Chapter 25

AlzPathway, an Updated Map of Curated Signaling Pathways: Towards Deciphering Alzheimer's Disease Pathogenesis

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Abstract

Alzheimer's disease (AD) is a complex neurodegenerative disorder in which loss of neurons and synaptic function causes dementia in the elderly. To clarify AD pathogenesis and develop drugs for AD, thousands of studies have elucidated signaling pathways involved. However, knowledge of AD signaling pathways has not been compiled as a pathway map. In this chapter, we introduce the manual construction of a pathway map in AD which we call "AlzPathway", that comprehensively catalogs signaling pathways in the field of AD. We have collected and manually curated over 100 review articles related to AD, and have built the AD pathway map. AlzPathway is currently composed of thousands of molecules and reactions in neurons, brain blood barrier, presynaptic, postsynaptic, astrocyte, and microglial cells, with their cellular localizations. AlzPathway provides a systems-biology platform of comprehensive AD signaling and related pathways which is expected to contribute to clarification of AD pathogenesis and AD drug development.

Key words Alzheimer's disease, Systems biology, Signaling pathway, Pathway map, Manual curation, AlzPathway, Drug discovery

1 Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disorder neuropathologically characterized by extracellular plaques of amyloid-beta (Aβ) peptide and intra-neuronal accumulation of neurofibrillary tangles (NFTs) [1]. AD causes dementia of the Alzheimer type in the elderly, with the number of patients increasing rapidly, which is becoming a serious social issue in the aging society. To address this issue, clarification of the pathogenic mechanisms of AD and development of AD drugs are urgently needed.

Genetic association studies with identification of putative AD susceptibility genes have been performed, and information collected in a publicly available database (AlzGene; http://www.alzgene.org/) [2].

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Efforts to clarify pathogenic signaling proteins and their signaling pathways in AD are also subject of continuous investigation. These are essential to understand two core pathological hallmarks of AD, amyloid plaques and neurofibrillary tangles (NFT) accumulation, with their underlying origin and exact role yet to be revealed. Several AD pathways associated with these two hallmarks have been studied in separate articles. However, they have not been properly compiled yet.

In this chapter, we introduce a manual construction of a pathway map in AD called "AlzPathway" that comprehensively catalogs signaling pathways in the field of AD [3]. We have collected and manually curated over 100 review articles related to AD, and manually elaborated an AD pathway map. AlzPathway is currently composed of thousands of molecules and reactions in neuron, brain blood barrier, presynaptic, postsynaptic, astrocyte, and microglial cells, with their cellular localizations.

Next generation high-throughput technologies (e.g. next generation sequencing (NGS), RNA-Sequencing (RNA-Seq), proteomics, metabolomics and others) are advancing rapidly, producing massive data contributing to identify e.g. pathogenic gene mutations, aberrant mRNA expression profiles, and aberrant protein interactions. AlzPathway allows not only to evaluate candidate risk genes listed by whole genome sequencing (WGS), but also to analyze 'omics data including e.g. RNA-Seq expression data to reveal patterns and pathways involved in pathogenesis of AD. AlzPathway provides a systems-biology platform of comprehensive AD signaling, which is expected to contribute to clarification of AD pathogenesis and AD drug development.

2 Materials

Over 100 carefully selected review articles involved in AD searched by PubMed were collected, to be manually curated, and pathogenic signaling proteins and their signaling pathways compiled as an AD pathway map (*see* **Note 1**).

3 Methods

According to our guideline of manual construction of AlzPathway (*see* **Note 1**), first, we collected selected AD review articles and conducted manual curation.

