Hippocampus-to-ventricle-ratio (HVR) as a novel biomarker for early diagnosis of Alzheimer disease (AD):

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Summary

As a progressive and irreversible disease, Alzheimer disease (AD) poses a serious health risk, as well as a heavy burden on caregivers, families, and society. Currently, AD cannot be cured, and the limited treatments available can only slow down the deterioration of symptoms. Therefore, diagnosis of AD in the preclinical stage is critical. Earlier diagnosis means a chance to slow down the disease at an earlier stage. However, AD has an insidious onset and can have a latent period of more than 10 years before symptoms appear. During this period, early onset biological markers of AD indexes can assist the diagnosis.

Structural imaging studies have demonstrated that the medial temporal lobe (MTL) is where the pathology of AD first appears. A central structure in the MTL is the hippocampus. The hippocampus has a clear anatomical structure, providing a practical basis for its use as a brain imaging marker. There are a large number of studies that have found a relationship between hippocampal volume and cognitive function, and the development of AD. Due to large interindividual variability, its clinical use was so far limited.

In this doctoral thesis, I used the hippocampal to ventricle ratio (HVR), and provided preliminary evidence that HVR is superior to pure hippocampal volume in indicating hippocampal integrity. I summarized previous AD markers in my first review paper, and presented the advantages of HVR. This became the basis for my second cross-sectional study and my third longitudinal study. In the cross-sectional study, I directly compared three groups of subjects (healthy, early stage, advanced stage) and used HV or HVR as markers for the statistical analysis. The results suggested that HVR can distinguish differences between groups better than HV. In the longitudinal study, all subjects were healthy at the start of the MRI examination. Over time, some remained healthy, some started to develop AD, and some developed the full AD syndrome. Here too, the results showed that even when all subjects were in the asymptomatic “healthy” phase, HVR was able to show significant differences between the groups.

In summary, HVR largely overcomes the disadvantages of pure hippocampal volume as a structural MRI marker for AD and its superiority and validity have been verified in this thesis. Although some important limitations are acknowledged, future studies should clarify whether this marker may also be useful in clinical practice.
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Part I

General introduction and overview
1. Introduction

Alzheimer disease (AD), which is the leading cause of dementia, is a progressive neurodegenerative disorder that leads to memory loss and cognitive decline. Although aging is not causing AD, the two are strongly correlated, and prevalence rates of AD are gradually increasing with advancing age. While around 1% of the population are diagnosed with AD before the age of 65, this number rises to 3% by 75, 17% by 85, and 34% for the population above 85. In the population cohort above 90 years of age, this number quickly approaches 50% (Zhao et al., 2020). The initial symptoms usually start with the impairment of episodic memory with people finding it difficult to recall old events and remember new ones. As neurodegeneration progresses with time, problems with language, executive function and spatial orientation are emerging. The disease continues to gradually worsen and eventually leads to death. Moreover, AD can also manifest with psychiatric symptoms that affect a person's mood, behavior, and personality, including depression, anxiety, agitation, psychosis, apathy, sleep disturbances and wandering (Landes et al., 2001; Lyketsos & Olin, 2002; Teri et al., 1999). There are also large variations in the course of the disease when it comes to subtypes of AD according to the symptoms and degeneration rate (Grøntvedt et al., 2018). The number of demented people is estimated to be 82 million in 2030 and 152 million in 2050 (Organization, 2019). Generally, AD poses a significant threat for human health in the elderly and adds heavy burden for caregivers, medical system, as well as for society as a whole. So far, there is no cure for AD. Although a large number of studies have investigated possible treatments for AD, the current understanding of its cause and the pathological mechanisms are still limited. In the following, I will provide an overview over different aspects of the disease.

1.1. The history of AD

The history of Alzheimer's disease can be traced back to the beginning of the 20th century. A German psychiatrist and neuropathologist named Alois Alzheimer was working in a Psychiatric Hospital. In 1901, Alois Alzheimer encounter a 51-year-old woman named Auguste Deter who exhibited severe memory loss, progression confusion, paranoia, and other cognitive impairments. After her death in 1906, Alzheimer performed an autopsy of her brain and identified abnormal clumps ( amyloid plaques) and tangled fibers ( neurofibrillary tangles) that have become characteristic hallmarks of the disease (Bondi et al., 2017). In 1910, Emil Kraepelin, a mentor of Alois Alzheimer and a prominent psychiatrist, used the term "Alzheimer's disease" to describe the neurological disorder characterized by the progressive cognitive decline that Alzheimer had studied.
In the years following Alzheimer's initial discovery, research on the disease remained relatively limited. However, by the mid-20th century, advancements in medical technology and neurology led to a deeper understanding of the disease's pathology and its effects on the brain. In 1980, The Alzheimer's Association, a nonprofit organization dedicated to raising awareness and supporting research on Alzheimer's disease, was founded in the United States. In 1990s, researchers identified specific genetic mutations associated with familial cases of AD, which lead to an early onset of the disease, typically in the sixth life decade. These discoveries provided crucial insights into the underlying causes of the disease and potential targets for therapeutic interventions. In the 21st century, with societies getting older, AD has become a significant public health concern. The World Alzheimer Report in 2019 estimated that over 50 million people worldwide were living with dementia, with AD accounting for a significant portion of these cases (Lynch, 2020).

1.2. The etiology of AD

As a complex neurodegenerative disorder with various contributing factors, the cause of Alzheimer's disease is not well understood. It is generally believed to be related to factors including genetics, amyloid-beta (Aβ), Tau protein, mitochondrial dysfunction, inflammation, lifestyle and environmental factors.

The amyloid hypothesis has been playing a central role in the beginning of the 21st century. According to this theory, as Aβ peptides accumulate, they begin to aggregate and form insoluble plaques outside the neurons in the brain (H. Ja & Ga, 1992). These amyloid plaques are one of the hallmark pathological features of AD and are believed to disrupt normal brain function. Amyloid plaques are thought to trigger a cascade of events progressively leading to neuronal damage and death, combined with cognitive decline and memory loss - hallmark symptoms of AD. Though still very influential, the amyloid hypothesis has faced challenges in recent years and has led researchers to explore other mechanisms of AD pathogenesis. The main problem with the amyloid hypothesis is that it is neither sufficient nor necessary for AD – there are people with high amyloid beta burden without AD, and there are people with AD how have little or no accumulation of amyloid beta in their brains.

In addition to Aβ, the aggregation of hyperphosphorylated tau protein in neurons appears as another critical aspect of AD (Leuzy et al., 2022). Tau protein plays an important role in maintaining the structural integrity of neuronal cells. When tau protein becomes abnormally phosphorylated, it forms neurofibrillary tangles (NFTs) inside the cell, disrupting normal function and communication
between neurons. NFTs are intracellular deposits of abnormally aggregated and hyperphosphorylated tau protein which are considered as another hallmark pathological feature of AD (T.-L. Ja et al., 2022), particularly in brain regions involving memory and cognitive functions, such as the medial temporal lobe (MTL).

Another prominent theory for the cause of AD is the mitochondrial cascade hypothesis, proposing that mitochondrial dysfunction is closely related to AD pathology. Mitochondria are organelles within cells responsible for producing adenosine triphosphate (ATP), the primary energy currency of the cell. In primary mitochondrial cascade hypothesis (Swerdlow, 2018), mitochondrial dysfunction results in energy metabolism failure that is the initial cause of AD pathology, while Aβ and tau protein, which are being aggregated in the process, are considered biomarkers but do not represent the original cause (Ashleigh et al., 2023). In the related but distinct secondary mitochondrial cascade hypothesis, mitochondrial damage is the consequence of Aβ and tau protein aggregation (Caspersen et al., 2005; Devi et al., 2006), although mitochondria could mediate Aβ toxicity in turn (Swerdlow, 2018).

Furthermore, neuroinflammation is recognized as a significant contributor to AD pathogenesis. While inflammation is a defense mechanism for protecting body and brain from harmful stimuli, excessive and dysregulated neuroinflammation in the brain can lead to AD pathology (Calsolaro & Edison, 2016). Microglia, the resident immune cells of the central nervous system, can be activated by the deposition of Aβ and tau protein. Overactive and prolonged activation of microglia and astrocytes can lead to the release of inflammatory molecules like cytokines and chemokines, which can contribute to neurodegeneration (Kwon & Koh, 2020). In addition, neuroflammation can also cause synaptic dysfunction and oxidative stress, which is also considered as contributing factor to AD.

Finally, lifestyle and environmental factors also have a substantial impact on the development of AD. The most important factor to list here is lack of physical activity, which is linked to an increased risk of developing AD, while regular exercise has been shown to be protective against AD (De la Rosa et al., 2020; Schuit et al., 2001; van Gelder et al., 2004). An unhealthy diet high in sugars, saturated fat and processed foods further increases the risk of developing AD, while a healthy and balanced diet that is rich in vegetables and fruits, whole grains, and healthy fat may help reduce the risk (Knight et al., 2016; Shannon et al., 2019). Smoking is a risk factor for numerous diseases including AD (Anstey et al., 2007; Barnes & Yaffe, 2011). The harmful chemicals in tobacco can lead to oxidative stress and inflammation in the brain, increasing the risk of AD. Social isolation is associated with cognitive decline in older people, while keeping a certain
level of social activities help protect against cognitive impairment (Penninkilampi et al., 2018). It has been shown that light to moderate alcohol consumption is associated with a decreased risk of cognitive decline, while heavy alcohol conception is associated with an increased risk of all kinds of dementia including AD (Koch et al., 2019; Rehm et al., 2019). Chronic exposure to certain toxins, such as metals and biotoxins, has been seen as the environmental factor that also plays a role in the progress of AD (Vasefi et al., 2020). Additionally, chronic stress and lack of intellectual stimulation are also factors contributing to AD pathology. Not only physical activity, but also cognitive stimulation can slow down the degenerative rate. Spatial navigation training in old people can significantly reduce the atrophy rate of hippocampus (Lövdén et al., 2012).

1.3. Other types of dementia

Although AD is the dominant cause of dementia, making up over 60% of all dementia cases when considered in isolation, and up to 75% of cases when combined with other forms of dementia, there are also several other types of dementia that should be mentioned, including vascular dementia, Lewy body dementia (LBD), Frontotemporal dementia (FTD), and Parkinson’s disease dementia (PDD).

Vascular dementia is an umbrella clinical term that includes a group of neurogenerative diseases caused by vascular disease. This type of dementia is usually caused by impaired blood flow to the brain, leading to cognitive impairment and problems on thinking, memory and other cognitive functions. A variety of vascular diseases have the potential to cause vascular dementia. Cerebrovascular diseases including stroke and small vessel disease, arteriosclerosis, hypertension, diabetes all can damage blood vessels or block sufficient blood flow in the brain, contributing to vascular dementia. Unlike the gradual progress of AD, vascular dementia symptoms may occur suddenly after a stroke, although they can also develop gradually.

LBD, the second common dementia after AD, is characterized by the presence of abnormal protein deposits called Lewy bodies, which contain deposition of the misfolded protein α-synuclein, in the brain. It shares some symptoms with both Alzheimer's and Parkinson's disease, including cognitive fluctuation, visual hallucinations, and Parkinsonism such as stiffness and tremors (Dubois et al., 2014). Since early LBD symptoms usually overlap with other forms of dementia, it can be easily misdiagnosed. Similar to AD, LBD also develops gradually, with a progressive worsening in cognition, mood and behavior.

Frontotemporal dementia is another common form of dementia, merely after AD and LBD, and is a
leading type of early-onset dementia particularly in people younger than 65 years (Bang et al., 2015). There are three clinical variants of frontotemporal dementia: behavioral-variant frontotemporal dementia which is characterized by early behavioral and executive deficits; non-fluent variant primary progressive aphasia which is characterized by progressive impairment in speech; and semantic-variant primary progressive aphasia which is characterized by semantic aphasia and associative agnosia (Neary et al., 1998). The initial symptoms of frontotemporal dementia can appear similar to some common psychiatric disorders, such as schizophrenia, depression, bipolar disorder, obsessive-compulsive behavior, adding to the difficulty of differential diagnosis. It has been shown that specific neurons in the frontal lobes, so called fork cells, are the initial targets of frontotemporal dementia.

PDD refers to the cognitive decline and impairment that occurs in individuals with Parkinson's disease. It has been shown that PDD and LBD share the pathological basis – abnormal processing of α-synuclein in the brain (Bohnen et al., 2017). However, PDD usually occurs after parkinsonism, while LBD usually begins with cognitive decline, or parkinsonism and cognitive decline occur and progress simultaneously. Apart from the symptoms of Parkinson's disease, PDD patients experience problems with cognitive and executive function which worsens gradually with the progress of Parkinson's disease. Treatment for PDD usually focus on both the motor symptoms of Parkinson's disease and the cognitive symptoms associated with dementia.

Actually many patients with LBD or PDD also have the pathology of Aβ and tau protein which are pathological hallmarks of AD. Similarly, vascular dementia and AD also often develop together as well as other combinations that include more than two types. These kinds of dementia that includes not only one pathology are called mixed dementia, which are common in clinical practice.

1.4. The potential factors affecting the progress of AD

Age

According to the onset age, AD is classified into two types – the early-onset AD (EOAD) and late-onset AD (LOAD). EOAD, which is rare and usually caused by familial genetic reasons, occurs in individuals before mid-60s, typically in their sixth life decade. LOAD is the most common type that affects people older than mid-60s. Although older age does not necessarily cause AD, it is the greatest risk of LOAD (Mielke et al., 2014; Tublin et al., 2019; Zhao et al., 2020). It has been shown that 3% people between ages 65 and 74, 17% of people between ages 75 and 84, and 32%
people older than 86 have AD (Zhao et al., 2020).

Aging is a progressive and natural process that occurs in all organisms, including humans. As people grow older, their bodies and brains undergo various changes which occur at the cellular, tissue, and organ levels including cellular damage, inflammation, vascular damage, production of unstable molecules called free radicals, energy expenditure decrease, and atrophy of certain parts of the brain, etc. As aging is a complex of many changes, it’s difficult to distinguish which one is the exact reason that causes AD, or probably several reasons contribute together. After all, while everyone gets old, not everyone gets AD or dementia. The underlying mechanisms of aging as the greatest risk factor for AD is still not fully understood, but when going into the age-related changes, we can find some of them have the potential to cause AD, such as cellular damage, inflammation, production of free radicals, even though not considering age.

From the point of view of homeostasis, people are in healthy state regarding the AD pathology when there is a balance between the Aβ production and clearance. If the Aβ production increases or the clearance decreases, the balance is broken which causes Aβ accumulation and deposition, forming a pathology that can lead to AD (Tarasoff-Conway et al., 2015). The aging process affects the protein clearance mechanism and further influences the proteostasis, increasing the risk of AD (Vilchez et al., 2014).

**Genetic factors**

As stated earlier, the greatest overall risk factor for LOAD is aging. When we come to the genetic factor, the most frequent genetic risk factor is the presence of the apolipoprotein E (APOE) ε4 allele (Arnold et al., 2020; Gamache et al., 2020; Shinohara et al., 2016; Zhao et al., 2020). The APOE has three different alleles: ε2, ε3, and ε4. The ε4 allele is considered a risk factor, as individuals carrying one copy of this variant are at a higher risk of developing AD, while carrying two copies of this variant has the highest risk of developing AD. In US about two thirds of AD patients have at least one copy of APOE ε4 allele (Riedel et al., 2016; Zhao et al., 2020). On the other hand, the presence of the ε2 allele is considered beneficial. Having one copy of ε2 allele is associated with a lower risk of developing AD compared to individuals without ε2 alleles, while having two copies of ε2 is considered to be the most protective genotype regarding AD. Generally, APOE ε4 increases the risk of having AD while APOE ε2 decreases the risk of having AD compared with APOE ε3.

Although it is not full understood, a variety of mechanisms have been shown to contribute to the role of APOE ε4 and the protective role of APOE ε2 for AD. APOE ε4 is associated with an increased accumulation of Aβ, while ε2 enhances the clearance of Aβ (Riddell et al., 2008). People
with APOE ε4 are found to have amyloidosis and AD earlier than those without ε4 alleles (Zhu et al., 2021). ε4 also enhances the toxic effect of tau protein pathology while ε2 is associated with a reduced burden of tau pathology, especially the formation of NFTs (Shi et al., 2017). Besides, ε4 impacts the lipid metabolism and the function of synapses which are essential in brain function and memory, while ε2 improves the lipid metabolism in the brain (Huang & Mucke, 2012). APOE also affects the cerebrovascular systems and neuroinflammation in the brain, in which ε4 plays a negative role for the brain health (Huang & Mucke, 2012).

While APOE ε4 is the most frequent genetic risk factor for LOAD, mutations of the precursor protein gene (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN 2) are genetic risk factors that will lead inevitably to EOAD (Khanahmadi et al., 2015). Those genes have been shown to cause an autosomal dominant form of AD which produces clinical symptoms before the seventh life decade, and typically in the sixth. While more severe, it is extremely rare.

When APP is cleaved by secretase, it can produce Aβ peptides of varying lengths. Mutation in the APP gene can lead to an increase in the production of certain forms of Aβ peptides, especially the Aβ 42 peptide, which is more likely to aggregate plaques in the brain (Oakley et al., 2006). Mutations in PSEN1 can alter the function of γ-secretase which is involved in the processing of APP, leading to an increased production of Aβ 42 and the development of plaques. Mutations in PSEN2 produce similar cases, but are less common, while mutations in PSEN1 are the most common cause of familial early-onset AD (Lanoiselée et al., 2017), accounting for a significant proportion of familial cases.

**Sex**

It is widely reported that the incidence of AD is higher in women than in men (Arnold et al., 2020; Dumitrescu et al., 2019; Ferretti et al., 2018; Laws et al., 2018; Mosconi et al., 2017; Tensil et al., 2018). Following age and ε4 allele, female sex is thus also considered a risk factor for AD. The well-known Framingham Heart Study has shown that the estimated lifetime risk for AD was about 10% for men and 20% for women both at age 45 and 65, although risks were slightly higher at age 65 (Chêne et al., 2015). A meta-analysis including 22 studies demonstrated that AD incidence is higher in women than in men, although not reaching statistical significance (Fiest et al., 2016). There is some evidence that greater longevity explains in part the higher AD incidence in women (Matyi et al., 2017; Zandi et al., 2002). However, some studies did not find a significant sex difference in AD risk (Fiest et al., 2016; Mielke et al., 2014), and there is even one study that found a higher AD risk in men (Matthews et al., 2016).
Notably, although APOE ε4 allele and female sex both are risk factors for AD, sex plays a significant role on the impact of APOE ε4 for AD. It has been shown that women with one copy of the APOE ε4 allele show a larger risk for AD, and a faster cognitive decline than men (Altmann et al., 2014; Ferretti et al., 2018; Heise et al., 2014). The females with APOE ε4 were also found to have a faster AD progression than any other men or women with other APOE allele (Beydoun et al., 2012; Buckley et al., 2018; Sundermann et al., 2018). These findings may suggest an interaction between APOE ε4 and estrogen in the higher risk of AD in women than in men. These findings indicate that interaction of estrogen and APOE4 may play a key role in elevated risks of AD development in women, compared to men.

Finally, in addition to biological reasons, socioeconomic factors also account for gender differences when considering the impact on AD risk. Women traditionally have lower income and lower access to education in most societies, and usually are primary caregivers in family with burden and higher possibility of unemployment, contributing to depression and sleep disorders which present psychological risk factors of AD (Zhu et al., 2021).

1.5. Existing treatments for AD

Although so far there is no cure for AD, plenty of treatments including pharmacological and non-pharmacological are available to relieve the symptoms of AD and improve quality of life of AD patients. Ongoing research efforts focus on developing new therapies to slow down or halt the progression of Alzheimer's, as well as improving early detection methods. The goal is to improve the quality of life of individuals affected by AD until ultimately a way to cure AD is found. Here I summarize some common approaches for the treatment of AD.

Medications

In 1984, the first drug that could temporarily improve symptoms was approved by the US food and drug administration (FDA). Since then, more drugs have been developed to treat Alzheimer's disease. Currently there are mainly two kinds of medicine to relieve the symptoms of AD: Cholinesterase inhibitors for mild to moderate AD and N-Methyl-D-aspartate (NMDA) receptor antagonists for moderate to severe AD (Breijyeh & Karaman, 2020). Cholinesterase inhibitors affect the cholinergic transmission to increase levels of acetylcholine (ACH), a neurotransmitter that is deficient in AD patients. There are three cholinesterase inhibitors: donepezil, rivastigmine, and galantamine. These medications aim to enhance the communication between nerve cells and
improve cognitive function by increasing ACH in the brain. Memantine is a typical example of an NMDA receptor antagonist, which helps regulate glutamate, a neurotransmitter involved in learning and memory, and reduce the excitotoxicity in the brain (S. Khan et al., 2020). However, there are also some medicines that experienced clinical trial failure without ever entering market, such as Beta Amyloid Cleaving Enzyme 1 (BACE) 1 inhibitors, Receptor for Advanced Glycation End (RAGE) inhibitors, Peroxisome Proliferator-Activated Receptor γ (PPAR-γ) agonist, and 5HT6 antagonist (S. Khan et al., 2020).

