

Role of altered cerebral proteolysis in the pathogenesis of sporadic Alzheimer's disease



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**Abstract**

Alzheimer’s disease (AD) is a common, age-related, neurodegenerative disease characterized by slowly-progressive brain shrinkage and cognitive decline. This global health challenge places a severe strain on both public health systems and patients’ families. Yet, there are still no effective treatments, owing at least in part to mechanistic uncertainty.

This research aims to conduct a multi-regional comparison of altered cerebral proteolysis in control and AD human brains, by employing mass spectrometry-based proteomics to obtain identity and expression data of proteases and inhibitors, followed by Bayesian modelling to compare relative levels of each protease between cases and control individuals. We hypothesize that proteolysis could play a crucial role in this form of brain atrophy.

We plan to identify and measure cerebral proteases and protease inhibitors whose expression levels are significantly altered in human AD brains. The total number of proteases with elevated expression levels in each region is expected to correlate with AD severity.

This research will provide insights into mapping of changes of proteases and protease inhibitors in human AD brains and will be of significant value in obtaining improved mechanistic knowledge of AD pathogenesis. The results of this study may contribute to discovering biomarkers and developing effective treatments for AD.

**Introduction**

General Background

Dementia is not a specific single disease. It is a syndrome that can be caused by multiple diseases that can destroy neurons and affect the brain gradually, leading to the decline of cognitive function (the ability to think, memorize, and reason) and the ability to perform everyday activities (National Insitute on Aging, 2022b; World Health Organization, 2023). Among the multiple risk factors for dementia, the increasing age process itself has by far the most impact (Centers for Disease Control and Prevention, 2019; World Health Organization, 2023). The elderly, aged 65 and over, are the most severely affected group (Centers for Disease Control and Prevention, 2019; World Health Organization, 2023). Nevertheless, dementia does not result from normal senescence (Centers for Disease Control and Prevention, 2019).

Common forms of dementia include Alzheimer’s disease (AD) dementia, Dementia with Lewy bodies (DLB, abnormal protein alpha-synuclein deposition in neurons), Parkinson’s disease dementia (PDD), Vascular dementia (damage to cerebral blood vessels or interruption of blood and oxygen flow to the brain), and Frontotemporal dementia (FTD, a group of diseases that are related to the abnormal quantities or forms of the proteins tau and TDP-43, then degenerating the frontal lobe) (Hanagasi, Tufekcioglu, & Emre, 2017; National Insitute on Aging, 2022b; Scholefield et al., 2023; World Health Organization, 2023). There are also some other causes of dementia or dementia-like symptoms, such as Huntington’s disease, Normal pressure hydrocephalus, HIV-associated dementia, Creutzfeldt-Jakob disease (CJD), Amyotrophic lateral sclerosis with dementia (ALS-D), Chronic traumatic encephalopathy, thyroid problems, prolonged alcohol abuse, head injury, emotional problems, medication side effects, vitamin deficiencies, and delirium (Alzheimer's Association, 2023b; National Insitute on Aging, 2022b; Yoshida, 2004). Among them, dementia caused by AD accounts for the largest proportion, approximately 60 - 70% of all cases (World Health Organization, 2023). Notably, different types of dementia can be combined, which is called “mixed dementia” (MD) (Alzheimer's Association, 2023a; Langa, Foster, & Larson, 2004). At present, dementia ranks seventh in global causes of death, and can cause disability and dependency in aged people (World Health Organization, 2023).