3.1 Collection of Review Articles and Manual Curation

We manually curated collected 123 review articles, and compiled pathogenic signaling proteins and their signaling pathways as an AD pathway map by using CellDesigner (http://www.celldesigner.org/) [4] (Fig. 1) (*see* **Note 2**). Molecules are distinguished, including

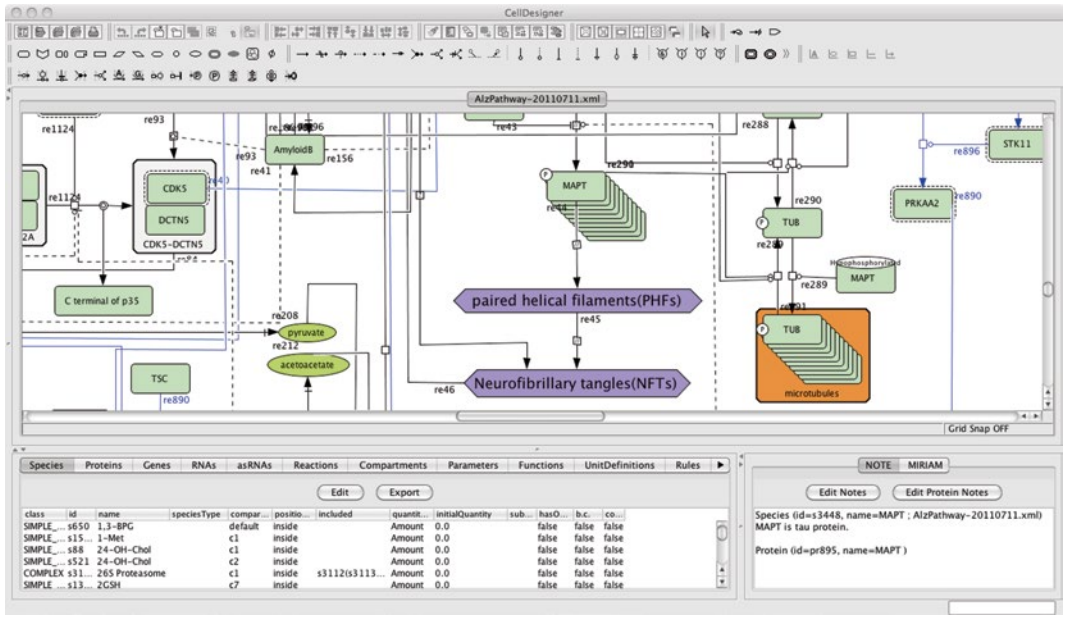

Fig. 1 Manual curation of collected review articles and compilation of AD pathogenic signaling proteins and signaling pathways using CellDesigner. 123 selected AD review articles were collected, manually curated and compiled as pathogenic signaling proteins and their signaling pathways. Visualization using CellDesigner

the following types: proteins, complexes, simple molecules, genes, RNAs, ions, degraded products and phenotypes. Reactions include the following categories: state transition, transcription, translation, heterodimer association, dissociation, transport, unknown transition, and omitted transition. Evidences/links to articles should be described as PubMed IDs using the MIRIAM scheme [5] for all reactions. Cellular types should be distinguished including the following: neuron, astrocyte, and microglial cells. Cellular compartments should include: brain blood barrier, presynaptic, postsynaptic, and cellular localizations. From here, we created a pathway model for AD by adding the molecules on a canvas, creating the reactions, with distinguishing molecule types, reaction categories, cellular types, and cellular compartments. We also added the notes and MIRIAMs to the molecules and the reactions, and tidying up the layout.

3.2 Update of AlzPathway

We have been incorporating new data and updating AlzPathway since the first release of the database [3]. As an example, in 2013 an association between the rs75932628 single nucleotide polymorphism (SNP) in the TREM2 gene and Alzheimer's disease was reported in persons of European ancestry [6, 7], a strong association comparable to those found for apolipoprotein E (APOE) gene variants. According to this new finding, three new species and six new reactions of the TREM2 gene and its related signaling molecules and relations were added to AlzPathway.

3.3 Web Service of AlzPathway

AlzPathway is available as a Systems Biology Markup Language (SBML) map for CellDesigner, i.e. compliant with the SBML language [8] for file exchange between different applications, and as a high resolution image map at http://alzpathway.org/.

