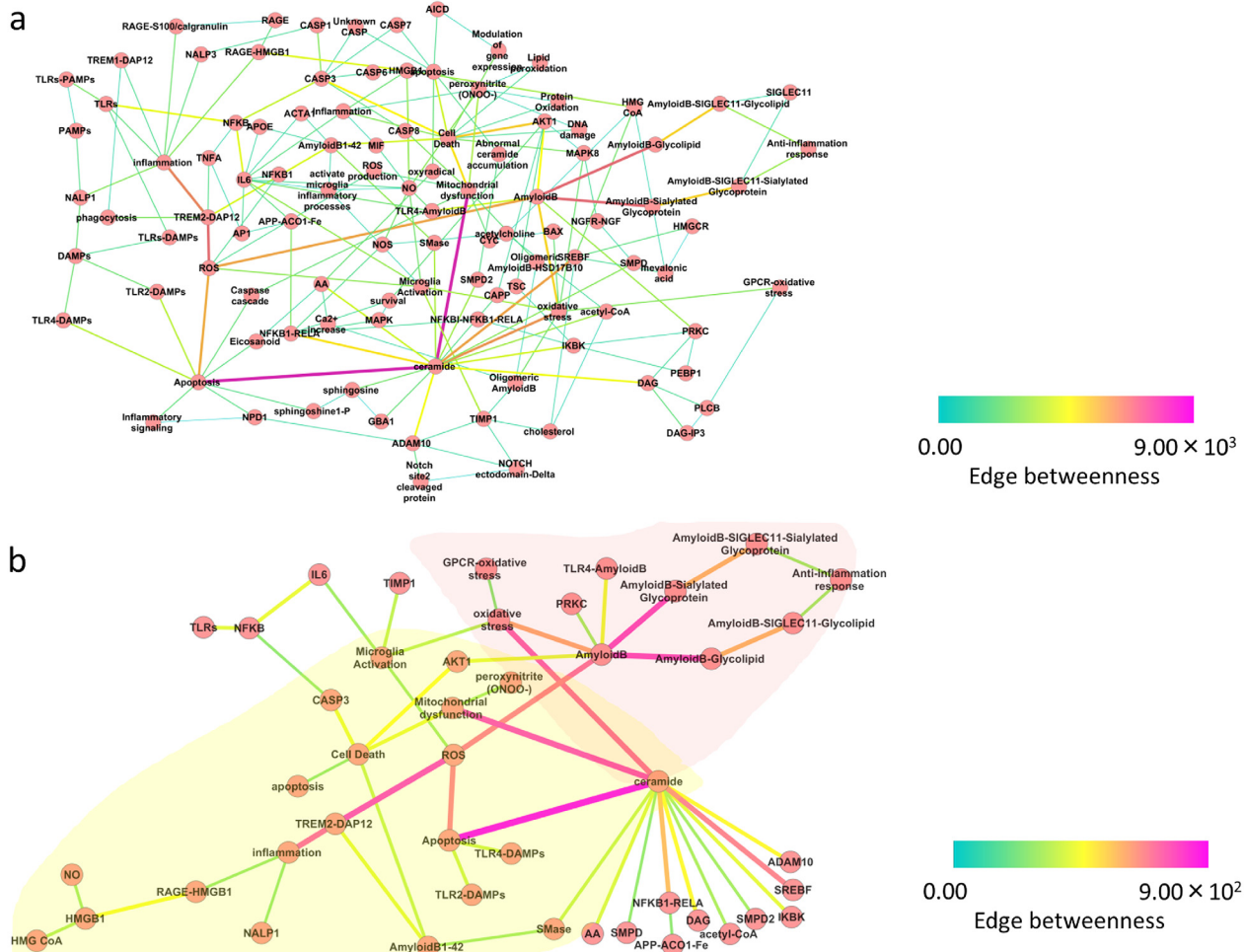
ceramide and amyloid-β. In the ranking of node betweenness (Fig 2b), amyloid-β is the highest centrality node (0.27) and ceramide is the second- highest (0.16).