Neurodegeneration is the process of losing structure and/or function for nerve cells (Przedborski, Vila, & Jackson-Lewis, 2003). Neurodegenerative diseases (ND) are a general term for several progressive neurological disorders characterized by neurodegeneration (Przedborski et al., 2003; Rivero-Segura, Guerrero-Cruz, & Barrera-Vázquez 2020). It results in the progressive loss of particular nerve cells, affecting increasingly more brain regions over time, resulting in progressive loss of cognitive and motor function (Fu, Hardy, & Duff, 2018; Gan, Cookson, Petrucelli, & La Spada, 2018; Rivero-Segura et al., 2020). Aging is also the strongest risk factor for the majority of neurodegenerative diseases (Hou et al., 2019). Common age-related neurodegenerative diseases (ARND) comprise AD, Parkinson’s disease (PD), FTD, amyotrophic lateral sclerosis (ALS), spinocerebellar ataxias (SCAs), and ischaemic stroke (Rivero-Segura et al., 2020). AD is the most prevalent ARND (Hou et al., 2019). Currently, there are few or no effective treatments for ageing-related neurodegenerative diseases (Hou et al., 2019).

AD was first reported in 1906 by Alois Alzheimer, a clinical psychiatrist and neuroanatomist (Hippius & Neundörfer, 2003; Möller & Graeber, 1998; Stelzma, Schnitzlein, & Murllagh, 1995). It is a progressive ARND that can cause dementia. Based on whether it is hereditary or not, AD can be divided into familial AD and sporadic AD. Familiar AD is an autosomal dominant disease; known related gene mutations occur in amyloid precursor protein (APP), presenilin 1 (PSEN1) and PSEN2, and the apolipoprotein E ε4 allele (APOE ε4), accounting for just 5% of all AD cases (Knopman et al., 2021); sporadic AD accounts for the remaining 95%, and is more related to age. Based on the age at which the symptoms/signs first appear, AD can be classified into late-onset and early-onset AD. Late-onset AD is the most common type, whose earliest signs typically appear in the mid-60s, while early-onset AD is rare, the earliest signs typically appearing between the 30s and mid-60s. Late-onset AD may be related to the APOE ɛ4 allele, and early-onset AD is frequently caused by gene mutations inherited from parents (National Insitute on Aging, 2019).

It is estimated that the number of people with AD currently numbers 416 million worldwide, accounting for 22% of people aged 50 and above (Gustavsson et al., 2023). The incidence and prevalence of AD are high globally, but numbers may still be underestimated due to underdiagnosis and misdiagnosis (Tahami Monfared, Byrnes, White, & Zhang, 2022).

AD is a global challenge for public health systems because patients can become severely disabled and must depend on others most of the time before death (Alzheimer's Association Report, 2023). AD also places a considerable strain on the patients’ families due to the time cost, economic cost, and emotional pressure of caregiving (Alzheimer's Association Report, 2023).

The symptoms of AD may vary from person to person. Symptoms will become increasingly severe with time because it is a progressive disease, patients will experience increased confusion and behavioural changes (National Insitute on Aging, 2022a). Clinically, AD can be divided into four stages. The first stage is preclinical AD, which is asymptomatic, and complex changes associated with AD start from this stage. The next stage is mild (sometimes called early-stage) AD, where patients begin to struggle to understand the surrounding world. Possible problems at this stage include loss of memory, spontaneity and initiative, weakness in judgment and solving problems, forgetting the date or location or places of items, spending more time finishing everyday activities, repeating questions or forgetting what has been learned recently, changes in emotions and personality, wandering and getting lost, and increased anxiety and/or aggression. The third stage is moderate AD, which is characterized by a series of symptoms that include increased confusion and memory loss; withdrawal from social activities; inability to learn something new; problems with language, reading, writing, handling numbers, performing familiar multi-step tasks, and thoughts and logical thinking; shortening of attention span; changes in sleep patterns; sometimes inability to recognize family and friends; illusions, delusions, and paranoia; impulsive behaviour; inappropriate emotional outbursts; irritation, agitation, anxiety, crying, wandering, especially in the late afternoon or evening; repeating statements or movements, occasional muscle twitching. The final stage is severe AD. In this stage, patients are completely unable to communicate and take care of themselves. They are not conscious of recent experiences or the surrounding environment, undergo weight loss, have little interest in diet, may suffer from seizures, decline in general physique, appear dysphagic (difficult in swallowing), vocalize by groaning, moaning, or grunting, sleep more and may lose control of intestinal and bladder function (National Insitute on Aging, 2022a). In the end, AD patients frequently die due to complications triggered by severe AD dementia, for instance, immobility, malnutrition, and swallowing disorders (Alzheimer's Association Report, 2023). The most common cause of death in AD is pneumonia due to dysphagia (Burns, Jacoby, Luthert, & Levy, 1990; Todd, Barr, & Passmore, 2013). The impact of AD on life span depends mainly on the age of the patient at diagnosis (Brookmeyer, Corrada, Curriero, & Kawas, 2002). AD patients survived an average (standard deviation, SD) duration of 5.9 (3.7) years and longer when the onset occurs at a younger age (Ganguli, Dodge, Shen, Pandav, & DeKosky, 2005).

