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Longitudinal Effects of Cognitive Reserve and Vascular Risks

in Aging and Dementia

By

Yen Yu Lo

A dissertation submitted in partial satisfaction of the

Requirements for the degree of

Doctor of Philosophy

in

Epidemiology

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of the

University of California, Berkeley

Committee in charge:

Professor William J. Jagust, Chair

Professor Alan E. Hubbard

Professor Robert W. Levenson

Spring 2012

Abstract

Longitudinal Effects of Cognitive Reserve and Vascular Risks

in Aging and Dementia

By

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Doctor of Philosophy in Epidemiology

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Professor William J. Jagust, Chair

The cognitive spectrum between normal aging and dementia is broad. Many terms including mild cognitive impairment (MCI) have been developed to identify a group of people at the transitional phase for early detection of Alzheimer disease (AD). The lack of biomarker based criteria and the dependence on the sociocultural context result in great variability in case definition.

Cerebrospinal fluid (CSF), positron emission tomography (PET) and magnetic resonance imaging (MRI) serve as three important tools to track biological changes in AD. The Alzheimer's Disease Neuroimaging Initiative (ADNI) provides the infrastructure for investigators to examine the longitudinal patterns of CSF, PET and MRI biomarkers at different cognitive stages. The dissertation first delineated the biomarker changes over time in relation to cognitive decline in ADNI and found that the trajectories support a hypothetical sequence of AD pathology, suggesting that biomarker prediction for cognitive change is stage dependent.

Missingness is common but often overlooked in longitudinal studies of AD. The mechanism of missing data is often assumed to be missing completely at random. The second aim of the dissertation is to test this assumption. The missing biomarker data in ADNI were found not completely at random but rather conditional on certain clinical features. Understanding the missing data structure may help in the design of future longitudinal studies and clinical trials in AD.

Cognitive reserve has been proposed to account for the discordance between cognitive performance and AD pathology. The long held viewpoint is that cognitive reserve affects the clinical expression but has no direct effect on AD pathology. This viewpoint was re-examined in the dissertation. The results showed that higher cognitive reserve indexed by education and other proxies was associated with slower rates of AD pathological deterioration, particularly among cognitively normal elderly people. These findings suggest that the pathological course of AD can be modified by cognitive reserve.

Many cardiovascular risk factors increase the risk of AD. Vascular dysfunction reduces brain reserve or threshold of cognitive impairment. Whether the underlying mechanism also involves impairment of cerebral amyloid clearance remains controversial. Vascular burden, indexed by

cardiovascular risk profile and MRI white matter hyperintensities, was not significantly associated with rates of AD biomarker changes, suggesting that typical AD pathology, presumably reflective of amyloid accumulation, appears to be independent of vascular burden.

In conclusion, CSF and imaging markers change over time at different rates in aging and dementia and the missing data are conditional on certain clinical features during follow-ups. Education and other cognitive reserve surrogates may have direct effects on AD pathological progression while vascular burden may influence cognitive function via its own pathway independent of amyloid deposition. Considering the longitudinal effect of cognitive reserve and the potential to control vascular risks, AD can be a preventable disease.

Dedicated in love and gratitude

to my wife and son in Taiwan,

Yu Chun and Yoyo.

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Preface

Alzheimer disease (AD) has become a major public health issue as the population ages globally. Despite the great success in understanding the basic science of AD, there is still no clinically effective treatment for patients with AD. The AD research community is moving towards early detection using biomarkers; the newly revised National Institute of Aging diagnostic criteria for AD, mild cognitive impairment (MCI) due to AD and preclinical AD incorporate biomarkers and exemplify this trend. Many previous AD studies using biomarkers were limited by cross-sectional design and very few followed up participants with repeated measurements. By contrast, longitudinal study design can avoid some unverifiable assumptions and better address causal inference. The Alzheimer's Disease Neuroimaging Initiative (ADNI) study provides such a unique opportunity to explore longitudinal changes of various AD biomarkers in a standardized way. I therefore applied epidemiological methods to investigate the relationships among biomarker dynamics, cognitive reserve and vascular risks in ADNI in an attempt to test existing hypotheses and find preventive strategies.

In the dissertation, I first review the limitations and implications of our ways to define dementia and MCI; then I delineate the longitudinal changes of AD biomarkers as well as their temporal inter-relationships; chapter 3 is focused on missing data, an often overlooked issue in longitudinal studies; after biomarker trajectories and missing data are handled, I investigate the effects of cognitive reserve and vascular burden on these AD biomarkers in chapter 4 and 5. Upon the completion of the dissertation, I am hoping we are getting closer even if just one more step towards AD prevention in the face of global aging.

Acknowledgments

I am indebted to many people throughout these years of research training in California. I want to first thank my mentor Professor Bill Jagust, whose humor and humility always inspire me to keep balance between research and life; the Jagust lab is created as a friendly and collaborative place, where I wish I could stay longer. I had the pleasure to enjoy the course 250C Epidemiologic Theory taught by Professor Ira Tager before his retirement; this course was the highlight of all my coursework and definitely "caused" my interest in causal inference. I had the privilege to have Professor Alan Hubbard to guide my biostatistics; he was always efficient to get statistical problems resolved. I am grateful to Professor Jack Colford, who provided very useful thoughts when I was stuck; in fact, the missing data analysis in the dissertation was the idea originated from the discussion with him. I benefitted much from my Epidemiology study cohort, which includes Hope, Aracely, Ling-I, Ben, Ann, Caitlin and Josh; without their comradeship the graduate program would have become dull and lonely. I deeply appreciate the invaluable and continuing support from my former mentor at the Parkinson's Institute, Dr. Carlie Tanner, who gave me the very opportunity to begin my career in clinical research, particularly in neuroepidemiology. Finally, this dissertation is dedicated to my dear wife, Yu Chun; her forbearance has been beyond praise and thanks.

Chapter 1

Between Normal Aging and Alzheimer Disease

Introduction

Alzheimer disease (AD) is a neurodegenerative disorder characterized by slowly progressive cognitive decline. It is, however, difficult to tell the onset of AD from normal aging. Mild cognitive impairment (MCI) is a concept originated from the attempt to detect AD early,[1] but again cognitive impairment is a spectrum rather than an event of clear onset. In this chapter, I review the development, implications and limitations of the concept of MCI.

In the past years, various kinds of drugs have been tested in clinical trials aimed to halt neurodegenerative processes in AD, such as estrogen,[2-4] testosterone,[5] aspirin,[6] non-steroidal anti-inflammatory drugs,[7,8] prednisolone,[9] omega-3 fatty acid[10] and dehydroepiandrosterone;[11] however, they all failed to effectively ameliorate cognitive deterioration. Scientists argue that neuronal death may have arrived at an irreversible state or end stage by the time AD is diagnosed, and it may be too late to intervene with these drugs. A hypothesis that these interventional strategies can protect against AD if patients are identified earlier and treated earlier therefore arises. Although to date, there is no evidence showing that we can change the clinical course if patients with AD are diagnosed earlier; identifying people at a higher risk seems to be a plausible step further towards finding treatment for AD.

This attempt resulted in tremendous enthusiasm in seeking biomarkers for early detection and defining at-risk people in clinical settings such as MCI, but also raised some concerns. At the individual level, people who are called at-risk for AD or MCI may become apprehensive about their health, especially knowing that there is no cure or anything they can do to make a change. Anxiety, despair and frustration are likely to influence these people as well as their family members, despite the fact that they are to some extent still functionally active in different social roles. At the population level, a new clinical entity like MCI, particularly when introduced to the public via media at varied levels of scientific rigor, tends to attract a large crowd of people, young and old, with mild forgetfulness flooding into clinics. Whether forgetfulness should always have clinical implication remains questionable. Presumably, only a small proportion of these people are the target population with prodromal AD we attempt to identify, but a considerable amount of medical resources would have to be spent on initial screening and regular follow-up visits. The economic impact of publicizing a new clinical entity warrants careful evaluation.

Further, a more fundamental issue is that the appropriateness of current nosology for MCI needs to be reconsidered. In the following paragraphs, I first review how the concept of MCI has evolved historically before being implemented with operational criteria; then I address the heterogeneity within the MCI group, particularly when applied in a community or general population; I argue that a substantial amount of variability in diagnosis of MCI stems from the environment, both physically and socially; lastly, I discuss why MCI as a nosological entity is problematic from viewpoints of clinical validity and categorization theory.

Evolution of MCI

Occasional forgetfulness or reduced cognitive capacity seems to be a common and natural feature in the elderly. In 1962, Kral proposed the concept of "benign senescent forgetfulness",[12] to contrast with a rather malignant form of memory impairment, with respect to clinical manifestation and prognosis. Forgetfulness with poor outcome was first recognized, though not necessarily as a precedent of AD. Later in 1986 by the group in National Institute of Mental Health, the term "age-associated memory impairment" was used to characterize very mild memory dysfunction in the elderly population based on formal memory tests comparing with young adults.[13] In 1989, Blackford and La Rue proposed a refined version of age-associated memory impairment, "late-life forgetfulness", as having a decrement greater than 50% of a specified test battery. In 1994, Levy proposed "age-associated cognitive decline", as memory impairment on any formal test in reference to norms for the elderly rather than the young.[14] The aforementioned syndromes were efforts trying to characterize memory impairment based on standardized tools in order to minimize variability in clinical judgment. However, some of these definitions compared older people to young, and many older people were categorized as "declining" when they were in fact normal for their age.

In 1994, the concept of MCI became recognized by the major international classification system. The Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) refers "age-related cognitive decline" to objective functional decline due to physiological aging process, but it has little practical value since no criteria or tests are specified. Another term is also proposed in DSM-IV is "mild neurocognitive disorder (MNCD)", which includes executive and linguistic function in addition to memory. A similar term, "mild cognitive disorder (MCD)", is encompassed in the tenth revision of the International Classification of Diseases (ICD-10); but it refers to memory and learning difficulty secondary to physical illnesses. Both MNCD and MCD are not designed for the elderly and thus not suitable for identifying the population at risk for AD.

In 1997, Graham proposed "cognitive impairment-no dementia (CIND)" in the context of the Canadian Study of Health and Aging to encompass primarily memory impairment but also other domains with a wider range of etiologies.[15] Both formal test and clinical examination are required to meet the criteria. Although not all people with CIND have a progressive course in memory impairment, studies implemented with CIND suggest that certain people with subclinical cognitive deficits are in fact at early stage of AD. In 1999, MCI as memory impairment beyond that expected for age and education yet not dementia, was characterized by Petersen and colleagues.[1] The diagnosis of MCI was made if the patient met the following criteria: (1) memory complaint, (2) normal activities of daily living, (3) normal general cognitive function, (4) abnormal memory for age, and (5) not demented. Since then, many studies applied these criteria for MCI and focused on how likely and how fast people with MCI would develop AD.

Although these different terms address a similar concept, the prevalence estimate varied across different operational criteria. A broader term such as age-associated memory impairment can give considerably inconsistent prevalence estimates ranging from 7% to 98% in the elderly population, depending on the specific cognitive test applied.[16-19] This suggests that the spectrum of cognitive function among the elderly between normal aging and dementia is wide, and the prevalence estimates simply reflect how sensitive or restrictive the particular cognitive test is in capturing cases meeting the operational criteria.

Memory is one dimension of the integrated cognitive function of human beings, and there are several types of memory: episodic, procedural, emotional and semantic, to name a few. Episodic memory refers to the ability to recall past events or personal experience, and it is also the major feature of AD. Isolated memory impairment is therefore a major focus of research. In a registry based study, Bowen and colleagues followed a group of people with new cognitive complaints and found that people with isolated memory loss have a higher risk of developing AD than those with non-memory cognitive complaints.[20] The diagnosis of MCI has also an emphasis on memory impairment as it requires memory complaints as well as objective memory dysfunction to meet criteria; however, not all MCI progress to AD or other dementia and many MCI even return to normal. The heterogeneity in the use of the term was subsequently recognized, and as a result, three subtypes as amnestic, multiple domains and single non-memory domain MCI were proposed.[21]

Amnestic MCI is thought to be the most common subtype and the most likely group which would convert to AD, whereas other subtypes may represent other types of dementia or normal aging. Whether a concept derived from patients who present to memory disorder clinics can be also applicable in the general population is questionable. Palmer and colleagues conducted a three-year study to determine the predictive value of each MCI subtype for identifying future AD.[22] They found that the majority of MCI were people with cognitive impairment of single non-memory domain; the subtype carrying the highest risk to convert to AD was not amnestic type but multiple domain type; and a substantial proportion of people with memory impairment did not complain. All these results suggest that people with MCI who come to the clinic seeking care for memory deficits may be different from people with MCI identified in the community. Amnestic MCI is likely overrepresented in memory clinics whereas MCI of other two subtypes may be seen in different specialties. It is conceivable that cognitive deficits other than memory can be attributable to a variety of medical and psychiatric illnesses, which in total may have a larger population than AD. Therefore, to identify people who are highly likely progress to AD simply based on the operational criteria for MCI may be inadequate.