**Gene therapy**

Gene therapy aims to introduce new genes into a person’s cells to correct genetic mutations or replace faulty proteins or modulate the expression of certain gene to achieve therapeutic effects. In AD therapy, gene therapy aims to modulate the molecular and cellular changes that contribute to AD progression. Neurotrophic factors are proteins that support the survival and function of neurons, but is insufficient in AD patients. Gene therapy could introduce genes that code for neurotrophic factors, such as neuron growth factor (NGF), into the brain. It has been shown that gene therapy focusing on NGF looks safe over long periods and appears as a promising treatment of neurodegenerative disorders (Tuszynski et al., 2015). The CRISPR-Cas9 gene editing technology has also shown promise in preclinical research for treating AD (Lu et al., 2021). It could potentially be used to edit genes associated with the pathology of AD, although this approach is still in its early stages of development and faces technical and ethical challenges.

**Immunotherapy**

Although there is no widely approved immunotherapy specifically for AD, research in this area is ongoing and has promising development. Immunotherapy for AD involves using the body’s immune system to hinder or slow down the pathology of AD, such as tau tangles and Aβ plaques, which are the primary target of immunotherapy for AD (Song et al., 2022). In recent years, the monoclonal antibodies are introduced to clear the Aβ and abnormal protein in the brain.

Aducanumab, developed by the US pharmaceutical company ‘Biogen’, was the first monoclonal antibody approved by the FDA for the treatment of AD in 2021 (Dhillon, 2021). However, it should be noted that using Aducanumab has the risk of experiencing amyloid-related imaging abnormalities (ARIA), which can cause discontinuation or modification of treatment. It has been reported that 35.2% patients experienced ARIA when taking aducanumab with the dosage of 10-mg/kg (Salloway et al., 2022). In general, developing immunotherapy for AD is challenging due to the complexity of the disease and the potential risk of severe sided effects. Given the importance of
safety, the immunotherapy for AD is still in a cautious progression.

**Probiotics**

The employment of probiotics is a novel area on the treatment of AD. Aging brings plenty of changes of body, including the gastrointestinal disturbances and disorders of central nervous system (CNS) like AD. The gut-brain connection has gained attention in recent year with research suggesting that the gut microbiota could influence brain and probably play an important role in the progress of neurodegenerative disease like AD (Angelucci et al., 2019; Jiang et al., 2017). Probiotics are live microbes that could provide health benefits when consumed in suitable amounts. They are considered as good bacteria to maintain the microbe balance in the gut and support a healthy suggestive function and overall health. Previous study on mice has revealed that probiotic treatment can slow down the progress of AD and relieve its symptoms (Abraham et al., 2019). In the case of human, the consumption of probiotic milk that contains various probiotic strains including *Lactobacillus acidophilus, L. casei, Bifidobacterium bifidum, and L. fermentum* improved the cognition and insulin metabolism in AD patients (Akbari et al., 2016). Another human study found that consumption of *B. breve* A1 for 24 weeks enhanced the cognition of elderly subjects suffering from mild cognitive impairment (Kobayashi et al., 2019). Generally, both animal and human clinical studies revealed that the probiotic interventions is a comparatively safe way to improve the cognitive function and relieve symptoms of MCI and AD patients, although further investigations are required.

**Behavioral and Cognitive Interventions**

Apart from medical treatments, behavioral and cognitive interventions play an essential role in the clinical practice of AD. These interventions focus on improving daily cognitive function, enhancing communicative and problem-solving ability, and supporting overall well-being. There are several common behavioral interventions including structured routine, validation therapy, music and art therapy. Structured routine is to build up a structured daily routine to form an atmosphere of familiarity and stability for AD patients. It could include regular sleeping time, mealtime, exercise time and so on. In a structured routine, AD patients can easily have a feeling of regularity and predictability which reduces anxiety and increases comfort. Validation therapy focuses on acknowledging and validating the feelings and emotions of AD patients (Atri et al., 2012), even if they are not consistent with reality. Compared with correcting their confused statement or behavior, acknowledging the feeling of AD patients could relieve their distress and enhance communication. Music and art therapy have been shown to activate the preserved part of cognitive function and
evoke positive emotion (Gómez Gallego & Gómez García, 2017). Listening to music and engaging in art activities can enhance mood and communication of AD patients. Besides, physical exercise is also a behavioral intervention that has positive effect on cognitive function (Herbert, 2022; Kirk-Sanchez & McGough, 2014) and well-being (Herbert, 2022). Common cognitive interventions include cognitive stimulation therapy (CST), reality orientation, and cognitive training. CST is mainly administered in a group to stimulate various domains of cognition like memory, attention, language, and reasoning (Stewart et al., 2017). CST offers group activities including discussion, puzzles and quizzes, which not only stimulate cognitive abilities but also provide social engagement. Reality orientation focuses on connecting individuals with reality. This intervention typically involves calendars, clocks, and reminders about the current location, time, and surroundings. Cognitive training takes cognitive abilities, such as memory, attention, reasoning, communication and executive function, as strength or skills that can be improved by exercising. Based on this precise, the training often involves computer-based programs targeting on enhancing memory, attention and problem-solving skills.

Clinical Trials

Clinical trials present ongoing research studies conducted with human volunteers to determine whether novel treatments are safe and effective. It is easy to understand the importance and benefit of clinical trials for the whole society. There will be no treatment and prevention for AD if there are no clinical trials. Actually there are also many benefits for the individuals participating in clinical trials. First, they get priority in accessing potentially cutting-edge technologies of treatment which has the potential to improve their symptoms, although at the risk of also experiencing side effects, or being in the placebo arm. Second, participants can have a sense of self-fulfillment for the meaningful contribution to scientific research, which is helpful to enhance their well-being. Third, participating in clinical trials can bring about the feeling of hope, which has the potential to relieve symptoms and improve the quality of life for those AD patients. Overall, clinical trials not only push forward the research process of AD, but also produce a valuable process for improving AD symptoms and enhancing well-being for the participants.

Occupational therapy

Occupations are daily activities such as cooking, painting, walking that make up of our life and help bring meaning to life. AD patients usually have difficulties to participate occupations. Occupational therapy help AD patients to engage in these activities to achieve a sense of meaning. In the process
of occupational therapy, patients receive an assessment of their cognitive, physical and emotional abilities along with their interests and goals firstly, then occupational therapist collaborate with patients and caregivers to produce customized plans (Matilla-Mora et al., 2016). Task simplification and environmental modification is quite recommended in the therapy process. Breaking down complex activities into smaller steps promotes task completion and increases the patients’ confidence and sense of accomplishment. Environmental modification includes setting visual cues and organizational systems to help patients handle their surroundings easily and maximize safety and independence (Pynoos et al., 2010). It should be noted that community occupational therapy is a successful and cost effective way, especially in terms of inform care giving. Overall, Occupational therapy serve as an effective method to improve cognitive function, independence, a sense of purpose and a higher quality of life for AD patients (Smallfield & Heckenlaible, 2017).

1.6. Diagnosis of AD

Due to the insidious course of AD, clinical symptoms are usually observed only after a long period of AD pathology progression in the brain. Although there is no cure for AD, the available pharmacological, cognitive, and behavioral treatments can slow down disease progression and relieve symptoms, especially when applied in the early stage. Given these characteristics of AD, the earliest possible diagnosis (ideally in the preclinical stage) is the best to treat the disease. However, early and accurate diagnosis for AD is not easy. A confirmative diagnosis of AD can only be made postmortem through neuropathological examination. Even in autopsy studies, it has been shown that about 30% patients meeting the clinical criteria of AD do not have typical AD pathology in brain, while many elderly individuals with normal cognitive function were found to have typical AD pathology (Aizenstein et al., 2008; Grøntvedt et al., 2018; Shaw et al., 2009). These studies demonstrate that brain pathology and clinical symptoms of AD not always keep in pace with each other, but follow a non-linear dynamic continuum (Jack et al., 2010).

Autopsy as the only definitive diagnosis of AD however has two fatal defects. First, autopsy can only demonstrate the specific status of the neural structure postmorten, but can not reflect the dynamic changes of the neuropathology and the correlations between clinical symptoms and brain pathology during the long period of AD progression. Second, since the patient is already dead when the autopsy is done, it greatly undermines, from a clinical point of aspect, the value of diagnosis. For these reasons, the core value of diagnosis of AD by autopsy is mainly for research rather than clinical purpose. But the ultimate purpose of AD research has to be the early detection and
identification of a treatment to slow or even halt disease progression. Thus, it is essential to find biomarkers that allow in vivo diagnosis of the early AD stage.

1.6.1. The continuum of AD pathology

A biomarker, short for “biological marker”, is an objective measure (specimen or imaging) that is used to indicate a biological state, condition or progress, usually for disease diagnosis, monitoring and prediction. When it comes to biomarkers of AD, it necessary to look into the pathological process of this disease. As a widely accepted assumption, the Aβ cascade hypothesis suggests that the accumulation of Aβ initiated a cascade of events that eventually lead to the occurrence of AD clinical symptoms. The production of Aβ is initiated by the amyloid precursor protein (APP), which is cleaved by enzymes including β-Secretase and γ-Secretase, leading to the production of various length of Aβ peptides including Aβ40 and Aβ42, while Aβ42 is more likely to aggregate and form plaques (Fagan et al., 2009). The toxic effect of Aβ plaques and oligomers causes a series of neural changes, such as synaptic dysfunction, tau aggregation and hyperphosphorylation, inflammatory response, cell death, and cerebral atrophy (Bloom, 2014). Tau is a protein that normally functions on stabilizing microtubules which are structures maintaining the shape and transport between nerve cells. Hyperphosphorylation of tau protein is essential to form neurofibrillary tangles (NFTs) which is a hallmark of AD. In healthy neurons, there is a dynamic balance between phosphorylation and dephosphorylation (Levy-Toledano et al., 1997). When the balance is broken, tau protein becomes hyperphosphorylated. Hyperphosphorylated tau loses the ability to bind to microtubules and is more likely to misfold and aggregate, gradually leading to the formation of NFTs within neurons. While the extracellular Aβ plaques and the intracellular tau NFTs are two well-known pathological correlates of AD, both of them interfere with normal cellular process, affect signaling at synapses, and ultimately contribute to synaptic dysfunction and loss (Bloom, 2014). Synapses are the connections between neurons for their communication with each other. These connections are essential for transmitting signals and information between neurons, which underlies various brain functions including memory, cognition and learning. As a prominent feature of neurodegeneration, synaptic loss has been shown as the significant substrate of clinical symptoms of AD. The neurodegeneration is manifest at both micro and macro levels in brain structure. At the micro level, neurodegeneration is characterized by the dysfunction and eventual death of neurons; at the macro level, neurodegeneration leads to the atrophy of brain structures. In AD, the significant atrophy is in the cerebral cortex, especially medial temporal lobe including the hippocampal formation, the
parahippocampal gyrus, amygdala, and the entorhinal cortex. The medial temporal lobe is involved in memory, spatial navigation and some other cognitive functions. It has been shown that the neurodegeneration has a much more direct correlation with AD clinical symptoms rather than Aβ (Holmes et al., 2008; Mormino et al., 2009).

1.6.2. Biomarkers of AD

Based on the continuum pathological changes described above, there are several biomarkers indicating AD in various pathological status and progression. According to various pathologies in the long progression of AD, here I categorize those biomarkers into four big groups: Aβ, tau protein, synaptic dysfunction and atrophy of brain structure.

**Aβ**

Aβ can be detected and measured through various methods, including CSF analysis, blood tests, and neuroimaging techniques like positron emission tomography (PET) scans with Aβ-specific tracers for different diagnostic purpose. CSF circulates within the ventricle of brain and spinal cord, therefore it reflects the biochemical alterations of brain and can be obtained by lumbar puncture. Because of good acceptability and cost-effectiveness and without the influence of blood-brain barrier, CSF specimen is usually considered the optimum biomarker of AD in vivo. Aβ42, a specific variant of Aβ, is defined as one of the best CSF biomarkers of AD, while the other one is phosphorylated tau. Reduced Aβ42 in CSF (perhaps a sign of impaired clearing) predicts pathological change and higher risk of AD occurrence in the future (Dubois et al., 2016; Olsson et al., 2016; Toledo et al., 2015). Many other studies also showed that the Aβ42/40 ratio may be a better CSF biomarker for differentiation of AD from non-AD disease including LBD, PDD and vascular dementia (Janelidze et al., 2016). However, invasive collection of CSF by lumbar puncture is a significant limitation which prevents its wide usage in early routine screening of the general population. In contrast, blood testing provides a less invasive and more accessible and convenient procedure. But blood Aβ is not a potent alternative to lumbar puncture as blood Aβ levels are influenced by other factors and not directly related to brain Aβ deposition – they are much less specific. Second, Aβ concentration is diluted in blood, and the concentration is much lower than CSF Aβ, making it challenging to detect subtle changes in the early stage of AD. That being said, encouraging findings on blood Aβ were revealed in recent studies. A lower plasma Aβ42/40 ratio was shown to be not only associated with the severe amyloidosis status of Aβ in CSF, but also has the potential to prescreen the pathological changes of AD in cognitively normal individuals (Pérez-Grijalba et al., 2019; Schindler et al., 2019; Verberk et al., 2018, 2020). In addition, its accuracy is
enhanced when age and APOE are taken into account. Recently, the developing of a fully automated instrument of plasma biomarker for AD facilitates its application (Palmqvist et al., 2023), although further validation and improvement are required. Of note, in blood Aβ studies, absolute value of Aβ42 was rarely used to predict the pathology of AD, perhaps because the ratio of Aβ42/40 is a more specific biomarker of AD than Aβ42, which could somewhat counter the characteristic of low specificity of blood Aβ. While CSF and plasma tests can only present an absolute value of Aβ level, Positron Emmission Tomography (PET) can inform about both severity and topographical information. Tracer binding with Aβ depositions allows visualization through PET scans. The Pittsburgh compound B (PIB) is one of the widely and earliest used Aβ tracer, although it has a relatively short half-life. The fibrillar Aβ deposition measured by PIB-PET could be indicated at least 15 years before the onset of AD symptoms (Bateman et al., 2012). However, amyloid PET is good at excluding AD but not good at confirming it. An individual who has negative amyloid PET is unlikely to have AD even when cognitive impairment is present, while up to 35% of cognitively normal older people have positive amyloid PET scans (Marchant et al., 2012).

**Tau protein**

Similar with Aβ, tau protein can be mainly detected by the three methods: CSF analysis, blood tests and PET scans. Those tau biomarkers in different test ways also have the similar pros and cons with their Aβ counterparts. However, tau, the component of NFTs spreading through the neocortex, can represent the neurodegeneration, which correlates with clinical symptoms more closely than Aβ (Hansson, 2021; Jack et al., 2013). Both total tau (t-tau) and phosphorylated tau (p-tau) are biomarkers for AD and other degenerative disease, either in CSF or blood. T-tau refers to the total concentration of tau protein including both normal and phosphorylated forms, while p-tau refers to the tau protein that has undergone phosphorylation. Increased t-tau levels are associated with many neurodegenerative diseases, such as AD, Creutzfeldt-Jakob disease, LBD, frontotemporal dementia, and some acute brain injuries. Generally, t-tau is not so specifically associated with AD, but is more associated with current neuronal injuries, reflecting neurodegenerative burden in a certain time (Blennow & Hampel, 2003; Gordon et al., 2016; Vos et al., 2016; Zetterberg et al., 2006). In contrast, increased P-tau levels reflects the chronic phosphorylated severity of tau that is usually only found in AD but not in other neurodegenerative disease or traumatic brain injuries (Blennow & Zetterberg, 2018). Thus, p-tau acts as a more specific biomarker of AD compared with t-tau both in CSF and blood. CSF P-tau 181 (tau phosphorylated at threonine 181) is a highly specific biomarker that consistently correlates with AD pathology (Skillbäck et al., 2015; Vanmechelen et al., 2000).
For a long time a reliable blood test for p-tau 181 was challenging due to the very low concentration in blood and the different processing ways of tau in CSF and blood. Recently a N-terminal form of tau was introduced, making the blood p-tau 181 assay has the potential to do early diagnosis and rapid screening test of AD (Karikari et al., 2020). Some other studies also supported the diagnostic value of blood p-tau 181 (Janelidze et al., 2020; Thijssen et al., 2020). In addition, p-tau 217 has also been found to be a decent biomarker to distinguish AD from other neurodegenerative diseases either in CSF (Janelidze et al., 2020) or blood (Janelidze et al., 2022; Mattsson-Carlgren et al., 2023; Palmqvist et al., 2020, 2021). Some studies even found a superiority of p-tau 217 over p-tau 181 in differentiating AD and frontotemporal neurodegenerative syndrome and predicting the results of tau-PET and Aβ-PET (Brum et al., 2023; Thijssen et al., 2021). In recent years plasma p-tau 231 also attracted the attention of researchers and was found to have decent performance on distinguishing AD and non-AD as well as identifying cases of MCI oR AD (Ashton et al., 2021). While Aβ PET imaging presents the accumulation and distribution of Aβ plaque in the brain, tau PET imaging presents the distribution and density of tau tangles in the brain. It has been shown that increased tau PET binding in medial temporal lobe and neocortex is significantly correlated with positive amyloid PET image, and with clinical symptoms across cognitively normal to AD dementia spectrum (Cho et al., 2016; Gordon et al., 2016; Johnson et al., 2016; Phillips et al., 2018; Schöll et al., 2016). Many studies have revealed that tau PET demonstrated much closer relationship with cognitive function than amyloid PET (Aschenbrenner et al., 2018; Brier et al., 2016; Wang et al., 2015), although the two interact with each other.

**Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET)**

In FDG-PET, the biomarker for AD is the distribution and uptake of fluorodexyglucose (FDG), a radioactive tracer molecule. FDG-PET is used to reflect the metabolism of cerebral glucose, which is the primary source of energy for neurons and glial cells. Decreased FDG-PET uptake is an index of synaptic dysfunction, indicating the magnitude of neuronal dysfunction (Gray et al., 2012; Jagust et al., 2007; Padilla et al., 2012; S. Teipel et al., 2015). FDG-PET studies have shown the decreased glucose uptake in the temporoparietal, posterior cingulate cortex, precuneus and frontal lobe distribution even before the atrophy starts (Jagust et al., 2007; Petrella et al., 2003; Walhovd et al., 2010). One major benefit of FDG-PET is that it can indicate both the cumulative loss and the functional impairment of neurons, while other biomarkers including Aβ, tau, and structural MRI can only present neural structure changes or topographic information. In addition, apart from many AD patients with typical symptoms like amnestic problems, some AD patients have atypical phenotypes including language, executive, behavioral or motor dysfunction. It has been found the
hypometabolism patterns in FDG-PET can reflect clinical impairment across atypical AD phenotypes and distinguish typical and atypical AD (Graff-Radford et al., 2021). Generally, FDG-PET is usually used to provide complementary information of the synaptic dysfunctions that relates to neurodegeneration (Jack et al., 2010; Walhovd et al., 2010), which aid in both differentiating AD from other neurodegenerative diseases and distinguishing different variants in AD.

**Brain structure atrophy**

According to the A/T/N classification system, CSF t-tau, FDG-PET and structural MRI all fall into the N category, indicating neurodegeneration or neuronal injury (Jack et al., 2016). It has been found that the biomarkers in the N group are more strongly associated with clinical symptoms compared with the biomarkers in the A (amyloid PET or CSF abeta42) and T (CSF p-tau or tau PET) groups (Jack et al., 2013). At the micro level, increased CSF t-tau indicates the impairment of neuronal structures, while FDG-PET shows the functional impairment like synaptic dysfunction and the topography of hypometabolism. The accumulation of the micro impairment and the loss of neuropils will eventually lead to the shrinkage of the brain structure which can be shown in imaging scans. At the macro level, structural MRI can indicate not only the structural atrophy but also the topographic distribution of the atrophy order and region. Based on the established understanding of brain region and function, structural MRI provides both information of structure and function of neurodegeneration from macro level. Neurodegeneration has been shown to be the proximate cause of AD clinical symptoms (Jack et al., 2010; Jack & Holtzman, 2013). Brain structure atrophy that can be shown in MRI is the ultimate embody of neurodegeneration in the macro level.

Apart from neurodegeneration, human brains undergo a natural atrophy rate of 0.2%-0.5% per year with aging (Enzinger et al., 2005; Fjell et al., 2014; Hedman et al., 2012). The atrophy rate will accelerate in individuals with neurodegenerative diseases including AD (Allen et al., 2005; Jack et al., 2000). The brain shrinkage develops most dramatically in medial temporal lobe (MTL) where the AD pathology begins. The MTL, which includes hippocampus, entorhinal cortex, perirhinal cortex, parahippocampal cortex and amygdala, plays a crucial role in various aspects of memory and cognitive function. According to the Braak & Braak staging system which is based on postmortem examination of brain tissues, the NFTs which are important index of neurodegeneration begin to appear in the transentorhinal region, then progress to hippocampus in the middle stage, and spread to neocortex in the advanced stage (Braak & Braak, 1991).
1.6.3. Hippocampal volume (HV)

The hippocampus, as one of the earliest affected brain structures by AD (Devanand et al., 2007; Jack et al., 1998, 2010), has been the focus of researchers’ attention in terms of its relationship to the cognitive function and the course of AD. Hippocampal volume (HV) is widely used as a biomarker to indicate AD because of the relatively clear delineation of the hippocampal anatomy. Plenty of previous studies have found that larger HV is positively associated with better cognitive function (Ezzati et al., 2016; Hardcastle et al., 2020; Konishi et al., 2017; O’Shea et al., 2016) and lower risk of developing dementia (Mungas et al., 2002; Tabatabaei-Jafari et al., 2020). Compared with healthy people, AD patients have significantly smaller HV, which is the best brain structure to distinguish AD patients from health controls (Jack et al., 1997). In addition, although the rates of change in different AD biomarkers are not consistent with each other, a smaller HV is positively associated with decreased Aβ42 (Fagan et al., 2009) and increased tau level (Aschenbrenner et al., 2018). Several longitudinal studies have shown that a smaller HV increased the risk of conversion from MCI to AD (Apostolova et al., 2006; Chupin et al., 2009; Eckerstrom et al., 2008; Fang et al., 2019; Hill et al., 2014; Risacher et al., 2009; Tabatabaei-Jafari et al., 2020). Moreover, the National Institute on Aging and the Alzheimer’s Association (NIAAA) (Albert et al., 2011) and the International Working Group (IWG) (Dubois et al., 2014) recommended HV as a supplementary biomarker for the diagnosis of AD. On the whole, a number of previous studies supported the potential of HV as a decent biomarker of AD.