The brains with AD are characterized by amyloid plaques (abnormal aggregation of beta-amyloid (Aβ) proteins), neurofibrillary tangles (NFTs, abnormal aggregation of tau proteins), chronic inflammation (may be related to the buildup of glial cells), vascular issues, and the loss of neuronal connections and neurons (National Insitute on Aging, 2017). First, AD typically disrupts neurons and their connections in the entorhinal cortex (ENT) and hippocampus (HP), which are parts of the brains responsible for memory. Thus, AD patients first suffer from memory loss. Afterwards, it destroys regions in the cerebral cortex responsible for reasoning, language, and social behaviour. Thus, AD patients will gradually undergo a loss of ability to care for themselves. Later, the damage extends to other brain regions, and patients gradually lose more of their ability to take care of themselves. Ultimately, the medulla oblongata is affected, causing difficulty swallowing and breathing, finally leading to death (National Insitute on Aging, 2017). It is noteworthy that AD neuropathologic changes may start a few years before symptoms occur, and before cognitive decline (Montine et al., 2012; Sperling et al., 2011).

The progression of pathological changes and the severity in AD brains can be described by the Braak staging system, which was proposed by Heiko Braak and Eva Braak in 1991 (Braak & Braak, 1991). The Braak staging system defines AD brains based on the distribution and severity of NFTs. In Braak Stage 0, the brain presents no observable AD pathology, corresponding to non-AD. In Braak Stages I and II, NFTs begin to accumulate mainly in the ENT, corresponding to early-AD. Then, in Braak Stages III and IV, NFTs spread to other brain regions, including the HP, corresponding to intermediate-AD. In parallel, cognitive decline often becomes noticeable. In Braak Stages V and VI, NFTs have already spread to the neocortex and impair higher cognitive functions such as language and reasoning, corresponding to late-AD. Thus, at these stages, patients suffer from severe cognitive decline.

Another widely used practical criteria for AD neuropathologic change was developed by The Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) (Mirra et al., 1991). This diagnostic neuropathologic criteria aimed to provide a comparatively impersonal, relatively easy to understand and use system, using common language, and a uniform diagnostic approach for AD. To develop this criterion, they collected the data from comprehensive post-mortem brain evaluations from 142 individuals with probable AD and eight control individuals. First, they made a semiquantitative assessment of the density of neocortical senile plaques (the major component is the Aβ protein) of the neuritic type and NFTs (the major component is the tau protein). Then, they combined the results of the semiquantitative assessment and the patient’s age at death to create a scoring table for age-related plaque (Table 1). Finally, they defined the level of certainty of the neuropathologic diagnosis for AD clearly based on the results of clinical diagnosis while alive and post-mortem brain examinations:

(1) normal brain: ①No clinical history of dementia, “0” age-related plaque score, and no other neuropathologic lesions likely to cause dementia; ②No clinical history of dementia and “A” age-related plaque score; ③Clinical history of dementia and no neuropathologic lesions likely to cause dementia;