Built environment and cultural context

In a population study, nearly half of the AD patients do not have complaints about cognitive function three years before diagnosis,[23] suggesting that there is a discrepancy between the objective cognitive impairment on tests and the subjective functional impairment in daily life. Aside from the fluctuating nature of cognitive dysfunction at certain points in the disease course, there are several external sources for this discrepancy: environmental support and cultural relativism.

People with episodic memory impairment are easily disoriented in the absence of many types of cues, and distracted when there are many stimuli. In the beginning stages, they often still have insight about their reduced memory capacity and may develop compensatory strategies to overcome the inconvenience due to forgetfulness and to avoid embarrassment in public, such as notes, timer, and calendar. This is a period when the level of disability very much relies on the environment; particularly the built environment and social support. If within the environment there are many options for strategies to optimize their cognitive performance, for instance, a portable global positioning system to navigate somewhere, then in spite of their deficits in topographic memory, they may still be able to venture out and buy groceries independently. Mental aids can be placed in bathrooms, bedrooms and kitchens for instructional purposes. The

use of household devices can be programmed and simplified into a few buttons to prevent unintended danger. The formation of adaptive behavior to cope with various cognitive challenges is greatly facilitated not only by advances in technology, but also by support from family members. Extended family remains the basic family unit conceptually, despite the fact that the nuclear family is becoming the dominant family type in many Asian societies. Children who have their own families may still live in the same neighborhood with their parents, so that they can take turns to care for old parents. Under such protected circumstances, daily lives are less affected by mild cognitive dysfunction such as memory impairment since meals, transportation, leisure, health and financial management can be taken care of by their children or other family members. They are not considered diseased until late stage of dementia, for example, when they no longer recognize people. The concept of MCI also reflects that cognitive demand is higher in a society like the United States, where even mild impairment can severely affect quality of life. For example, driving skill is almost a requisite to be mobile and a slight decrease in visuospatial attention or topographic memory may put drivers at risk. As a consequence, MCI becomes an important issue as it can lead to driving disability and thus individual immobility. On the contrary, for elderly who live in a rather self-contained community with no need to drive on their own, MCI is less relevant with respect to individual mobility. Both hardware and software in the built environment contribute to determine how much cognitive capacity is necessary to live an independent life. The difference in the built environment for individuals with comparable cognitive impairment can result in heterogeneity in defining their state of disability. While performance on neuropsychological tests may be less subjective in determining cognitive function, the reliance on these standardized tests may lose sight of the importance of the local or micro-environment which poses different levels of cognitive demand. In other words, when lower cognitive capacity is required to be able to function well in certain situations, MCI becomes meaningless.

Culture refers to a collective set of values and beliefs practiced and shared by a group of people. Conceivably, MCI or memory impairment is viewed in different ways depending on the cultural context. In a society which values people who "lift themselves up by their own bootstraps", even mild impairment in cognitive performance can hurt individual competence in daily activities, and therefore brings these individuals to the clinic for evaluation. The less than ideal cognitive performance is considered abnormal when the normal range is rescaled. Similar trends can also be seen in other medical fields, such as hypertension.[24] The criteria become more and more stringent for defining normal blood pressure (systolic blood pressure <120, diastolic blood pressure < 80) and a new category such as pre-hypertension is created to denote the borderline between normal and hypertension (systolic blood pressure 120-139, diastolic blood pressure 80-89). From a disease prevention perspective, there is no doubt that this approach will increase the sensitivity to capture patients at a pre-morbid state, but what is the meaning of "pre-hypertension" if community hygiene and infectious diseases are major concerns of a society where more people die of diarrhea and parasitic infection than cardiovascular events. Likewise, it is ironic to talk about obesity in a country that suffers from poverty and famine. In a different culture, forgetfulness may be regarded as part of the aging process, just as we do not expect elderly people to act as swiftly as the young or exercise as intensely as the young. MCI is not suitable to apply to people with cognitive function "appropriate" to their age in certain cultural contexts. Moreover, in some cultures, cognitive impairment may be conceptualized, studied and experienced in a totally different way from the aging processes. For example, in Cohen's book

"No aging in India", aging or dementia is explained beyond individual health status and the old person is seen as a metaphor for the moral decay of the family and the nation.[25]

Heterogeneity in dementia

The diagnosis of MCI is tied to what we know about dementia. Dementia has to be excluded to fulfill the diagnostic criteria of MCI. But dementia is also a diagnosis with great heterogeneity. The disagreement among several common criteria for dementia can differ by a factor of 10 in the prevalence estimate.[26] The disagreement can be attributed to different primary cognitive tests used in diagnosis. In addition, the social and occupational aspects in diagnostic criteria for dementia are weighed differently. The DSM-IV criteria for dementia of Alzheimer's type, for example, require that the cognitive deficits cause significant impairment in social and occupational functioning.[27] This is where clinical judgment comes into play as we do not have a standardized tool to measure this dimension. The research-oriented diagnostic criteria for AD, established by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA), has less to do with social and occupational functioning and also excludes other psychiatric or medical causes to increase its specificity.[28] Studies show that NINCDS-ADRDA criteria provide only fair reliability (interrater agreement kappa = 0.64)[29] and validity (sensitivity = 0.92, specificity = 0.65).[30] Blacker and colleagues further examined the sources of disagreement and found that the majority of disagreement originated from complicated medical, neurological and psychiatric illnesses.[31] Besides, the medical records were not always detailed in clinical information, making the interpretation and inferences less consistent across different reviewers. The authors recognized that no diagnostic tool is perfect and the consensus process may improve the diagnostic accuracy.

Functional status remains the core criterion in clinical settings. For example, the Clinical Dementia Rating (CDR) scale is often applied to the operational case definition. The CDR scale is composed of features in problem solving, community affairs and hobbies, which largely depend on personal educational background and facilities in the community. If the subject has fewer years of education to adequately do problem solving and lives in an unfriendly community where he or she is more reluctant to join any program, then this subject is more likely to be considered functionally impaired and thus MCI or dementia. Education has been proposed to be protective against AD.[32] It is speculated that education in early life can increase cognitive reserve. However, it is also argued that education can enhance performance on various cognitive tests because students are trained and given tests of similar formats during normal education. All in all, we should recognize that there are subjective components in the diagnostic process for AD. These components are not measured, not measurable or quasi-quantitative. These all add to the complexity and variability of diagnosing dementia. MCI is not even a diagnosis with a pathological basis like AD but rather a concept. It is therefore conceivable that MCI is a diagnosis with more heterogeneity and instability.

Biomedicalization of dementia

Although the current focus of AD is on the elderly population, the first case of AD reported by Alois Alzheimer at the beginning of the 20th century was a 51-year-old woman, who presented with symptoms including impaired memory, aphasia, hallucination and bizarre behavior. Obviously it was very unusual to see a patient with such clinical manifestations at this age, but

how the patient would be viewed and treated if her age of onset was more than 80 years old was not known. Senility has long been deemed as an inevitable condition of old age until the 1980s, when the public awareness of dementia grew and funds from National Institute of Health for AD research dramatically increased. Since then, senile dementia is no longer a natural phenomenon when people age but has become a medical problem, which is attributable to biological causes and subject to drug treatment. A similar example can be seen in the diagnosis of attention-deficit and hyperactivity disorder (ADHD) in children. Hyperactive behavior in children used to be considered normal, or at least, not a pathological condition; however, ADHD is now an established clinical entity with formal diagnostic assessment, theory in pathophysiology and medical treatment. AD refers to a pathological condition involving loss of cognitive functions and memory in particular and was in effect originally intended to illustrate onset before very old age. Through the process of biomedicalization, senility, which has been considered appropriate to age, is now a deviance, a medical problem and a clinical entity with distinct pathology and requiring specific treatment.[33] As a result, all coexisting symptoms or illnesses are unsurprisingly brought in under the umbrella of dementia, such as depression, regardless that they may simply reflect a normal emotional response to this social construct. All features that come along with dementia tend to be seen as part of the constellation of symptoms and signs belong to AD or an indicator of the disease stage.

Ever since the paradigm of AD was established, more and more evidence supported the notion that AD is a disease entity. However, aside from some rare genetic causes of AD, there has never been a definite etiology. Some studies also demonstrated that not all cases with brain plaques and tangles typically shown in AD would develop dementia;[34,35] and conversely, disseminated vascular lesions or small infarcts in brain seemed no less contributory than amyloid deposition to typical presentations of AD.[36] The amyloid hypothesis of AD pathophysiology is no longer certain. The discrepancy between pathology and clinical presentation further increases the complexity of what we know about AD. Our understanding of AD has arrived at the stage where AD is unlikely a single disease, and instead, a general category. Biomedicalization does not seem lead us to a definite biological answer.

MCI is a diagnosis made on the top of our understanding of AD. Since the biological underpinning of AD is even somewhat undecided, transforming MCI or the concept of transitional phase into a biomedical entity is even more challenging. As a recent study showed, many patients with clinically diagnosed amnestic MCI exhibited mixed pathologies.[37] What has been neglected throughout the course of biomedicalization is how the sociocultural context frames the nosology of AD or MCI. The implication of MCI diagnosis needs to be reconsidered.

MCI as a nosological entity

A clinical syndrome consists of a cluster of symptoms and signs placed in a distinctive time course. The constituents of MCI are not derived from a group of patients with unique clinical features observed in clinical settings but rather a conceptual set of attributes. This makes MCI diagnosis different from how we define AD. To be qualified as a clinical syndrome, there should be ways we can ensure the validity beyond a cluster of symptoms and signs.

To validate a clinical syndrome, several validators are proposed: (1) identification and description by "clinical intuition" or by cluster analysis, (2) demonstration of boundaries between related syndromes by discriminant function analysis, (3) follow-up studies establishing

a distinctive course or outcome, (4) therapeutic trials establishing a distinctive treatment response, (5) evidence of familial clusters, (6) association with more fundamental abnormalities-histological, biochemical or molecular.[38] MCI diagnosis is based on artificial criteria but not identified by clinical intuition. Boundaries for MCI are blurred and related syndromes are distributed all over the spectrum of symptomatology without "point of rarity". People with MCI are more likely to develop AD during follow-ups but it is also common to see reversion to normal among MCI.[39] Overall clinical course of MCI may be rather more heterogeneous than distinctive. There is no documented treatment for MCI, although whether current therapeutic options for AD can be effectively applied to MCI remains unknown. MCI is not considered a familial or inheritable disorder, and transition to AD does not vary with family history.[40] Lastly, unlike AD, there is no way we can validate MCI by histological pathology. Therefore, MCI seems well formulated but lacks validators; MCI becomes a syndrome that cannot be accurately identified. Although accurately identifying a clinical syndrome does not always precede etiological discovery, it undoubtedly increases the likelihood of successful elucidation of etiology.

Furthermore, there is a lack of prototype to make MCI an independent category. The prototype theory was first introduced to cognitive psychology by Eleanor Rosch.[41] She concluded that the natural way we categorize objects is based on recognizing the prototype but not on logical classification. Take AD as an example, the prototype is the first case reported by Alois Alzheimer. Alzheimer noticed the unique pattern of cognitive impairment and behavioral change, and he correlated these clinical features with pathological findings in the brain. The typical case or prototype was then established, which allowed following physicians to diagnose patients by comparing with the AD prototype. Lots of experience from AD experts accumulated over time and subsequently formed the basis of consensus criteria. Although current concepts of AD are much different from that in Alzheimer's era, the origin can be traced back to that prototype case. Another good example is Parkinson's disease. When James Parkinson first described cases with paralysis agitans, he thought these patients were cognitively intact with pure motor dysfunction. To date, there is a growing body of evidence showing that dementia, depression, sleep disorders and autonomic dysfunction are likely part of the disease course of Parkinson's disease. The concept may evolve and branch into different categories, but there is always a prototype fertilizing the nosology.

Current criteria of MCI are still broad and not specific. At the clinical level, it is difficult to distinguish which cases among MCI will develop AD and which cases will return to normal. There is no prototype MCI case as a reference for physicians to compare, contrast and comprehend. Although it is known that many MCI patients have AD pathology and subsequently convert to AD, treating MCI as a nosological entity is still of great debate. Assume clinical diagnosis of MCI in combination with other biomarkers is highly predictive of AD, this is in reality a diagnosis of AD or preclinical AD but not something unique and separable from AD.

Early detection of dementia

As mentioned earlier, clinical neuroscientists are striving to identify the at-risk group who will develop AD in the future. However, defining the transitional phase between normal aging and AD brought both hope and complexity, especially when the sociocultural context was taken into consideration. During the past two decades, several biomarkers of AD have emerged, such as

amyloid and tau protein in cerebrospinal fluid (CSF), hippocampal atrophy on brain magnetic resonance imaging (MRI), glucose metabolism and even amyloid on positron emission tomography (PET), and are ready to be incorporated into new diagnostic criteria.[42] Patients with AD may be differentiated from normal elderly people by measuring these biomarkers; however, when these markers begin to progress and how fast these markers change over time are not known. There has not been enough evidence from prospective studies to show trajectories of these biomarkers. Tracking AD biomarkers over time together with repeated cognitive tests may allow us to capture the earliest pathological change and evaluate the possibility of using biomarkers for early detection of AD, thereby limiting our dependence on the imperfectly defined syndrome of MCI.