However, apart from AD pathology, there are a variety of factors affecting HV, which undermines the association between HV and AD. First, individuals have an inherent variation of size of brain structures. Genetic factors and stature both account for variations in brain size. A number of studies report a positive association between brain size and stature of human (Chirachariyavej et al., 2006; Heymsfield et al., 2007; Koh et al., 2005; Nopoulos et al., 2000; Witelson et al., 2006). It can be speculated that hippocampal size scales to brain size which varies inherently without linkage to AD pathology. For example, it has been reported that there is significant variation of HV among young adults independent of AD pathology. Second, given that the hippocampus is a structure with environmental susceptibility, there are some developmental factors other than aging that influence HV in middle to old population, including environmental effects (Sullivan et al., 2001; Taylor et al., 2020), chronic stress (Mirescu et al., 2004; Pruessner et al., 2005; Zainuddin & Thuret, 2012), nutritional supply (Zainuddin & Thuret, 2012), alcohol abuse (Agartz et al., 1999; Nixon et al., 2010), obesity (Jagust et al., 2005) and head trauma (Ariza et al., 2006; Beauchamp et al., 2011). Although there are such a few factors of influence, HV in cross-sectional studies is merely an
absolute value that can not indicate the inherent and developmental factors, which may explain why HV was merely recommended as a supplementary biomarker for AD and only a moderate relationship between HV and cognitive function was found. Moreover, a meta-analysis of 33 studies on HV and cognition found little support for the “bigger-is-better” hypothesis, while the relationship between HV and memory is slightly positive in old people (Van Petten, 2004). In a cross-sectional study, the association between HC and cognitive decline was even not found (Aschenbrenner et al., 2018).

In general, HV seems not a perfect cross-sectional biomarker for AD. However, HV have a set of irreplaceable advantages compared to other AD biomarkers. First, the non-invasiveness of MRI is absent in all CSF indices, and to a lesser degree, also in blood sampling; second, the cost is much more affordable compared with PET imaging; third, the excellent spatial resolution and topographic information that is absent in blood biomarkers; forth, the stronger association with clinical symptoms of AD than other biomarker. Taken together, the non-invasiveness, cost-effectiveness, good spatial resolution and closest relationship with AD clinical symptoms suggest that HV can’t be ignored as a AD biomarker, although it has some limitations. Hence, structural MRI research has been investing in identifying MRI biomarkers with better accuracy and validity.

1.6.4. The Hippocampal-to-ventricle ratio (HVR)

Both aging and AD pathology cause hippocampal atrophy (HA). HA involving longitudinal changes of HV should be more informative than absolute HV alone. While HV shows a static status of hippocampus, HA indicates dynamic change. Numerous previous studies have found accelerated HA to predict a high possibility of MCI-to-AD conversion (Henneman et al., 2009; Jack et al., 2004; McRae-McKee et al., 2019; Vemuri et al., 2009). MCI and AD patients were found to have significantly higher rate of HA compared to healthy controls (Fang et al., 2019; Jack et al., 2008; Ridha et al., 2006; Schuff et al., 2009). Some studies argued that HA is the direct cause (Mormino et al., 2009) or at least coupled to cognitive decline (Evans et al., 2018; Jack et al., 2004; Schuff et al., 2009; Thompson et al., 2004), especially the memory deficit. HV can be influenced by inherent and developmental factors listed above, while HA indicates the dynamic change in the AD spectrum, which is probably stronger related to AD pathology. Although HA could be influenced by other non-AD factors in the atrophy period, it should be much more reliable than a static HV. Previous studies supported the view that combining HV and HA significantly enhances the prediction of AD in preclinical individuals (McRae-McKee et al., 2019). However, the strong
limitation of HA is that repeated measurements are required over time. Repeated assessments over long periods are acceptable in controlled clinical trials but not practical in the general population.

A promising solution for an improved MRI biomarker is to combine the HV and its surrounding ventricle, filled with CSF, into one assessment. Ventricular expansion (VE) is an ongoing progress coupled with HA. Previous studies focusing on the VE found it can indicate the pathology of MC and AD (Apostolova et al., 2013; Bartos et al., 2019; Coutu et al., 2016). Some studies even found a better performance of VE than HA on indicating the progressive development of AD (Macdonald et al., 2013; Thompson et al., 2004). It can be speculated that, when HV is maximal, the surrounding ventricular space is minimal (Schoemaker et al., 2019). In the progression of HA, as the hippocampus shrinks, its surrounding ventricle expands accordingly. From this point of view, the increased ventricle space can be considered a previous part of the hippocampus, which is now lost. Thus, the core idea to improve existing MRI biomarkers, is to combine HV and surrounding ventricle into an index together to indicate the hippocampal integrity. It is assumed that the ratio of HV to the volume of its surrounding ventricle, abbreviated as hippocampal-to-ventricle ratio (HVR), is such a promising biomarker of AD. There are several obvious advantages of HVR. First, it is an integrity index containing both information of hippocampus and its adjacent ventricle. HVR is established cross-sectionally, but to some extent can act as a dynamic index to reflect the atrophy degree of hippocampus, providing the information that normally derived from longitudinal studies. Second, since HVR is a ratio index, the individual variation of HV in the general population is not influencing it, and is naturally controlled for. Third, there is no requirement for repeated assessments, which makes HVR practical in clinical applications, which is promising for the routine screening of AD among population.

Currently there are preliminary evidences supporting the priority of HVR over HV on indicating AD. Bartos et al. (2019) showed that the ratio of hippocampus and the inferior part of the lateral ventricle distinguished AD and control groups better than absolute HV. Silhan et al. (2021) showed that the ratio of hippocampal area and temporal horn area of the lateral ventricle has high specificity and sensitivity on the diagnosis of LOAD, although only one suitable MRI slice was chosen for each subject in this study. Schoemaker et al. (2019) proposed the concept of HVR explicitly and elaborated a manual segmentation protocol for HVR. In this study, the validity of HVR was preliminarily demonstrated: age and memory were shown to have stronger association with HVR compared to HV. Since all participants in this study were cognitively normal individuals, this study provided an initial validation of HVR among healthy population. Whether HVR is effective in discriminating between AD, MCI, and healthy controls remains to be seen. In addition, AD usually
has an insidious onset with a latency period up to one or two decades. The ideal biomarker of AD should be able to predict its occurrence in the preclinical period. Could HVR serve this purpose?

1.7. Overview of current three researches

To address these questions about HVR, I contributed three papers as part of my PhD.. The first one is a review study, the latter two are two experimental studies, including one cross-sectional study and one longitudinal study.

The first paper: Challenges and opportunities of diagnostic markers of Alzheimer’s disease based on structural magnetic resonance imaging

This article has been published in “Brain and behavior”. This paper first summarized early biomarkers of AD including biomarkers derived from cerebrospinal fluid (e.g., Amyloid-β and tau protein), and from functional imaging techniques (e.g., PET). It then described previously proposed structural magnetic resonance imaging markers of AD, especially hippocampal volume (HV). Although a majority of studies showed a significant relationship between HV and the AD progression, absolute HV is not very sensitive in picking up the disease in its early stages. This might have to do with the large inter-individual variation of naturally occurring volume differences across individuals, in the absence of disease. In fact, the early neurological approach of atrophy assessment in the medial temporal lobe was based on a visual comparison between gray matter volume and its directly adjacent ventricular space, realizing that as gray matter atrophies, ventricular volume enlarges. Acknowledging this notion, approaches that take into consideration gray matter volume to ventricle ratios might overcome the limitations of absolute volume and be more sensitive in picking up disease progression at an early stage. Finally, it put forward the updated MRI biomarker ‘hippocampal-to-ventricle ratio’ (HVR) and described conceptual details of it. At last, it reviewed preliminary evidence showing that HVR predicts memory functions better than HV alone in an elderly preclinical sample.

Overall, this review first made a concise summary of different kinds of AD biomarkers and then focused on the MRI biomarker – HV, based on which eventually proposing its promising refined successor - HVR, the core of my PhD thesis topic.

The second paper: A higher sensitivity of the hippocampus-to-ventricle-ratio (HVR) compared with pure hippocampal volume (HCvol) on differentiating AD, MCI and normal controls

HVR was proposed in the first review article with only preliminary evidence in cognitively healthy individuals. In the second article the aim was to confirm the superiority of HVR over HV in
differentiating AD, MCI and healthy controls. A total of 244 subjects were selected from the Open Access Series of Imaging Studies 3 (OASIS-3) dataset, including three groups: AD, MCI and healthy control. Structural MRI data, clinical data and age were included in statistical analyses to compare the group differences of HV and HVR respectively. We hypothesized that HVR and HV would show the same trend in results, but that HVR would be more sensitive in showing group differences, and to reveal relationships with age and cognitive function.

The MMSE scores between the three groups were significantly different. The difference between average left HV between MCI and AD group was not significant, while all other results of HV between groups were significant. All differences with HVR between groups were significant. HVR and age correlations were significantly greater than HV and age correlations, while HVR and MMSE correlations were significantly greater than HV and MMSE correlations (Steiger's Z tests, p < .001). The superior validity of HVR diagnosing AD and MCI was confirmed in this study. HVR was proved to be a more sensitive MRI biomarker than HV in indicating AD.

Overall, in this cross-sectional study our hypotheses were validated. Although the performance of HV on differentiating AD, MCI and normal control (NC) could also be shown, HVR had better diagnostic value than than HV. Moreover, both with age and cognitive function, HVR was more strongly correlated than HV.

The third paper: Hippocampal-to-ventricle (HVR) predicts mild cognitive impairment (MCI) and Alzheimer’s disease (AD) in cognitively normal individuals

The second study was a cross-sectional study merely directly showing the excellent validity of HVR on differentiating AD, MCI and normal controls. But the predictive function of an AD biomarker is what is ultimately required in clinical practice. To achieve this goal, we performed the third study, aiming to test predictive power of HVR. This study contributed a preliminary validation of the predictive efficacy of α (the visualization of HVR, tan α =CSFvol/HCvol) in a longitudinal study. 174 healthy people (mean age= 70.36 years [SD = 7.98], range: 51.3 to 92.4 years) were selected from OASIS-3. Over the course of OASIS-3, 93 subjects stayed healthy; 38 subjects developed mild cognitive impairment (MCI); and 43 subjects developed AD. We hypothesized that the α of the three groups were significantly different even at the time that they were cognitively normal in the beginning: thus, we predicted that the α of AD is greater than that of MCI, the α of MCI is greater than that of normal control.

As a result, we could show that the initial α of AD group was significantly greater than that of MCI group, and the initial α of MCI group was significantly greater than that of healthy group. We also
found that $\alpha$ of left hemisphere is larger than that of right hemisphere, and that $\alpha$ is larger in males than in females. For the MCI and AD patients, we showed that their brain degeneration accelerates evenly from the cognitively normal stage. The key finding of this study is that $\alpha$ was shown to be a valid neuroimaging biomarker for predicting MCI and AD, although further quantitative research is needed.

Overall, our hypothesis was confirmed in this longitudinal study. The introduction of $\alpha$ realized the visualization of HVR, and $\alpha$ was initially shown to have a good performance on predicting AD, MCI and keeping healthy.
Part II

Study 1: the review of existing biomarkers of AD and HVR was proposed
2. Article 1: Challenges and opportunities of diagnostic markers of Alzheimer’s disease based on structural magnetic resonance imaging

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2.1. Abstract

Alzheimer's Disease (AD) is the most common form of dementia, which is irreversibly progressive. Early diagnosis is difficult but if successful beneficial to slow down the progression of the disease at an early stage. This paper first summarizes early biomarkers of AD including biomarkers derived from cerebrospinal fluid (e.g., Amyloid-β and tau protein), and from functional imaging techniques (e.g., PET). It then describes previously proposed structural magnetic resonance imaging markers of AD, especially hippocampal volume (HV). Although a majority of studies show a significant relationship between HV and the AD progression, absolute HV is not very sensitive in picking up the disease in its early stages. This might have to do with the large inter-individual variation of naturally occurring volume differences across individuals, in the absence of disease. In fact, the early neurological approach of atrophy assessment in the medial temporal lobe was based on a visual comparison between gray matter volume and its directly adjacent ventricular space, realizing that as gray matter atrophies, ventricular volume enlarges. Acknowledging this notion, approaches that take into consideration grey matter volume to ventricle ratios might overcome the limitations of absolute volume and be more sensitive in picking up disease progression at an early stage. Finally, we describe one such marker, the hippocampal to ventricle ratio (HVR). We review preliminary evidence showing that HVR predicts memory functions better than HV alone in an elderly preclinical sample. Future studies will have to show if these ratio markers are better in predicting disease than absolute volume assessments.

Key words: Alzheimer's Disease (AD); Biomarker; Structural Magnetic Resonance Imaging (MRI); Hippocampus; Ventricle
2.2. Introduction

Dementia encompasses various diseases that are associated with a progressive and irreversible decline in cognitive capacity. According to the world health organization (WHO), around 50 million people worldwide are presently being affected by dementia, with 10 million new cases being reported every year (Organization, 2019). The total number of people suffering from dementia is projected to be 82 million in 2030 and 152 million in 2050. In 2015, the total global societal cost of dementia was estimated to be US$ 818 billion, accounting for 1.1% of the global gross domestic product. Overall, dementia exerts a huge burden on the public health system, with the situation turning for the worse as the aging population is growing, if no effective treatments are identified.

There are different forms of dementia including vascular dementia, dementia with Lewy bodies, frontotemporal dementia, and Alzheimer disease (AD). AD is the most common form of dementia contributing to 60–70% of the cases. Typically, diagnosis of AD is based on its clinical manifestation, encompassing a range of cognitive symptoms with mild but progressive anterograde amnesia being the most typical early sign. Yet, there is a long pre-symptomatic phase, likely lasting decades, between the beginning of neuronal degeneration in the brain, and the first clinical symptoms (Braak & Braak, 1991; DeKosky & Marek, 2003; Delacourte et al., 1999a; Jack et al., 2013). Although the progress of AD is irreversible until an effective treatment can be identified, studies have shown that pharmacological and behavioral intervention at the preclinical stage can prolong the time until clinical symptoms appear. This is important because AD is a disease that occurs in old age. An argument can be made that instead of finding a treatment for AD, it is sufficient to postpone the onset of the disease past the natural life expectancy of the individual. Even if that will not immediately be possible, every year that the onset of the disease can be pushed back will be one year where subjects can enjoy living independently with a high quality of life, without any burden on health care system and caregivers. Among the pharmacological treatments that are currently being investigated are non-steroidal anti inflammatory drugs (NSAI), that show some promise (Szekely et al., 2004). Among the behavioral interventions are regular exercise (Schuit et al., 2001; van Gelder et al., 2004), quitting smoking (Anstey et al., 2007; Barnes & Yaffe, 2011), not drinking alcohol excessively (Koch et al., 2019), keeping a healthy weight (Horie et al., 2016), healthy diet (Knight et al., 2016; Shannon et al., 2019), normotension (de Heus et al., 2019; C. Qiu et al., 2005) and normal blood sugar levels (Zheng et al., 2018).

The pre-symptomatic period is the ideal stage for both pharmacological and behavioral
interventions, for those at risk of developing AD. While most of the behavioral interventions are sound recommendations for anybody, the pharmacological interventions are costly and carry potential side-effects. As such, these interventions should target predominantly those at risk. This creates the need to find reliable tools to identify persons, in which the disease has begun to take hold in the brain, but who are not yet symptomatic. Currently, a myriad of studies have tested the reliability and validity of various biomarkers in predicting the insidious degeneration in the brain at an early, pre-symptomatic stage of AD (Aschenbrenner et al., 2018; Blennow & Zetterberg, 2018; Davis et al., 2018; Dubois et al., 2014; S. Teipel et al., 2015; Vemuri et al., 2009). In this context, several biomarkers have been shown to be sensitive for the prediction of AD, and there exist several reviews that give an overview of these biomarkers (McGhee et al., 2014; Olsson et al., 2016; Risacher & Saykin, 2013; Shui et al., 2018). Depending on complexity and cost, these markers vary in their ease to implement them in daily clinical practice.

Here, we summarize early diagnostic markers of AD with a focus on ease of use and cost in clinical practice. We end by putting forward that a ratio between brain region volume and its directly adjacent space, which would occupy the region in case of atrophy, is a more informative marker compared to absolute brain region magnitude alone. We summarize promising first evidence from the hippocampal-to-ventricle ratio (HVR) and elaborate on its principle and possible advantages compared to other markers.

2.3. Early diagnostic markers of Alzheimer disease based on pathology on the micro level

Neuropathological changes occur many years before clinical manifestations of AD (Braak & Braak, 1991; DeKosky & Marek, 2003; Delacourte et al., 1999a; Jack et al., 2013). Already at pre-symptomatic stages of AD, pathological neurofibrillary tangles (NFTs) composed of phosphorylated tau protein accumulate in brain cells. Further, different isoforms (T. Qiu et al., 2015) of amyloid deposits of amyloid-β (Aβ) peptides accumulate in the extracellular space. Those brain proteins are secreted to the cerebrospinal fluid (CSF) (Seubert et al., 1992; Wolozin & Davies, 1987), where they can be detected in CSF (Blennow et al., 2010).

On the basis of these pathological changes on the micro perspective, a variety of biomarkers have been developed to detect beginning AD. First, there is tau protein, which is a group of highly soluble protein isoforms that stabilize microtubules in axons. When looking at NFTs, total tau (T-tau) and phosphorylated tau (P-tau) are usually assessed. Hyperphosphorylation of tau proteins can
cause NFTs which contribute to the pathology of AD (Iqbal et al., 2005). CSF T-tau is associated with acute neuronal injuries, reflecting current neurodegeneration burden from days to weeks (Blennow & Hampel, 2003; Zetterberg et al., 2006), while P-tau does not change with acute brain injury (Zetterberg et al., 2006). High P-tau levels reflect the chronic phosphorylation degree of tau which is only found in AD but not in other neurodegenerative disorders (Blennow & Zetterberg, 2018). In view of this, CSF P-tau represents a more specific biomarker for AD as compared to CSF T-tau, although the two are often highly correlated.

Next, there is amyloid beta (Aβ), which represents peptides of amino acids which mainly constitute amyloid plaques found in the brain of AD patients (Hamley, 2012). When focusing on Aβ, total Aβ peptide accumulation does not seem to be a convincing marker to detect AD. Total Aβ CSF concentration is usually indexed by the most represented isoform – Aβ40 (Aβ with 40 residues; other isoforms are named analogously) (Hansson et al., 2019). Aβ40 levels have however been shown to not be specific enough to differentiate between AD and control groups (Shoji et al., 1998). In contrast, focusing on other isoforms of Aβ seems to be more promising. For example, decreased Aβ42 harbors a much stronger potential to reveal imminent AD, since it likely reflects the deposition of amyloid plaque in the brain which gradually leads to brain atrophy (Fagan et al., 2009). Further, some studies showed that the CSF Aβ42/Aβ40 ratio is more clearly linked to AD than Aβ42 alone (Lewczuk et al., 2004; Wiltfang et al., 2007). The reason for the improved performance of Aβ42/Aβ40 ratio is still unclear, but it is hypothesized that Aβ40 can serve as an indicator of total Aβ and the ratio offsets the individual differences of total Aβ level in CSF (Lewczuk et al., 2015).

Besides measuring Aβ directly from CSF, proton emission tomography (PET) has been used to detect areas in which amyloid deposition is accumulated in the brain at early stages of AD (Blennow & Zetterberg, 2018). Both the Food and Drug Administration (FDA) and European Medicines Agency (EMA) have approved this method to rule out AD as the etiology of mild cognitive impairment (MCI) (Ritchie et al., 2017). For this purpose, an agent named Pittsburgh compound-B ([11C]-PIB) is used as ligand in PET imaging to indicate the amyloid deposition in the brain by inferring from the distribution area of ligand retention (Klunk et al., 2004). Apart from amyloid, tau agents are also used with PET imaging in numerous clinical trials (Brosch et al., 2017). Besides that, Fluorodeoxyglucose (FDG) PET is used to detect decreased brain metabolism, which indicates the magnitude of neurodegeneration (Gray et al., 2012; Padilla et al., 2012; S. Teipel et al., 2015).