(2) possible AD: ①Clinical history of dementia, “A” age-related plaque score, and presence or absence of other neuropathologic lesions likely to cause dementia; ②“B” or “C” age-related plaque score and absence of clinical manifestations of dementia;

(3) probable AD: Clinical history of dementia, “B” age-related plaque score, and presence or absence of other neuropathologic disorders likely to cause dementia;

(4) definite AD: Clinical history of dementia, “C” age-related plaque score, and presence or absence of other neuropathologic lesions likely to cause dementia.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| the age of the patient at death (years) | Frequency of plaques | | | |
| None | Sparse | Moderate | Frequent |
| <50 | 0 | C | C | C |
| 50-75 | 0 | B | C | C |
| >75 | 0 | A | B | C |

Table 1. Age-related plaque scores for CERAD diagnostic neuropathologic criteria for AD. 0 = NO histologic evidence of AD, A = Histologic findings are UNCERTAIN evidence of AD, B = Histologic findings SUGGEST the diagnosis of AD, C = Histologic findings INDICATE the diagnosis of AD. This table from the CERAD neuropathologic assessment of AD by Mirra et al. in 1991 (Mirra et al., 1991).

The third assessment criteria for neuropathologic changes in AD brains is the “ABC” score approach from the guidelines of the National Institute on Aging (NIA) and Alzheimer’s Association (Montine et al., 2012). In this approach, “A” stands for the histopathologic assessments of Aβ aggregation, described by a modified version of Thal phases (this approach divides progression of β-amyloidosis into five phases: in phase 1, Aβ only deposits in the neocortex; in phase 2, Aβ deposits spread to allocortical brain regions; in phase 3, Aβ deposits further spread to the striatum, diencephalic nuclei, and the cholinergic nuclei of the basal forebrain; in phase 4, a few distinct brainstem nuclei, including red nucleus, substantia nigra, central gray, inferior olivary nucleus, superior colliculus and inferior colliculus, and intermediate reticular zone, also exhibit Aβ deposits; in phase 5, cerebellum and additional brainstem nuclei (locus coeruleus, pontine nuclei, parabrachial nuclei, dorsal tegmental nucleus, reticulo-tegmental nucleus, and oral and central raphe nuclei) involves in Aβ deposition) (Thal, Rüb, Orantes, & Braak, 2002); “B” stands for staging scheme of NFTs, described by the Braak staging system; and “C” stands for neuritic plaque scoring, described by the CERAD criteria. The scores of “ABC” are shown in Table 2. Afterwards, “ABC” scores are utilized to report the neuropathologic changes in AD brains. The fixed standard report format is “Alzheimer Disease Neuropathologic Change: Ax, By, Cz”. Finally, the system in Table 3 is utilized to transform the “ABC” scores to levels of AD neuropathologic change: Not, Low, Intermediate or High.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| “A” | Aβ plaques | “B” | NFTs | “C” | neuritic plaques |
| 0 | 0 | 0 | None | 0 | None |
| 1 | 1 or 2 | 1 | Ⅰ or Ⅱ | 1 | Sparse |
| 2 | 3 | 2 | Ⅲ or Ⅳ | 2 | Moderate |
| 3 | 4 or 5 | 3 | Ⅴ or Ⅵ | 3 | Frequent |

Table 2. Scores for AD neuropathologic change in “ABC” approach. This table was from the guidelines of the NIA and Alzheimer’s Association (Montine et al., 2012).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Levels of AD neuropathologic change | | B | | |
| A | C | 0 or 1 | 2 | 3 |
| 0 | 0 | Not | Not | Not |
| 1 | 0 or 1 | Low | Low | Low |
| 2 or 3 | Low | Intermediate | Intermediate |
| 2 | Any C | Low | Intermediate | Intermediate |
| 3 | 0 or 1 | Low | Intermediate | Intermediate |
| 2 or 3 | Low | Intermediate | High |

Table 3. The transforming system of “ABC” scores and levels of AD neuropathologic change. This table was taken from the guidelines of the NIA and Alzheimer’s Association (Montine et al., 2012).