Biomarkers seem to be reliable and objective tools for diagnosis of AD, but defining AD purely on a biological basis is not without concern. For example, previous studies have shown the discordance between clinical severity and pathological severity in AD and found that non-biological factors, such as education and occupation, also play an important role in cognitive expression. Cognitive reserve theory was therefore developed to account for the observed discordance.[43] Based on the theory, given the same amount of AD pathological burden, people with higher education or greater reserve are more resistant to cognitive impairment. However, the neural basis of cognitive reserve remains elusive and whether the progression of AD pathology can be altered by cognitive reserve is not clear.

In addition to our lack of understanding of AD biomarker dynamics and clinicopathological discrepancy, early detection of AD is also hampered by the fact that many elderly people have multiple comorbidities, particularly cardio- and cerebrovascular diseases. Either overt stroke or microinfarcts in the brain can lead to cognitive dysfunction. Typical vascular pathology is commonly found in postmortem brain examinations of patients with AD diagnosis and in fact mixed pathologies accounts for most dementia cases in the community.[44] To better define dementia of the Alzheimer type, it is warranted to further clarify the role of vascular risks.

Summary

MCI is a concept attempting to identify patients with AD early in the disease course. This attempt reflects the failure of multiple clinical trials for AD and the hope for effective treatment if given earlier. Various terms including MCI have been proposed to signify the transition phase between normal aging and dementia. Among people with MCI defined by the same criteria, there are several subtypes and MCI patients seen in clinic are different from those in community. The diagnosis of MCI or dementia relies on not only cognitive tests but also the interaction between each individual and his or her local environment and sociocultural context. The level of required cognitive capacity varies with the cognitive demand in the environment, physically and socially. Different cultures have their own interpretation of cognitive impairment and the border separating normalcy from deviance is quite blurred.

Dementia of the Alzheimer type represents a referent diagnosis for MCI; however, the established criteria for AD are sensitive but not specific. Biomedicalization of AD is intended to explain the disease on a more biological and objective basis, but in fact this approach has created more complexity. Although cerebral amyloid deposition and neurofibrillary tangles are the key

components in AD pathology, the etiology of AD is still under investigation. MCI is a diagnosis without validators and the lack of prototype MCI case makes the diagnosis unstable. Treating MCI as a nosological entity to feature the intermediate stage between normal aging and dementia may be an intuitive but also complicated approach.

The development of biofluid and imaging markers has improved our understanding of the temporality of AD pathological progression. Longitudinal research design is thus crucial in studying cognitive decline in relation to pathological change in aging and dementia. Low cognitive reserve and high vascular burden may contribute to dementia through different pathways, and to understand their roles will have enormous impact on AD prevention. In the following chapters, normal aging, MCI and AD are presumably on the same cognitive spectrum but carefully treated as three independent groups. The theme of the dissertation is outlined by the following causal diagram:

```
┌──────────┐        ┌──────────┐
│ Vascular │        │Cognitive │
│  burden  │        │ reserve  │
└──────────┘        └──────────┘

┌──────────────┐        ┌──────────┐
│Alzheimer disease│ ───▶ │Cognitive │
│   pathology   │        │ function │
└──────────────┘        └──────────┘

          ┌──────┐
          │ Age  │
          └──────┘
```
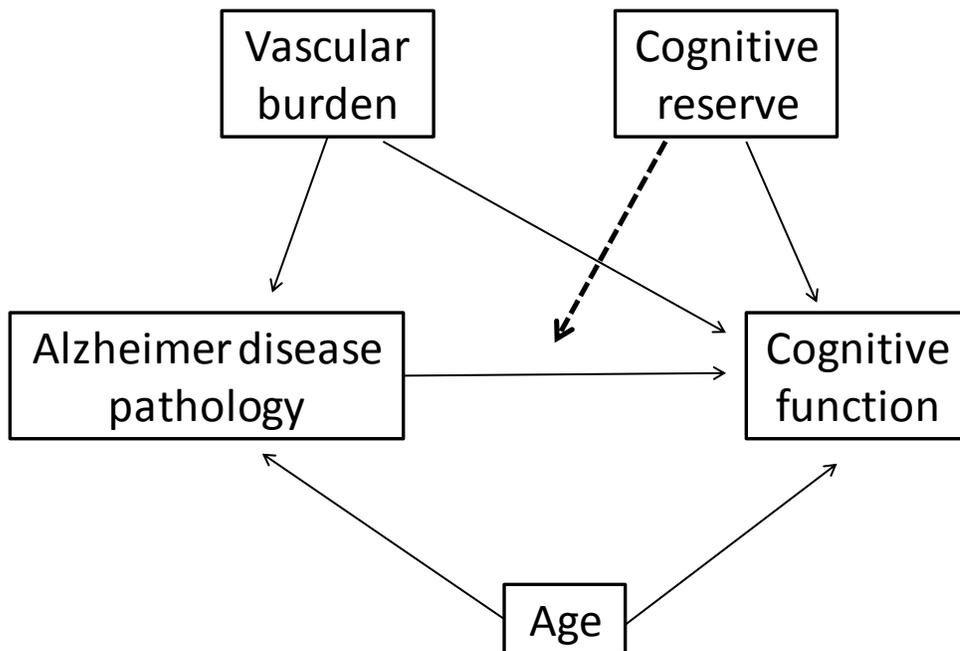
The causal diagram represents that cognitive function deteriorates as Alzheimer disease (AD) progresses and cognitive reserve may not only influence cognitive performance but also modify the pathological effect of AD (dashed line); whereas vascular burden in the brain may have an indirect effect on cognitive function through its interaction with typical AD pathology or amyloid deposition.

9

Chapter 2

Longitudinal Change of Biomarkers in Cognitive Decline

Introduction

Using biomarkers for the early detection of Alzheimer disease (AD) is crucial for developing potential treatment. Previous studies have shown that CSF levels of β-amyloid 42 peptide ($A\beta_{42}$) and tau protein,[45] region-specific fluoro-deoxy-glucose uptake on PET (FDG-PET)[46] and MRI of hippocampal volume[47] were markers associated with AD. Postmortem examinations further demonstrated that the burden of AD pathology was reflected by antemortem CSF $A\beta_{42}$,[48] region-specific FDG-PET,[49] and MRI hippocampal volume,[50] suggesting that these markers are indicative of the altered biological states in AD.

Although lower levels of CSF $A\beta_{42}$ are associated with the risk of incipient AD,[51] CSF biomarkers appear to be relatively stable over time within individuals.[52,53] Greater hippocampal atrophy rates measured by serial MRI correlated with faster cognitive decline in normal aging and early conversion to dementia in MCI in previous studies.[54-57] Several longitudinal FDG-PET studies also suggested that regional hypometabolism predicted clinical progression or conversion to AD.[58-61] Since these time-varying biomarkers as well as the APOE 4 gene are all associated with AD or cognitive impairment, it is conceivable that they are correlated with one another.[62-65] However, very few studies have examined the dynamic change of two or more biomarkers simultaneously.[66,67] Longitudinal comparison of biomarker change is an important approach to assess the relative importance and pathological significance of each biomarker.

In this chapter, we aimed to delineate the trajectories of CSF, PET and MRI biomarkers as well as the influence by APOE 4 gene and then evaluated their relative associations with cognitive function in participants with normal cognition (NC), MCI and AD.

Methods

Study population A total of 819 research participants (NC: 229; MCI: 397; AD: 193) were enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI) study from 59 centers in the United States and Canada during 2005– 2007. Full inclusion/exclusion criteria are detailed at www.adni-info.org. Briefly, screening criteria for entry into the study included the Mini-Mental State Examination score, Clinical Dementia Rating scale and an education-adjusted cutoff score on delayed recall of one paragraph from the Logical Memory subtest of the Wechsler Memory Scale-Revised.[68] All participants were recruited between the ages of 55 and 90, and had at least 6 years of education. Specific psychoactive medications or other neurological disorders were excluded. After the baseline visit, subsequent visits took place at six or 12 month intervals in person. Participants with NC or MCI were followed up for three years, while those with AD for two years at maximum.

Standard protocol approvals, registrations, and patients consents The study procedures were approved by institutional review boards of all participating institutions. Written informed consents to blood sampling, lumbar puncture, neuropsychological testing and neuroimaging were obtained from all research participants or their representatives.

Genetic marker Blood samples at baseline were collected and APOE genotyping was carried out at the University of Pennsylvania Alzheimer's Disease Biomarker Laboratory. APOE 4 gene carriers were participants who had at least one APOE 4 allele.

CSF proteins CSF samples were collected in the morning after overnight fast, shipped to the University of Pennsylvania Alzheimer's Disease Biomarker Laboratory and analyzed using a standardized protocol.[69] $A\beta_{42}$, total-tau (t-tau), phosphorylated-tau (p-tau$_{181p}$) were measured (pg/ml) in each of the CSF aliquots using the multiplex xMAP Luminex (Luminex Corp, Austin, TX) platform with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use-only reagents) immunoassay kit-based reagents. About 50% of all participants underwent lumbar puncture at baseline and annually repeated lumbar punctures up to three years were available for 106 participants.

FDG-PET The protocol to acquire ADNI PET data at sites nationwide is detailed at www.loni.ucla.edu/ADNI/Data/ADNI_Data.shtml, and methods for FDG-PET analysis have been described previously.[70] Briefly, PET images were acquired 30-60 minutes postinjection. Images were averaged, spatially aligned, interpolated to a standard voxel size, intensity normalized and smoothed to a common resolution of 8-mm full width at half maximum. PET volumes were intensity normalized to a single region comprised of the cerebellar vermis and the pons defined by the Montreal Neurological Institute template. We used pre-defined regions of interest (FDG-ROIs) to reflect glucose metabolism. Mean FDG uptake was extracted and averaged from five ROIs (right/left temporal gyrus, right/left angular gyrus and posterior cingulate gyrus) for each participant. Baseline PET images were available for 404 participants and over 60% of these participants were followed up for two additional years with repeated PET scans.

MRI hippocampal volume The 1.5-T MRI protocol was described elsewhere,[71] which was standardized across all sites: 2 T1-weighted MRI scans, using a sagittal volumetric magnetization-prepared rapid gradient echo sequence, with an echo of 4 msec, repetition time of 9 msec, flip angle of 8°, and acquisition matrix size of $256 \times 256 \times 166$ in the x-, y- and z-dimensions with a nominal voxel size of $0.94 \times 0.94 \times 1.2$ mm. The images were aligned, skull-stripped, and segmented. A quality control center was designated to exclude scans with serious motion artifacts. FreeSurfer software (http://surfer.nmr.mgh.harvard.edu) was applied to obtain bilateral hippocampal volumes in mm^3 from this segmentation. Baseline MRI images were available for 811 participants and over 60% of these participants were followed up for two more years with multiple MRI scans.

Cognitive function assessment The Alzheimer's Disease Assessment Scale- Cognitive Subscale (ADAS-cog) was used as a dependent measure to examine relationships between biomarkers and cognitive change. This test contains 11 items covering language, memory, praxis and comprehension function. The total score ranges from 0 to 70 and higher scores indicate poorer cognitive function. Baseline and multiple follow-up ADAS-cog assessments were available for all participants.

Statistical analyses Participants with two or more repeated measures were entered into analyses. We first delineated the trajectories of different biomarkers and used repeated measures linear regression (an exchangeable working within subject correlation model via a generalized estimating equation, GEE)[72] to estimate population average rates of change in CSF proteins,

FDG-PET ROIs and MRI hippocampal volume as well as ADAS-cog scores for NC, MCI and AD participants. To account for the residual correlation due to repeated measures on the same subject, we could have also used a more parametric, mixed model approach. However, given that our focus was on the average rate of change in biomarkers (and not on the variance components) as well as wanting to derive robust inference (standard errors not sensitive to the specified correlation model), we chose the GEE approach, rather than a parametric maximum likelihood approach.[73] Time-varying biomarkers were treated as the outcome and modeled by time and baseline age in the regression. In these models, a significant time coefficient indicated a non-zero rate of change. We also made inter-group comparisons of rates of change. In a separate analysis, we included APOE 4 allele carrier status in the model to evaluate its influence on the rate of change for each biomarker, reflected by the coefficient of the interaction term (APOE 4 $\times$ Time).

We then examined the relation between the change of cognitive function and the change of different biomarkers. Time-varying ADAS-cog scores were treated as the outcome of interest and modeled by time and the change in biomarkers after adjusting for baseline age and baseline biomarker value. R-squares were calculated for each longitudinal model to represent the goodness of fit or the extent to which the marginal variance of cognitive function was explained by the model. Models differed by biomarker of interest and sample size, because only a limited number of participants had all three biomarkers available. We conducted model comparisons by restricting participants to those with two biomarkers available (CSF and PET; CSF and MRI; PET and MRI) so as to make models more comparable.