AlzPathway is also available as a web service, online map implemented using Payao [9] (Fig. 2). Payao is a community-based, collaborative web service to enable a community to work on the same gene-regulatory and biochemical pathway model simultaneously, insert tags to the model, exchange comments, record discussions and update models. Payao will allow AD researchers not only to browse reactions and their references in PubMed ID but also to comment, correct and update AlzPathway in a community-wide collaboration.

3.4 Overview of AlzPathway

An overview of AlzPathway is shown in Fig. 3. The AlzPathway map consists of 1,347 species, 1,070 reactions, and 129 phenotypes. The molecules are classified as follows: 650 proteins, 232 complexes, 223 simple molecules, 32 genes, 36 RNAs, 24 ions, and 21 degraded products. The reactions are classified as 401 state transitions, 22 transcriptions, 30 translations, 172 heterodimer associations, 49 dissociations, 87 transports, 20 unknown transitions, and 228 omitted transitions. The map consists of the AD hallmark pathways and canonical pathways. The AD hallmark pathways are amyloid β cleavage, amyloid β degradation, APOE-cholesterol pathway and NFT accumulation, which are major pathological

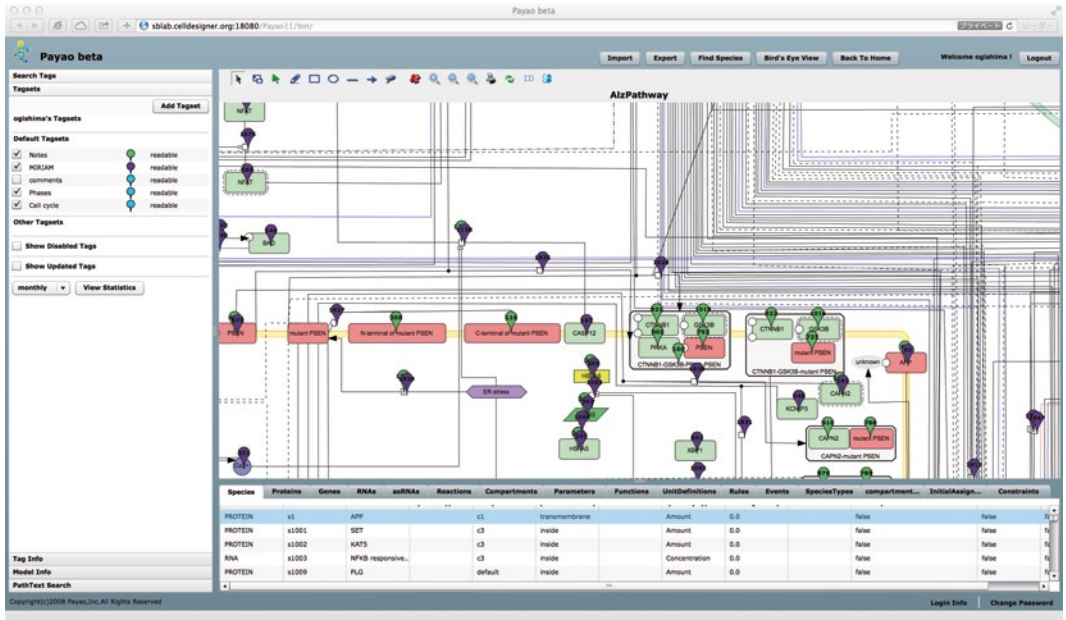

Fig. 2 Community curation of AlzPathway using Payao. Payao system provides a community-based, collaborative web service (online map) platform for pathway manual curation