These results for the centralities of binary-relation notation AlzPathway 3 show that pathways related to ceramide are some of the primary pathways, as are those involving amyloid plaques and neurofibrillary tangle accumulation. In addition, the results of our network analysis suggest that ceramide is one of the important players in the pathogenesis of AD, as shown in previous studies [46–48].

### Spingolipids and inflammation mediators in AlzPathway

Ceramide is one of the highest centrality nodes in binary-relation notation AlzPathway 3. We extracted a sub-network consisting of two hops from “Ceramide” and “Inflammation” from binary-relation notation AlzPathway 3 to model the comprehensive relationships among ceramide and inflammatory mediators (Fig 3a). The sub-network termed as ‘simplified binary-relation notation AlzPathway 3.’ Based on the edge betweenness centrality of each reaction, the high-centrality relations were highlighted as primary relations. The top 50 high-centrality relations in simplified binary-relation notation AlzPathway 3 are shown in Fig 3b.

We found two inflammation-related pathways in simplified binary-relation notation AlzPathway 3: (1) an inflammatory pathway connecting ceramide and “Inflammation” (overlaid by yellow), and (2) an anti-inflammatory pathway connecting ceramide and “Anti-inflammation response” (overlaid by red). The inflammatory pathway includes several proteins and phenotypes: TREM-DAP12 complex, sphingomyelinase, amyloid-β1–42, mitochondria dysfunction, microglia activation and apoptosis. TREM-DAP12 complex is the starting point in the inflammatory pathway, which is a well-known trigger of inflammation [49]. Previous



**Fig 3. Factors potentially associated with ceramide and inflammation.** (a) Extracted sub-network from the binary-relation notation AlzPathway 3. The simplified binary-relation notation AlzPathway 3 consists of two hops from “Ceramide” and “Inflammation.” (b) The top 50 high-centrality relations of the simplified binary-relation notation AlzPathway 3.

doi:10.1371/journal.pone.0148431.g003

studies showed that the secretion of sphingomyelinase is triggered by inflammatory cytokines [50] and that the expression of sphingomyelinase is upregulated in tissues/cells of inflammatory diseases including AD [7, 51]. Amyloid-β1–42 is known as a stimulator of neuroinflammation in the AD brain [52]. These studies suggest that TREM-DAP12 complex, sphingomyelinase and amyloid-β1–42 play roles in inflammatory signaling in the AD brain.

In contrast, the anti-inflammatory pathway includes several nodes showed as amyloid-β-, glycolipid- and glycoprotein-involved relations. In these relations, amyloid-β-glycolipid, amyloid-β-sialylated glycoprotein, and amyloid-β-sialic acid-binding immunoglobulin-type lectin (iglec) 11-glycolipid and amyloid-β-siglec 11-sialylated glycoprotein complexes were found. These siglec 11 complexes lie on the membrane of microglia and reduce the induction of interleukin (IL)-1β and inducible nitric oxide synthase [53]. This reduction activity leads to a suppression of microglial inflammation.

These studies reveals that ceramide has both proinflammatory and anti-inflammatory effects in microglia. Our network analysis suggests two possibilities regarding inflammation in AD: (1) ceramide is centered in both inflammatory and anti-inflammatory pathways of AD, and (2)

several factors such as sphingomyelinase and siglec 11 are associated with inflammation or anti-inflammation, respectively.

The regulatory mechanisms of inflammation and anti-inflammation by ceramide and inflammatory mediators will be revealed by further studies.