All statistical analyses and graphics were performed in R version 2.11.1.

Results

Demographic features of all participants are summarized in Table 2-1. The sample size declined over time and the number of repeated measures available for longitudinal analysis varied across different biomarkers and diagnostic groups (Table 2-2). CSF Aβ_{42} (pg/ml/month) appeared to decrease faster in NC (-0.46) than in MCI (-0.26) and AD (-0.29), but inter-group differences were not significant; changes in CSF total and p-tau for the most part were not significantly different from zero (Table 2-2). Brain regional glucose metabolic decline (normalized intensity/month) was significantly slower in NC (-7.0×10^{-4}) than in MCI (-1.9×10^{-3}) and AD (-4.2×10^{-3}), and slower in MCI than AD (Table 2-2). The rate of MRI hippocampal atrophy (mm^3/month) was also significantly slower in NC (-3.0) than MCI (-5.4) and AD (-7.8) and slower in MCI than AD (Table 2-2). Cognitive function assessed by ADAS-cog declined (increased in ADAS-cog score) in MCI and even faster in AD, but improved (decreased in ADAS-cog score) a little in NC. The hypothetical average changes of these biomarkers and the ADAS-cog for a 75-year old person in the three diagnostic groups are illustrated (Figure 2-1).

The mean level of CSF Aβ_{42} was 53.4 pg/ml lower in cognitively normal APOE 4 carriers than non-carriers at baseline, and the difference did not change significantly at follow ups (-57.2 (95% CI: -86.1,-30.3) pg/ml at 1 year and -61.0 (95% CI:-87.0,-37.1) pg/ml at 2 year). The mean difference in CSF Aβ_{42} between APOE 4 carriers and non-carriers became smaller in the MCI group (-46.4 (95% CI:-70.3,-24.5) pg/ml at baseline, -44.0 (95% CI:-66.8,-23.2) pg/ml at 1 year and -41.7 (95% CI: -63.9,-21.4) pg/ml at 2 year) and no longer significant in the AD group. The mean difference in FDG uptake between APOE 4 carriers and non-carriers was 0.05 unit

lower in the NC group, 0.04 unit in the MCI group and not significant in the AD group. The mean hippocampal volume was 189.8 (95% CI: -288,-93.2) mm^3 smaller in APOE 4 carriers than non-carriers in the MCI group at baseline and the difference increased over time (-214 (95% CI: -315,-114) mm^3 at 1 year and -237 (95% CI: -342,-135) mm^3 at 2 year). A similar effect of APOE 4 on hippocampal atrophy was also seen in AD (-148.8 (95% CI: -315,15.0) mm^3 smaller at baseline, -173 (95% CI: -338,-10.3) mm^3 at 1 year and -198 (95% CI: -363,-34.1) mm^3 at 2 year). The associations between APOE 4 status and the baseline value of biomarkers were significant in the NC group for CSF $A\beta_{42}$ and FDG-PET and in the MCI group for all three biomarkers. Positive APOE 4 gene status did not appear to modify the rate of change in CSF $A\beta_{42}$ or glucose metabolism in all three groups, but it accelerated hippocampal atrophy in MCI and AD (Table 2-3).

For NC participants, although changes in cognitive function were not captured by any of these time-varying biomarkers, CSF $A\beta_{42}$ ($R^2 = 0.12$) appeared to be better in explaining the total variance of ADAS-cog scores over time than PET ($R^2 = 0.07$) and MRI ($R^2 = 0.03$) (Table 2-4). In MCI, changes in cognitive function were associated with all of these biomarkers; such that cognitive decline (increase in ADAS-cog score) was associated with the decrease of CSF $A\beta_{42}$ level, FDG-PET regional metabolism and MRI hippocampal volume. Cognitive function at the MCI stage was about equally well modeled by PET ($R^2 = 0.18$) and MRI ($R^2 = 0.16$). For participants with mild AD, cognitive decline was still captured by PET and MRI though no longer by CSF $A\beta_{42}$. The variance of ADAS-cog score during the course of dementia seemed better modeled by PET ($R^2 = 0.36$) than MRI ($R^2 = 0.19$). We further conducted head-to-head comparisons in sample-size matched groups (CSF vs. PET; CSF vs. MRI; PET vs. MRI) and their relative contributions to model cognitive decline remained largely unchanged (Table 2-5).

Discussion

Annualized changes of CSF, PET and MRI biomarkers as well as cognitive function during the first 12-month follow-ups in ADNI have been reported.[74] We extended the follow-up study to up to 36 months and found evidence of significant change in biomarkers of $A\beta_{42}$, glucose metabolism, and hippocampal volume in all three groups of subjects – NC, MCI and AD. These biomarker trajectories showed that rates of change in $A\beta_{42}$ were not different among the groups, but changes in glucose metabolism and hippocampal volume accelerated as cognitive function deteriorates. In normal subjects, cognitive change was not related to change in any of these biomarkers, although a model that included CSF $A\beta_{42}$ captured more variance than models that contained other biomarkers. The lack of association between cognitive change and biomarker dynamics in NC may be due to only subtle functional difference at this stage or the limitation of our cognitive measurement tool. In MCI patients all three categories of biomarkers were related to cognitive decline, while in AD only glucose metabolism and hippocampal atrophy, and not CSF $A\beta_{42}$ were related to cognitive decline. These findings imply that CSF $A\beta_{42}$ declines prior to the onset of cognitive impairment, in relation to aging or preclinical AD; whereas measures of neuronal dysfunction and injury (glucose metabolism and hippocampal atrophy) change with disease severity and stage.

Previous studies showed that prior to cognitive impairment, APOE 4 carriers have accelerated memory decline,[75] greater MRI hippocampal atrophy rates[76] and faster decline in regional FDG-PET.[77] Our data in Table 2-3 demonstrated that APOE 4 was associated with baseline CSF $A\beta_{42}$ and FDG-PET (but not baseline MRI hippocampal volume) in NC; whereas in MCI and AD,

APOE 4 accelerated MRI hippocampal atrophy (but not CSF $A\beta_{42}$ or FDG-PET). The influence of the APOE 4 gene on CSF $A\beta_{42}$ and FDG-PET regional metabolism appeared to begin earlier than on hippocampal atrophy. There is evidence from pathological examinations and amyloid PET imaging showing that the APOE 4 gene increases the risk of AD through $A\beta$ accumulation in the brain.[78,79] Therefore, the effect of APOE 4 on biomarkers at different stages may reflect the pathological sequence led by the pivotal event in AD, β-amyloid deposition.

The decrease in CSF $A\beta_{42}$ as an early event shown in our biomarker trajectories and the influence of APOE 4 on hippocampal atrophy which occurred after CSF $A\beta_{42}$ and FDG-PET both imply that the FDG-PET marker changes after CSF $A\beta_{42}$ but before MRI hippocampal atrophy. Our study supports the hypothetical model of the AD pathological cascade proposed by Jack et al.,[80] in which brain $A\beta$ deposition heralds the onset of the entire AD pathological process, is followed by regional synaptic dysfunction or glucose hypometabolism which eventually culminates in cell loss or brain atrophy.

One of the unique features in the study is that we have follow-up information on CSF, PET, and MRI biomarkers as well as ADAS-cog scores of study participants to address the dynamics of the pathological course of AD. These biomarker dynamics have been examined in ADNI using a cross-sectional approach;[81] however, to translate cross-sectional results into actual patterns of change requires a strong assumption that all participants follow the same pattern of disease progression from normal all the way to dementia. We understand that this assumption may hold true for MCI converters and AD but it is unlikely for NC and MCI non-converters. Nearly half of the MCI participants converted to AD during follow-up but very few people changed from NC to MCI or AD in ADNI. NC may be a very different group from those who used to be cognitively normal but currently have MCI or AD. Ideally, longitudinal change of biomarkers could be best delineated had the study continued with follow up that was long enough to observe the same group of participants from NC transitioning to MCI and AD. Limited by this design, we might be observing biomarker dynamics in aging but not necessarily disease progression in AD; therefore, we should be conservative about making inferences from participants who remained cognitively intact.

Previous longitudinal CSF studies showed that the decrease of CSF $A\beta_{42}$ correlated with cognitive decline in normal elderly[82] but the decrease might be too slight to detect later in the disease course,[66,83,84] suggesting the level of CSF $A\beta_{42}$ might stabilize long before symptomatic dementia. These longitudinal CSF studies were, however, limited by at most two repeated measures and relatively small sample size. Our longitudinal study of CSF biomarkers is based on up to three repeated measures, which is the minimum number of time points allowing us to evaluate the variance of change. Baseline and one follow-up measure can only generate one single slope or change for each individual, and therefore there is no variance of slope to evaluate. The two-point difference may result from either actual change or simply measurement error. In addition, if CSF biomarker measurement error exists, which is very likely for all laboratory tests, the magnitude of difference can be subject to the "regression towards the mean" effect. In other words, the more the baseline value deviates from the population mean, the larger the change is likely to be.

We used the ADAS-cog score to monitor cognitive function and mapped the change of biomarkers to ADAS-cog as a way to assess the extent to which pathological markers correlated with clinical progression over time. There is no gold standard for measurement of cognitive

function, particularly when our outcome of interest includes multiple stages of AD from normal to overt dementia. We noticed that ADAS-cog in NC even improved over time and recognized that the possible learning effect might hinder us from using the ADAS-cog to track cognitive change among normal elderly people. Nevertheless, ADAS-cog is still the standard tool in many clinical trials to assess AD, which allows our results to be more interpretable across different studies.

There are several limitations in our study. First, research participants in ADNI were volunteer-based and clinic-based but not drawn from the general population. Although they all met inclusion/ exclusion criteria for NC, amnestic MCI or mild AD, they were not newly diagnosed or incident cases. Within the same diagnostic group, participants were enrolled in the study at different stages in the disease course. Baseline evaluation did not adequately reflect their clinical states when they first had the disease. Therefore, we want to be clear that our target population is patients who come to the clinic rather than the general community; and we applied a GEE approach to avoid the unverifiable assumption about their biological states at the beginning of cognitive impairment. Second, not all ADNI research participants underwent all biomarker examinations, especially lumbar puncture for CSF. Like many longitudinal studies, we had substantial missing data for biomarkers during the 36-month follow ups. Although a GEE approach can handle missing time points within individuals, there is no way we can recover the actual biomarker profiles for those individuals who did not end up being in the analyses. The differences in sample size, particularly the smaller samples of individuals with longitudinal CSF samples compared to the other biomarkers, may limit our ability to draw inferences about the relative changes in these biomarkers. Participants present in the analyses might be different from those who were not included or who dropped out; we do not know whether this is informative censoring or random missing data. Nevertheless, we focused on the relative rates of change or associations with cognitive change but not true rates. The calculated biomarker values would be biased by informative censoring but the inter-relationship among these biomarkers might not be affected.

In sum, longitudinal patterns of biomarkers suggest that CSF, PET and MRI capture AD pathological states sequentially and their predictive values for cognitive decline depend upon the stage of the disease. Repeated measurement of these candidate biomarkers provides a potential approach for early diagnosis of AD.

Table 2-1 Demographic features of 819 participants in ADNI at enrollment

	ADNI diagnostic group		
	NC	MCI	AD
Sample size	229	397	193
Mean age (SD)	75.1 (5.0)	74.0 (7.5)	74.6 (7.5)
M : F	119 : 110	256 : 141	102 : 91
Years of education (SD)	16.0 (2.9)	15.7 (3.0)	14.7 (3.1)
Mean MMSE (SD)	29.1 (1.0)	27.0 (1.8)	23.3 (2.1)
APOE 4 carrier (%)	61(26.6)	212 (53.4)	127 (65.8)

ADNI: Alzheimer's Disease Neuroimaging Initiative; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; MMSE: mini-mental state examination.