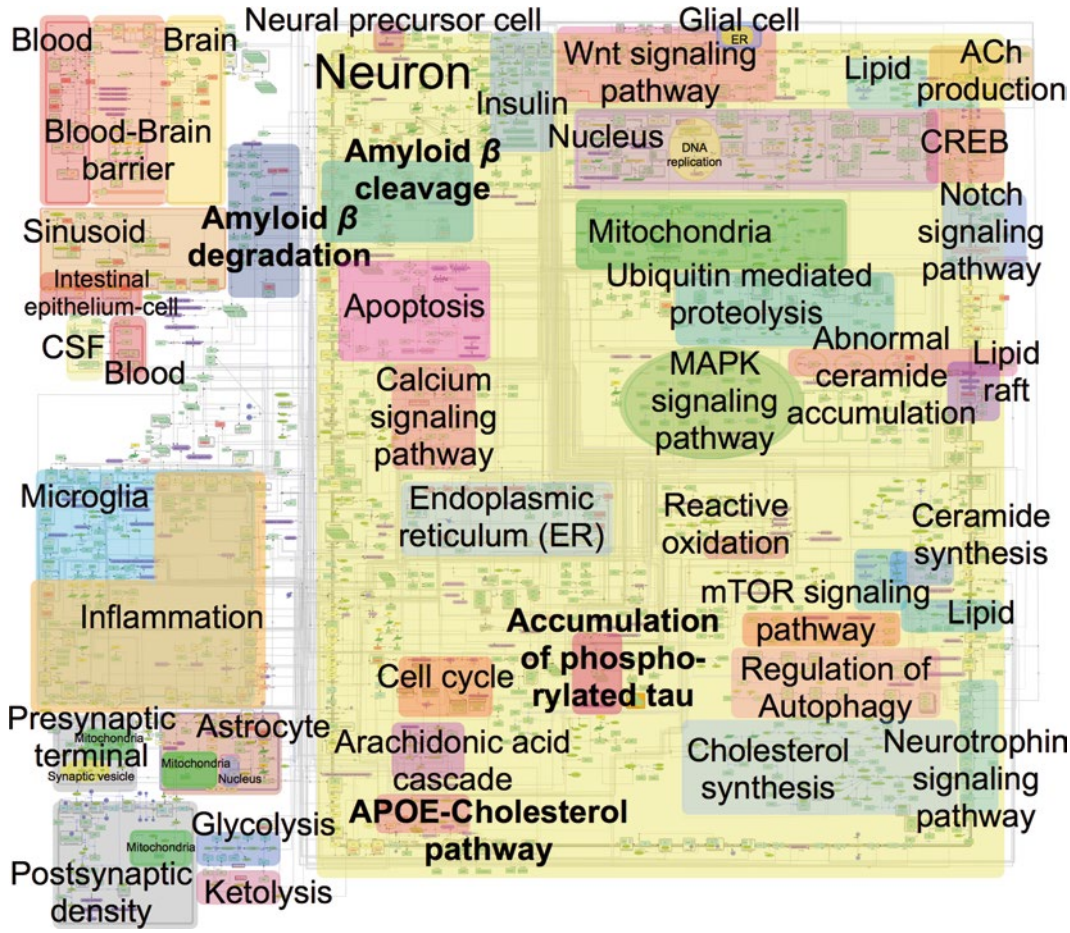

Fig. 3 Overview of AlzPathway overlaid with canonical pathway annotations AlzPathway consists of 1,347 molecules, 1,070 reactions, and 129 phenotypes. AlzPathway is available at the http://alzpathway.org website

pathways of AD. The canonical pathways are acetylcholine production, cholesterol synthesis, Wnt signaling pathway, Notch signaling pathway, Ubiquitin mediated proteolysis, apoptosis, calcium signaling pathway, ER stress, MAPK signaling pathway, abnormal ceramide accumulation, ceramide synthesis, reactive oxidation process, regulation of autophagy, neurotrophin signaling pathway, cell cycle, arachidonic acid cascade, mTOR signaling pathway, lipid pathway, lipid raft, inflammation pathway, insulin pathway, and CREB pathway. Manual elaborations of comprehensive maps have been made before, e.g. for epidermal growth factor receptor (EGF) signaling, toll-like receptor signaling network, RB/E2F signaling and mTOR signaling pathways [10–13], which are individual signaling pathways, but not compilations of pathways/maps involved in a particular disease. Therefore, AlzPathway is the first comprehensive map of a particular disease manually constructed,