The mechanism of AD still remains unknown, but it is undoubtedly a multifactorial disease. Scientists are still tirelessly exploring it from multiple perspectives. Here, I introduce some recent studies published in authoritative international journals to give a few examples. A paper published on August 2nd in Nature (Xie et al., 2023) demonstrated that the activation of cyclic GMP-AMP synthase–stimulator of interferon genes (cGAS–STING) signalling pathway induces ageing-related inflammation and neurodegeneration in the brain and peripheral organs, contributing to brain ageing; thus blocking this pathway is a potential strategy for ARND, including AD (Gulen et al., 2023; Xie et al., 2023). A recent article published on September 14th in Science (Balusu et al., 2023) extrapolated that the strong up-regulation of the neuron-specific long noncoding RNA maternally expressed gene 3 (MEG3) in human neurons may be implicated in neuronal cell loss in AD (Balusu et al., 2023). A set of four papers published on September 28th in Cell analyzed the genomic, transcriptomic, and epigenomic changes in every cell type in the AD brains broadly, drawing a series of conclusions, including: (1) Among different subtypes of excitatory neurons, alterations related to AD pathology are conserved, and differentially expressed genes (DEGs) related to global cognitive function overlap significantly (Mathys et al., 2023); (2) the somatostatin inhibitory neuron (in particular three of its subtypes) is especially susceptible to AD pathology (Mathys et al., 2023); (3) The abundance of inhibitory neurons (especially in two distinct groups which are related to high cognitive function) are higher in the prefrontal cortex of individuals who maintain good cognitive function into their later years (Mathys et al., 2023); (4) In the late stage of AD, human brains undergo global epigenomic dysregulation and erosion, and widespread cell identity loss, accompanied by damaged 3D genome structure and decreased 3D genome compartmentalization (Xiong et al., 2023); (5) Different transcriptional states of microglia play a critical role in neuroinflammation and AD (Sun et al., 2023); (6) DNA double-strand breaks (DSBs) in neurons help damage genome stability and 3D genome organization in neurodegenerative diseases including AD, and the disruption of the integrity of genome and epigenome may have a significant impact in the pathogenesis of AD (Dileep et al., 2023; Mathys et al., 2023).

The biomarkers of AD are the accumulation of Aβ and tau, whose biological changes can indicate the risk or existence of AD. Analysis for the diagnosis of AD includes variables that can be measured in cerebrospinal fluid (CSF; the fluid surrounding the brain), including Aβ and tau protein; and positron emission tomography (PET), a scanning technique that can produce images to indicate the location of Aβ and tau aggregation (Alzheimer's Association Report, 2023).

There is no cure for AD, at least in part due to mechanistic uncertainty. Until 2023, seven drugs have been approved by the U.S. Food and Drug Administration (FDA) to treat AD. Five of these include donepezil (a kind of cholinesterase inhibitor), galantamine, rivastigmine, memantine (a type of NMDA receptor antagonist), and memantine combined with donepezil. All of these can only help manage the symptoms of AD (Alzheimer's Association Report, 2023; World Health Organization, 2023). The other two drugs are aducanumab and lecanemab, whose therapeutic objective is to remove Aβ from early AD patients’ brains. However, they are unable to cure AD completely, and they are not suitable for all AD patients. All of these drugs may have substantial side effects (Alzheimer's Association Report, 2023). At present, many novel drugs are still under clinical trials. As at 25 January 2022, 143 novel therapeutic agents with diverse targets for AD were under clinical trials in the United States (Cummings et al., 2022).