Table 2-2 Population monthly change of biomarkers and inter-group rate comparison

	ADNI diagnostic group Monthly change (SE)			Inter-group comparison Coefficient (SE) of (Group × Time)		
CSF (pg/ml)	NC (n = 36)	MCI (n = 54)	AD (n = 16)	NC (ref) vs. MCI	NC (ref) vs. AD	MCI (ref) vs. AD
$A\beta_{42}$	-0.46*** (0.14)	-0.26** (0.08)	-0.29** (0.10)	0.20 (0.16)	0.16 (0.17)	-0.04 (0.13)
t-tau	0.05 (0.07)	-0.04 (0.15)	-0.41 (0.25)	-0.09 (0.17)	-0.46 (0.26)	-0.38 (0.28)
$p\text{-tau}_{181p}$	0.05* (0.02)	-0.01 (0.02)	-0.09 (0.06)	-0.05 (0.03)	-0.14* (0.06)	-0.09 (0.06)
FDG-PET (normalized intensity)	NC (n = 104)	MCI (n = 203)	AD (n = 97)			
	$-7.4\times10^{-4*}$ (3.0×10^{-4})	$-1.9\times10^{-3***}$ (2.1×10^{-4})	$-4.2\times10^{-3***}$ (4.6×10^{-4})	$-1.2\times10^{-3**}$ (3.6×10^{-4})	$-3.4\times10^{-3***}$ (5.4×10^{-4})	$-2.2\times10^{-3***}$ (5.0×10^{-4})
Hippocampal volume (mm^3)	NC (n = 228)	MCI (n = 390)	AD (n = 191)			
	-2.95*** (0.19)	-5.52*** (0.23)	-8.01*** (0.34)	-2.57*** (0.29)	-5.03*** (0.39)	-2.49*** (0.41)
ADAS-cog (point)	NC (n = 228)	MCI (n = 390)	AD (n = 190)			
	-0.02* (0.01)	0.12*** (0.01)	0.40*** (0.04)	0.14*** (0.02)	0.41*** (0.04)	0.28*** (0.04)

Population average rate (unit per month) was calculated using a GEE approach adjusting for baseline age. Sample sizes were limited to subjects with two or more repeated measures during 3-year follow-ups. Biomarker change is statistically significant if different than zero. Inter-group rate comparisons were tested in the longitudinal models with two diagnostic groups each time and the inter-group rate difference was reflected by the coefficient of the interaction term (Group × Time). ADNI: Alzheimer's Disease Neuroimaging Initiative; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; FDG-PET: [18]F- fluorodeoxyglucose PET; ADAS-cog: Alzheimer's Disease Assessment Scale- cognitive subscale. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2-3 APOE 4 influence on biomarkers

	ADNI diagnostic group		
CSF Aβ$_{42}$	NC (n = 36)	MCI (n = 54)	AD (n = 16)
Age	2.45	0.30	0.92*
Time	-0.38*	-0.36**	-0.41
APOE 4	-52.34**	-46.24***	-0.71
APOE 4 × Time	-0.32	0.20	0.15
FDG-PET	NC (n = 104)	MCI (n = 203)	AD (n = 97)
Age	$-9.4\times10^{-3***}$	$-3.1\times10^{-3*}$	$8.0\times10^{-3***}$
Time	$-7.7\times10^{-4*}$	$1.7\times10^{-3***}$	$-3.7\times10^{-3***}$
APOE 4	$-5.0\times10^{-2*}$	$-4.3\times10^{-2*}$	2.9×10^{-2}
APOE 4 × Time	1.6×10^{-4}	5.0×10^{-4}	-6.4×10^{-4}
MRI hippocampus	NC (n = 228)	MCI (n = 390)	AD (n = 191)
Age	-33.19***	-26.96***	-25.18***
Time	-2.72***	-4.44***	-6.56***
APOE 4	-67.50	-188.19***	-152.47
APOE 4 × Time	-0.81	-1.98***	-2.04**

Time-varying biomarkers of CSF Aβ$_{42}$ (pg/ml), FDG uptake (normalized intensity) and MRI hippocampal volume (mm^3) were modeled by baseline age (year), time (month), APOE 4 gene carrier status (1 or 0) and interaction between APOE 4 and time using a GEE approach. Values in the table were coefficients in GEE models. Coefficients of the interaction term (APOE 4×Time) represented the influence of APOE 4 on rates of change. Sample sizes were limited to subjects with two or more repeated measures during 3-year follow-ups. ADNI: Alzheimer's Disease Neuroimaging Initiative; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; FDG-PET: ^{18}F- fluorodeoxyglucose PET. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2-4 Goodness of fit of regressions modeling cognitive change by biomarkers

	NC	MCI	AD
CSF model R^2	0.12	0.12	0.26
	(n = 36)	(n = 54)	(n = 16)
Age	0.06	-0.01	-0.12
Time	-0.02	0.05	0.43^{***}
Baseline	-0.02^{**}	-0.03^{**}	0.12
Change	-0.02	-0.08^{*}	-0.15
PET model R^2	0.07	0.18	0.36
	(n = 104)	(n = 203)	(n = 96)
Age	0.02	0.03	0.13
Time	-0.02	0.08^{***}	0.18^{***}
Baseline	-6.27^{**}	-15.39^{***}	-29.83^{***}
Change	-3.11	-11.45^{***}	-36.00^{***}
MRI model R^2	0.03	0.16	0.19
	(n = 228)	(n = 390)	(n = 190)
Age	0.08^{*}	-0.05	-0.18^{*}
Time	-0.03^{**}	0.05^{**}	0.24^{***}
Baseline	2.0×10^{-4}	$-4.0\times10^{-3***}$	$-4.1\times10^{-3***}$
Change	-4.6×10^{-3}	-0.01^{***}	-0.02^{**}

Time-varying ADAS-cog scores were modeled by baseline age (year), time (month) and baseline value and change of biomarkers of CSF $A\beta_{42}$ (pg/ml), FDG uptake (normalized intensity) and MRI hippocampal volume (mm^3). R-square was the percentage of the outcome variance explained by the model. Values below R-square were coefficients in GEE models and they represented an estimated mean change in ADAS-cog score for change covariate value of 1 unit while keeping others fixed. NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2-5 Sample-size matched inter-biomarker comparisons of goodness of fit in generalized estimating equations models

CSF vs. PET

	NC (n = 17)	MCI (n = 28)	AD (n = 8)
CSF model R^2	0.23	0.02	0.28
Age	0.09	0.01	-0.07
Time	-0.06[*]	0.04	0.49[***]
Baseline	-0.03[*]	-0.01	0.02
Change	-0.02	-0.03	-0.01
PET model R^2	0.30	0.14	0.66
Age	-0.02	0.01	0.19
Time	-0.07[**]	0.01	0.29[***]
Baseline	-15.67[**]	-11.38[**]	-39.57[*]
Change	-17.35[*]	-22.80[*]	-35.27[**]

CSF vs. MRI

	NC (n = 36)	MCI (n = 53)	AD (n = 16)
CSF model R^2	0.13	0.10	0.25
Age	0.03	-0.01	-0.07
Time	-0.02	0.06	0.37[**]
Baseline	-0.018[**]	-0.02[*]	0.08
Change	-0.01	-0.09[*]	-0.26
MRI model R^2	0.02	0.10	0.18
Age	-0.03	-0.05	0.07

Time	-0.02	0.02	0.31
Baseline	-5.3×10^{-4}	-2.7×10^{-3}	-3.3×10^{-3}
Change	-3.1×10^{-3}	-0.01	-0.01

PET vs. MRI

	NC (n = 102)	MCI (n = 198)	AD (n = 94)
PET model R^2	0.08	0.16	0.38
Age	0.01	0.02	0.15
Time	-0.03	0.07[***]	0.18[***]
Baseline	-6.47[***]	-14.80[***]	-31.53[***]
Change	-4.46	-11.20[**]	-38.79[***]
MRI model R^2	0.05	0.19	0.21
Age	0.07	-0.06	-0.25[**]
Time	-0.05[**]	0.04	0.22[**]
Baseline	1.8×10^{-4}	-4.3×10^{-3}[***]	-5.4×10^{-3}[**]
Change	-0.01	-0.01[**]	-0.01[*]

Time-varying Alzheimer's Disease Assessment Scale- cognitive subscale (ADAS-cog) scores were modeled by baseline age (year), time (month) and baseline value and change of biomarkers of CSF Aβ_{42} (pg/ml), FDG uptake (normalized intensity) and MRI hippocampal volume (mm^3). R-square was the percentage of the outcome variance explained by the model. Values below R-square were coefficients in GEE models and they represented an estimated mean change in ADAS-cog score for change covariate value of 1 unit while keeping others fixed. NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
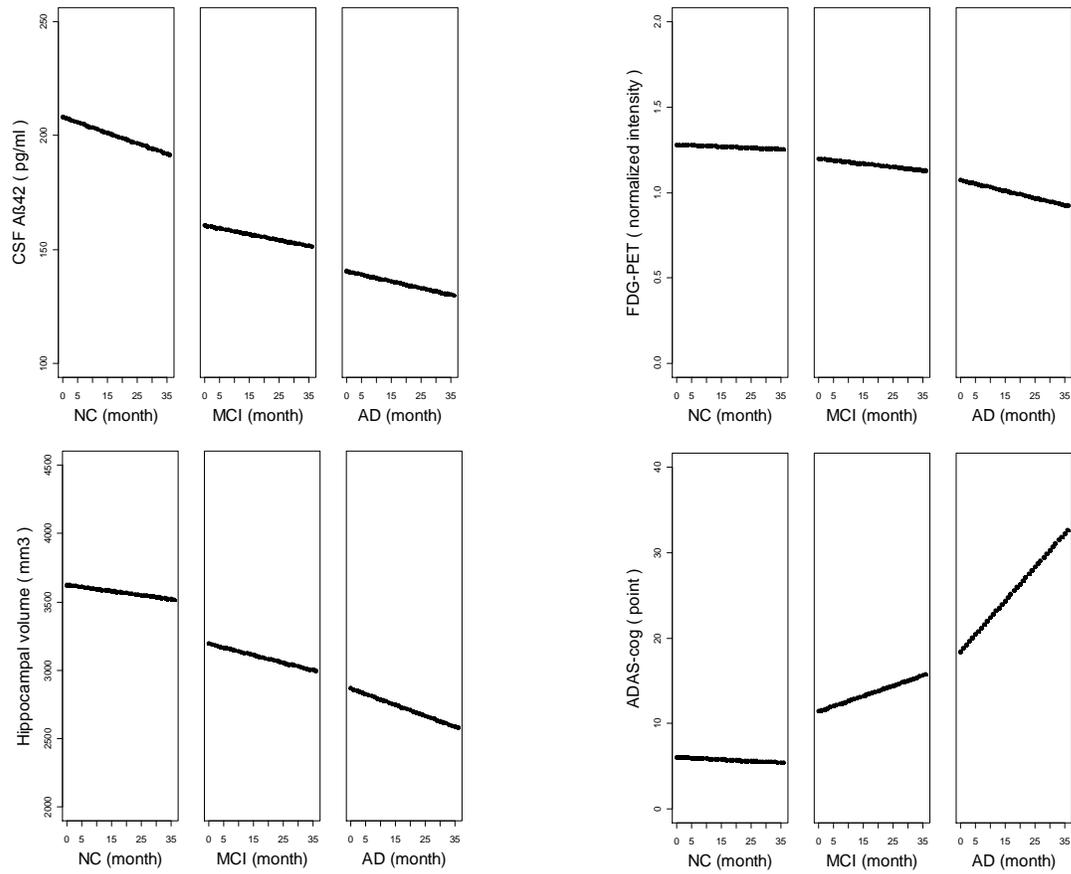
Figure 2-1 Hypothetical longitudinal changes of CSF Aβ, FDG-PET, MRI hippocampal volume and ADAS-cog score for a 75-year-old person at different cognitive states.

Chapter 3

Longitudinal Missing Biomarker Data in Alzheimer Disease

Introduction

Missing data are common in cohort studies, particularly in Alzheimer disease (AD) research.[85] Higher mortality risk and cognitive impairment hinder older adults from staying in studies requiring multiple visits and thus result in incomplete data.[86] Although statistical methods have been developed to handle missing data in repeated measures studies,[87-89] the underlying mechanism for missing data is rarely examined in actual studies.

Most longitudinal studies of AD use complete data for analysis and ignore missing data, assuming the complete data are a random sample drawn from the entire study population, so-called missing completely at random (MCAR).[90] A less stringent assumption, missing at random (MAR),[90] may be satisfied if missingness does not depend on the variable itself, conditional on observed covariates. If missingness does depend on the variable itself, even after accounting for observed covariates, then data are said to be missing not at random (MNAR).[90] Analysis methods should be used which are appropriate to the type of missingness at work. However, it is important to note that it is not possible to distinguish between MAR and MNAR based on observed data, suggesting sensitivity analyses ought to ideally be performed.

In this chapter I examine the missing data structure of the ADNI in an attempt to understand the direction of bias due to drop-outs, which is essential to developing strategies to retain cases in future longitudinal studies and to inform how the ADNI data themselves are analyzed.

Methods

Study Population The ADNI population has been described in chapter 2. Briefly, a total of 819 research participants (NC: 229; MCI: 397; AD: 193) between the ages of 55 and 90 were enrolled from 59 centers in the United States and Canada during 2005– 2007. Full inclusion/exclusion criteria are detailed at www.adni-info.org. Screening criteria for entry into the study included the Mini-Mental State Examination score (MMSE), Clinical Dementia Rating scale (CDR) and an education-adjusted cutoff score on delayed recall of one paragraph from the Logical Memory subtest of the Wechsler Memory Scale-Revised.[68]

Follow-up Timeline Detailed schedules of assessment for NC, MCI and AD are posted in the general procedure manual on the ADNI website:
http://adni.loni.ucla.edu/wpcontent/uploads/2010/09/ADNI_GeneralProceduresManual.pdf

Briefly, after the baseline visit, subsequent visits took place at 6 or 12 month intervals in person. Participants with NC or MCI were followed up for three years, while those with AD for two years at maximum. The visit schedules for collecting biomarkers were similar but not the same for NC, MCI and AD groups. Participants might visit the research clinic for other assessments without consenting or completing certain biomarker tests. We used the data from ADNI up to the date 4/19/11.