which catalogs not only intra- but also inter- and extracellular signaling pathways among neurons, glial cells, microglia, presynaptic cells, postsynaptic cells, astrocytes, and the blood–brain barrier. The brain and spinal cord are made up of various regions and cells, including neurons and glial cells. To clarify pathogenic mechanisms of AD, complex signaling pathways among neurons, glial cells, microglia, presynaptic and postsynaptic cells, astrocytes, and the blood–brain barrier should be elucidated.

3.5 AlzPathway Applications

AlzPathway provides most relevant reported pathogenic signaling proteins and their complex relations (*see* above). Using AlzPathway, we could explore e.g. relationships between two core pathological hallmarks of AD, amyloid plaques and neurofibrillary tangles (NFT) accumulation, and find key molecules in complex signaling pathways. To explore key molecules, network analysis is efficient. We converted the AlzPathway in SBGN PD notation (Systems Biology Graphical Notation-Process Description) notation [14], to a binary-relation notation as a simple interaction format (SIF) file, which can be opened using Cytoscape [15] (the SBGN PD notation [14] is a precise notation for describing pathways but is unsuitable for network analyses). We then calculated edge betweenness centrality, which is defined as the number of the shortest paths that go through an edge in a graph or network V [16]. The edge betweenness centrality is formulated as follows:

3.5.1 Key Molecules Discovery

$$C_b(e) = \sum_{u,w \in V, u \neq w} \frac{\sigma_{uw}(e)}{\sigma_{uw}}$$

where $\sigma_{uw}(e)$ denotes the total number of shortest path between u and w that pass through edge e, and σ_{uw} denotes the total number of shortest paths between u and w. According to their centralities, high centrality relations were obtained and are highlighted in Fig. 4. Highlighted binary relations found were e.g. amyloid plagues formation (amyloid β accumulation) and NFT accumulation (hyperphosphorylated tau accumulation), two AD hallmark pathways. The γ-secretase mediates e.g. amyloid β 1–40 production, which aggregates to form oligomeric amyloid β (amyloid β accumulation) crucial for AD progression. Together with this, microtubule-associated protein tau (encoded by the MAPT gene) is phosphorylated by mutant presenilins (PSEN) and APC-AXIN-GSK3B-CTNNB1, which leads to tau hyperphosphorylation and aggregates to result in NFTs accumulation.

3.5.2 Pathway-Based Drug Discovery

As described above, high centrality relations can be highlighted, and their molecular components, e.g. amyloid β, γ-secretase, APP, APOE, and MAPT, are considered key molecules in AD pathogenesis. A key molecule might be a drug target. A drug targeting a key molecule can affect (e.g. inhibit) its functioning, which might be