In conclusion, we showed the importance of ceramide in the pathogenesis of AD. We have collected and manually curated review articles related in AD, and we updated AlzPathway using Cell Designer named it AlzPathway 3. Our network analysis revealed that ceramide in particular among the sphingolipids is a key molecule in AD. Ceramide could play important roles in both inflammatory and anti-inflammatory pathways. The results of our network analysis also suggest mediators of inflammation and anti-inflammation in addition to ceramide in AD. These results contribute to a new hypothesis that ceramide play a dual role in the regulation of inflammation in AD brain and need further investigation as a mechanism of inflammatory mediation in the AD brain. The result of network analysis provides a clue to clarify inflammatory and anti-inflammatory pathway related sphingolipids in AD. Our comprehensive knowledge repository and network analysis approach will be used to increase the database for AD drug discovery and development.

## Comparing with other knowledge driven strategies

Recently, a study with network analysis of knowledge driven protein-protein interaction (PPI) network has been reported [54]. In this study, they calculated reliability scores of PPI with knowledge and discovered 'Knowledge cliff' which includes new therapeutic target of AD. Their research was specialized to discover therapeutic target with PPI rather than clarify AD related molecular mechanisms. Our AlzPathway focuses on not only PPI but also on signaling cascade including small molecules such as sphingolipids. Because of this completeness, AlzPathway is providing clues to clarify comprehensive molecular mechanisms such as network analysis on this research.

## Supporting Information

**S1 File. SBML map file of AlzPathway3.** The SBML map file `alzpathway_3` can be browsed using CellDesigner. Please download CellDesigner at <http://www.celldesigner.org/>, install it, and open the SBML map file `alzpathway_sbml_map.xml` to browse AlzPathway map by CellDesigner.

(XML)

**S2 File. Binary-relation notation file of AlzPathway 3.** The `cys` file `alzpathway_br.cys` is the binary-relation notation of AlzPathway 3 which can be opened by using Cytoscape 3.

(ZIP)

**S1 Table. Gene names and their functional categories which have been newly added to the AlzPathway 3.**

(XLSX)

## Acknowledgments

We are grateful for the helpful comments from the editor and anonymous referees. None of the authors has any conflict of interest related to this study. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

## Author Contributions

Conceived and designed the experiments: SM SO KK. Performed the experiments: SM. Analyzed the data: SM. Contributed reagents/materials/analysis tools: SM SO MK. Wrote the paper: SM SO KK HT NY JN.