Biomarkers Missing data for blood homocysteine, CSF $A\beta_{42}$ and tau proteins, [^{18}F]fluorodeoxyglucose PET (FDG-PET) and volumetric MRI were examined in ADNI. Blood

and CSF samples were collected and analyzed using a standardized protocol.[69] Biochemical profiles including homocysteine in blood samples and $A\beta_{42}$, total-tau and phosphorylated-tau in CSF were measured. The study was targeted to acquire baseline CSF samples for at least 20% of total participants by approaching any potential subject who might be interested. The sources of protocols to acquire ADNI PET and MRI data have been indicated in chapter 2. The study was targeted to acquire baseline PET scans for 50% of total participants. While inclusion in the PET protocol was randomly assigned, participants were free to decline to enter this arm of the study. The study was targeted to acquire baseline MRI scans for all participants; individuals who refused MRI could not enroll.

Predictors of Missing Biomarkers Predictors of interest were baseline demographic and clinical features that were likely associated with both cognitive impairment (study outcome) and loss of follow up (missingness).

Demographic features Age, sex, years of formal education, smoking, and family history of AD were recorded at enrollment. Occupation types were recorded and classified into three levels: 1. professional or managerial; 2. skilled; 3. partly-skilled or unskilled occupations according to The National Statistics Socio-economic Classification.[91] APOE genotyping was carried out at the University of Pennsylvania Alzheimer's Disease Biomarker Laboratory. APOE 4 gene carriers were participants who had at least one APOE 4 allele. Premorbid intelligence indicated by number of errors (range: 0-50) in American National Adult Reading Test (ANART) was evaluated at baseline as part of the neuropsychological battery.[92]

Clinical assessments Body mass index (BMI) was measured at baseline. The number of comorbid illnesses was documented regardless of severity or chronicity. Cardiovascular risk score was calculated using the office-based cardiovascular risk profile prediction function from the Framingham Heart Study;[93] higher scores indicate higher risks of cardiovascular events. Gait function was assessed as part of the neurological examination. Functional assessment questionnaire (FAQ),[94] geriatric depression scale (GDS)[95] and neuropsychiatric inventory questionnaire (NPI-Q)[96] were all included to reflect the global function and behavior of participants.

Cognitive measures CDR scale[97], MMSE score and Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog) were used to evaluate cognitive performance at enrollment.

Statistical Analysis Predictors were treated as continuous variables, except sex, smoking, family history of AD, APOE 4 carrier and gait, which were dichotomous. We first examined factors that influenced whether biomarkers were obtained at baseline. The outcome was the indicator (missing = 1; non-missing = 0) of missing data for biomarkers (blood, CSF, PET and MRI) in each diagnostic group (NC, MCI and AD) and the aforementioned demographic, clinical and cognitive predictors were entered into the logistic regression model one at a time for univariate analyses. Odds ratios (ORs) were calculated; ORs > 1 indicated increased probability of missingness and ORs < 1 indicated increased probability of remaining in the study for each unit increase of predictors. Significant predictors in univariate models were subsequently pooled into a multivariable model to test the robustness as some of these predictors might correlate with one another. MCAR assumptions would be violated if the missingness was associated with any of these predictors.

Secondly, we were interested in factors associated with loss to follow up once participants enrolled in biomarker studies. For participants who had baseline biomarkers, we defined longitudinal missingness as having only baseline without further lumbar puncture for CSF biomarkers and having only measures within the first year for blood, PET and MRI biomarkers without longer follow-ups. In addition to the predictors above, we included baseline biomarker values (blood homocysteine, CSF $A\beta_{42}$ and tau, FDG-PET ROIs, MRI hippocampal volume) in these longitudinal analyses.

All statistical analyses and graphics were performed in R version 2.11.1. All tests of statistical significance were conducted at the two-tailed alpha level of 0.05.

Results

Baseline demographic and clinical features, biomarker values and year of last visit in ADNI are shown in Table 3-1. Regardless of whether biomarkers were obtained at the visit, most participants were followed up for over a year (NC: 93%, MCI: 85%, AD: 81%); there were 8 participants (NC: 1; MCI: 4; AD: 3) who died during the first year and 23 who died during the 3-year observation. All participants had at least one blood test (819/819, 100%) with the majority having a MRI scan (814/819, 99%), and more than half of participants in each diagnostic group had at least one CSF study (418/819, 51%) or one PET scan (455/819, 56%). Although the sample size in general shrank over time, the majority of participants who had baseline tests had biomarkers repeatedly measured longer than a year.

In CSF studies, a family history of AD was associated with having CSF measured at baseline for participants with MCI or AD, but no evidence was found against MCAR for the NC group at enrollment (Table 3-2). During follow-ups for CSF biomarkers, higher baseline ADAS-cog scores (worse cognitive performance) predicted drop-outs for NC and higher levels of baseline β-amyloid in CSF predicted drop-outs for MCI (Table 3-3). Thus the NC group tended to keep cognitively normal participants; while the MCI group tended to recruit individuals with an AD family history and retain those who were more AD-like in the longitudinal CSF study.

In PET studies, we found no evidence against MCAR for the NC group at enrollment. MCI participants with lower ADAS-cog scores (better cognitive performance) as opposed to AD participants with more neuropsychiatric complaints and higher CDR scores were more likely to be included in PET studies (Table 3-4). During follow-ups for PET, female normal participants were more likely to drop out, depression and lower cognitive performance predicted missing data in the MCI group, while family history of AD, APOE 4 carrier and higher cardiovascular risk scores were associated with drop-outs in the AD group (Table 3-5). Baseline FDG-PET results did not predict missing data in subsequent visits for all three groups.

During follow-ups for MRI after the first year, poor cognitive performance (lower MMSE scores and higher ADAS-cog scores) was predictive of missing data even for the NC group; depression stood out among all other factors in a multivariable model to be associated with drop-outs in MCI; and a family history of AD and higher CDR scores characterized AD participants who stayed in the study. Baseline MRI hippocampal volume was not predictive of missing data during follow-ups (Table 3-6).

For blood tests, lower cognitive performance predicted missing data for NC and MCI during follow-ups. Higher cardiovascular risk scores and higher baseline levels of serum homocysteine were associated with drop-outs in AD (Table 3-7).

Discussion

The missing data structure varied across different biomarkers that were repeatedly measured in ADNI. For at least some of the measured parameters we show that missingness is not MCAR, although whether it is MAR or MNAR cannot be determined based on the observed data. Our findings indicate that using complete data analysis may result in biased estimates and that handling missing data must be tailored to the target biomarker.

MCI participants with positive family histories of AD and lower premorbid verbal intelligence were more likely to be included in CSF studies and a similar pattern was also seen in AD; these findings suggest that MCI/AD recruitment for CSF donation likely captured people with more AD characteristics. Subjects with positive family histories of AD may have learned about AD from family experience and thus be more motivated to participate in AD studies even though the study procedure is invasive. The motivation may be further enhanced when subjects themselves are cognitively impaired, have hopes of finding effective treatments, or in the case of MCI be apprehensive about converting to dementia. During CSF follow-ups, poor cognitive performance in NC and higher baseline CSF $A\beta_{42}$ in MCI predicted missingness, suggesting the NC group tended to retain relatively normal subjects and the MCI group would retain subjects with lower CSF $A\beta_{42}$ who have a higher likelihood of converting to AD. Thus using CSF biomarkers to track clinical progression in MCI would be predicted to result in an overestimation of the proportion of converters in longitudinal studies or clinical trials.

Better cognitive function was associated with PET enrollment in MCI. This association, however, did not extend to the AD group who were more likely to enroll if more impaired. The AD group tended to retain APOE 4 positive individuals, those with positive family histories, and those with lower cardiovascular risk, suggesting that following up AD patients using PET scans may capture more purely AD than those with more vascular risk factors. This demonstrates that the missing data structure in MCI and AD should not be assumed to be the same.

Cognitive impairment, particularly decision-making impairment, may reduce the willingness to participate in research;[98] and this may explain our observations in the MCI group. But for AD patients who are overtly demented, surrogates may have more involvement in the decision-making process[99] which would explain the associations between greater impairment and participation and retention in the PET and MRI components. However, for patients with comorbid illnesses, such as cardiovascular diseases, surrogates may be concerned that the overall benefit/risk ratio does not favor longer participation[100] or such subjects may be more likely to drop out due to medical illness. We cannot confirm these explanations without interviewing both patients and study partners, but our observation at least demonstrates that retained MCI and AD patients in a follow-up study belong to two selected groups. These data suggest that caution is required when assuming that MCI and AD represent the same cognitive spectrum, especially when using PET scans to track disease progression.

Loss of follow up in MRI studies was conditional on poor cognitive performance in both NC and MCI but not in AD, which again suggests that cognitive impairment may have differential

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influence on following participants with MCI and AD. In line with CSF studies, baseline cognitive performance despite the limited variability among people considered cognitively normal is still associated with the long term drop outs in MRI studies. Similar to PET studies, depression was also associated with missingness in follow-up MRI scans, suggesting that depression is the major factor driving longitudinal missingness of imaging markers among all covariates considered in the study.

Since repeated blood tests are the standard source of biomarkers in population health studies, blood biomarkers can serve as a control variable to compare missing data patterns across different biomarkers. Poor cognitive function seemed to affect participation in long term follow-ups in NC and MCI groups. After a diagnosis of AD, cognitive function was no longer critical in determining the missingness. Interestingly, similar to the results from the PET studies, higher baseline homocysteine and higher cardiovascular risk in AD were associated with loss of follow-up, suggesting that AD patients with vascular risk factors may be more likely to drop out of longitudinal studies per se.

Our study has several strengths. First, the design of ADNI emulates a typical clinical trial in terms of case enrollment criteria, multi-center setting, standardized outcome measures and follow-up protocols, making our results generalizable to other AD clinical trials. However, we recognize that ADNI is not a clinical trial; missingness related to adverse drug effects or hope of improvement cannot be addressed in this observational study. Second, biomarkers in ADNI have been demonstrated to be useful in tracking AD progression. Future clinical trials for AD will likely incorporate these biomarkers to track cognitive decline and similar missing data challenges may be encountered; therefore our ADNI case study is of high reference value. Third, the ADNI study provides comprehensive data on demographic features, laboratory tests and clinical assessments, allowing us to systematically examine the missing data structure and plausibly test MCAR and MAR assumptions.

There are also several limitations in the study. First, despite the comprehensive approach taken in ADNI, we can never be certain whether missing data is MAR or MNAR based on the observed data. Second, we acknowledge that some ORs were just barely statistically significant and results might be due to multiple comparisons as we included more than a dozen potential predictors in the models. However, all of these predictors were selected based on *a priori* hypotheses and most of these significant predictors were coherent with the missingness across biomarkers and diagnostic groups rather than reflecting a random set of variables. Third, although one can hypothesize plausible reasons why certain predictors might predict drop-out, we could not confirm these, being neither able to interview the individuals nor to collect information on the reasons for missingness. Fourth, three diagnostic groups had different visit schedules, making the missing data structures of NC, MCI and AD less comparable. Thus we should be conservative in making inferences about inter-group difference.

How best to handle missing data is the subject of considerable interest and debate. Ideally the method chosen should be based on the assumptions one is willing to make regarding missingness. For example, popular methods such as multiple-imputation, maximum likelihood or weighted estimating equation methods are typically based on the missing at random assumption.[87,90,101] A possible alternative is to stratify by biomarker-specific missingness predictors and perform a complete case analysis, although this increases the complexity of trial design, and assumes that predictors of missingness are consistent across studies.

Longitudinal missingness in ADNI is not completely at random and CSF and imaging markers may bias longitudinal parameters in different directions. Poor cognitive performance at baseline is predictive of missingness even for cognitively normal participants but may be less critical for AD patients. Depression is a strong predictor for missingness of imaging biomarkers. Patterns of longitudinal missingness may reflect their different levels of accessibility, invasiveness, public awareness, and surrogate decision-making in relation to dementia. Dealing with the missing data in a cohort study or clinical trial for dementia should be tailored to the target biomarker and cognitive stage.