Taken together, on a micro perspective, the neurobiological, pathological progression of AD is
accompanied by the occurrence of tau, and/or isoforms of the Aβ protein in the brain. Early
diagnostic markers of AD either try to assess the concentration at which these pathological
neurobiological (by)products are present in the brain, or try to assess their distribution within the
brain. However, CSF biomarkers are collected through lumbar puncture and the cost of PET testing
is expensive. While these “micro” biomarkers of AD are currently the most sensitive, their costly
methodologies and related high patient burden hinder the implementation of these practices in
routine clinical examinations. As such, identifying less costly and burdensome alternatives could be
beneficial for clinical practice.

2.4. Early diagnostic markers of Alzheimer disease based on pathology on
the macro level

We can look into the pathological progression of AD from both macro and micro perspectives. On
the micro neurobiological level, Aβ and/or tau accumulation damage the neurons day by day. On
the macro level these pathological changes cause atrophy of certain brain structures, which can be
detected by neuroimaging techniques such as magnetic resonance imaging (MRI).

The medial temporal lobe (MTL, including the hippocampus and its adjacent parahippocampal
cortex, and the temporopolar cortex) is the brain region where the neuropathology of AD emerges
and develops dramatically (Braak & Braak, 1991; Hyman et al., 1984). Braak and Braak
demonstrated typical stages in which AD spreads through the brain. Earliest neurodegeneration
seems to always occur in the entorhinal cortex (part of the parahippocampal cortex), and spread
from there to the hippocampus. According to Braak stage theory (Stages I and II: transentorhinal
region is affected with mild involvement of hippocampus; stages III and IV: both the entorhinal and
transentorhinal regions are conspicuously affected with mild-to-moderate hippocampal and a low
isocortical involvement; stages V and VI: the hippocampus is infested with NFTs and all isocortical
association areas are severely affected.), NFTs start to aggregate in the hippocampus from stage II
onward and progressively damage it. From there, the disease spreads to the entire temporal lobe,
and eventually reaches the isocortex at stage VI (Braak & Braak, 1991). It is not actually clear why
the disease seems to follow these distinct stages, but it might have to do with the central role the
medial temporal lobe plays in contextualization, and memory consolidation, reconsolidation and
retrieval, making it one of the metabolically most active areas in the CNS.

Microscopic injuries chronically give rise to macroscopic atrophy which can then be visualized by
in vivo techniques like MRI. In the revised edition of NINCDS–ADRDA criteria (Dubois et al.,
MTL atrophy is proposed as a crucially supportive diagnostic criteria for AD in addition to clinical symptoms. Because of the relatively clear delineation of hippocampus anatomy, hippocampal volume (HV) estimated from MRI is considered a better structural biomarker compared to total MTL volume, or entorhinal cortex volume. The European Federation of the Neurological Societies (EFNS) (Hort et al., 2010), the EMA (Hill et al., 2014), the National Institute on Aging and the Alzheimer’s Association (NIAAA) (Albert et al., 2011), and the International Working Group (IWG) (Dubois et al., 2014) recommended HV as a supplementary biomarker indicating neuronal damage, and facilitating the clinical diagnosis of AD. Moreover, studies have linked hippocampal degeneration to CSF biomarkers of AD. For example, a smaller HV is positively correlated with decreased Aβ42 (Fagan et al., 2009), increased amyloid and increased tau (Aschenbrenner et al., 2018). HV has further been found to be smaller in AD patients, and is summarized to be the best brain structure to discriminate AD patients from healthy controls (Jack et al., 1997). A bigger hippocampus is usually associated with better cognitive function (Ezzati et al., 2016; Hardcastle et al., 2020; Konishi et al., 2017; O’Shea et al., 2016) and lower risk of developing dementia (Mungas et al., 2002; Tabatabaei-Jafari et al., 2020). An autopsy study showed significantly larger HV in patients not cognitively impaired within one year of their death (Erten-Lyons et al., 2009). Further, old patients with large hippocampi show preserved cognitive function despite pathological deterioration in the brain (Fotuhi et al., 2012). Taken together, these results confirm the notion that hippocampal volume assessment can support the diagnosis of AD in the presence of clinical symptoms.

Since the concept of MCI was introduced in the 1990s (Flicker et al., 1991; Petersen et al., 1995, 1999), much research has taken place to determine whether it is a preclinical stage of AD. MCI describes symptoms of slight cognitive dysfunction; yet people suffering from MCI do not fulfill the criteria of AD. However, compared to healthy controls, people with MCI are more likely to develop AD, with a conversion rate from 35% to 50.5% within 3 years (Luis et al., 2004; Palmer et al., 2002). It has further been suggested that the conversion rate within one year lies above 10% (Petersen et al., 2001), although a recent meta-analysis argued that the conversion rate might be less than 10% (Mitchell & Shiri-Feshki, 2009). On the transition from normal cognitive functioning to AD, MCI is a critical stage to focus on for the purpose of slowing down neurodegenerative progression, either by behavioral interventions or drug administration. Not everyone diagnosed with MCI goes on to develop AD, however, and not everyone developing AD went through a stage of MCI. Many other factors can cause MCI, including metabolic, vascular, dehydration, and inflammation. If the cause is reversible, the stage of MCI can then also revert to normal again.
Recently, the term ‘amnestic mild cognitive impairment’ (aMCI) has been introduced as it appears that this form of MCI, with a focus on cognitive impairment in the memory domain, is more strongly connected to subsequent development of AD. This would make sense in the light of the critical role both entorhinal cortex and hippocampus play in memory function.

Clearly, having MRI biomarkers that are sensitive at the preclinical stage (perhaps even pre-MCI) would be beneficial to aid in the identification of those at risk. Unfortunately, the existing MRI biomarkers all fail to be sensitive enough for this purpose. On the transition from MCI to clinical AD, structural markers, which directly reflect neurodegeneration, have closer relationships with clinical symptoms than CSF biomarkers (like amyloid deposition) (Jack et al., 2009). Several longitudinal studies could demonstrate that decreased HV is positively related to increased risk of conversion from MCI to AD (Apostolova et al., 2006; Chupin et al., 2009; Eckerstrom et al., 2008; Fang et al., 2019; Jack et al., 1999; Risacher et al., 2009; Tabatabaei-Jafari et al., 2020). A meta-analysis including 27 studies also supports the notion that HV is a good predictor of MCI-to-AD conversion (Hill et al., 2014). Overall, HV seems to be a good predictor of AD, at least at the MCI stage of the disease.

Since the pathological changes related to AD induce brain atrophy, changes in HV should be more informative compared to HV alone. Hippocampal atrophy through repeated assessment in longitudinal MRI designs is a marker closely associated with HV and harbors the advantage of a dynamic feature reflecting the progression of change. One previous study showed that combining two hippocampal metrics (HV and hippocampal atrophy) enhances the prediction of AD progression in preclinical individuals (McRae-McKee et al., 2019). In accordance with the disease progression, significantly higher rates of hippocampal loss were found in AD and MCI patients compared to healthy controls (Fang et al., 2019; Jack et al., 2008; Ridha et al., 2006; Schuff et al., 2009). In harmony with HV, accelerated hippocampal atrophy also predicts a high possibility of conversion from MCI to AD (Henneman et al., 2009; Jack et al., 2004; McRae-McKee et al., 2019; Vemuri et al., 2009). Several studies argue that hippocampal atrophy is the direct cause of (Mormino et al., 2009) or at least coupled to (Evans et al., 2018; Jack et al., 2004; Schuff et al., 2009; Thompson et al., 2004) cognitive decline, especially memory impairment. A clear disadvantage is however the need for repeated assessments sufficiently spaced in time to allow accurate assessment of atrophy.

A possible alternative biomarker is the assessment of adjacent hippocampal ventricular space in combination with the assessment of the gray matter volume. It can be argued that with hippocampal volume loss, surrounding CSF space increases accordingly (Schoemaker et al., 2019). Previous
studies have shown that ventricular expansion can correctly indicate presence of AD and MCI (Apostolova et al., 2013; Bartos et al., 2019; Coutu et al., 2016). Some studies even reported a better performance in measuring AD progress by assessing ventricular expansion compared with hippocampal atrophy (Macdonald et al., 2013; Thompson et al., 2004).

Overall, HV, the atrophy of the hippocampus, and the expansion of adjacent CSF space should be highly correlated with each other. These structural markers could thus be considered valid markers of MCI, AD and their progression (Frisoni et al., 2010). Apart from the noninvasive and economic features of these measures, which is a clear advantage compared with CSF based markers of AD, MRI biomarkers present good anatomical features of degenerated regions, and the longitudinal assessment of MR images can display the dynamics of disease progression straightforwardly. As noted above, relying on a longitudinal assessment, diagnoses cannot be made promptly and the process of targeted intervention is delayed, which questions the applicability of these measures in the clinical context.

These limitations raise the question of whether there is a comprehensive biomarker embracing the features and information of the three markers together (HV, hippocampal atrophy and ventricular expansion), while at the same time avoiding repeated measurements.

2.5. The ratio between gray matter structures and directly adjacent ventricular volumes as superior diagnostic marker of beginning neurodegeneration?

Normally, HV decreases with aging, with the decline accelerating through the pathology of AD (Jack et al., 1997; Pruessner et al., 2001). However, a variety of factors also affect HV and cause variations among individuals, decreasing the diagnostic validity of HV on AD. Firstly, different individuals have inherent variations in their hippocampal sizes. Initial hippocampal development early in life is typically independent of neuropathology, and related to a variety of different factors – genetic influences, availability of nutritional resources, amount of cognitive stimulation, etc. It can however be speculated that a larger generic HV might serve as a protective factor against neurodegeneration in old age, potentially providing a cognitive reserve. Be that as it may, there is significant variation of HV in young adulthood across the general population independent of disease (Lupien et al., 2007). Secondly, there are also some developmental factors other than aging that affect HV in middle to old adulthood (Schoemaker et al., 2019), e.g. obesity (Jagust et al., 2005), environmental effects (Sullivan et al., 2001; Taylor et al., 2020), stress (Mirescu et al., 2004;
Pruessner et al., 2005), chronic alcohol abuse (Agartz et al., 1999; Nixon et al., 2010), nutrition (Zainuddin & Thuret, 2012), and head trauma (Ariza et al., 2006; Beauchamp et al., 2011).

However, HV in cross-sectional studies merely indicates the current status without incorporating inherent and developmental factors causing additional variation, as mentioned above. As a result, studies investigating the association between HV and cognitive function show only moderate associations. On the one hand, a series of studies reported a positive correlation between HV and memory function (Ezzati et al., 2016; Hardcastle et al., 2020; Konishi et al., 2017; O’Shea et al., 2016); on the other hand, a meta-analysis of 33 studies found little support for the “bigger-is-better” hypothesis, while the relationship between HV and memory is positive but weak in old people (Van Petten, 2004). We believe that a lot of this has to do with the inability of pure HV to differentiate the many factors contributing to the size of the structure at a given point in time.

A promising biomarker that can possibly overcome the limitations of the established structural markers associated with HV and hippocampal atrophy is the volumetric ratio of the hippocampus and its surrounding ventricle, abbreviated as hippocampal-to-ventricle ratio (HVR) (Schoemaker et al., 2019). We believe there are clear advantages of HVR compared to HV: Firstly, HVR takes into account information about HV and relates it to surrounding ventricular enlargement. Thus, HVR presents a dynamic feature that to some extent provides information that is normally available only through longitudinal data (i.e., a marker of change over time). As such, it could possibly provide information about the progress of hippocampal volume loss even though it is established cross-sectionally. By providing a ratio, variations in hippocampal size that might be unrelated to volume loss caused by neurodegenerative factors are automatically controlled for.

There is first evidence that supports the view that HVR might be a superior measure to predict AD compared to HV alone. Bartos et al. (Bartos et al., 2019) showed that the ratio of hippocampus and the inferior part of the lateral ventricle allowed better discrimination of AD and control groups than absolute HV. This result partially provides the preliminary evidence of the superiority of HVR compared to HV. We expanded on this by taking the entire surrounding ventricle mass into account, and articulated the core rationale of the HVR (84):

“To compute the HVR, we were guided by the assumption that the ventricle space directly surrounding the hippocampus increases as a function of atrophy or neurodegeneration of the hippocampus proper. Calculating a ratio combining a structural estimation of the target structure together with the ventricle space surrounding the target structure could provide an integrity index, which will indicate the preservation of the given structure (Schoemaker et al., 2019)(Page 116108).”
In this paper, the validation of the HVR was first performed in a preclinical sample: both age and memory showed stronger negative correlations with HVR than compared to HV alone. Thus, the usefulness of HRV needs next to be confirmed in clinical studies that aim to discriminate AD from MCI and controls. As HVR assessment comes at low structural and economic costs, and low burden for the patient, its application in clinical practice would be highly feasible in the course of routine assessments.

Taken together, we argue that the pathological decline of hippocampal volume and surrounding structures often occur in MCI and AD. However, pure volume assessment of these brain structures is not sufficient to predict AD progression accurately. From preliminary results, HVR is introduced as potentially better index to represent structural integrity of the hippocampus. Thus, the consistent implementation of HVR in future studies might hold the potential to decrease the inconsistencies of results between studies. The possibly improved accuracy introduced by HVR would perhaps make it a better biomarker to predict MCI and prodromal AD in the preclinical period, which would be beneficial in clinical practice. Figure 1 is inspired by the illustration by Sperling et al. (Sperling et al., 2011) and Jack et al. (Jack et al., 2010) illustrating the sensitivity of different biomarkers of AD in predicting MCI and dementia at a preclinical stage. We suggest that a ratio of volume to ventricle marker has an improved sensitivity, which will shift the value of structural MRI to the left, into the proximity and perhaps past some of the established wet marker for early diagnosis of AD.

Figure 1. Schematic of how brain structure ratio might increase sensitivity for early diagnosis, based on Sperling et al. and Jack et al.
A potential downside of HVR is the high cost in time and labor, as segmentation in the initial study was performed manually. This is a downside of all (manual) segmentation paradigms, that the exact delineation of the individual anatomy takes considerable amounts of time. The segmentation includes the parcellation of two structures - the hippocampus and surrounding ventricle, consuming 1.5-2 hours per brain for the expert rater. Therefore, automating the segmentation can largely reduce the overall time devoted to the segmentation. From pilot trials we know that by using automated algorithms, for example the Multiple Automatically Generated Templates (MAGeT) (Chakravarty et al., 2013; Pipitone et al., 2014) package, segmentation time can be reduced to around 30 minutes, only requiring quality control of the automated segmentation. Thus, for studies in large populations samples the implementation of the automated pipelines can largely enhance the practicability of utilizing the HVR index. If employed in individual subjects in the context of clinical assessments, this can be considered less of a concern though.

2.6. Conclusion

In this opinion paper, we first reviewed the current status of dementia and AD in the population and showed the necessity and importance for sensitive biomarkers already at the preclinical stage. Then we reviewed existing early diagnostic markers commonly used for AD: CSF biomarkers including Aβ42, T-tau and P-tau, PET imaging of amyloid and tau, Pittsburgh compound-B, and FDG PET. Next, we focused on a structural neuroimaging biomarker, HV, summarizing previous results. Here, we described the relationship between HV and MCI/AD as well as the predictive value of HV on MCI-to-AD conversion. Also, we presented two markers related to AD, hippocampal atrophy and ventricular expansion. At last, we put forward that ratios between structural volumes, e.g. the HVR, might be promising diagnostic markers of AD. Finally, we elaborated the rationale of HVR and showed the evidence for preliminary validation of the HVR.

It remains to be seen whether HVR has the potential to increase the early diagnostic accuracy of hippocampal structural integrity in AD and MCI and decrease the discrepancy of research findings. Clearly, HVR needs to be applied to clinical studies with MCI and AD patients. Finally, the implementation of HVR in longitudinal studies investigating the possibility of conversion from normal to MCI/AD would allow validating its predictive power in direct comparison with longitudinal analyses.

In conclusion, we suggest that depicting the ratio between atrophied structures and direct adjacent regions that have occupied the atrophied region can be a more informative measure compared to absolute structural volumes of atrophied regions alone. Extending that argument, ratio measures of
regions that are affected by AD even earlier than the hippocampus (i.e., the transentorhinal region) could even be more informative compared to the proposed HVR. Overall, we think that this line of research holds the promise of providing valuable clinical information, could help with the diagnosis of AD at an early preclinical stage, and could thus help in the prevention and intervention and the lowering of individual, as well as societal costs associated with dementia.
Part III

Study 2: a cross-sectional study showing a higher sensitivity of HVR than pure hippocampal volume
3. **Article 2:** A higher sensitivity of the hippocampus-to-ventricle-ratio (HVR) compared with pure hippocampal volume (HV) on differentiating AD, MCI and normal controls

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3.1. Abstract

Large interindividual variations in hippocampal volume (HCvol) among populations are likely to undermine the validity of pure HCvol on early Alzheimer's disease (AD) diagnosis, whereas combining the hippocampus and its adjacent ventricle to calculate the ratio could compensate for this drawback. Our aim was to confirm the HVR's superiority over pure HCvol in detecting mild cognitive impairment (MCI) and AD. A total of 244 subjects were selected from the OASIS-3 dataset, including three groups: normal control (NC), MCI and AD. MRI data, clinical data and age were included in statistical analyses to compare the differences between groups as well as HCvol and HVR. The MMSE scores between the three groups are significantly different. The difference between average left HCvols in MCI and AD group is not significant, while all the other results of HCvol between groups are significant. All the differences of HVR between groups are significant. HVR and age correlations are significantly greater than HCvol and age correlations, while HVR and MMSE correlations are significantly greater than HCvol and MMSE correlations (Steiger's Z tests, p.001). We validated the reliability of HVR and confirmed that HVR is more sensitive than pure hippocampal volume on indicating hippocampal integrity, though the final validity of HVR on predicting MCI and AD should be confirmed in follow-up longitudinal studies.

**Key words:** Alzheimer's Disease (AD); Biomarker; Structural Magnetic Resonance Imaging (MRI); Hippocampus; hippocampus-to-ventricle-ratio (HVR)
3.2. Introduction

The brain region where Alzheimer's Disease (AD) pathology develops earliest and most extensively is the medial temporal lobe (MTL) (Hyman et al., 1984; Jack et al., 1997; Tabatabaei-Jafari et al., 2015; Tomlinson et al., 1970). Hippocampus (HC) is the core structure related to memory and spacial function in MTL. The volume of HC is typically regarded as a structural biomarker of AD due to its distinct delineation. Normally, HC volume is positively associated with better cognitive function (Ezzati et al., 2016; Jack et al., 2004; O’Shea et al., 2016) and lower rate of MCI to AD conversion (Chetelat & Baron, 2003; de Leon et al., 2007; Geuze et al., 2005).

The performance of memory and cognitive functions is the focal point of an AD diagnosis. However, by the time clinical symptoms appear, the neuropathology has already advanced to an irreversible middle or even advanced stage (DeKosky & Marek, 2003; Delacourte et al., 1999b; Jack et al., 2013) In order for patients to receive treatments to stop or delay cognitive decline as well as clinical manifestations, an early diagnosis of MCI/AD is crucial. Despite the fact that certain Cerebrospinal Fluid (CSF) biomarkers, such as amyloid- and tau protein, can identify the early stages of AD, the invasiveness of lumber puncture prevents its promotion in routine clinical examination. In light of this, a neuroimaging biomarker like HC volume is a better option due to its noninvasiveness.

Numerous longitudinal studies revealed a positive correlation between decreased HC volume and an increased risk of MCI to AD conversion (Apostolova et al., 2006; Eckerstrom et al., 2008; Jack et al., 2009; Risacher et al., 2009). Additionally, numerous studies demonstrated the connection between cognitive decline and hippocampal atrophy (Evans et al., 2018; Jack et al., 2004; Mormino et al., 2009; Schuff et al., 2009). The majority of studies demonstrating a connection between HC volume and memory function have been criticized for only using patient populations in their analyses, and a meta-analysis of 33 studies' findings found little evidence to support the idea that bigger is better (Van Petten, 2004). In this meta-analysis, the positive correlation between HC volume and memory function in older people was surprisingly weak, while even the negative correlation between HC volume and memory function was found in children, adolescents, and young adults (Van Petten, 2004). Additionally, a recent meta-analysis found that the pure volume of the hippocampus was insufficient for the early diagnosis of AD (Lombardi et al., 2020).

Large interindividual variations in HC volumes among the population are likely the main cause of the insufficiency of pure HC volume for early AD diagnosis. Hippocampal volumes vary from
person to person due to genetic variability, developmental factors, pathological causes, etc. AD is only one of many pathological factors that affect hippocampal size, so it cannot account for all of them. As a typical neuroimaging biomarker of AD, HC volume does have some clear benefits, but it seems insufficiently valid to serve as a trustworthy diagnostic biomarker of AD. As a result, this volumetric index needs to be updated or improved.

The volumetric index of hippocampal integrity may be enhanced by accounting for the ventricle surrounding the hippocampus. The CSF-filled ventricle grows as hippocampal atrophy worsens. In aging and dementia, ventricular expansions and hippocampal volume atrophy progressed together over time (Thompson et al., 2004). It has been discovered that atrophy starts in the hippocampal formation before spreading elsewhere (Price et al., 1991; Price & Morris, 1999). Previous studies have shown that ventricular expansion can decently indicate the occurrence of AD and MCI (Apostolova et al., 2013; Bartos et al., 2019).