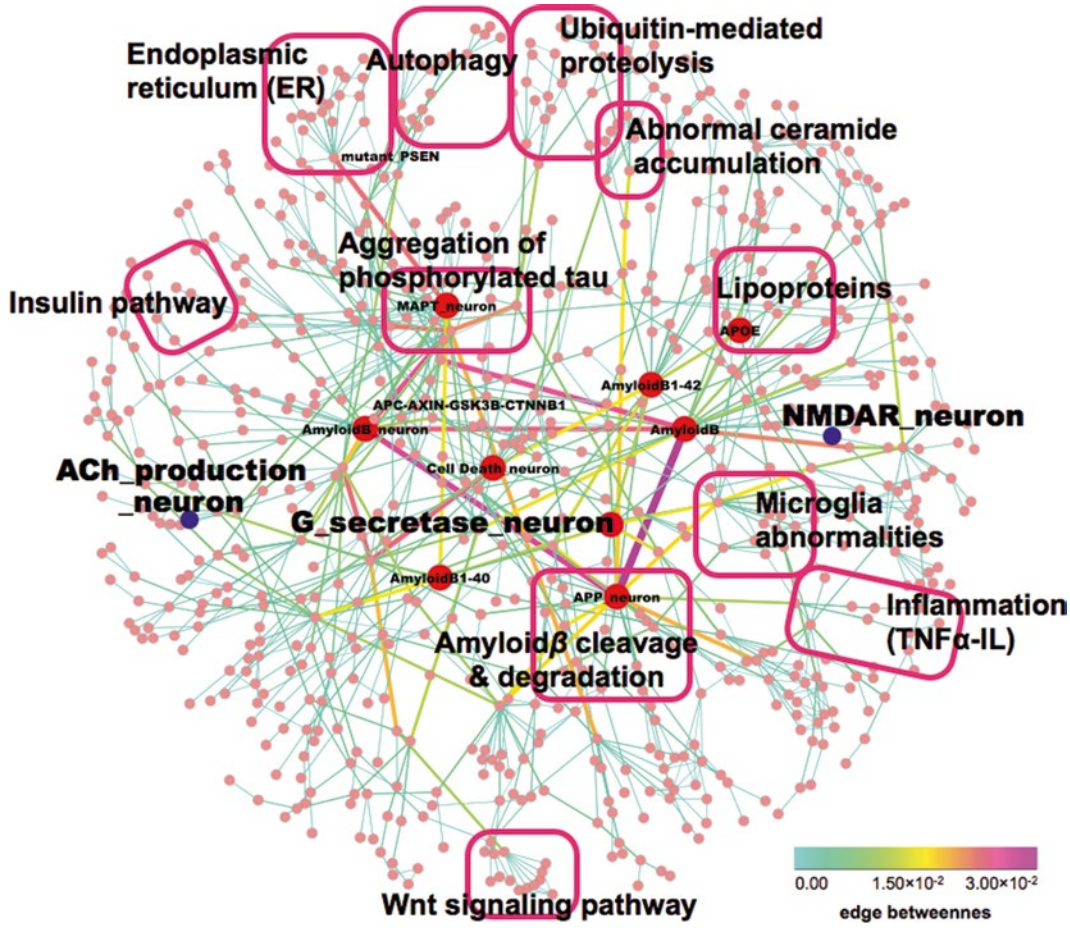

Fig. 4 High centrality relations of AlzPathway and key molecules and pathways. Overview of AlzPathway in binary-relation notation. High centrality relations and key molecules and pathways

investigated as a candidate drug for AD. At the same time, a drug WWtargeting a key molecule might develop significant side effects by affecting off-targets. Could a drug targeting a key molecule be a curative drug for AD?

Currently approved drugs by the U.S. Food and Drug Administration (FDA) are only able to treat, palliate AD symptoms. These are for example, tacrine, rivastigmine, galantamine, donepezil, and memanatine. Tacrine, rivastigmine, galantamine and donepezil are cholinesterase inhibitors, and memanatine is an *N*-methyl-D-aspartic acid (NMDA) receptor antagonist. Interestingly, according to the AlzPathway map, cholinesterase and NMDA receptor are peripheral, not key molecules (Fig. 4), no major interacting pathway appears involved and no interacting compensatory pathways are observed either (Fig. 4). This may implies that these drugs might not cause significant side effects due to off-target effects (focusing on the

AD map only). For these drugs, it can be predicted to have relatively specific effects, due to the low centrality of their target molecules in the AD signaling network. These drugs are dementia-suppressing, palliative drugs, not AD curative drugs. Drugs targeting key molecules with minimum side effects might be investigated as candidate curative drugs.