Table 3-1 Baseline characteristics of 819 participants in ADNI

	ADNI diagnostic group		
	NC	MCI	AD
Sample size	229	397	193
Demographic features			
Mean age, y (SD)	75.1 (5.0)	74.0 (7.5)	74.6 (7.5)
M : F, n	119 : 110	256 : 141	102 : 91
Education, y (SD)	16.0 (2.9)	15.7 (3.0)	14.7 (3.1)
Occupation, n (%)			
I	138 (60.3)	190 (47.9)	75 (38.9)
II	54 (23.6)	115 (29.0)	59 (30.6)
III	37 (16.2)	92 (23.2)	59 (30.6)
Smoker, n (%)	85 (37.1)	163 (41.1)	75 (38.9)
AD family history, n (%)	59 (25.8)	101 (25.4)	45 (23.3)
ANART error, n, mean (SD)	9.5 (8.8)	13.6 (9.9)	15.8 (10.0)
APOE 4 carrier, n (%)	61(26.6)	212 (53.4)	127 (65.8)
Clinical features			
Body mass index, mean (SD)	26.7 (4.4)	26.0 (4.0)	25.6 (3.9)
Comorbidity, n, mean (SD)	5 (3.0)	5 (3.0)	5 (3.3)
CVD risk score, mean (SD)	18.9 (3.6)	18.4 (3.9)	18.7 (4.1)
FAQ score, mean (SD)	0.1 (0.6)	3.9 (4.5)	13.0 (6.8)
GDS score, mean (SD)	0.8 (1.1)	1.6 (1.4)	1.7 (1.4)
NPI-Q score, mean (SD)	0.4 (0.9)	1.9 (2.7)	3.5 (3.3)
Abnormal gait, n (%)	12 (5.2)	36 (9.1)	35 (18.1)
Cognitive performance			
CDR scale, mean (SD)	0 (0)	0.5 (0.03)	0.7 (0.3)
MMSE score, mean (SD)	29.1 (1.0)	27.0 (1.8)	23.3 (2.1)
ADAS-cog, mean (SD)	6.2 (2.9)	11.5 (4.4)	18.6 (6.3)
Mean biomarker value			

Blood homocysteine, µM/L	10.0(n=227)	10.6 (n=393)	10.8 (n=193)
CSF Aβ$_{42}$, pg/ml	205.6 (n=114)	163.7 (n=198)	143.0 (n=102)
CSF Tau, pg/ml	69.7 (n=114)	103.6 (n=195)	121.6 (n=100)
FDG-PET ROIs, normalized intensity	1.28 (n=103)	1.20 (n=203)	1.08 (n=97)
MRI hippocampal volume, mm^3	3633 (n=228)	3233 (n=393)	2895 (n=193)
Year of last visit			
Within 1st year, n	16	59	37
Within 2nd year, n	8	45	140
Within 3rd year, n	91	152	14
After 3rd year, n	114	141	2

ADNI: Alzheimer's Disease Neuroimaging Initiative; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; Occupation: I: professional/managerial; II: skilled; III: partly skilled/unskilled. ANART: American national adult reading test; CVD: cardiovascular disease; FAQ: functional assessment questionnaire; GDS: geriatric depression scale; NPI-Q: neuropsychiatric inventory questionnaire; CDR: clinical dementia rating; MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale- cognitive subscale; FDG-PET ROIs: fludeoxyglucose F18- PET region-of-interest.

Table 3-2 Univariate association with missing CSF at baseline

	Odds ratios (95% CI)		
	NC	MCI	AD
Missing n / total	113 / 229	197 / 397	91 / 193
Demographic features			
Age, y	1.03 (0.97-1.08)	1.01 (0.99-1.04)	1.02 (0.98-1.06)
Female	0.91 (0.54-1.54)	1.25 (0.83-1.89)	1.53 (0.87-2.72)
Education, y	1.07 (0.98-1.18)	0.97 (0.91-1.03)	0.91 (0.82-0.99)*
Occupation type	0.88 (0.62-1.24)	0.98 (0.77-1.25)	1.18 (0.84-1.67)
Smoker	0.86 (0.50-1.48)	1.05 (0.70-1.56)	0.68 (0.38-1.22)
Family history of AD	0.99 (0.55-1.79)	0.55 (0.34-0.87)*†	0.47 (0.23-0.94)*
APOE 4 carrier	1.42 (0.79-2.57)	0.95 (0.64-1.41)	0.70 (0.38-1.27)
ANART error, n	0.99 (0.96-1.02)	0.98 (0.96-0.99)*†	1.02 (0.99-1.05)
General clinical features			
Body mass index	0.99 (0.94-1.06)	1.02 (0.97-1.08)	0.99 (0.93-1.07)
Comorbidity, n	1.04 (0.95-1.13)	0.99 (0.93-1.06)	0.99 (0.90-1.08)
CVD risk score	1.01 (0.94-1.09)	1.04 (0.99-1.09)	1.04 (0.97-1.11)
FAQ score	0.91 (0.56-1.43)	1.01 (0.96-1.05)	1.01 (0.97-1.06)
GDS score	0.98 (0.78-1.23)	0.91 (0.78-1.05)	1.00 (0.82-1.22)
NPI-Q score	1.34 (0.98-1.93)	1.03 (0.96-1.11)	1.04 (0.95-1.13)
Abnormal gait	0.50 (0.13-1.62)	0.80 (0.40-1.59)	0.81 (0.38-1.69)
Cognitive performance			
CDR scale	NA	NA	2.00 (0.64-6.26)
MMSE score	1.02 (0.79-1.33)	1.07 (0.95-1.19)	0.90 (0.78-1.03)
ADAS-cog	0.96 (0.88-1.05)	0.98 (0.94-1.03)	1.03 (0.98-1.08)

NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; ANART: American national adult reading test; CVD: cardiovascular disease; FAQ: functional assessment questionnaire; GDS: geriatric depression scale; NPI-Q: neuropsychiatric inventory questionnaire; CDR: clinical dementia rating; MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale- cognitive subscale. NA: not applicable. In logistic regression models: sex: 1 = male; 2 = female; occupation: 1 = professional/managerial; 2 = skilled; 3 = partly skilled/unskilled; gait: 1 = normal; 2 = abnormal. The dependent variable is the indicator (missing = 1; non-missing = 0) for missing biomarkers. Odds ratios > 1 indicate increased probability of missingness for each unit increase of predictors; while odds ratios < 1 indicate increased probability of remaining in the study for each unit increase of predictors. * $p < 0.05$. † Statistical significance remained in a multivariable model.

Table 3-3 Univariate association with missing CSF during follow-up

	Odds ratios (95% CI)		
	NC	MCI	AD
Missing n / total	20 / 116	45 / 200	28 / 102
Demographic features			
Age, y	0.99 (0.91-1.10)	0.96 (0.92-1.01)	0.97 (0.92-1.03)
Female	1.04 (0.39-2.76)	1.16 (0.57-2.31)	1.04 (0.43-2.50)
Education, y	1.05 (0.88-1.26)	0.91 (0.81-1.01)	0.95 (0.84-1.09)
Occupation type	0.74 (0.36-1.38)	1.13 (0.75-1.69)	0.74 (0.42-1.25)
Smoking	0.82 (0.29-2.20)	0.97 (0.49-1.91)	0.98 (0.40-2.36)
Family history of AD	0.45 (0.10-1.48)	0.88 (0.41-1.79)	1.20 (0.45-3.02)
APOE 4 carrier	1.12 (0.33-3.27)	0.97 (0.50-1.89)	0.89 (0.36-2.35)
ANART error, n	1.01 (0.96-1.07)	1.02 (0.99-1.05)	1.01 (0.96-1.06)
General clinical features			
Body mass index	0.94 (0.83-1.06)	1.08 (0.99-1.17)	1.03 (0.92-1.16)
Comorbidity, n	0.94 (0.78-1.11)	1.01 (0.90-1.12)	1.02 (0.90-1.15)
CVD risk score	1.02 (0.89-1.17)	0.98 (0.90-1.07)	1.00 (0.90-1.12)
FAQ score	1.12 (0.45-2.08)	1.03 (0.96-1.11)	0.99 (0.92-1.05)
GDS score	1.20 (0.78-1.79)	1.14 (0.90-1.44)	1.31 (0.95-1.80)
NPI-Q score	1.18 (0.52-2.28)	1.12 (0.98-1.28)	1.09 (0.96-1.25)
Abnormal gait	1.67 (0.23-7.93)	1.17 (0.36-3.22)	0.86 (0.25-2.50)
Cognitive performance			
CDR scale	NA	NA	1.29 (0.22-7.46)
MMSE score	1.06 (0.67-1.79)	1.06 (0.88-1.29)	1.02 (0.81-1.29)
ADAS-cog	1.22 (1.03-1.45)*	0.97 (0.90-1.04)	0.98 (0.91-1.06)
Baseline CSF			
$A\beta_{1-42}$ ‡	1.00 (0.92-1.09)	1.08 (1.02-1.15)*†	1.07 (0.96-1.19)
Tau ‡	0.99 (0.83-1.16)	0.99 (0.92-1.04)	0.98 (0.90-1.06)

NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; ANART: American national adult reading test; CVD: cardiovascular disease; FAQ: functional assessment questionnaire; GDS: geriatric depression scale; NPI-Q: neuropsychiatric inventory questionnaire; CDR: clinical dementia rating; MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale- cognitive subscale. NA: not applicable. In logistic regression models: sex: 1 = male, 2 = female; occupation: 1 = professional/managerial, 2 = skilled, 3 = partly skilled/unskilled; gait: 1 = normal, 2 = abnormal. The dependent variable is the indicator (missing = 1; non-missing = 0) for missing biomarkers. Odds ratios > 1 indicate increased probability of missingness for each unit increase of predictors; while odds ratios < 1 indicate increased probability of remaining in the study for each unit increase of predictors. * $p < 0.05$. † Statistical significance remained in a multivariable model. ‡ Odds ratios for each 10 pg/ml increase.

Table 3-4 Univariate association with missing PET at baseline

	Odds ratios (95% CI)		
	NC	MCI	AD
Missing n / total	96 / 229	173 / 397	95 / 193
Demographic features			
Age, y	1.01 (0.96-1.07)	1.01 (0.98-1.03)	0.99 (0.95-1.03)
Female	1.53 (0.90-2.60)	1.40 (0.93-2.12)	1.83 (1.04-3.26)*†
Education, y	1.07 (0.98-1.18)	0.96 (0.90-1.03)	1.01 (0.92-1.11)
Occupation type	0.89 (0.62-1.26)	1.22 (0.96-1.56)	0.97 (0.69-1.36)
Smoking	0.95 (0.55-1.64)	1.18 (0.79-1.77)	1.56 (0.87-2.81)
Family history of AD	0.85 (0.46-1.55)	0.90 (0.57-1.41)	1.76 (0.90-3.52)
APOE 4 carrier	1.37 (0.76-2.47)	1.03 (0.69-1.53)	1.05 (0.58-1.90)
ANART error, n	0.99 (0.96-1.03)	1.01 (0.99-1.03)	1.01 (0.98-1.04)
General clinical features			
Body mass index	1.02 (0.96-1.09)	0.97 (0.92-1.02)	0.97 (0.90-1.05)
Comorbidity, n	0.97 (0.88-1.06)	0.98 (0.92-1.05)	0.97 (0.89-1.06)
CVD risk score	0.99 (0.92-1.07)	1.01 (0.96-1.06)	1.00 (0.93-1.07)
FAQ score	0.83 (0.46-1.30)	1.04 (0.99-1.09)	0.98 (0.94-1.02)
GDS score	0.87 (0.68-1.10)	0.99 (0.86-1.15)	0.94 (0.77-1.15)
NPI-Q score	0.79 (0.54-1.08)	0.99 (0.92-1.07)	0.91 (0.83-0.99)*
Abnormal gait	1.41 (0.43-4.65)	1.51 (0.76-3.03)	0.63 (0.30-1.32)
Cognitive performance			
CDR scale	NA	NA	0.16 (0.05-0.51)*†
MMSE score	1.14 (0.87-1.50)	0.91 (0.81-1.02)	0.92 (0.80-1.05)
ADAS-cog	0.95 (0.87-1.04)	1.09 (1.04-1.14)*	0.98 (0.94-1.03)

NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; ANART: American national adult reading test; CVD: cardiovascular disease; FAQ: functional assessment questionnaire; GDS: geriatric depression scale; NPI-Q: neuropsychiatric inventory questionnaire; CDR: clinical dementia rating; MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale- cognitive subscale. FDG: fludeoxyglucose. NA: not applicable. In logistic regression models: sex: 1 = male; 2 = female; occupation: 1 = professional/managerial; 2 = skilled; 3 = partly skilled/unskilled; gait: 1 = normal; 2 = abnormal. The dependent variable is the indicator (missing = 1; non-missing = 0) for missing biomarkers. Odds ratios > 1 indicate increased probability of missingness for each unit increase of predictors; while odds ratios < 1 indicate increased probability of remaining in the study for each unit increase of predictors. * $p <$ 0.05. † Statistical significance remained in a multivariable model.