In general, the ventricle, in addition to the hippocampal volume, is a key indicator of MCI and AD. HC deteriorates as the surrounding CSF enlarges. Instead of just looking at hippocampal volume, a better indicator might be the ratio of HC to CSF. According to fundamental mathematical theory, this ratio can probably attenuate the individual variations in HC volumes, better indicating changes in hippocampal integrity. In relation to that, a few studies made some preliminary attempts to take the ventricle into account. An early study found that coronal hippocampal and perihippocampal CSF together were more accurate at predicting AD than any single index (Convit et al., 1993). Hippocampal volume and hippocampal atrophy work together to improve the prediction of AD progression in preclinical individuals, according to (McRae-McKee et al., 2019). Furthermore, Bartos et al. (2019) discovered that AD patients have the greatest enlargement of the inferior portions of both lateral ventricles, and that the ratio of the hippocampi to the inferior lateral ventricle was the most sensitive and specific indicator of AD compared to healthy controls. Additionally, Schoemaker et al. (2019) presented the manual segmentation protocol for computing the hippocampal-to-ventricle ratio (HVR), a new index of hippocampal integrity. The hippocampus and the CSF that surrounds it are thought to have a completely opposing relationship: when hippocampal volume is maximal, surrounding CSF space is minimal (Schoemaker et al., 2019). When examining correlations between aging and cognitive function, the HVR with both the volumetric data of the hippocampus and CSF demonstrated an advantage over the standard hippocampal volume (Schoemaker et al., 2019). However, none of the participants in that study have cognitive impairments, whereas cohorts with MCI and AD were not present. HVR needs additional testing in various data sets as a new index. Furthermore, it is important to validate and
utilize HVR, a promising hippocampal index that not only has the benefit of a neuroimaging biomarker but also effectively overcomes the drawback of individual variation.

In the current study, we selected the participants from the database known as The Open Access Series of Imaging Studies 3 (OASIS-3) and compared the hippocampal volume (HCvol) and HVR differences in three groups, including normal control, MCI, and AD, as well as the associations between age/(cognitive function), (HCvol)/HVR, and the three groups' differences. We hypothesized that HVR and HCvol would show the same trend in results, but that HVR would be more sensitive to group differences and the relationship with age and cognitive function.

3.3. Methods

Subjects

The data was collected from the OASIS-3 database (oasis-brains.org). OASIS-3 is a compilation of 1098 participants' MRI, PET imaging, and associated clinical data that was gathered over a 15-year period from several ongoing studies at the Washington University Knight Alzheimer Disease Research Center. Participants range in age from 42 to 95 years old and include 605 adults with normal cognitive function and 493 people who are in various stages of cognitive decline. The OASIS-3 dataset contains over 2000 MR sessions, including multiple structural and functional sequences (LaMontagne et al., 2019). For this study, 264 participants were selected from the OASIS-3 database. 20 of these individuals were ultimately excluded from the analysis due to MRI image flaws. As a result, the final sample for this analysis consisted of 244 subjects, including 121 men and 123 women, with a mean age of 69.6 years (SD=6.95), a range of 50 to 88 years. There are 219 right-handed subjects and 25 left-handed subjects.

Grouping of Normal Control, Mild Cognitive Impairment and Alzheimer’s Disease

The current study includes three groups: 99 participants in the normal control (NC) group have a mean age of 67.2 years (SD: 7.05), ranging from 52 to 85 years; 49 participants in the mild cognitive impairment (MCI) group have a mean age of 74.1 years (SD: 7.39), ranging from 57 to 88 years; 96 participants in the Alzheimer's disease (AD) group have a mean age of 69.8 years (SD: 5.30), ranging from 50 to 79 years.

Participants completed clinical assessment protocols in accordance with the Uniform Data Set (UDS) of the National Alzheimer Coordinating Center (Beekly et al., 2007; Morris et al., 2006). The UDS assessments included a physical exam, medical history, neurological evaluation, and family history of AD. The Clinical Dementia Rating (CDR) Scale (Morris, 1993) was used to
determine a participant's dementia status for the UDS. CDR 0 indicates normal cognitive function, CDR 0.5 very mild impairment, CDR 1 mild impairment, and CDR 2 moderate dementia; participants who reached CDR 2 were no longer eligible for in-person assessments. Clinical staff conducted a diagnostic impression intake and interview that resulted in a coded dementia diagnosis that included the terms "cognitively normal" and "AD dementia" (LaMontagne et al., 2019). The MCI group should satisfy the following two requirements: 1. A CDR score of 0.5 or 1; 2. A person without dementia. The following conditions are considered exclusion criteria for the NC group and MCI group: 1. severe head injury; 2. active drug or alcohol abuse; 3. active hypothyroidism; 4. Parkinson's disease or any significant psychiatric disorder.

**The Mini Mental State Examination (MMSE) test**

A 30-point self-report questionnaire called the Mini Mental State Examination (MMSE) (Folstein et al., 1975) is frequently used in clinical and research settings to assess cognitive impairment. It can be applied to evaluate the severity and progression of dementia. The better cognitive function is indicated by a higher score. In the current study, the MMSE test results are recorded alongside the MR session.

**MR Image Acquisition**

All neuroimaging scans were performed by the Knight Alzheimer Research Imaging Program at Washington University in St. Louis. Three different Siemens scanner models—Vision 1.5T, TIM Trio 3T (there were two of these scanners) and BioGraph mMR PET-MR 3T—were used to collect the MRI data. In order to reduce motion, foam pad stabilizers were placed next to the ears of participants in 20-channel head coils on 3T scanners and 16-channel head coils on 1.5T scanners. The MRI scans used in this study are T1-weighted, high resolution structural sequences.

**MRI preprocessing**

The Minc-toolkit software program, created by the Brain Imaging Centre of the Montreal Neurological Institute (https://bic-mni.github.io), was used for all preprocessing operations on the raw MRI images.

Data in Nifti format was downloaded from OASIS-3. First, the NifTI format files were converted into Minc format, which is compatible with the Minc-toolkit, and then the denoising and intensity non-uniformity correction processes (Coupe et al., 2008)) were performed (Sled et al., 1998). The brain images were registered to the Montreal Neurological Institute Standard Space template (MN152) in order to account for variations in brain size and shape (Collins et al., 1994). The area of
interest's absolute volume from various brains is then comparable. After that, the quality of the preprocessed images was checked visually, and the poor-quality images were discarded.

**Volumetric assessment of the hippocampus (HC) and Cerebrospinal Fluid (CSF)**

The Minc-toolkit software DISPLAY was used to view images of the brain. The qualified volumetric assessment is based on decent HC and CSF segmentations, and both the segmentation and the volumetric assessment were carried out in DISPLAY. Five brains (2/1/2 from the NC/MCI/AD groups, respectively) were used as samples to feed the automatization algorithm developed using Multiple Automatically Generated Templates (MAGeT). At first, a trained expert manually segmented HC and CSF average values in each of the five brains (Pipitone et al., 2014). When given a set of (in the current study, five) labeled MR images (atlases), the MAGeT algorithm semi-automatically segments specific brain regions for each subject, saving a considerable amount of time and labor. Following MAGeT, trained experts implemented quality controls on the automatically segmented brains. The segmentation protocol of HC and CSF was described in detail, and tested with high intra-rater and inter-rater reliability in Schoemaker et al. (2019).

**Calculation of the hippocampus-to-ventricle ratio (HVR)**

The average volumes of HC and CSF were calculated by their voxel amount in Display. According to the hypothesis of Schoemaker et al.(2019), as the hippocampus shrinks, the adjacent ventricle space (which is filled with CSF) expands. An integrity index that takes into account both the structure of the hippocampus and the area around the ventricle is provided by the ratio of the two structures. The HVR is calculated by the formula:

\[ HVR = \frac{HC_{vol}}{(HC_{vol} + CSF_{vol})} \]

HVR = hippocampal-to-ventricle ratio, HCvol = Volume of the HC, CSFvol = Volume of CSF surrounding the HC.

**Age-adjusted HC and HVR**

The average HCvol and HVR were divided by age to create age-adjusted indices, HCvol/age and HVR/age, to account for age.

**Statistical analyses**

One-way ANOVA was carried out to compare the three groups concerning MMSE scores, as well as HCvol, CSFvol and HVR average values. Further, we followed up with post-hoc t-test (Bonferroni corrected) t to check for pairwise differences. Steiger’s Z tests was used to compare
two dependent correlation coefficients with another variable in common.

The Statistical Package for the Social Sciences (SPSS 26.0, IBM Deutschland GmbH, Ehningen, Germany) was used to perform statistical analyses. The Hotelling’s t and Steiger’s Z tests calculator was used to implement Steiger’s Z tests (Weiss, B.A., 2011, Available from https://blogs.gwu.edu/weissba/teaching/calculators/hotellings-t-and-steigers-z-tests/). R 3.6.3 was used to plot the graphs.

3.4. Results

The average means of MMSE scores and basic demographics (age, sex and handness) of three groups are presented in Table 1.

The results of one-way ANOVA showed that the differences of MMSE scores between the three groups are significant (F(2,241) = 110.8, p < .001). The post hoc test (Bonferroni) showed that: the difference between NC and MCI (p = .001), the difference between MCI and AD are significant (p < .001), and the difference between NC and AD are significant (p < .001).

The results of a one-way ANOVA showed that the differences of ages between the three groups are significant (F(2,241) = 18.7, p < .001). The post hoc test (Bonferroni) showed that: the difference between NC and MCI (p = .001), the difference between MCI and AD are significant (p = .001), and the difference between NC and AD are significant (p = .017).

Table 1
The MMSE scores and basic demographics (age, sex and handness) of three groups

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>MCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE (SD)</td>
<td>29.6 (.7)</td>
<td>27.9 (1.8)</td>
<td>24.1 (3.8)</td>
</tr>
<tr>
<td>Age (SD)</td>
<td>67.2 (7.0)</td>
<td>74.1 (7.4)</td>
<td>69.8 (5.3)</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>45/54</td>
<td>27/22</td>
<td>49/47</td>
</tr>
<tr>
<td>Handness (Left/Right)</td>
<td>11/88</td>
<td>7/42</td>
<td>7/89</td>
</tr>
</tbody>
</table>

The average volumes of HCs and average values of HVRs of three groups

The average volumes of the three groups are depicted in Figure 1(a) as left, right, and total (left + right) HCvols. The differences between the average left HCvols in three groups are significant, (F(2,241) = 40.2, p < .001); The differences between the average right HCvols in three groups are
significant, \(F(2,241) = 50.2, \ p < .001\); The differences between the average total HCvols in three groups are significant, \(F(2,241) = 46.5, \ p < .001\). The post hoc test (Bonferroni) showed that: the difference between the average left HCvols in MCI and AD group is not significant, while all the other results between groups are significant.

The average HVR of the three groups are depicted in Figure 1(b) as left, right, and total (left + right) HVR. The differences between the left HVRs in three groups are significant, \(F(2,241) = 144.5, \ p < .001\); The differences between the right HVRs in three groups are significant, \(F(2,241) = 173.3, \ p < .001\); The differences between the total HVRs in three groups are significant, \(F(2,241) = 171.9, \ p < .001\). The post hoc test (Bonferroni) showed that: all the differences between two groups are significant.

The average volumes of CSFs of three groups

Figure 2 shows the average volumes of left, right and total (left + right) CSFs of three groups respectively. The differences between the average volumes of left CSFs in three groups are significant, \(F(2,241) = 81.1, \ p < .001\); The differences between the average volumes of right CSFs in three groups are significant, \(F(2,241) = 104.8, \ p < .001\); The differences between the average volumes of total CSFs in three groups are significant, \(F(2,241) = 99.9, \ p < .001\). The post hoc test (Bonferroni) showed that: all the differences between two groups are significant.

The average values of HCvol/age and HVR/age of three groups

Figure 3(a) shows the average values of left, right and total HCvol/age of three groups respectively. The differences between the average values of left HCvol/age in three groups are significant, \(F(2,241) = 41.8, \ p < .001\); The differences between the average values of right HCvol/age in three groups are significant, \(F(2,241) = 48.3, \ p < .001\); The differences between the average values of total HCvol/age in three groups are significant, \(F(2,241) = 45.9, \ p < .001\). The post hoc test (Bonferroni) showed that: the differences between values of HCvol/age in MCI and AD group is not significant in both left and right brains, while all the other results between two groups are significant.

Figure 3(b) shows the average values of left, right and total HVR/age of three groups respectively. The differences between the average values of left HVR/age in three groups are significant, \(F(2,241) = 95.3, \ p < .001\); The differences between the average values of right HVR/age in three groups are significant, \(F(2,241) = 102.3, \ p < .001\); The differences between the average values of total HVR/age in three groups are significant, \(F(2,241) = 102.2, \ p < .001\). The post hoc test
(Bonferroni) showed that: only the difference between value of HVR/age in MCI and AD group is not significant in left brain, while all the other results between two groups are significant.

Figure 1. The average volumes (left, right, and total) of HCs (a) and average values of HVRs (b) and in NC, MCI and AD groups. LHC = left HC; RHC = right HC; TotalHC = total HC. LHVR = left HVR; RHVR = right HVR; TotalHVR = total HVR. Note: **p < 0.01, NS = Not significant.

Figure 2. The average volumes (left, right, and total) of CSFs in NC, MCI and AD groups. LCSF = left CSF; RCSF = right CSF; TotalCSF = total CSF. Note: **p < 0.01
Figure 3. The average values (left, right, and total) of HCvol/age (a) and HVR/age (b) in NC, MCI and AD groups. LHC/age = left HCvol/age; RHC/age = right HCvol/age; TotalHC/age = total HCvol/age. LHVR/age = left HVR/age; RHVR/age = right HVR/age; TotalHVR/age = total HVR/age. Note: *p < 0.05, **p < 0.01, NS = Not significant.

Correlations between age/MSSE and the volumetric measures (HCvol, CSF, and HVR)

All volumetric evaluations were significantly correlated with age and MMSE scores in the correlation analysis (Table 2). Although all of the correlations between age or MMSE and volumetric measures are significant, HVR and age correlations are notably greater than HCvol and age correlations and CSFvol and age correlations (Steiger’s Z tests, p = .011), while HVR and MMSE correlations are significantly better than HCvol and MMSE correlations and CSFvol and MMSE correlations (Steiger’s Z tests, p = .001), respectively.

Comparisons of HCvol, CSFvol and HVR between left and right hippocampus.

The results of a paired-samples t test showed that: right hippocampal volume is significantly larger than left one ( t = -11.2, p < .001); right CSF volume is significantly smaller than left one ( t = 5.7, p < .001); right HVR is significantly bigger than left HVR ( t = -8.9, p < .001)

Table 2

<table>
<thead>
<tr>
<th>Left</th>
<th>Right</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCvol</td>
<td>CSFvol</td>
<td>HVR</td>
</tr>
<tr>
<td>(3617)</td>
<td>(2377)</td>
<td>(.608)</td>
</tr>
<tr>
<td>Age</td>
<td>-.329**</td>
<td>.390**</td>
</tr>
</tbody>
</table>
3.5. **Discussion**

When compared to pure hippocampal volume, HVR, which includes information about the CSF surrounding the hippocampus, is a more comprehensive biomarker for predicting AD, likely compensating for the large individual variation in the population. However, Schoemker's study only compared the relationship between age/(memory performance) and HCvol as well as the relationship between age/(memory performance) and HVR in healthy participants, providing preliminary evidence that HVR is superior to HCvol. As a cross-sectional study, it is insufficiently convincing without MCI and AD patients to support Schoemaker's findings as a generalization. A new promising biomarker also needs to be validated in follow-up studies using data from various databases. The current study mainly confirmed the validity and superiority of HVR over pure HCvol.

The HVRs and HCvols in the three groups (NC, MCI, and AD) were contrasted in the current study. The performance of HCvol was not bad - the differences between three groups were differentiated by HCvol except the difference of left HCvol between MCI and AD group, but all the group differences were differentiated well by HVR. Additionally, the F value for groups differing in an ANOVA using HCvol is approximately 45, whereas the F value for groups differing in an ANOVA using HVR is approximately 170. Although not a conclusive proof, this difference could help to imply the higher sensitivity of HVR. It is not surprising that the HCvols are significantly different in the three groups given that the current study is a cross-sectional one with clear group classification and that the large group differences between healthy individuals, those with MCI, and AD patients could easily cover the individual variation in participants. Numerous studies have revealed a link between HCvol and the severity of AD process development that is negative (Chupin et al., 2009; Hill et al., 2014; Jack et al., 1999; Tabatabaei-Jafari et al., 2020). However, the ultimate purpose of introducing HVR is to apply it into clinical prediction of MCI and AD, which has no clear grouping and naturally requires higher level of sensitivity. In this instance, using HVR as the neuroimaging biomarker with higher sensitivity will have a more pronounced benefit on MCI and AD prediction.

In addition to pathological degeneration, aging is a major factor contributing to the atrophy of the hippocampus. In light of this, age was included in the statistics. After taking age into account, we
discovered that the HCvols (either left side or right side, or total) of MCI group and AD group have no significant difference, while only the left HVRs of MCI group and AD group have no significant difference but the right and total HVRs of that have significant differences. Previous studies found the loss of hippocampus starts from left side, then the right side (Rahman et al., 2016), which is probably an important reason causing bigger right hippocampus than left. The current study confirmed the findings of a previous study (Pedraza et al., 2004a) that the left CSF volume is significantly larger than the right one. We can imagine that, in MCI phase, the degree of left hippocampus degeneration is already similar with the degree in AD phase due to its early development, while the degree of right hippocampus degeneration is reasonably better than the degree in AD due to its later development. This is probably the reason why the left HVR of AD group is not significantly different with that of MCI group but the right HVR of AD group is significantly smaller than that of MCI when taking age into account. When we use the HCvol as an index, this right side difference was not distinguished. From this, we could see the advantage of higher sensitivity of HVR that differentiated the right side difference between MCI group and AD group well, which is also consistent with our hypothesis.

3.6. Conclusion

In current study, we validated the reliability of HVR using participants from the OASIS-3 database, confirming that HVR is more sensitive than pure hippocampal volume to detect AD. After taking age into account, we discovered that while both hippocampal volumes cannot distinguish between the MCI group and the AD group, the right HVR can, demonstrating the benefit of its higher sensitivity. We also discovered through correlation analysis that there is a much stronger correlation between HVR and age or cognitive function than that between hippocampal volume and either of these variables. In general, we further confirmed that HVR is a promising index with better sensitivity than pure hippocampal volume on indicating hippocampal integrity, though the final validity of HVR on predicting MCI and AD should be confirmed in follow-up longitudinal studies.
Part IV

Study 3: a longitudinal study showing the predictive function of HVR for MCI and AD
4. Article 3: Hippocampal-to-ventricle (HVR) predicts mild cognitive impairment (MCI) and Alzheimer’s disease (AD) in cognitively normal baseline

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¹Department of Psychology, Neuropsychology, University of Constance, Constance, 78464, Germany
4.1. Abstract

Hippocampus-to-ventricle-ratio (HVR) appears to be a promising neuroimaging biomarker of Alzheimer’s disease (AD) that overcomes some disadvantages of pure hippocampal volume. This article contributed a preliminary validation of the predictive efficacy of \( \alpha \) (the visualization of HVR) in a longitudinal study. 174 healthy people (mean age= 70.36 years, SD = 7.98, range: 51.3 to 92.4 years) were selected from OASIS-3. Over the course of OASIS-3, 93 subjects stayed healthy; 38 subjects developed mild cognitive impairment (MCI); and 43 subjects developed AD. We showed that the initial \( \alpha \) of AD group is significantly greater than that of MCI group, and the initial \( \alpha \) of MCI group is significantly greater than that of healthy group. We also found that \( \alpha \) of left hemisphere is larger than that of right hemisphere, and that \( \alpha \) is larger in males than in females. For the MCI and AD patients, we showed that their brain degeneration accelerates from the preclinical stage. The key finding of this study is that \( \alpha \) was proved to be a decent neuroimaging biomarker for predicting MCI and AD, although further quantitative research is required.

Key words: Alzheimer's Disease (AD); mild cognitive impairment (MCI); Hippocampus; Predict, Structural Magnetic Resonance Imaging (MRI)
4.2. Introduction

Alzheimer’s disease (AD) is a progressive neurological disorder that causes cognitive decline and memory loss. The harm caused by AD extends beyond the individuals and have great impact on their family, caregiver and the whole society. To date, the process of AD appears irreversible and the symptoms of AD usually emerge a long period after the pathology starts (Bateman et al., 2012; Gordon et al., 2018; Reiman et al., 2012; Villemagne et al., 2013). Given the variety of causes of dementia, the only conclusive way of diagnosing AD is through autopsy of brain tissue to determine the neuropathology (S. Khan et al., 2020), which is unfortunately too late for treatment. Therefore, the optimum way is to use some biomarkers to detect real AD at early stage as far as possible, allowing for timely intervention in the preclinical stage of the disease. According to a mitochondrial cascade hypothesis, AD neuropathologies such as Aβ and tau begin with a cellular phase involving mitochondrial dysfunction, and the neurodegeneration may begin sooner than previously assumed (Weidling & Swerdlow, 2020). Enhancing the diagnostic accuracy and bringing forward the diagnostic timing of AD biomarkers are required.