## References

1. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet*. 2011; 377(9770):1019–31. doi: [10.1016/S0140-6736\(10\)61349-9](https://doi.org/10.1016/S0140-6736(10)61349-9) PMID: [21371747](https://pubmed.ncbi.nlm.nih.gov/21371747/).
2. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, et al. Inflammation and Alzheimer's disease. *Neurobiol Aging*. 2000; 21(3):383–421. PMID: [10858586](https://pubmed.ncbi.nlm.nih.gov/10858586/); PubMed Central PMCID: [PMC3887148](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC3887148/).
3. Lukiw WJ, Bazan NG. Inflammatory, apoptotic, and survival gene signaling in Alzheimer's disease. A review on the bioactivity of neuroprotectin D1 and apoptosis. *Mol Neurobiol*. 2010; 42(1):10–6. doi: [10.1007/s12035-010-8126-4](https://doi.org/10.1007/s12035-010-8126-4) PMID: [20414817](https://pubmed.ncbi.nlm.nih.gov/20414817/).
4. McGeer PL, McGeer EG. The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy. *Acta Neuropathol*. 2013; 126(4):479–97. doi: [10.1007/s00401-013-1177-7](https://doi.org/10.1007/s00401-013-1177-7) PMID: [24052108](https://pubmed.ncbi.nlm.nih.gov/24052108/).
5. Rubio-Perez JM, Morillas-Ruiz JM. A review: inflammatory process in Alzheimer's disease, role of cytokines. *ScientificWorldJournal*. 2012; 2012:756357. doi: [10.1100/2012/756357](https://doi.org/10.1100/2012/756357) PMID: [22566778](https://pubmed.ncbi.nlm.nih.gov/22566778/); PubMed Central PMCID: [PMC3330269](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC3330269/).
6. Pernber Z, Blennow K, Bogdanovic N, Mansson JE, Blomqvist M. Altered distribution of the gangliosides GM1 and GM2 in Alzheimer's disease. *Dement Geriatr Cogn Disord*. 2012; 33(2–3):174–88. doi: [10.1159/000338181](https://doi.org/10.1159/000338181) PMID: [22572791](https://pubmed.ncbi.nlm.nih.gov/22572791/).
7. Filippov V, Song MA, Zhang K, Vinters HV, Tung S, Kirsch WM, et al. Increased ceramide in brains with Alzheimer's and other neurodegenerative diseases. *J Alzheimers Dis*. 2012; 29(3):537–47. doi: [10.3233/JAD-2011-111202](https://doi.org/10.3233/JAD-2011-111202) PMID: [22258513](https://pubmed.ncbi.nlm.nih.gov/22258513/); PubMed Central PMCID: [PMC3643694](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC3643694/).
8. Teichgraber V, Ulrich M, Endlich N, Riethmuller J, Wilker B, De Oliveira-Munding CC, et al. Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. *Nat Med*. 2008; 14(4):382–91. doi: [10.1038/nm1748](https://doi.org/10.1038/nm1748) PMID: [18376404](https://pubmed.ncbi.nlm.nih.gov/18376404/).
9. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. *Nature*. 2014; 510(7503):58–67. doi: [10.1038/nature13475](https://doi.org/10.1038/nature13475) PMID: [24899305](https://pubmed.ncbi.nlm.nih.gov/24899305/); PubMed Central PMCID: [PMC4320971](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC4320971/).
10. Yuyama K, Mitsutake S, Igarashi Y. Pathological roles of ceramide and its metabolites in metabolic syndrome and Alzheimer's disease. *Biochim Biophys Acta*. 2014; 1841(5):793–8. doi: [10.1016/j.bbaliip.2013.08.002](https://doi.org/10.1016/j.bbaliip.2013.08.002) PMID: [23948264](https://pubmed.ncbi.nlm.nih.gov/23948264/).