Similarly, there is no effective prevention method for AD. However, circumvention of some of the risk factors of AD, including midlife hypertension, diabetes, midlife obesity, lack of physical activity, cognitive inactivity or low educational attainment, depression, and smoking, might potentially help prevent development of AD (Barnes & Yaffe, 2011; Norton, Matthews, Barnes, Yaffe, & Brayne, 2014; Ogino, Manly, Schupf, Mayeux, & Gu, 2019). As estimated, a reduction in the seven risk factors above may reduce 184,000-492,000 AD cases in the United States and 1.1-3.0 million cases globally (Barnes & Yaffe, 2011).

Brain atrophy is one of the main clinical characteristics of AD, manifesting as a reduction in brain weight (Figure 1). According to Xu et al., the average brain weight of AD patients was significantly lighter than control individuals, about 200 - 300 grams (Xu et al., 2016). During the normal ageing process, the brain usually shrinks to a certain extent also, but does not undergo massive loss of neurons. However, in AD brains, neurons are injured and dying throughout the entire brain, interrupting connections between neural networks, finally causing brain atrophy across multiple brain regions and massive loss of brain volume (National Insitute on Aging, 2017). In the clinic, magnetic resonance imaging (MRI) techniques are utilized to assess the extent of brain atrophy and help diagnose AD (Pini et al., 2016).

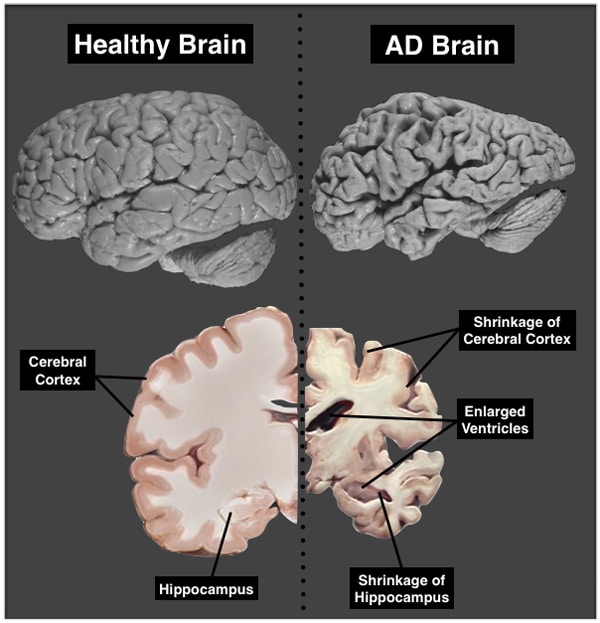


Figure 1. Brain atrophy phenomenon in AD brains. The left part showcases the typical structure of healthy brains, while the right part showcases the structure of atrophic AD brains. This figure was taken from the Knowing Neurons website (Knowing Neurons, 2012).

In addition, metabolism in AD-affected brains was also disturbed. For instance, according to Xu et al. (Xu et al., 2016), compared with the control group, the urea abundance in AD brains was several-fold higher in multiple brain regions (Table 4). Urea is one of the primary product of proteolysis (the breaking down of proteins or peptides into simpler substances such as amino acids, especially by the action of enzymes) (Sakami & Harrington, 1963).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Regions | HP | ENT | MTG | SCX | MCX | CG | CB |
| Urea | 6.5 | 5.6 | 4.7 | 4.9 | 5.0 | 5.3 | 4.9 |

Table 4. The altered abundance of urea in AD-brain tissue. Abbreviations indicate brain regions: hippocampus (HP), entorhinal cortex (ENT), middle-temporal gyrus (MTG), sensory cortex (SCX), motor cortex (MCX), cingulate gyrus (CG), and cerebellum (CB). Numbers indicate fold-changes of the urea (AD/controls). This figure was taken from Xu et al. in 2016 (Xu et al., 2016).

**Hypothesis & Aims**

Problem:

To this day, the mechanism of AD remains unclear. The mechanistic uncertainty is an obstacle to the preventive strategy and the development of AD treatment.

Hypothesis:

Based on previous studies, we hypothesize that brain atrophy in AD brains may be related to cerebral proteolysis and consequent elevation of brain urea.