According to the ATN classification system, the diagnostic biomarkers of AD were categorized into A (amyloid), T (p-tau) and N (neurodegeneration) by the biological process (Jack et al., 2018). Low CSF Aβ_{42} and CSF P-tau are typically regarded as biomarkers of a pathological state of AD; PET imaging likely indicates the cumulative impairment of neurons and the magnitude of neurodegeneration, but the invasiveness of lumbar puncture and high cost of PET hinder their implementations in preliminary screening. In addition, neither amyloid PET nor tau PET has a detectable threshold in vivo, meaning that Aβ or P-tau that is below the threshold can not be present (Jack et al., 2018). Hippocampal volume (HV) estimated from structural MRI could be a better biomarker for routine examination of potential AD patients, given its acceptable cost and noninvasiveness. Multiple studies have demonstrated that a smaller HV is positively correlated with poorer cognitive performance and increased risk of developing AD (Apostolova et al., 2006; Fang et al., 2019; Tabatabaei-Jafari et al., 2020). Structural MRI biomarkers present clear anatomical features in brain, and the longitudinal measure of MR images could present the dynamic change of brain structure. Now that an increasing number of individuals desire to know their brain status in order to maintain and improve their brain health, sensitive biomarkers to indicate the brain health state and AD diagnosis are urgently needed (Scheltens et al., 2021). Unfortunately, relatively sensitive biomarkers such as CSF markers and PET markers have their inherent weaknesses, the existing MRI biomarkers are not sensitive enough for this purpose (Hu et al., 2023). Consequently, it
is necessary to increase the sensitivity of MRI biomarkers.

Hippocampal atrophy (HA) is caused by both aging and pathology, and is the most robust MRI marker during the prodromal phase of AD (Frisoni et al., 2008; Jack et al., 2010; Risacher et al., 2009). As a dynamic index, HA includes two components: a decrease of HV and an enlargement of the surrounding ventricle. In comparison to the static index – HV, HA, which reflects the change of hippocampal integrity, is more informative. However, measuring atrophy requires at least two separated by a long interval. Repetitive measurements lasting months or years are common in scientific research, but are impractical in clinical practice. Moreover, the time lag between two measures may delay the timely treatment of potential AD patients. In light of this, we are attempting to develop a new index that can reflect both atrophy and does not require repeated measurements. Given the decrease of HV and the expansion of surrounding ventricle are two opposing processes occurring at the same time, it would be useful to combine the ratio of HV to surrounding ventricle into a comprehensive index – hippocampus-to-ventricle-ratio (HVR). There is currently a scarcity of research on HVR, with Schoemaker (2019) indicating that the correlation between HVR and cognitive function/age is significantly higher than the correlation between HV and cognitive function/age. Previously, Bartos (2019) discovered the ratio of hippocampus and lateral ventricle distinguishes AD patients and health control participants better than pure HV, demonstrating the superiority of relative ratio over pure volume. However, the two both are cross-sectional studies, which indicated that ratio performed better but did not reflect its predictive effect. What is really required in clinical practice is prediction, which should be supported by a longitudinal study.

In this study, longitudinal data from Open Access Series of Imaging Studies – 3 (OASIS-3) were used to validate the prediction of HVR. In addition, α, the visualization of HVR, was introduced in this study. Our colleague Hartmann proposed it in an article that is still in revision. HVR can be represented by angle α of a trigonometric function with HC volume being the adjacent and CSF volume being the opponent to α (figure 1). α will increase with both aging and pathology. Larger α implies older age or severer pathology. Age is known, hence we can infer the severity of AD pathology based on this logic. Our first hypothesis is that α could predicts the future incidence of AD in the preclinical stage. The second hypothesis is that α increases faster in AD patients than in health controls even at the asymptomatic stage.
4.3. Methods

Subjects

The data was collected from the OASIS-3 database (oasis-brains.org). OASIS-3 is a compilation of MRI, PET imaging and related clinical data for 1098 participants from several ongoing studies in the Washington University Knight Alzheimer Disease Research Center over a 15-year period. Participants range in age from 42 to 95 years and include 605 cognitively normal adults and 493 adults in various stages of cognitive decline. The OASIS-3 dataset contains over 2000 MR sessions, including multiple structural and functional sequences. Compared with other available Alzheimer databases such as ADNI, which only enrolled participants with dementia or MCI, OASIS-3 is the initial enrollment focused on preclinical cohort with longitudinal progression. (LaMontagne et al., 2019). Participants were recruited from the community through flyers, word of mouth and community organizations. For the current study, only the OASIS-3 participants who were initially diagnosed as healthy control were included. Exclusion criteria for the current study were: 1) severe trauma on head; 2) active alcoholism or drug abuse; 3) active hypothyroidism; and 4) Parkinson’s or any other major psychiatric disorder. Finally, 174 subjects (mean age = 70.36 years [SD=7.98], range: 51.3 to 92.4 years) who met the inclusion criteria were chosen. There are 80 male subjects.

Over the course of OASIS-3, 93 subjects (mean age = 67.25 years [SD=7.0], range: 51.9 to 85.3 years) stayed healthy; 38 subjects (mean age = 71.54 years [SD=8.14], range: 51.3 to 86.3 years) developed into MCI; and 43 subjects (mean age = 76.03 years [SD=6.4], range: 64.5 to 92.4 years) were diagnosed with AD.
Classification of Normal control (NC), Mild Cognitive Impairment (MCI) and Alzheimer’s Disease (AD)

Over the long course of OASIS-3, the 174 initially healthy subjects developed into 3 groups: NC, MCI, and AD.

Only those who participated the course at least 84 months and stayed healthy were included in HC to ensure we chose “real” healthy control subjects who would not develop MCI or AD in a short time span. NC subjects must not have any cognitive impairment in their daily lives.

For the classification of MCI, the subjects did not meet the criteria of AD but at least demonstrated impairments on memory function, while some also demonstrated impairment on other cognitive function including language, attention, executive function and visuospatial function.

For the classification of AD, the subjects should meet the revised criteria of NINCDS-ADRDA (McKhann et al., 2011) for probable AD.

MR Image Acquisition

MRI scans were carried out by the Knight Alzheimer Research Imaging Program at Washington University in St. Louis. Structural images were obtained using 3 different Siemens scanner models (Siemens Medical Solutions USA, Inc): Vision 1.5T, TIM Trio 3T (2 different models), and BioGraph mMR PET-MR 3T. Participants wore a 16-channel head coil on 1.5T scanners and a 20-channel head coil on 3T scanners with foam pad stabilizers placed next to their ears to reduce motion (LaMontagne et al., 2019). The MRI scans used in this study are high resolution structural T1-weighted images.

Each subject in the current study had two MR images taken– the first at the beginning of OASIS-3 process, and the second months (mean = 60.5 months [SD=26.6], range from 13.1 to 126.5 months) later.

MRI preprocessing

The Minc-toolkit package developed by the Brain Imaging Centre of the Montreal Neurological Institute (https://bic-mni.github.io/) was used to preprocess the MRI images.

Firstly, the raw MRI data downloaded from OASIS-3 were converted from NifTI format to Minc format compatible with the Minc-toolkit, then denoised (Coupe et al., 2008) and corrected for intensity non-uniformity (Sled et al., 1998). The brain images were then registered to the Montreal Neurological Institute Standard Space template (MN152) to control for variations in brain size and
shape (Collins et al., 1994). Finally, defective images were discarded after a visual inspection to ensure the quality of the preprocessed images.

**Volumetric assessment of the hippocampus (HC) and ventricle**

The segmentations of HC and ventricle as well as the volumetric assessments were carried out in DISPLAY which is a visual tool of Minc-toolkit. Firstly, a trained expert manually segmented HC and surrounding ventricles of 5 brain images that were used as templates to feed the automatization algorithm of Multiple Automatically Generated Templates (MAGeT) (Pipitone et al., 2014). After being given a set of (odd number, in current study is 5) labeled MR images (atlases), MAGeT was used to carry out automatic segmentations of the labeled brain areas for all the subjects, saving large amount of labor and time. Quality controls of the results of MAGeT were implemented by trained experts. The segmentation protocol of HC and surrounding ventricle was described in Schoemaker et al. (2019) and was confirmed with high intra-rater and inter-rater reliability.

**Calculation of the hippocampus-to-ventricle ratio (HVR) and HVR α**

To compute HVR alpha, trigonometric rules were applied. Alpha is calculated by the arctangent of CSF volume divided by HC volume (equation 1). This was done separately for the left and right hemisphere as well as the combined hemispheres for each subject.

\[ \alpha = \arctan \left( \frac{\text{OPP}}{\text{ADJ}} \right) = \arctan \left( \frac{\text{CSFvol}}{\text{HCvol}} \right) \]

*Equation 1.1* Calculation of alpha. \( \alpha = \) alpha, OPP = opponent, ADJ = adjacent, HCvol = HC volume, CSFvol = CSF volume.

**Age-adjusted HVR α**

To take age into account, the average HVR α was divided by age to yield an age-adjusted index - \( \alpha_{age} \).

**Statistical analyses**

JASP (Version 0.16.4) (JASP Team, 2022) was used for the statistical analysis. To compare the differences of α between groups and genders, three-way mixed ANOVA was carried out, with groups and sex as between-subjects factors and hemisphere (left and right) as the within-subjects factor. If there are significant differences of them, the predictive function of α is confirmed. To compare the changes of α between groups with time, three-way mixed ANOVA was carried out, with groups as the between-subjects factor and hemisphere (left and right) and time points (time 1
and time 2) as the within-subjects factors. To compare the increasing rate of α between groups, another three-way mixed ANOVA was carried out, with groups and sex as the between-subjects factors and hemisphere (left and right) as the within-subjects factor. We then used a post-hoc t-test (Bonferroni corrected) to look for pairwise differences. The graphs were plotted with R 4.2.2.

### 4.4. Results

**Descriptive statistics of age and volumetric data for three diagnostic groups respectively.**

Table 1 shows the means and standard deviations of age, HC volume, CSF volume and α_age, and rate of monthly increased α in three diagnostic groups.

Table 1. *Means and standard deviations (in brackets) of age, HC volume, CSF volume, α_age and monthly increased rate of α*

<table>
<thead>
<tr>
<th></th>
<th>NC(N=93)</th>
<th>MCI(n=38)</th>
<th>AD(n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time 1</td>
<td>Time 2</td>
<td>Time 1</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>67.2(7.0)</td>
<td>73.2(7.1)</td>
<td>71.5(8.1)</td>
</tr>
<tr>
<td><strong>HCvol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>4151.5(64)</td>
<td>3688.0(54)</td>
<td>3805.9(6)</td>
</tr>
<tr>
<td>Right</td>
<td>4454.7(58)</td>
<td>3942.3(56)</td>
<td>4102.9(6)</td>
</tr>
<tr>
<td><strong>CSFvol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>1596.1(46)</td>
<td>2610.0(64)</td>
<td>2001.6(8)</td>
</tr>
<tr>
<td>Right</td>
<td>1428.3(38)</td>
<td>2394.7(65)</td>
<td>1723.9(5)</td>
</tr>
<tr>
<td><strong>α_age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>5.5(1.6)</td>
<td>8.4(1.7)</td>
<td>6.7(2.2)</td>
</tr>
<tr>
<td>Right</td>
<td>4.7(1.3)</td>
<td>7.4(1.7)</td>
<td>5.6(1.9)</td>
</tr>
<tr>
<td>Rate of α increase( monthly)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>3.7(2.2)</td>
<td>5.0(3.6)</td>
<td>5.5(2.1)</td>
</tr>
<tr>
<td>Right</td>
<td>3.6(2.1)</td>
<td>4.9(3.2)</td>
<td>5.7(2.4)</td>
</tr>
</tbody>
</table>

Note. Volumes in mm³, α_age=1000 *α/age, Rate of α increase(monthly) = 1000 *α/(number of months from time 1 to time 2)

**Male and female α_age differences between groups and both hemispheres at time 1.**

The main effect of group on α_age is significant, F(2,168)=37.35, p<.001 with ηp² = 0.308, while the Post hoc test results showed that the α_age of AD group is significantly greater than that of the
MCI group, \( p < 0.001 \) with Cohen’s \( d = 0.91 \), and the \( \alpha \_age \) of MCI group is significantly greater than that of NC group, \( p = 0.005 \) with Cohen’s \( d = 0.57 \). The \( \alpha \_age \) of male is significantly greater than the \( \alpha \_age \) of female, \( F(1,168) = 19.64, p < .001 \) with \( \eta^2 = 0.105 \); the \( \alpha \_age \) of left hemisphere is significantly greater than the \( \alpha \_age \) of right hemisphere, \( F(1,168) = 81.38, p < .001 \) with \( \eta^2 = 0.326 \).

There is no significant interaction effect of \( \alpha \_age \) between group and sex. There is no significant interaction effect of \( \alpha \_age \) between group and hemisphere. There is no significant interaction effect of \( \alpha \_age \) between hemisphere and sex. Figure 2 depicts the \( \alpha \_ages \) at time 1 for three diagnostic groups and both hemispheres in male and female.

![Figure 2. The \( \alpha \_ages \) for three diagnostic groups and both hemisphere in male and female at time 1.](image)

**The \( \alpha \_age \) differences between groups and both hemispheres at time 1 and time 2.**

The main effect of group on \( \alpha \_age \) is significant, \( F(2,171) = 32.19, p < 0.001 \) with \( \eta^2 = 0.172 \), while the results of Post hoc test showed that the \( \alpha \_age \) of AD group is significantly greater than that of MCI group, \( p < 0.001 \) with Cohen’s \( d = 0.87 \), and the \( \alpha \_age \) of MCI group is significantly greater than that of NC group, \( p = 0.026 \) with Cohen’s \( d = 0.45 \). The \( \alpha \_age \) of time 2 is significantly higher than the \( \alpha \_age \) of time 1, \( p < 0.001 \) with \( \eta^2 = 0.847 \). The \( \alpha \_age \) of Left hemisphere is significantly greater than the \( \alpha \_age \) of right hemisphere, \( p < 0.001 \) with \( \eta^2 = 0.315 \). There is significant interaction effect of \( \alpha \_age \) between group and time, \( p = 0.007 \) with \( \eta^2 = 0.057 \), while the post hoc test results shows that the \( \alpha \_age \) of MCI group is significantly greater than the \( \alpha \_age \) of NC group at time 1, \( p = 0.02 \) with Cohen’s \( d = 0.57 \), but there is no significant difference of the \( \alpha \_age \) between MCI and NC group at time 2.
There is no significant interaction effect of α_age between group and hemisphere. There is no significant interaction effect of α_age between time and hemisphere. Figure 3 depicts the α_ages for three diagnostic groups and both hemispheres at time 1 and time 2.

Figure 3. The α_ages for three diagnostic groups and both hemisphere at time 1 and time 2.

Male and female differences of increased rates (monthly, from time 1 to time 2) of α between groups and both hemispheres.

The main effect of group on increased rates of α is significant, F(2,168) = 10.82, p<.001 with ηp² = 0.11, while the Post hoc test results showed that the increased rates of α of AD group are significantly greater than that of NC group, p < 0.001 with Cohen’s d =0.79, the increased rates of α of MCI group is significantly greater than that of NC group, p = 0.016 with Cohen’s d = 0.53, while the differences of increased rates of α between MCI and AD group are not significant. There is no significant difference in the increased rates of α between left and right hemisphere. There is no significant difference in the increased rates of α between men and women.

There is no significant interaction effect of increased rates of α between group and sex. There is no significant interaction effect of increased rates of α between group and hemisphere. There is no significant interaction effect of increased rates of α between hemisphere and sex. Figure 4 shows the increased rate of α for three diagnostic groups and both hemisphere in male and female.
4.5. Discussion

This is the first longitudinal study to predict the occurrence of MCI and AD using HVR α. At the onset of the study, all of the participants were in good health. After a period of time, the participants developed into three groups: staying health, MCI, AD. Based on the theoretical foundation of α, we hypothesized that participants with high α are prone to develop AD, participants with second-highest α are prone to develop MCI, and participants with low α are more likely to stay health. Given the prominent effect of aging on the atrophy of hippocampus (Bakhtiari et al., 2023; Boldrini et al., 2018; Fotuhi et al., 2012), we included age in the HVR α calculation in this study. Those participants who later developed AD had the highest initial α, followed by those who later developed MCI, and those who stayed healthy had the lowest initial α. The results confirmed our hypothesis, meaning that a high α indicates a high chance to develop AD, low α indicates a high chance to stay healthy, and MCI lies in the middle. According to the findings of current study, a α_age that is larger than 7.5 predicts a high probability of converting to AD in future, a α_age that is around 6 roughly indicates a high probability of converting to MCI in future, and a α_age less than 5 normally forecasts a healthy future.

Although a good deal of studies have shown that accelerated HA indicates a high risk of conversion from MCI to AD (Henneman et al., 2009; Risacher et al., 2009; Vemuri et al., 2009), and that combining HC and HA the two indices together obviously improves the predictive effect of AD in the preclinical stage (McRae-McKee et al., 2019), there is a lack of studies investigating HV on the straightforward prediction of AD incidence in the future. The International Working Group (IWG)
and the US National Institute on Aging–Alzheimer’s Association also reported that HV is not sensitive and specific enough for AD diagnosis (Dubois et al., 2014), however repeated measures of HV are reliable (Giedd et al., 1995). HVR $\alpha$ incorporates the combined information of HV and surrounded ventricle, which reduces inter-individual variation in HV across subjects and provides more information about volume change (Schoemaker et al., 2019). Given the superiorities of $\alpha$, we employed longitudinal data from OASIS-3 in this study to confirm the predictive effect of $\alpha$, and obtained a set of primary quantitative data.

Apart from the primary finding of $\alpha$ prediction on AD, current study also includes secondary and tertiary findings. We found that the $\alpha$ of left hemisphere is greater than the right side, implying that the right hemisphere has a bigger HV and smaller surrounding ventricle compared with the left side, which is consistent with earlier studies (Maller et al., 2007; Pedraza et al., 2004b; Ystad et al., 2009). It appears that the left hippocampus atrophies earlier than the right hippocampus. The right hippocampus is prominent on visuospatial memory and spacial orientation, whereas the left hippocampus is prominent on episodic memory especially content-related memory and autobiographical memory (Burgess et al., 2002; Ezzati et al., 2016; Kühn & Gallinat, 2014). The typical symptoms of AD begin with episodic memory dysfunction, and progress to other cognitive regions, while spatial memory dysfunction, such as disorientation, begins later (Balasa et al., 2011; Stopford et al., 2008). It remains to be seen whether the above description of structure and function of hippocampus is a consequence or a cause of the pre-atrophy of the left brain, or an interaction of structure and function. In addition, a rodent study revealed that chronic stress affects the left hippocampus significantly earlier than the right hippocampus (Rahman et al., 2016), which may also account for the larger left $\alpha$.

The value of $\alpha$ increases with aging. We compared the $\alpha$ growth rate of three groups, and found that the $\alpha$ growth rates of AD and MCI group were significantly faster than those of NC group, while the $\alpha$ growth rate of AD group was numerically higher than that of MCI group, but this difference did not reach statistical significance. In accordance, previous studies have discovered a quicker rate of hippocampal loss in AD and MCI patients than in healthy controls (Fang et al., 2019; Fleisher et al., 2008; Schuff et al., 2009). Although current study began with healthy participants, the results are still consistent with previous findings, which indicate an accelerated degeneration in AD and MCI patients even from the early preclinical stage. As to why it was not statistically significant between the AD and MCI groups, we speculate that due to the preponderance of elderly subjects in the current study, it may have been too late to detect a difference in the rate of structural change between MCI and AD. This may require testing with younger subjects to confirm.
In this study, $\alpha$ was found to be higher in males than in females, which does not appear to be consistent with the higher risk of AD incidence in females in previous studies (Kukull et al., 2002; Nebel et al., 2018; Niu et al., 2017). But actually age is the greatest risk factor of AD (Chêne et al., 2015; Zhu et al., 2021), and the fact that women live longer than men may be an important reason. The inclusion of age in the calculation in this study, which excluded the influence of longevity, may be an important reason for the absence of a higher female risk. Moreover, a previous study showed women have an innate language advantage in verbal memory despite showing moderate HA. This advantage may facilitate a cognitive reserve in females (Sundermann et al., 2016). In accordance, two studies from Mayo clinic showed a higher incidence rate of MCI in male (Petersen et al., 2010; Roberts et al., 2012). A previous study showed that HA rate is slower in women (Christova & Georgopoulos, 2023), which is consistent with the results of this study. These studies listed above support the lower $\alpha$ of women in current study and provide a source of explanation for the results.

There are several limitations of current study. First, the time span from NC to MCI or AD varied a lot among individuals, and this study simply classified them as converted or not, without taking into account how long it took to convert, which can affect the accuracy of the results. Second, although this study provided primary quantitative results, the classification threshold for the three groups is not precise enough. This study did not directly predict the likelihood of conversion to MCI or AD by $\alpha$, which limited the applicability of this study.