11. Oda K, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. *Mol Syst Biol*. 2005; 1:2005 0010. doi: [10.1038/msb4100014](https://doi.org/10.1038/msb4100014) PMID: [16729045](https://pubmed.ncbi.nlm.nih.gov/16729045/); PubMed Central PMCID: [PMC1681468](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC1681468/).
12. Oda K, Kitano H. A comprehensive map of the toll-like receptor signaling network. *Mol Syst Biol*. 2006; 2:2006 0015. doi: [10.1038/msb4100057](https://doi.org/10.1038/msb4100057) PMID: [16738560](https://pubmed.ncbi.nlm.nih.gov/16738560/); PubMed Central PMCID: [PMC1681489](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC1681489/).
13. Calzone L, Gelay A, Zinovyev A, Radvanyi F, Barillot E. A comprehensive modular map of molecular interactions in RB/E2F pathway. *Mol Syst Biol*. 2008; 4:173. doi: [10.1038/msb.2008.7](https://doi.org/10.1038/msb.2008.7) PMID: [18319725](https://pubmed.ncbi.nlm.nih.gov/18319725/); PubMed Central PMCID: [PMC2290939](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC2290939/).
14. Patil S, Pincas H, Seto J, Nudelman G, Nudelman I, Sealfon SC. Signaling network of dendritic cells in response to pathogens: a community-input supported knowledgebase. *BMC Syst Biol*. 2010; 4:137. doi: [10.1186/1752-0509-4-137](https://doi.org/10.1186/1752-0509-4-137) PMID: [20929569](https://pubmed.ncbi.nlm.nih.gov/20929569/); PubMed Central PMCID: [PMC2958907](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC2958907/).
15. Fujita KA, Ostaszewski M, Matsuoka Y, Ghosh S, Glaab E, Trefois C, et al. Integrating pathways of Parkinson's disease in a molecular interaction map. *Mol Neurobiol*. 2014; 49(1):88–102. doi: [10.1007/s12035-013-8489-4](https://doi.org/10.1007/s12035-013-8489-4) PMID: [23832570](https://pubmed.ncbi.nlm.nih.gov/23832570/); PubMed Central PMCID: [PMC4153395](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC4153395/).
16. Mizuno S, Iijima R, Ogishima S, Kikuchi M, Matsuoka Y, Ghosh S, et al. AlzPathway: a comprehensive map of signaling pathways of Alzheimer's disease. *BMC Syst Biol*. 2012; 6:52. doi: [10.1186/1752-0509-6-52](https://doi.org/10.1186/1752-0509-6-52) PMID: [22647208](https://pubmed.ncbi.nlm.nih.gov/22647208/); PubMed Central PMCID: [PMC3411424](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC3411424/).
17. Van Dooren T, Princen K, De Witte K, Griffioen G. Derailed intraneuronal signalling drives pathogenesis in sporadic and familial Alzheimer's disease. *Biomed Res Int*. 2014; 2014:167024. doi: [10.1155/2014/167024](https://doi.org/10.1155/2014/167024) PMID: [25243118](https://pubmed.ncbi.nlm.nih.gov/25243118/); PubMed Central PMCID: [PMC4160617](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC4160617/).
18. Kwak JW, Jeong H, Han SH, Kim Y, Son SM, Mook-Jung I, et al. Phosphokinase antibody arrays on dendron-coated surface. *PLoS One*. 2014; 9(5):e96456. doi: [10.1371/journal.pone.0096456](https://doi.org/10.1371/journal.pone.0096456) PMID: [24802362](https://pubmed.ncbi.nlm.nih.gov/24802362/); PubMed Central PMCID: [PMC4011796](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC4011796/).