The total number of proteases and protease inhibitors may relate to AD severity.

The different regions may have distinct protease and protease-inhibitor alteration patterns in AD brains.

Aims:

This research aims to conduct a regional comparison of altered cerebral proteolysis in human control and AD brains.

Specifically, we plan to solve these questions, by using the following approaches:

1. Develop a comprehensive understanding of the nature and amounts of proteases and protease inhibitors in human control brains in the six brain regions to be studied.
2. Compare and contrast the status of protease and protease-inhibitor pathways that are significantly altered in expression between human control and AD brains in each of the brain regions under study, which together account for approximately 90% of the human brain mass.
3. Identify, then measure the levels and distribution of each of the critical proteases and protease inhibitors in the human brain.
4. Compare and contrast the levels of individual proteases and protease inhibitors between control and AD brains.

Objective:

1. Collect samples including AD brains (experimental group) and control brains (control group), and record their clinical characteristics (including age, sex, ante-mortem brain/mental state, cause of death, Braak stage, amyloid load, post-mortem delay (PMD), and brain weight) into a table. This has already been done by our team.
2. Plot a complete workflow of the whole study.

3. Process and analyze the comprehensive protease and protease-inhibitor expression data to develop a comprehensive understanding of protease and protease-inhibitor expression changes in human control and AD brains in six regions.

4. Identify proteases and protease inhibitors that show differential expression, then compare and contrast protease and protease-inhibitor pathways that are significantly altered in expression in each of the brain regions in human control and AD brains.

5. Use Bayesian modelling to measure and contrast the levels and distributions of key proteases and protease inhibitors across brain regions.

6. Visualize the results.

**Methods**

Research design

This research will investigate the role of cerebral proteolysis in sporadic AD-affected brains. First, we collected high-quality patient information (sample size = 18), which was well-matched in gender and age, with short PMD and no significant differences between cases and controls except for brain weight, which was lighter in cases compared with controls. This finding is consistent with AD in the cases and probable differences in proteolysis between AD and controls. The experimental group was collected from AD donors (n = 9 AD cases), and the control group was collected from asymptomatic donors (n = 9 asymptomatic controls). The task of patient information collection has already been done by our team. Then, we used the isobaric tags for relative and absolute quantitation (iTRAQ) technique (a technique to use stable isotope-labelled molecules that can be covalently bonded to the NH2-terminus and side-chain amines of proteins) to label all accessible proteins, followed by two-dimensional liquid chromatography and mass spectrometry-based proteomics (the study of all proteins in an organism/organ/tissue) to obtain relative protease and protease-inhibitor expression data (levels of all accessible proteins). Then, we will process and analyze the comprehensive protease and protease-inhibitor expression data, to develop a comprehensive understanding of these changes. We will thus identify critical proteases and protease inhibitors, allowing identification of pathways responsible for all altered proteases and protease inhibitors in the AD brain. Finally, we will employ Bayesian modelling to obtain posterior probability distribution data for key proteases and protease inhibitors that are differentially expressed between cases and controls. We plan to conduct our research in six functionally distinct brain regions, which are hippocampus (HP), entorhinal cortex (ENT), middle-temporal gyrus (MTG), sensory cortex (SCX), motor cortex (MCX), cingulate gyrus (CG), and cerebellum (CB). These six brain regions can be divided into three groups: HP, ENT, and CG, which are highly affected by AD; MCX and SCX, which are moderately affected by AD; and CB, which is thought to be “relatively spared.”

This study is designed to address the following questions.

Question 1. What are the proteases and protease inhibitors in human control brains?

The following strategy is designed to conduct a regional comparison of the total number of proteases and protease inhibitors in human control brains. To our knowledge, a list of such proteases and protease inhibitors in human control brains is not available in the literature.

Strategy:

Use the database to identify proteases and protease inhibitors present in control human brains, which would, to our knowledge, provide for the first time, a comprehensive list of proteases and protease inhibitors in control human brain regions that has not previously been available in the literature.