Overall, this is the first study to employ HVR $\alpha$ to predict MCI and AD. $\alpha$ offers its inherent benefits as a visual update of hippocampal integrity. The key contribution of this study is the preliminary confirmation of the predictive effect of $\alpha$ on MCI and AD, which is a step forward in improving neuroimaging biomarkers, particularly in reducing individual differences and enhancing accuracy. Future studies could concentrate on resolving the problem of large variation in conversion time among individuals using appropriate methods, whether large samples or statistics, thereby enabling the direct quantification of the incidence of MCI and AD.
Part V

Overall Discussion
5. Overall Discussion

Since AD is still a disease that can not be cured currently, early detection, diagnosis and intervention to the currently possible extent are the best strategies to cope with it. Among the available diagnostic biomarkers of AD, structural MRI has the advantages of noninvasiveness, cost-effectiveness, and accessibility. The medial temporal lobe (MTL) is the earliest strongly affected area of the brain in AD (Braak & Braak, 1991; Hyman et al., 1984), including hippocampus, parahippocampal cortex, entorhinal cortex, perirhinal cortex, and amygdala. Currently, hippocampus volume is mostly used as a brain imaging marker for AD because of its relatively clear anatomic delineation that is essential for segmentation and volume measurement. In fact, apart from the hippocampus, other brain structures within the MTL, as well as some brain structures outside of the MTL, also are progressively implicated in the development of AD. In the introduction, I mainly described in detail the relationship between the hippocampus and cognitive function as well as the relationship with AD and MCI. Here, I plan to describe other brain structures that are affected by AD, aiming to explore the effects of AD on related brain structures (inside and outside MTL) in a widely spectrum.

5.1. The brain structures within MTL

**Entorhinal cortex (EC)**

The Entorhinal cortex is a structure in the MTL connecting the hippocampus to the neocortex which is responsible for higher-order cognitive functions. The EC is further divided into two subregions including the medial entorhinal cortex (MEC) and the lateral entorhinal cortex (LEC). While the MEC is mainly responsible for calculating and transferring spatial information to the hippocampus, the LEC is mainly in charge of information related to objects and the environment surrounding the objects (Eichenbaum et al., 2012; Sasaki et al., 2015). In MEC, specialized neurons called grid cells have been identified. These cells are involved in spatial processing and play an essential role in the representation of the spatial environment of the brain (Hafting et al., 2005; Jacobs et al., 2013). The EC, hippocampus, perirhinal cortex and parahippocampal cortex together form the so-called "MTL memory system" (Nadel & Hardt, 2011; Pini et al., 2016). The EC has a total of 6 layers, 2-3 of which contain the perforant pathway, an important structure that transmits information from the neocortex to the hippocampus (Hyman et al., 1986). Sensory and associative information from different brain regions is filtered through the EC before it reaches the hippocampus. The EC helps to decide which information is relevant for long-term storage in the hippocampus and which
information can be discarded, as well as guide the consolidation of memories, playing an important role in transferring information from the short-term to the long-term memory. EC is also involved in the process of pattern separation (Poli et al., 2018), which ensures that similar memories are stored in different representations, avoiding interference between memories.

Results from post-mortem studies suggested that the EC is the earliest brain structure in which AD pathology begins. Tau protein deposits have been found in the EC in the preclinical period (Braak & Del Tredici, 2015), especially in the layer 2 of EC tissue (Braak & Braak, 1991, 1992). A number of studies have found that Aβ and tau proteins in the EC are transported to the hippocampus via synaptic connections (de Calignon et al., 2012; Harris & Milton, 2010; Lee et al., 2022). Accordingly, NFTs aggregate early in the EC of AD patients (Braak & Braak, 1995; Hyman et al., 1984; Price et al., 2001; Van Hoesen et al., 1991). The formation of NFTs in the EC leads to apoptosis of neuronal cells, with the severest cellular decline in the layer 2 of the EC (Gómez-Isla et al., 1996; Van Hoesen et al., 1991). The effects of AD on the medial and lateral parts of the EC are not synchronized. A fMRI study found that it is the LEC that is predominantly affected in the EC during the preclinical stage of AD (U. A. Khan et al., 2014). Many studies have found the atrophy of the EC is indicative of the developmental course of AD, and is strongly associated with declines in cognitive functions, including memory and language (Du et al., 2003; Li et al., 2012; Velayudhan et al., 2013). Interestingly, although the AD pathology in EC starts earlier than that in hippocampus, the hippocampus is more widely used as a structural MRI marker than EC due to its clear boundary for segmentation, whereas the EC usually has an ambiguous anatomic boundary, which poses challenges for volumetric analysis. Although the segmentation of the EC and the accuracy of its volume measurements are still somewhat challenging, a number of studies have found that EC volume decreases with AD progression, and the degree of EC volume reduction in individuals with MCI lies between that of NC and AD (Pennanen et al., 2004; Stoub et al., 2006; Velayudhan et al., 2013; Xu et al., 2000)

Overall, the EC is an essential structure in the "MTL memory system" that is responsible for transmitting information between the neocortex and hippocampus. It is also the earliest structure where the pathogenesis of AD starts, and its severity of atrophy is related to cognitive function and the progression of AD. However, it is not as widely discussed as the hippocampus as an MRI marker due to its ambiguous edges in structural MRI.

**Parahippocampal cortex**

The parahippocampal cortex (PHC) is another brain structure in “MTL memory system”, which is
located in the posterior subregion of MTL, inferior to the hippocampus, and posterior to the EC (Raslau et al., 2015). The PHC comprises anterior and posterior regions, where the anterior regions mainly functionally connect with parietal cortex and retrosplenial cortex, and the posterior regions mainly create functional connection with visual areas (Baldassano et al., 2013). The PHC has a wide range of projections in the MTL, including information transferring with the perirhinal cortex, the temporal pole, and with the PHC itself (Aminoff et al., 2013). On the one hand, the PHC provides most of the information transferring into the EC, which is then transferred to the hippocampus. On the other hand, PHC also interacts with amygdala as well as the CA1 area and presubiculum of the hippocampus for direct information exchange (Blatt et al., 2003). Outside MTL, the PHC has close functional connections with the insula and the medial prefrontal cortex, orbitofrontal cortex, and dorsolateral prefrontal cortex (Suzuki, 2009). The strong connectivity of PHC allows it to involve in many cognitive functions. Generally there are two main categories of cognitive functions in which the PHC is involved: episodic memory and visuospatial processing. Previous studies have found that the PHC is involved in those cognitive activities including recollection and source memory (Davachi et al., 2003; Diana et al., 2007), spatial representation (Park et al., 2011), navigation (Janzen et al., 2007), and visuospatial processing related to environment (Ekstrom et al., 2003). Apart of being involved in visual processing, it has been found that the PHC is also involved in auditory information (Arnott et al., 2008; Gosselin et al., 2006; Mt et al., 2007), odor stimulation (Cerf-Ducastel & Murphy, 2009; Kjelvik et al., 2012), and emotional stimuli (Gosselin et al., 2006, 2006; Van den Stock et al., 2014). Aminoff et al. (2013) summarized that all stimuli associated with contextual association process is probably to activate the activity of PHC.

In typical AD, accumulations of NFTs begin in the transentorhinal cortex, then progress to the EC and the hippocampus, before they finally transcend into the isocortical region (Braak & Braak, 1991; Kordower et al., 2001). While some studies have found significant atrophy in the PHC of AD patients (Dickson, 2009; S. J. Teipel & Hampel, 2006), others have found no significant atrophy in the PHC of either MCI or AD individuals compared to normal control (NC) individuals, although significant atrophy was found in the perirhinal cortex and EC at the same time (Krumm et al., 2016). It was further hypothesized that early AD does not lead to the cognitive deficits associated with PHC. The extent to which PHC is affected during the course of AD is not fully understood, and how much the corresponding pathogenic sources of AD such as Aβ and tau proteins can affect PHC remains unknown. However, Hrybouski et al. found that age-related declines in functional connectivity occurred predominantly around the PHC, and PHC connectivity was also more
susceptible to the decline of age-related connectivity. Therefore, we speculate that the PHC is a brain structure that is more sensitive to age rather than AD pathology.

Overall, as a structure in the "MTL memory system", PHC is responsible for information processing in situational memory and processing, although it is not typically used as a MRI biomarker for AD.

**Perirhinal cortex (PRC)**

PRC is located in the anterior part of MTL and lies at the border of ventral visual pathway and MTL. PRC consists of several layers of neurons, and it is divided into two primary subregions known as Brodmann area 35 and Brodmann area 36 (Suzuki & Naya, 2014). Structurally, on the one hand, the PRC is closely connected to its neighboring structures within MTL, including the EC, PHC, amygdala, and hippocampus; on the other hand, the PRC has extensive unimodal and multimodal connections with cortical areas outside the visuospatial MTL (Suzuki & Naya, 2014).

Functionally, the PRC plays a role in a range of higher cognitive functions, while many studies have found that the prominent role of the PRC is in recollection memory (Ranganath & Ritchey, 2012; Suzuki & Amaral, 2003). Recognition memory is usually thought to comprise two important elements: recollection and familiarity. Recollection usually refers to the memory of a specific thing in a particular context, whereas familiarity refers to the degree of awareness of that thing, regardless of the context. A number of studies have found that PRC is primarily involved in the processing of familiarity information (Bowles et al., 2007; Davachi, 2006; Diana et al., 2007; Eichenbaum et al., 2007; Mayes et al., 2007). PRC-based memories are primarily thought to be used to encode information about individual objects, whereas the hippocampus is often responsible for associating this information with contextual information (Davachi, 2006; Staresina & Davachi, 2008). Overall, these studies suggest that the two forms of memory, recollection and familiarity, are influenced by different brain structures, with the hippocampus being primarily responsible for recollections that are closely related to context and associations, whereas the PRC is primarily responsible for familiarity with the information about the object itself. The PRC is essential for encoding, storing, and retrieving information about objects, as well as distinguishing and recognizing different objects, including visual and tactile stimuli. There are also studies that have found an important role of the PRC for declarative memory (Squire et al., 2007; Wixted & Squire, 2010). Apart from memory functions, the PRC is also responsible for some other cognitive functions, especially perception (Bussey & Saksida, 2007; Graham et al., 2010; Shrager et al., 2006). When there is a high degree of similarity between objects, the PRC can function as a perceptual discriminator to distinguish these similar features. Although the PRC is a member of the "MTL memory system", it also functions as a perceptual region for both perception and memory (Murray & Wise, 2012). A review of the PRC...
showed that the PRC is a multifunctional memory area that can transmit and process a variety of information, including familiarity, associative learning, memory, and recollection, as well as synchronizing with the amygdala to regulate information transmitted to the hippocampus related to the emotional salience of the current situation (Suzuki & Naya, 2014).

Although PRC is one of the brain regions early affected by AD pathology, few studies have directly addressed the volume of the PRC as a marker for structural MRI. In the post-mortem study it has been found PRC has dense NFTs and moderate Aβ plaques deposition of the people with preclinical AD (Hof et al., 1992). Degradation of the PRC during the AD progression also affects the function of the hippocampus. The PRC acts as a pathway to transmit information from the neocortex to the hippocampus, which is simultaneously modulated by the emotional salience of the current situation. It has been shown that the volume of the PRC in MCI and AD patients predicts their ability to name living things - there are more similarities to distinguish between living individuals than non-living ones (Kivisaari et al., 2012). In individuals with MCI, the structural integrity of the PRC has been found to significantly affect familiarity-based memory functions (Westerberg et al., 2013; Wolk et al., 2011). Atrophy of the PRC has been associated with both impairment of memory differentiation of unitary representations and individual objects in AD patients, as well as impairment of overall perceptual functioning (Delhaye et al., 2019).

Overall, the PRC plays an important role in recognition and perception, and its atrophy can also significantly affect the cognitive function of AD patients, especially in identifying similarities between objects. However, since structural and functional deterioration of the PRC is not typically indicative of the most typical AD pathology, and delineation of the PRC varies in literature according to different researchers (Yushkevich et al., 2015), so that there is not a uniform segmentation protocol and the PRC volume is seldom utilized as a MRI biomarker of AD.

**Amygdala**

The amygdala is a pair of almond-shaped cluster of nuclei located on each side of the brain within the MTL. It is a crucial part of the brain’s limbic system, involving in processing and regulating emotions, motivation and memory (Hensler, 2006). It is widely accepted that the amygdala is structurally comprised of two main parts: an pre-evolved cortico-medial region, associated with the olfactory system, and a later evolved basolateral region, associated with the neocortex (LeDoux, 2007). The amygdala, as an emotion-centered structure under the cerebral cortex, constantly evaluates and integrates sensory information from the surrounding environment and assigns appropriate emotional values to it (Šimić et al., 2021). Daily experience tells us that events with
strong emotional experiences are usually the most memorable known as emotional memories. A number of studies on animals and humans have shown that the amygdala plays a central role in emotional memory (Babinsky et al., 1993; Cahill & McGaugh, 1998; Mori et al., 1999; Sun et al., 2020), and also plays a key role in fear conditioning as well as in various forms of psychopathological behavior (Dolan, 2002). The amygdala is involved in encoding, consolidation, and retention of memories associated with emotional experiences, both positive and negative (Hamann, 2001). Emotional memories tend to be more vivid and long-lasting compared with other kinds of memories. One of the most well-known functions of the amygdala is its involvement in detecting and responding to threats and potential dangers. Stressful situations can activate the amygdala (McGaugh & Roozendaal, 2002), leading to the release of stress hormones that prepare the body to respond to a perceived threat. This activation of the amygdala is part of the body's "fight or flight" response (Adolphs, 2013; Nader et al., 2000), which is a physiological reaction to stress. The amygdala was confirmed as the key role to associate with the fear response (Dolan, 2002; Larson et al., 2006; Wang et al., 2005), which acts as an alarm system in the brain, detecting potential threats and preparing for a rapid response. Some mental illnesses including social phobias, panic disorder, generalized anxiety disorder, and PTSD can lead to an overreaction of the amygdala. MRI brain imaging findings in patients with autism and bipolar disorder suggested increased amygdala volume (Li et al., 1998; Ma et al., 2000). Damage of the amygdala can not only cause impairments in fear processing and the recognition of emotional facial expressions (Indovina et al., 2011; Pessoa, 2010; Phelps & LeDoux, 2005), but also have an impact on eating habit changes, behavioral disorders, and emotional deficits (Adolphs et al., 1994; Bechara et al., 1995).

As a member of the "MTL memroy system", the amygdala is one of the early brain regions to become permeated with NFTs, a pathologic hallmark of AD. Previous post-mortem studies have shown that AD causes atrophy of the amygdala due to loss of neuronal cells, accumulation of neurolipid plaques and NFTs, and gliosis in the subnuclei of the amygdala (Scott et al., 1991, 1992). However, previous findings were not always consistent in terms of the relationship between the degree of amygdala atrophy and AD severity. Some studies have found a significantly positive correlation between the degree of atrophy of the amygdala and symptom severity in patients with MCI and AD (Poulin et al., 2011; Yi et al., 2016), while others failed to find a significant correlation (Deweer et al., 1995; Horínek et al., 2006). The inconsistency of these results may be due to inconsistency in symptoms across AD subtypes on the one hand, and limitations of amygdala segmentation methods on the other. The most widely used amygdala segmentation techniques are based on the Watson’s criteria (Watson et al., 1992), which uses an arbitrary measurement to
exclude some parts of amygdala. Some disadvantages of the segmentation of amygdala, including the ambiguous boundaries, the proximity to the hippocampus and the similarity of near tissues, affect the consistency of different research results (Klein-Koerkamp et al., 2014). Correspondingly, the diagnostic effect of amygdala volume studies for AD has varied from study to study. Some studies have not found a diagnostic effect of amygdala volume for AD (Laakso et al., 1995); however, some studies have found a consistent degree of volume loss in the amygdala and hippocampus in early AD, implying that the amygdala may have a similar diagnostic role as the hippocampus (Basso et al., 2006). Of note, Atrophy of the amygdala has been found to be more associated with cognitive decline rather than behavioral changes (Horínek et al., 2006). Furthermore, atrophy of the amygdala can also indicate frontotemporal lobe degeneration. It has been shown that patients with frontotemporal lobe degeneration show significant amygdala atrophy relative to controls (Rabinovici et al., 2007), and the degree of atrophy is greater than that of the amygdala in patients with AD (Möller et al., 2015). A number of studies have compared the symmetry and degree of atrophy of the amygdala between frontotemporal lobe disorders and AD (Bocti et al., 2006; Ridha et al., 2006; Whitwell et al., 2005), and found that the amygdala is more asymmetric and atrophied in frontotemporal lobe disorders. Therefore, asymmetry of the amygdala is also frequently used as a differential diagnosis between frontotemporal lobe disorders and AD. Additionally, the amygdala atrophied at a different pace in EOAD and LOAD. LOAD was found to have significantly more amygdala atrophy than EOAD, all in regions that connect with the limbic system (Cavedo et al., 2014).

Overall, the amygdala is a critical brain structure involved in emotion processing, emotional memory, the fear response, social and emotional learning, and decision-making. The amygdala looks like a better diagnostic tool for frontotemporal lobe degeneration, however the relationship between amygdala atrophy and the neuropsychiatric symptoms of AD has not been fully investigated, and together with some technical limitations inherent to the amygdala segmentation, the amygdala volume is not used as a typical structural MRI marker for AD. Some neuropsychiatric symptoms of AD may not be caused by amygdala damage alone, but more likely due to a broader neurological disorder that encompasses the amygdala (Horínek et al., 2007).

5.2. The brain structures outside MTL

In addition to the brain structures in MTL that have been discussed above including the entorhinal cortex, parahippocampal coetex, perirhinal cortex and amygdala, there are also some structures outside MTL that are associated with AD, such as neocortex, fornix, basal ganglia and thalamus. I
will provide a brief discussion of them next.

**Neocortex**

The neocortex, also known as the cerebral cortex, is the outermost layer of the brain. It is the most highly developed and largest part of the brain involving many high-class cognitive functions. The neocortex consists of two cerebral hemispheres, the left and right halves of the brain, each of which is divided into four main lobes: the frontal, parietal, temporal, and occipital lobes. The accumulation of amyloid plaques, NFTs and neuronal loss results in reduced brain volume, particularly in regions of the neocortex associated with memory and higher cognition. Previous studies have demonstrated that the topological distribution of atrophy in neocortex generally aligns with the stage of the development of NFTs (Braak & Braak, 1991). AD causes atrophy of brain structures first appearing in the MTL, such as the hippocampus, EC and PRC mentioned above, then spreading to the rest of the temporal lobe before going to the parietal lobe, and finally to the frontal lobe (Apostolova et al., 2007; Dickerson et al., 2009; Frisoni et al., 2009; Prestia et al., 2010; Vemuri et al., 2008). Frisoni et al. (2009) demonstrated a vivo mapping on cortical atrophy from early to late AD. In this study, the early stage of AD was structurally characterized with the atrophy of medial temporal and posterior cingulate/retrosplenial cortex, as well as milder atrophy of orbitofrontal cortex. In the stage of mild AD, extensive gray matter loss spread to lateral temporal cortex, dorsal parietal cortex and frontal cortex. In the late stage of AD, the newly atrophied area was not as much as that of the mild AD stage, but still spread to sensorimotor and visual cortex (Frisoni et al., 2009). Speaking of the function impairment, episodic and spacial memory deficit was prominent with the MTL atrophy at early stage; at mild AD stage, cognitive impairments mainly occurred in semantic memories and discriminatory sensory perception; at late AD stage, symptoms of motor deficits started to appear. Normal aging also causes atrophy of the cerebral cortex, but this atrophy is different from the atrophy caused by AD pathology, both in terms of severity and topographic distribution (Bakkour et al., 2013). AD-related loss is much greater than aging-related loss in magnitude (Bakkour et al., 2013; Dickerson et al., 2009). Areas of atrophy associated with both age and AD were predominantly in the parietal angular cortex and dorsolateral prefrontal lobes, but atrophy associated with aging alone was predominantly distributed in the sensorimotor and visual cortices and a small fraction of frontal regions (Bakkour et al., 2013). In addition, the topographic distribution of cortical atrophy differs between EOAD and LOAD. EOAD usually has severe and widespread neocortical atrophy, mainly resulting in parietal-frontal cortex atrophy, diffuse lateral temporal lobe atrophy, and relatively less pronounced MTL atrophy, whereas LOAD is mainly manifested in moderate-to-severe MTL atrophy (Frisoni et al., 2007). And previous studies have
also found that the neocortex atrophies faster in eoad than in load (cho et al., 2013), and that the
threshold for the degree of atrophy is higher when eoad presents with clinical symptoms, reflecting
the stronger cognitive reserve capacity of eoad (pini et al., 2016), although the mechanisms behind
this are not yet fully understood.