19. Caberlotto L, Lauria M, Nguyen TP, Scotti M. The central role of AMP-kinase and energy homeostasis impairment in Alzheimer's disease: a multifactor network analysis. *PLoS One*. 2013; 8(11):e78919. doi: [10.1371/journal.pone.0078919](https://doi.org/10.1371/journal.pone.0078919) PMID: [24265728](https://pubmed.ncbi.nlm.nih.gov/24265728/); PubMed Central PMCID: PMC3827084.
20. Wu C, Schwartz JM, Nenadic G. PathNER: a tool for systematic identification of biological pathway mentions in the literature. *BMC Syst Biol*. 2013; 7 Suppl 3:S2. doi: [10.1186/1752-0509-7-S3-S2](https://doi.org/10.1186/1752-0509-7-S3-S2) PMID: [24555844](https://pubmed.ncbi.nlm.nih.gov/24555844/); PubMed Central PMCID: PMC3852116.
21. Ogishima S, Mizuno S, Kikuchi M, Miyashita A, Kuwano R, Tanaka H, et al. A map of Alzheimer's disease-signaling pathways: a hope for drug target discovery. *Clin Pharmacol Ther*. 2013; 93(5):399–401. doi: [10.1038/clpt.2013.37](https://doi.org/10.1038/clpt.2013.37) PMID: [23511713](https://pubmed.ncbi.nlm.nih.gov/23511713/).
22. Funahashi A, Matsuoka Y, Jouraku A, Morohashi M, Kikuchi N, Kitano H. CellDesigner 3.5: A versatile modeling tool for biochemical networks. *P IEEE*. 2008; 96(8):1254–65. doi: [10.1109/Jproc.2008.925458](https://doi.org/10.1109/Jproc.2008.925458) WOS:000257860800003.
23. Horres CR, Hannun YA. The roles of neutral sphingomyelinases in neurological pathologies. *Neurochem Res*. 2012; 37(6):1137–49. doi: [10.1007/s11064-011-0692-y](https://doi.org/10.1007/s11064-011-0692-y) PMID: [22237969](https://pubmed.ncbi.nlm.nih.gov/22237969/).
24. Yu RK, Tsai YT, Ariga T. Functional roles of gangliosides in neurodevelopment: an overview of recent advances. *Neurochem Res*. 2012; 37(6):1230–44. doi: [10.1007/s11064-012-0744-y](https://doi.org/10.1007/s11064-012-0744-y) PMID: [22410735](https://pubmed.ncbi.nlm.nih.gov/22410735/); PubMed Central PMCID: PMC3895947.
25. Cai Z, Yan LJ, Li K, Quazi SH, Zhao B. Roles of AMP-activated protein kinase in Alzheimer's disease. *Neuromolecular Med*. 2012; 14(1):1–14. doi: [10.1007/s12017-012-8173-2](https://doi.org/10.1007/s12017-012-8173-2) PMID: [22367557](https://pubmed.ncbi.nlm.nih.gov/22367557/).
26. van Echten-Deckert G, Walter J. Sphingolipids: critical players in Alzheimer's disease. *Prog Lipid Res*. 2012; 51(4):378–93. PMID: [22835784](https://pubmed.ncbi.nlm.nih.gov/22835784/).
27. Car H, Zendzian-Piotrowska M, Fiedorowicz A, Prokopiuk S, Sadowska A, Kurek K. [The role of ceramides in selected brain pathologies: ischemia/hypoxia, Alzheimer disease]. *Postepy Hig Med Dosw (Online)*. 2012; 66:295–303. PMID: [22706115](https://pubmed.ncbi.nlm.nih.gov/22706115/).
28. Voronkov M, Braithwaite SP, Stock JB. Phosphoprotein phosphatase 2A: a novel druggable target for Alzheimer's disease. *Future Med Chem*. 2011; 3(7):821–33. doi: [10.4155/fmc.11.47](https://doi.org/10.4155/fmc.11.47) PMID: [21644827](https://pubmed.ncbi.nlm.nih.gov/21644827/); PubMed Central PMCID: PMC3292348.