Question 2. What are the proteases and protease inhibitors that display altered expression in human AD brains?

The following strategy is designed to conduct a regional comparison of the total number of proteases and protease inhibitors with altered expression levels in AD human brains. To our knowledge, a list of such proteases and protease inhibitors in human AD brains has not previously been available in the literature.

Strategy:

Use the database to identify proteases and protease inhibitors present in AD human brains, which would be, to our knowledge, a comprehensive list of proteases and protease inhibitors in the AD human brain has not previously been available in the literature.

Question 3. What are the protease and protease-inhibitor pathways that are significantly altered in expression in human AD brains?

This aims to identify the alterations of protease and protease-inhibitor pathways with significantly altered expression levels in AD brains, which may be more critical for AD research. The following strategy is designed to obtain data of protease and protease-inhibitor pathways with significantly altered expression levels in human AD brains.

Strategy:

Use the database to identify pathways that contain the proteases and protease inhibitors that are significantly altered in expression in Question 2 in control and AD human brains.

Question 4. What are key individual proteases and protease inhibitors with altered expression in AD brains? These proteases and protease inhibitors are members of the above pathways.

Proteases and protease inhibitors with significant expression alterations triggered by AD may play a vital role in brain atrophy and consequential AD pathogenesis. The following strategy is designed to identify critical protease and protease inhibitors whose expression alterations are triggered by AD.

Strategy:

Use the database to identify key individual proteases and protease inhibitors which are significantly altered in expression in Question 3 in control and AD human brains.

Question 5. Measure the levels and distributions of key proteases and protease inhibitors across brain regions.

The following strategy is designed to analyze the levels and distribution of these key proteases for AD.

Strategy:

We plan to apply Bayesian modelling to measure the differences in the levels and distributions of key proteases and protease inhibitors.

Requirements for equipment and facilities

The basic equipment and facilities include the 8-plex iTRAQ kit (AB Sciex), liquid chromatography/tandem mass spectrometry (LC-MS/MS) system, Agilent high-performance liquid chromatography (HPLC) 1200 system (Agilent, Santa Clara, California), and the necessary computing systems which are available in Professor Cooper’s laboratories.

The low-PMD human brain samples were obtained from the New Zealand Neurological Foundation Human Brain Bank, University of Auckland, and chemically analyzed as described (Waldvogel et al., 2008). All of the specified biological reagents are commercially obtainable.

**Discussion**

This research proposal is based on the discoveries of our team’s previous programme, which found that the urea abundance in AD brains was several-fold higher than in the control brains (Table 4). We have designed a series of strategies using state-of-the-art bioinformatics to characterize the role of cerebral proteolysis in sporadic AD. There are still no effective treatments, owing at least in part to mechanistic uncertainty. This research will provide insights into the mapping of protease changes in human AD brains, of significant value in obtaining detailed mechanistic knowledge of AD pathogenesis. The results of this study may contribute to discovering biomarkers and developing effective treatments for AD.

Compared with previous studies, this research has notable strengths. First, the samples studied in this research are high-quality human brains, which were age-matched, and had short PMD. One important strength is that AD is poorly modelled in animal models as opposed to human brain tissues. Second, this research analyzes and compares AD’s impact on different brain regions independently, while most previous studies have just focused on a single brain region. Third, this study utilized appropriate methods, which are reliable and accurate.

However, certain limitations of this research are acknowledged. First, the techniques have their limitations. For instance, iTRAQ is time-consuming, costly, and sensitive to salt contamination. Two-dimensional liquid chromatography requires complex instruments and data processing, with potential reproducibility challenges due to differences in materials and operation. Second, the sample size is limited (n = 18), although Bayesian modelling has shown that it is sufficient.

In conclusion, this study is expected to provide expression data of proteases and protease inhibitors in human AD brains, thereby improving our understanding of the mechanism of AD.

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