**Fornix**

The fornix is a C-shaped bundle of nerve fibers (white matter) in the brain that plays a role in
memory formation and retrieval. Previous studies on animals and humans have shown that the
lesions in the fornix lead to the memory deficits (Tsivilis et al., 2008; Vann et al., 2009). As a
susceptible structure which demonstrated changes in early AD, fornix acts as the major outflow
tract of the hippocampus, connecting the MTL to hypothalamus to allow for the transmission of
information related to memory. Previous study found that PSEN 1 and PSEN 2, the genetic risks for
EOAD, mutation carriers had significantly more reduced fornix fractional anisotropy (FA)
(Ringman et al., 2007), which is measured by diffusion tensor imaging (DTI) to reflect the
structural integrity of nerve fibers. Reduced FA values can be indicative of disruptions in white
matter tracts, which can occur due to neurodegenerative diseases like AD. It has been found fornix
FA was reduced in MCI and early AD patients compared with cognitively normal people (Mielke et
al., 2009). Furthermore, Mielke (2012) found fornix FA and hippocampal volume were both
longitudinally predictive of of memory decline and progression to AD, also, a significant
correlation between fornix FA and hippocampal volume. In a phase I trial study, It was found that
fornix deep brain stimulation produced strong biological benefits for the mild AD patients (Laxton
et al., 2010). Axons are more sensitive than neurons to response to electrode stimulation (Ranck,
1975), which is probably the reason why deep brain stimulation is more effective for fornix than for
hippocampus. Overall, a number of studies have supported that the fornix integrity can not only
serve the diagnostic purpose, for AD, but also can be used as a therapeutic target (Badea et al.,

**Basal Ganglia (BG)**

the basal ganglia (bg) is a group of nuclei located deep within the brain. The basal ganglia is
composed of several structures including: Striatum, globus pallidus, subthalamic nucleus and
substantia nigra, while striatum comprises dorsal striatum (caudate nucleus and putamen) and
ventral striatum (nucleus accumbens and olfactory tubercle). BG plays a critical role in motor
control, including the initiation and coordination of movements, as well as the suppression of
unwanted or involuntary movements (Riva et al., 2018). The BG is also involved in various
cognitive and emotional processes, including decision-making, habit formation, reward processing, and emotional regulation (Stocco et al., 2010; Yahya, 2021). The sub-structures inside BG have their own particular function and some of them are associated with the AD pathology. The caudate nucleus and putamen are involved in sensorimotor function, while the nucleus accumbens is associated with memory and behavior functions (Alexander et al., 1986). Braak and Braak (1990) showed that the caudate nucleus is a region where Aβ and tau protein accumulates in the progression of AD. Madsen et al. (2010) found that caudate volumes were significantly lower in MCI and AD when compared with normal control, while Liu et al. (2010) found the caudate volume is predictive for the conversion from MCI to AD. However, there are also studies that found no significant reduction of the caudate volume in AD (Roh et al., 2011), or even that found slightly bigger caudate nucleus in AD when compared with HC (Tang et al., 2014), although the reason is still not clearly understood. Some previous studies found caudate atrophy was associated with memory and cognitive function (Cho et al., 2014; Yi et al., 2016), while other study failed to find any association between cognitive function and caudate atrophy (de Jong et al., 2008). The putamen was found to be involved in learning and working memory (Bellebaum et al., 2008), while the putamen volume was found significantly decreased in patients with moderate AD (de Jong et al., 2008; Roh et al., 2011). Besides, a number of studies reported a correlation between the atrophy of putamen and declined cognitive performance in MCI and AD patients (Cho et al., 2014; Yi et al., 2016). Striatum is a region that was found to be crept with NFTs, which especially accumulates in the nucleus accumbens (Engelhardt & Laks, 2008). Similar to caudate nucleus, the decreased volume of nucleus accumbens is also predictive to the higher rate of MCI-to-AD conversion (Yi et al., 2016). Accordingly, the volume of nucleus accumbens was found to be positively related with cognitive function in MCI and AD patients (de Jong et al., 2008; Yi et al., 2016). As a structure more related to motor function rather than cognitive function, the volume of globus pallidus was seldom found to be indicative in the progression of AD. Previous studies found limited accumulations of Aβ and NFTs in the globus pallidus (Braak & Braak, 1991; Engelhardt & Laks, 2008), and several studies reported no obvious volume changes of globus pallidus during the progression of AD (Cho et al., 2014; Roh et al., 2011). Overall, among those sub-structures of basal ganglia, several subregions of striatum including caudate nucleus, putamen and nucleus accumbens seems susceptible to the pathology of AD, while the globus pallidus seems sheltered from the affect of AD mostly.

**Thalamus**

the thalamus is a highly interconnected structure located deep within the brain, situated between the
cerebral cortex and the midbrain. Thalamus is involved in several functions including processing of sensory signals and the regulation of consciousness and alertness. It has been shown that the connection between thalamus and hippocampus via fornix was essential for episodic memory (Aggleton & Brown, 1999), and the thalamus played an important role in the attention direction and declarative memory (Newman, 1995; Van der Werf et al., 2000). The AD pathologies initially begin in the MTL, and then spreads to the other regions like limbic system including thalamus (Braak & Braak, 1991; Mann, 1991). Although the thalamus is not a structure primarily accumulated with amyloid plaques and NFTs like the MTL, it could be affected by AD in some indirect ways. The thalamus relies on intact neural circuits with other brain regions to relay sensory information effectively. Disruptions in these circuits caused by NFTs can lead to sensory processing deficits and cognitive dysfunction. Previous studies have found that the volume of thalamus significantly decreased bilaterally in AD patients (de Jong et al., 2008; Štěpán-Buksakowska et al., 2014; Tang et al., 2014; Yi et al., 2016; Zarei et al., 2010). Individuals with MCI and EOAD patients were also found with thalamic atrophy (Pedro et al., 2012; Pievani et al., 2013; Susanto et al., 2015; Yi et al., 2016). A longitudinal study lasting three year found that AD patients demonstrated regional atrophy in the thalamus at baseline when compared to normal controls, and the atrophy rate is faster in left thalamus for AD patients (Cho et al., 2014). Overall, the impact of AD pathology on the thalamus is part of the broader disruption of neural circuits and communication networks within the brain. The thalamus is indeed affected by the progression of AD, but probably not particularly.

5.3. Summary of current studies

By discussing the above brain structures related to AD, we found that the structures inside the MTL are more indicative of AD, especially early AD pathology, than the bodies outside the MTL. The boundaries of EC, PRC, PHC, and amygdala inside the MTL are not as clear as those of the hippocampus, and in addition may involve some complex functions which are likely to go beyond the scope of AD pathology. Therefore, the hippocampus was selected as the optimal brain structure to investigate its role in AD. Overall, structural MRI has the advantages of being non-invasive, economical, and available compared to other AD markers. Among the structural MRI markers, the hippocampus is the most viable, and the fact that there is a large amount of previous research on the hippocampus and AD available for us to retrieve and read proves it in itself.

That being said, hippocampal volume is not widely recognized as a reliable diagnostic criterion for AD, but as an ancillary marker. Some of the shortcomings of hippocampal volume are likely to
affect its accuracy and validity in the diagnosis of AD. The most important drawbacks include two major ones: first, the large individual differences in hippocampal volume between populations; and second, the limitation that hippocampal volume as a cross-sectional index cannot reflect the dynamic changes of hippocampal atrophy. The advantages of structural MRI markers make them attractive for routine primary screening of early AD; at the same time, the two disadvantages of hippocampal volume mentioned above affect their clinical use and promotion. Therefore, improving the shortcomings of hippocampal volume as an MRI biomarker is required. On this basis, we proposed the HVR that more comprehensively reflects the information of hippocampal integrity. However, previously our team only conducted some preliminary validation of the HVR in individuals with normal cognitive function, and its validity in distinguishing between AD, MCI, and healthy individuals had not been investigated. Furthermore, in AD research, an AD marker that can predict disease onset is what is more needed in clinical practice. Can HVR predict the occurrence of MCI and AD? To address these questions, this doctoral dissertation has contributed a review study, a cross-sectional study, and a longitudinal study.

In the review study, we first summarized the different types of AD markers and summarized their advantages and disadvantages. Second, we focus on the brain imaging marker hippocampal volume (HV). It has been placed as a complementary biomarker due to some of its limitations, such as large individual differences, and the fact that it is only a static volume metric but does not dynamically reflect information about brain atrophy. In contrast, dynamic hippocampal atrophy better reflects the progression of AD than a static hippocampal volume. Finally the article introduced HVR as an MRI marker reflecting hippocampal atrophy and demonstrated preliminary evidence of its validity.

In the cross-sectional study, we selected 244 subjects from the OASIS-3 database, including three groups: NC, MCI, and AD. MRI data, clinical data, and age were collected for all subjects. We used both HV and HVR, respectively, as MRI markers for AD for statistical analysis. The results suggested that when HV was used as an MRI index, there was no significant difference in left-sided HC between MCI and AD, while all other groups differed significantly from each other. When HVR was used as an MRI index, the bilateral hemispheric differences between each group were significant. In addition, the results of Steiger's Z test also suggested a significantly stronger correlation between HVR and age compared to HV, as well as a significantly stronger correlation between HVR and cognitive function scores. Overall, this one simple cross-sectional study demonstrated that the HVR performs better than the HV in indicating the structure and function of the hippocampal complex as well as in differentiating between AD and MCI. In addition, HVR and age were also more closely correlated than HV.
In the longitudinal study, we selected 174 healthy subjects from the OASIS-3 database. Over the course of OASIS-3, 93 subjects stayed healthy, 38 subjects developed MCI, and 43 subjects developed AD. Based on this change, we categorized the subjects into 3 groups: NC, MCI, and AD. In this study we used a visualization form of HVR, $\alpha$ (\(\tan \alpha = \text{CSFvol}/\text{HCvol}\)), which can visualize HVR in an angular form. The results suggested that although all three groups of subjects were clinically healthy at baseline, there were already significant between-group differences in their baseline levels of $\alpha$: the AD group had significantly higher $\alpha$ than the MCI group, and the MCI group had significantly higher $\alpha$ than the NC group. In addition, we found higher $\alpha$ in the left brain than in the right, and higher $\alpha$ in men than in women. In addition, the MCI and AD groups had significantly faster brain deterioration relative to healthy controls, even during the asymptomatic phase of cognitive normalization. The major value of this study is that $\alpha$ proved to be a reliable MRI biomarker for predicting the occurrence of MCI and AD in the preclinical stage. Moreover, we combined $\alpha$ and age in the statistical analysis and obtained a rough quantitative prediction: when an individual's $\alpha_{age}$ is greater than 7.5, it predicts a higher probability of developing AD, when an individual's $\alpha_{age}$ is around 6, it predicts a higher probability of converting to MCI, and when an individual's $\alpha_{age}$ is less than 5, it predicts a higher likelihood of staying healthy over the long term.

There were some limitations in this study. The first one is about the segmentation scheme of HVR. The brain segmentation of HVR involves two parts: the hippocampus and its adjacent ventricle. Regarding the segmentation protocol of the hippocampus, it is relatively uniform; but for the segmentation of the ventricles, we still need to establish a better uniform standard. A broadly standardized approach would greatly facilitate scientific and clinical exchanges between different institutions on a given topic. Second, in the longitudinal study, we simply classified the three groups of subjects as NC, MCI, and AD. The NC group was included only if they stayed healthy after 84 months to ensure that the "health" of the subjects was real and not simply due to insufficient follow-up time in the study. However, the other two groups had large individual variations in the time of conversion to MCI/AD, ranging from two or three years to more than ten years. In this study, they were simply categorized as "converted" or "stay healthy", which may have affected the accuracy of the results. Third, the quantitative prediction results in Study 3 only provided a general range, which is relatively rough. It can provide evidence in scientific research, but when applied to clinical practice, its accuracy seems to be insufficient to support clinical validity.

Considering the obvious advantages of volume ratio of cortex to its adjacent ventricle, recently our team also tried another promising biomarker, cortex-to-sulcus-ratio (CSR), for AD in a not peer
reviewed paper (Waldraff et al., 2023). In line with the logic with HVR, CSR represents the volume ratio of parahippocampal cortex (PHC) to collateral sulcus. When PHC shrinks, the collateral sulcus is expanding. Parahippocampal atrophy caused by AD pathology was found to be associated with the enlargement of sulcus (Im et al., 2008). The results of this study suggested that CSR overcame the shortcomings of pure volume measurement and could reflect the course of AD more accurately than the standard parahippocampal volume in cross-sectional studies. Thus the results of this study also support the rationality and validity of HVR from another aspect.

5.4. Overall Summary

As a progressive and irreversible disease, AD poses a serious health risk, as well as a heavy burden on caregivers, families, and society. Currently, AD cannot be completely cured, and the limited treatments available can only stop or slow down the deterioration of symptoms. Therefore, diagnosis of AD in the preclinical stage is critical. Earlier diagnosis means a chance to control the disease at an earlier stage. However, AD has an insidious onset and can have a latent period of up to more than 10 years before symptoms appear (Braak & Braak, 1991; DeKosky & Marek, 2003; Delacourte et al., 1999b; Jack et al., 2013). During this period, clinical observation is not useful, and we need some AD indexes to assist in screening or diagnosis.

It is now widely accepted that there are two main initial neuropathogens of AD: the abnormal deposition of β-amyloid outside neuronal cells (Hamley, 2012; Hansson et al., 2019), and the formation of neurofibrillary tangles formed by the abnormal aggregation of tau proteins inside neuronal cells (Blennow & Zetterberg, 2018). These two pathogens subsequently lead to synaptic dysfunction and decreased energy metabolism in the brain, which in turn leads to degeneration of neuronal structure and function. At a macroscopic level, this leads to structural atrophy of the relevant brain regions. So theoretically, pathological changes at any point in this disease process can be used as markers for AD diagnosis as long as they can be accurately detected by instruments or devices invented by humans. Based on the developmental process of AD neuropathology, the markers of AD can be mainly classified into four major categories: CSF biomarkers, blood biomarkers, PET markers and structural MRI markers.

The CSF index mainly reflects the concentration level of Aβ and tau proteins in CSF, which in turn reflects the lesion level of Aβ and tau proteins in the brain. The advantage of CSF is that it can reflect the pathology of AD at an early stage and is affordable, but its fatal disadvantage is that it needs to be acquired through an invasive lumbar puncture. Apart from the pain and discomfort of the acquisition process, it also carries a higher risk of infection and complications in the elderly.
The risk is only worth taking if CSF collection is necessary. If our goal is to develop biomarkers that can be widely used in clinical screening for AD, it is clear that the invasive and risky CSF test is not applicable to this requirement. Blood markers, which also reflect biochemical parameters, have the advantages of being economical to collect and noninvasive. However, due to the complexity of blood components, the accuracy of the results has been less than satisfactory. But in recent years there have been new advances of studies on p-tau 181 (Janelidze et al., 2020; Thijssen et al., 2020) and p-tau 217 (Janelidze et al., 2022; Mattsson-Carlgren et al., 2023; Palmqvist et al., 2020, 2021) in blood, which enhanced the diagnostic validity of blood biomarkers, adding possibilities for the promotion of clinical routine screening for AD. The PET assays most commonly used to indicate AD are PiB-PET and FDG-PET. PiB-PET is primarily used to detect the topographic distribution and severity of Aβ in the brain, whereas FDG-PET reflects synaptic function and detects the onset of neurodegeneration by demonstrating the level of glucose metabolism in the brain. A great advantage of PET testing is that it reflects the topographic distribution of neurologic lesions in the brain, rather than being limited to the values of biochemical indicators. However, the major drawback of PET is its high cost and low availability, which makes it difficult to be used for routine screening in clinical practice. AD structural imaging indices are a manifestation of morphologic changes of micro neurodegenerative lesions at the macroscopic level. Structural MRI has the advantages of being noninvasive, relatively cost-effective, highly available, and reflective of topological changes in brain structure. Overall, these strengths of structural MRI make it both valuable and possible for the promotion of the routine screening for AD at early stage.

Early studies have demonstrated that the MTL is where the pathology of AD, including Aβ and NFTs, earliest aggregates (Braak & Braak, 1991). So it is also where the earliest brain atrophy affected by AD occurs. Although other structures in the MTL including the PHC, PRC, and EC also have atrophy, none of their structural edges are clear enough. In contrast, the hippocampus has a clear anatomical structure, providing a practical basis for its use as a decent brain imaging marker. There are a large number of studies that have found a relationship between hippocampal volume and cognitive function (Ezzati et al., 2016; Hardcastle et al., 2020; Konishi et al., 2017; O’Shea et al., 2016), the development of AD diseases (Aschenbrenner et al., 2018; Jack et al., 1997; Tabatabaei-Jafari et al., 2020), and MCI-to-AD conversion (Apostolova et al., 2006; Chupin et al., 2009; Eckerstrom et al., 2008; Fang et al., 2019; Hill et al., 2014; Risacher et al., 2009; Tabatabaei-Jafari et al., 2020). But there have also been review study of the hippocampus that do not support the above associations (Van Petten, 2004). We speculate that one possibility is the large individual variation of hippocampal volume in the population. It is possible that the differences of
hippocampal volume between individuals are due to genetic factors or personal developmental factors rather than AD pathology. In addition, hippocampal atrophy (HV) leads to its smaller size, which is a dynamic process of change. However, HV can only provide a cross-sectional static indicator. Moreover, according to Jack (2010), the diagnostic timing of structural MRI is later than biological markers of body fluids as well as PET markers. Therefore, the next three directions for improvement of HV as an AD marker for structural MRI may be: 1) controlling for individual differences in the population; 2) reflecting the dynamic changes of hippocampal atrophy; and 3) advancing the diagnostic timing of HV in the long process of AD development. To address these issues, based on HV, we introduced a new brain imaging marker, the Hippocampal-to-ventricle-ratio (HVR).

Schoemarker (2019) provided a description of the concept of HVR and gave preliminary evidence that HVR is superior to pure hippocampal volume on indicating the hippocampal integrity. With aging or the pathological effects of AD, the hippocampal volume shrinks progressively, and in parallel with this is the expansion of its surrounding ventricles. When people are young and healthy, the hippocampus is large and the surrounding ventricles are small; with aging and the long-term effects of AD pathology, the hippocampus shrinks smaller and its surrounding ventricles expand larger. Thus, using the volume ratio of the hippocampus to the surrounding ventricles as a composite indicator of hippocampal integrity could be a superior strategy. I summarized previous AD markers in my first review study and presented the advantages of structural MRI and the shortcomings of hippocampal volume as a structural MRI marker. Based on this, HVR was introduced and preliminary evidence supporting its validity was presented (Hu et al., 2023). This became the basis of the literature for my second cross-sectional study and my third longitudinal study. In the second cross-sectional study, I directly compared three groups of subjects with NC, MCI and AD, using HV and HVR as MRI markers for statistical analysis, respectively. The results suggested that HVR could better distinguish between-group differences than HV, especially between MCI and AD. Comparison of the strength of the correlation coefficients revealed that HVR was significantly more strongly correlated with age and cognitive function than HV. In the third longitudinal study, all subjects were healthy at baseline. Over time, some stayed healthy, some developed MCI, and some developed AD. In this study, we used a visualized form of the HVR, α. The results suggested that even when they were all in the asymptomatic "healthy" period, the baseline α was higher for AD than MCI, and the baseline α was higher for MCI than NC. Stated differently, a larger α predicts a greater risk of developing AD, while a smaller α predicts a safer and healthier future. Moreover, not only the baseline level, but the rate of brain atrophy was already
faster in the preclinical period in MCI and AD than in the NC group. But there are some limitations in the study. Firstly, the segmentation protocol of HVR, especially the standard of segmentation of ventricles around the hippocampus, has yet to be promoted and standardized. Second, in the longitudinal study, there was a large individual variation on the time of conversion to MCI or AD among different subjects, but this variation was not reflected in the present study. Again, the prediction of HVR for the occurrence of MCI or AD in longitudinal studies is still relatively rough and does not meet the standard of quantitatively predicting the probability of AD occurrence. Based on these limitations, future research can focus on these directions: 1) promotion and standardization of HVR, especially its ventricular segmentation scheme; 2) adopting experimental or statistical methods to control the time from the initial measurement to the conversion to MCI/AD, so as to reduce the impact of its variability; 3) on the basis of solving the problem of last issue, then improving the quantitative accuracy of prediction, aiming to reach such a stage: by inputting a HVR or \( \alpha \) value, together with blood biomarkers, age, gender, and then the risk probability of converting to MCI or AD after a certain number of years will be shown directly by pipeline.

Overall, Study 1 laid the literature and theoretical foundation for the proposal of HVR through an OPINION review; then, Study 2 directly reflected the better discriminatory function of HVR compared to HV for patients with MCI and AD in a cross-sectional study; finally, Study 3 validated the predictive function of HVR for AD through a longitudinal study, which is a core value of the researches on AD biomarker. In summary, HVR mostly overcomes the drawbacks of pure hippocampal volume as a structural MRI marker for AD, and its superiority and validity were verified in this doctoral dissertation. HVR, as a superior structural MRI marker, is promising to be promoted for utilization in clinical practice. If HVR and blood biomarkers are combined to utilize in routine screening for AD in the preclinical stage, they can complement the strengths and weaknesses of each other, which is believed to greatly improve the timeliness and accuracy of routine screening for AD.
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7. Personal academic contributions


Hu, X., Meier, M., & Pruessner, J. A higher sensitivity of the hippocampus-to-ventricle-ratio (HVR) compared with pure hippocampal volume (HV) on differentiating AD, MCI and normal controls. (article in revision)

Hu, X., Meier, M., & Pruessner, J. Hippocampal-to-ventricle (HVR) predicts mild cognitive impairment (MCI) and Alzheimer’s disease (AD) in cognitively normal baseline. (article in revision)

Johanna Hartmann, Xiang Hu & Jens Pruessner. Age-related trajectories of Hippocampus-to-ventricle-ratio (HVR) as an early diagnostic marker of mild cognitive impairment and Alzheimer disease. (article in revision)
8. Curriculum Vitae

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