29. Taki T. An approach to glycobiology from glycolipidomics: ganglioside molecular scanning in the brains of patients with Alzheimer's disease by TLC-blot/matrix assisted laser desorption/ionization-time of flight MS. *Biol Pharm Bull*. 2012; 35(10):1642–7. PMID: [23037154](https://pubmed.ncbi.nlm.nih.gov/23037154/).
30. Mielke MM, Lyketsos CG. Alterations of the sphingolipid pathway in Alzheimer's disease: new biomarkers and treatment targets? *Neuromolecular Med*. 2010; 12(4):331–40. doi: [10.1007/s12017-010-8121-y](https://doi.org/10.1007/s12017-010-8121-y) PMID: [20571935](https://pubmed.ncbi.nlm.nih.gov/20571935/); PubMed Central PMCID: PMC3129545.
31. Matsuzaki K, Kato K, Yanagisawa K. Aβ polymerization through interaction with membrane gangliosides. *Biochim Biophys Acta*. 2010; 1801(8):868–77. doi: [10.1016/j.bbali.2010.01.008](https://doi.org/10.1016/j.bbali.2010.01.008) PMID: [20117237](https://pubmed.ncbi.nlm.nih.gov/20117237/).
32. Colell A, Fernandez A, Fernandez-Checa JC. Mitochondria, cholesterol and amyloid beta peptide: a dangerous trio in Alzheimer disease. *J Bioenerg Biomembr*. 2009; 41(5):417–23. doi: [10.1007/s10863-009-9242-6](https://doi.org/10.1007/s10863-009-9242-6) PMID: [19784764](https://pubmed.ncbi.nlm.nih.gov/19784764/).
33. Salminen A, Kaamiranta K. Siglec receptors and hiding plaques in Alzheimer's disease. *J Mol Med (Berl)*. 2009; 87(7):697–701. doi: [10.1007/s00109-009-0472-1](https://doi.org/10.1007/s00109-009-0472-1) PMID: [19390836](https://pubmed.ncbi.nlm.nih.gov/19390836/).
34. Ariga T, McDonald MP, Yu RK. Role of ganglioside metabolism in the pathogenesis of Alzheimer's disease—a review. *J Lipid Res*. 2008; 49(6):1157–75. PMID: [18334715](https://pubmed.ncbi.nlm.nih.gov/18334715/); PubMed Central PMCID: PMC2386904.
35. Matsuzaki K. Physicochemical interactions of amyloid beta-peptide with lipid bilayers. *Biochim Biophys Acta*. 2007; 1768(8):1935–42. PMID: [17382287](https://pubmed.ncbi.nlm.nih.gov/17382287/).
36. Hartmann T. Role of amyloid precursor protein, amyloid-beta and gamma-secretase in cholesterol maintenance. *Neurodegener Dis*. 2006; 3(4–5):305–11. doi: [10.1159/000095271](https://doi.org/10.1159/000095271) PMID: [17047372](https://pubmed.ncbi.nlm.nih.gov/17047372/).
37. Soreghan B, Thomas SN, Yang AJ. Aberrant sphingomyelin/ceramide metabolic-induced neuronal endosomal/lysosomal dysfunction: potential pathological consequences in age-related neurodegeneration. *Adv Drug Deliv Rev*. 2003; 55(11):1515–24. PMID: [14597144](https://pubmed.ncbi.nlm.nih.gov/14597144/).
38. Han X, Gross RW. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. *J Lipid Res*. 2003; 44(6):1071–9. doi: [10.1194/jlr.R300004-JLR200](https://doi.org/10.1194/jlr.R300004-JLR200) PMID: [12671038](https://pubmed.ncbi.nlm.nih.gov/12671038/).
39. Haughey NJ, Bandaru VV, Bae M, Mattson MP. Roles for dysfunctional sphingolipid metabolism in Alzheimer's disease neuropathogenesis. *Biochim Biophys Acta*. 2010; 1801(8):878–86. doi: [10.1016/j.bbali.2010.05.003](https://doi.org/10.1016/j.bbali.2010.05.003) PMID: [20452460](https://pubmed.ncbi.nlm.nih.gov/20452460/); PubMed Central PMCID: PMC2907186.
40. Brandes U. A faster algorithm for betweenness centrality. *J Math Sociol*. 2001; 25(2):163–77. WOS:000171753600002.