Semagacestat, a γ-secretase inhibitor targeting a key molecule, γ-secretase, was expected to be a promising drug for AD. Semagacestat had been developed for AD treatment, and was in Phase III. However, it was found to increase the risk of skin cancer, with significant side effects due to its off-targets. Semagacestat targets not only γ-secretase but also peripheral Notch signaling pathway, which heightens a risk of skin cancer compared to placebo. In fact, in the AlzPathway, γ-secretase is as a key molecule showing high centrality, which, if inhibited, could affect unintended downstream molecules and pathways (Fig. 4). Also γ-secretase has a clear relationship with Notch signaling. Thus, AlzPathway collects and provides comprehensive knowledge of AD pathways, and is able to show the possibility of significant side effects according to the structure/topology and relations of the AD signaling pathways.

3.5.3 Cross-Pathway Analysis Between Neurodegenerative Diseases

The rs75932628 SNP in the TREM2 gene was reported to be very strongly associated with Alzheimer's disease, and association comparable to those found for apolipoprotein E (APOE) gene variants. Therefore, recently the TREM2 and its related signaling molecules and reactions were added to the AlzPathway as the latest key molecules and reactions. Interestingly, the TREM2 gene is also reported to be associated with another neurodegenerative disease, Parkinson's disease (PD), and a PD map is available as a PD pathway map. (http://minerva.uni.lu/pd_map) [17]. We could explore the possibility that AlzPathway and PD maps may have common pathogenic signaling molecules and reactions. We conducted cross-pathway analysis between AlzPathway and PD maps. The TREM2 gene could not appear as a common pathogenic signaling molecule because the TREM2 gene has not been compiled in the PD map yet. However, we found several common pathogenic signaling molecules and reactions including amyloid β precursor protein (APP) and tau (MAPT gene). Cross-pathway analysis may clarify pathogenic signaling molecules and reactions common between Alzheimer's disease and other neurodegenerative diseases at different stages of the disease.

AlzPathway is the first comprehensive map of intra, inter and extra cellular signaling pathways networks of a particular disease, towards deciphering pathogenesis of AD and to assist in the developing of AD drugs. AlzPathway is currently composed of 1,347 molecules, 1,070 reactions, and 129 phenotypes in neuron, brain

blood barrier, presynaptic, postsynaptic, astrocyte, and microglial cells and their cellular localizations. We are planning to update AlzPathway with support of natural language processing (NLP). AlzPathway is freely available and can be updated by the AD research community. AlzPathway provides a comprehensive resource to the AD community towards deeper insights into AD pathogenesis and identification of novel therapeutic targets.

4 Notes

1. The guideline for manual construction of AlzPathway can be summarized as follows: (1) Collection of review articles searched in PubMed. To manually elaborate AlzPathway, manual curation of ca. 100,000 AD research articles published after 2000 was needed, which was discarded. Instead of this, careful selection and manual curation of review articles with current state and understanding of AD pathogenic signaling proteins and signaling pathways was performed; (2) Manual curation of collected review articles. Manually curate AD review articles, and compile AD pathogenic signaling proteins and their signaling pathways using CellDesigner. Molecules should be distinguished, including the following types: proteins, complexes, simple molecules, genes, RNAs, ions, degraded products and phenotypes. Reactions should be also distinguished, including the following categories: state transition, transcription, translation, heterodimer association, dissociation, transport, unknown transition, and omitted transition. Evidences/links to articles should be described as PubMed IDs using the MIRIAM scheme for all reactions. Cellular types should be distinguished including the following: neuron, astrocyte, and microglial cells. Cellular compartments should include: brain blood barrier, presynaptic, postsynaptic, and cellular localizations.
2. CellDesigner is a structured diagram editor for drawing gene-regulatory, biochemical and signaling networks. Intuitive user-interface allows to draw a diagram in rich graphical notation. Notation is compliant with the PD (Process Description) of SBGN (Systems Biology Graphical Notation) [14].

Acknowledgements

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