41. Joy MP, Brock A, Ingber DE, Huang S. High-betweenness proteins in the yeast protein interaction network. *J Biomed Biotechnol.* 2005; 2005(2):96–103. doi: [10.1155/JBB.2005.96](https://doi.org/10.1155/JBB.2005.96) PMID: [16046814](https://pubmed.ncbi.nlm.nih.gov/16046814/); PubMed Central PMCID: PMC1184047.
42. Potapov AP, Voss N, Sasse N, Wingender E. Topology of mammalian transcription networks. *Genome Inform.* 2005; 16(2):270–8. PMID: [16901109](https://pubmed.ncbi.nlm.nih.gov/16901109/).
43. Girvan M, Newman ME. Community structure in social and biological networks. *Proc Natl Acad Sci U S A.* 2002; 99(12):7821–6. doi: [10.1073/pnas.122653799](https://doi.org/10.1073/pnas.122653799) PMID: [12060727](https://pubmed.ncbi.nlm.nih.gov/12060727/); PubMed Central PMCID: PMC122977.
44. Newman ME, Girvan M. Finding and evaluating community structure in networks. *Phys Rev E Stat Nonlin Soft Matter Phys.* 2004; 69(2 Pt 2):026113. PMID: [14995526](https://pubmed.ncbi.nlm.nih.gov/14995526/).
45. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 2003; 13(11):2498–504. doi: [10.1101/gr.1239303](https://doi.org/10.1101/gr.1239303) PMID: [14597658](https://pubmed.ncbi.nlm.nih.gov/14597658/); PubMed Central PMCID: PMC403769.
46. Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, et al. Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proc Natl Acad Sci U S A.* 2004; 101(7):2070–5. doi: [10.1073/pnas.0305799101](https://doi.org/10.1073/pnas.0305799101) PMID: [14970312](https://pubmed.ncbi.nlm.nih.gov/14970312/); PubMed Central PMCID: PMC357053.
47. He X, Huang Y, Li B, Gong CX, Schuchman EH. Deregulation of sphingolipid metabolism in Alzheimer's disease. *Neurobiol Aging.* 2010; 31(3):398–408. doi: [10.1016/j.neurobiolaging.2008.05.010](https://doi.org/10.1016/j.neurobiolaging.2008.05.010) PMID: [18547682](https://pubmed.ncbi.nlm.nih.gov/18547682/); PubMed Central PMCID: PMC2829762.
48. Mielke MM, Haughey NJ. Could plasma sphingolipids be diagnostic or prognostic biomarkers for Alzheimer's disease? *Clin Lipidol.* 2012; 7(5):525–36. PMID: [23606909](https://pubmed.ncbi.nlm.nih.gov/23606909/); PubMed Central PMCID: PMC3627378.
49. Tessarz AS, Cerwenka A. The TREM-1/DAP12 pathway. *Immunol Lett.* 2008; 116(2):111–6. doi: [10.1016/j.imlet.2007.11.021](https://doi.org/10.1016/j.imlet.2007.11.021) PMID: [18192027](https://pubmed.ncbi.nlm.nih.gov/18192027/).
50. Wong ML, Xie B, Beatini N, Phu P, Marathe S, Johns A, et al. Acute systemic inflammation up-regulates secretory sphingomyelinase in vivo: a possible link between inflammatory cytokines and atherogenesis. *Proc Natl Acad Sci U S A.* 2000; 97(15):8681–6. doi: [10.1073/pnas.150098097](https://doi.org/10.1073/pnas.150098097) PMID: [10890909](https://pubmed.ncbi.nlm.nih.gov/10890909/); PubMed Central PMCID: PMC27008.
51. Jenkins RW, Canals D, Hannun YA. Roles and regulation of secretory and lysosomal acid sphingomyelinase. *Cell Signal.* 2009; 21(6):836–46. PMID: [19385042](https://pubmed.ncbi.nlm.nih.gov/19385042/); PubMed Central PMCID: PMC3488588.
52. Terrill-Usery SE, Mohan MJ, Nichols MR. Amyloid-beta(1–42) protofibrils stimulate a quantum of secreted IL-1beta despite significant intracellular IL-1beta accumulation in microglia. *Biochim Biophys Acta.* 2014; 1842(11):2276–85. PMID: [25125050](https://pubmed.ncbi.nlm.nih.gov/25125050/); PubMed Central PMCID: PMC4188733.
53. Fernandes A, Miller-Fleming L, Pais TF. Microglia and inflammation: conspiracy, controversy or control? *Cell Mol Life Sci.* 2014; 71(20):3969–85. doi: [10.1007/s00018-014-1670-8](https://doi.org/10.1007/s00018-014-1670-8) PMID: [25008043](https://pubmed.ncbi.nlm.nih.gov/25008043/).
54. Malhotra A, Younesi E, Sahadevan S, Zimmermann J, Hofmann-Apitius M. Exploring novel mechanistic insights in Alzheimer's disease by assessing reliability of protein interactions. *Sci Rep.* 2015; 5:13634. doi: [10.1038/srep13634](https://doi.org/10.1038/srep13634) PMID: [26346705](https://pubmed.ncbi.nlm.nih.gov/26346705/); PubMed Central PMCID: PMC4562155.