Table 3-5 Univariate association with missing PET during follow-up

	Odds ratios (95% CI)		
	NC	MCI	AD
Missing n / total	46 / 133	62 / 224	39 / 98
Demographic features			
Age, y	0.97 (0.88-1.06)	0.99 (0.95-1.05)	0.98 (0.92-1.03)
Female	3.47 (1.41-9.19)*	0.68 (0.31-1.40)	0.82 (0.35-1.87)
Education, y	0.92 (0.79-1.06)	0.93 (0.83-1.04)	0.99 (0.88-1.13)
Occupation type	1.64 (0.95-2.81)	1.00 (0.64-1.53)	0.92 (0.56-1.50)
Smoking	1.13 (0.45-2.74)	1.83 (0.93-3.59)	2.70 (1.15-6.51)*
Family history of AD	0.31 (0.07-0.97)	0.48 (0.19-1.09)	0.15 (0.02-0.58)*†
APOE 4 carrier	0.37 (0.08-1.18)	1.45 (0.74-2.89)	0.40 (0.17-0.95)*
ANART error, n	1.04 (0.99-1.09)	0.99 (0.95-1.02)	1.01 (0.97-1.05)
General clinical features			
Body mass index	1.06 (0.95-1.19)	1.03 (0.95-1.12)	0.94 (0.84-1.05)
Comorbidity, n	0.94 (0.80-1.08)	0.98 (0.87-1.10)	0.90 (0.78-1.02)
CVD risk score	1.07 (0.95-1.23)	1.02 (0.94-1.11)	1.13 (1.01-1.28)*
FAQ score	NA	1.05 (0.96-1.13)	1.01 (0.95-1.08)
GDS score	1.04 (0.72-1.45)	1.34 (1.07-1.67)*†	0.97 (0.72-1.29)
NPI-Q score	0.92 (0.51-1.38)	1.06 (0.95-1.19)	1.05 (0.93-1.18)
Abnormal gait	NA	1.33 (0.36-3.98)	0.42 (0.13-1.19)
Cognitive performance			
CDR scale	NA	NA	1.20 (0.23-6.42)
MMSE score	0.68 (0.46-1.01)	0.94 (0.77-1.15)	0.93 (0.77-1.12)
ADAS-cog	1.14 (0.99-1.32)	1.10 (1.02-1.20)*†	1.04 (0.98-1.11)
Baseline FDG uptake, normalized intensity	0.29 (0.00-19.8)	0.47 (0.03-6.71)	0.13 (0.01-2.90)

NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; ANART: American national adult reading test; CVD: cardiovascular disease; FAQ: functional assessment questionnaire; GDS: geriatric depression scale; NPI-Q: neuropsychiatric inventory questionnaire; CDR: clinical dementia rating; MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale- cognitive subscale. FDG: fludeoxyglucose. NA: not applicable. In logistic regression models: sex: 1 = male; 2 = female; occupation: 1 = professional/managerial; 2 = skilled; 3 = partly skilled/unskilled; gait: 1 = normal; 2 = abnormal. The dependent variable is the indicator (missing = 1; non-missing = 0) for missing biomarkers. Odds ratios > 1 indicate increased probability of missingness for each unit increase of predictors; while odds ratios < 1 indicate increased probability of remaining in the study for each unit increase of predictors. * $p <$ 0.05. † Statistical significance remained in a multivariable model.

Table 3-6 Univariate association with missing MRI during follow-up

| | Odds ratios (95% CI) | | |
	NC	MCI	AD
Missing n / total	47 / 228	85 / 393	86 / 193
Demographic features			
Age, y	1.03 (0.97-1.10)	1.01 (0.98-1.05)	0.99 (0.96-1.03)
Female	1.15 (0.61-2.20)	0.87 (0.52-1.44)	1.04 (0.59-1.84)
Education, y	0.93 (0.83-1.04)	0.93 (0.86-0.99)*	0.93 (0.85-1.02)
Occupation type	1.29 (0.85-1.93)	1.12 (0.83-1.50)	1.24 (0.88-1.76)
Smoking	0.84 (0.42-1.62)	1.28 (0.78-2.07)	1.15 (0.64-2.06)
Family history of AD	0.36 (0.13-0.85)*†	0.67 (0.36-1.18)	0.36 (0.17-0.74)*†
APOE 4 carrier	0.59 (0.25-1.25)	1.15 (0.71-1.88)	0.59 (0.32-1.08)
ANART error, n	1.01 (0.98-1.05)	1.01 (0.98-1.03)	0.99 (0.97-1.02)
General clinical features			
Body mass index	0.93 (0.86-1.01)	1.02 (0.96-1.08)	0.96 (0.89-1.04)
Comorbidity, n	0.99 (0.88-1.10)	0.97 (0.89-1.05)	0.96 (0.88-1.05)
CVD risk score	0.97 (0.89-1.06)	1.02 (0.96-1.09)	1.04 (0.97-1.12)
FAQ score	0.67 (0.20-1.29)	1.01 (0.96-1.06)	0.99 (0.95-1.03)
GDS score	1.03 (0.77-1.35)	1.23 (1.03-1.45)*†	0.97 (0.79-1.19)
NPI-Q score	0.96 (0.63-1.34)	1.09 (1.01-1.19)*	1.02 (0.94-1.11)
Abnormal gait	0.76 (0.11-3.01)	1.04 (0.43-2.27)	0.51 (0.23-1.08)
Cognitive performance			
CDR scale	NA	NA	0.28 (0.09-0.89)*†
MMSE score	0.69 (0.51-0.93)*†	0.93 (0.81-1.06)	1.01 (0.88-1.16)
ADAS-cog	1.21 (1.08-1.36)*†	1.06 (1.01-1.12)*	0.99 (0.95-1.04)
Baseline MRI hippocampal volume, mm^3	1.00 (0.99-1.00)	1.00 (0.99-1.00)	0.99 (0.99-1.00)

NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; ANART: American national adult reading test; CVD: cardiovascular disease; FAQ: functional assessment questionnaire; GDS: geriatric depression scale; NPI-Q: neuropsychiatric inventory questionnaire; CDR: clinical dementia rating; MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale- cognitive subscale. NA: not applicable. In logistic regression models: sex: 1 = male; 2 = female; occupation: 1 = professional/managerial; 2 = skilled; 3 = partly skilled/unskilled; gait: 1 = normal; 2 = abnormal. The dependent variable is the indicator (missing = 1; non-missing = 0) for missing biomarkers. Odds ratios > 1 indicate increased probability of missingness for each unit increase of predictors; while odds ratios < 1 indicate increased probability of remaining in the study for each unit increase of predictors. * $p < 0.05$. † Statistical significance remained in a multivariable model.

Table 3-7 Univariate association with missing blood sample during follow-up

	Odds ratios (95% CI)		
	NC	MCI	AD
Missing n / total	27 / 229	100 / 397	66 / 193
Demographic features			
Age, y	1.03 (0.95-1.11)	0.99 (0.96-1.02)	1.02 (0.98-1.06)
Female	1.19 (0.53-2.69)	1.22 (0.76-1.95)	0.99 (0.54-1.80)
Education, y	0.95 (0.83-1.10)	0.93 (0.87-1.00)	1.02 (0.92-1.12)
Occupation type	1.49 (0.90-2.43)	1.12 (0.85-1.48)	1.12 (0.78-1.61)
Smoking	0.68 (0.27-1.59)	1.05 (0.66-1.66)	1.52 (0.83-2.79)
Family history of AD	0.33 (0.08-0.98)	0.78 (0.45-1.32)	0.63 (0.29-1.30)
APOE 4 carrier	0.59 (0.19-1.53)	1.15 (0.73-1.82)	0.64 (0.34-1.19)
ANART error, n	1.01 (0.97-1.06)	1.01 (0.98-1.03)	0.97 (0.94-1.00)
General clinical features			
Body mass index	0.91 (0.81-1.01)	1.01 (0.95-1.07)	0.99 (0.92-1.07)
Comorbidity, n	0.99 (0.86-1.12)	0.96 (0.88-1.04)	1.02 (0.93-1.11)
CVD risk score	0.98 (0.88-1.10)	1.02 (0.97-1.09)	1.09 (1.01-1.17)*
FAQ score	NA	1.02 (0.96-1.07)	1.02 (0.98-1.07)
GDS score	1.01 (0.69-1.40)	1.15 (0.98-1.35)	0.99 (0.81-1.23)
NPI-Q score	0.98 (0.55-1.43)	1.05 (0.97-1.14)	1.03 (0.94-1.12)
Abnormal gait	1.54 (0.23-6.26)	0.69 (0.27-1.55)	0.73 (0.31-1.59)
Cognitive performance			
CDR scale	NA	NA	0.72 (0.22-2.36)
MMSE score	0.72 (0.50-1.04)	0.86 (0.75-0.98)*	0.99 (0.86-1.15)
ADAS-cog	1.24 (1.08-1.42)*	1.09 (1.03-1.14)*†	1.01 (0.96-1.06)
Baseline blood Hcyt, micromoles/L	0.87 (0.73-1.02)	1.02 (0.94-1.10)	1.10 (1.01-1.21)*

NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; ANART: American national adult reading test; CVD: cardiovascular disease; FAQ: functional assessment questionnaire; GDS: geriatric depression scale; NPI-Q: neuropsychiatric inventory questionnaire; CDR: clinical dementia rating; MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale- cognitive subscale. Hcyt: homocysteine. NA: not applicable. In logistic regression models: sex: 1 = male; 2 = female; occupation: 1 = professional/managerial; 2 = skilled; 3 = partly skilled/unskilled; gait: 1 = normal; 2 = abnormal. The dependent variable is the indicator (missing = 1; non-missing = 0) for missing biomarkers. Odds ratios > 1 indicate increased probability of missingness for each unit increase of predictors; while odds ratios < 1 indicate increased probability of remaining in the study for each unit increase of predictors. * $p < 0.05$. † Statistical significance remained in a multivariable model.

Chapter 4

Effect of Cognitive Reserve on Alzheimer Pathological Progression

Introduction

Reserve is a hypothetical construct proposed to explain the disjunction between the burden of Alzheimer disease (AD) pathology and the degree of cognitive dysfunction in some older people.[34] Two kinds of reserve have been proposed: brain reserve and cognitive reserve; here we use cognitive reserve to refer to both types.[43]

Education, a common proxy of cognitive reserve, is robustly and consistently associated with a lower risk of AD across studies.[32,102,103] Similar findings were also reported in studies using occupation, premorbid intelligence or brain size as a reserve proxy.[32,104-107] Once cognitive impairment begins, people with higher reserve appear to have faster cognitive deterioration.[108-110] It is assumed that by the time dementia is diagnosed, more AD pathology has accumulated in people with higher reserve, and therefore accelerated clinical deterioration occurs. Cognitive reserve may reduce the risk of symptomatic expression of AD or modulate the course of cognitive decline but reserve *per se* supposedly has no biological effect on AD pathology and thus is independent of AD pathological progression.[111]

In previous chapters, I have described stage-dependent trajectories of three biomarkers of AD pathology: CSF $A\beta_{42}$, [^{18}F] fluorodeoxyglucose PET (FDG-PET) uptake and MRI hippocampal volume[112] and the patterns of longitudinal missing biomarker data;[113] but how these pathology markers change over time in relation to individual reserve status is not known. In this chapter I aim to examine the influence of cognitive reserve, indexed by education, occupation, American National Adult Reading Test (ANART) and intracranial volume (ICV), on longitudinal change of AD pathology in ADNI.

Methods

Study Population ADNI is supported by the NIH, private pharmaceutical companies, and non-profit organizations, with the primary goal of examining the utility of serial biomarker measurement in AD and pre-AD stages. The sources of ADNI full enrollment criteria and screening cognitive tests have been indicated in previous chapters. The study procedures were approved by institutional review boards of all participating institutions.

Biomarkers of AD Pathology

Protocols and acquisitions of CSF, FDG-PET and MRI biomarkers can be referenced from previous chapters.

Proxy Measures of Reserve

Education The number of completed years of formal education was recorded. Educational attainment in the entire ADNI population was divided into tertiles: high (> 17 years), intermediate (15-17 years) and low (< 15 years) reserve levels.

Occupation Occupation types were recorded and classified into three levels: I. professional or managerial, II. skilled and III. partly-skilled or unskilled occupations according to The National Statistics Socio-economic Classification.[91] These three levels were used to approximate reserve status.

Premorbid intelligence American National Adult Reading Test (ANART) was used to estimate premorbid intelligence.[114] Participants were tested by asking to pronounce a total of 50 English words that did not follow regular grapheme-phoneme and stress rules. The number of mispronounced words was then recorded. More errors predict lower premorbid intelligence. ANART in the entire ADNI population was stratified into tertiles: high (< 8 errors), intermediate (8-16 errors) and low (> 16 errors) reserve levels.

Intracranial volume Intracranial volume (ICV) was estimated by the automated MRI method, which combined three tissue classes of segmentation: gray matter, white matter and CSF spaces. The ICV (cm^3) information is available in the ADNI image database. ICV in the entire ADNI population was stratified into tertiles: high (> 1626 cm^3), intermediate (1475-1626 cm^3) and low (< 1475 cm^3) reserve levels.

Statistical Analyses

Biomarker rates of change Participants with repeated measures were entered into analyses. We delineated biomarker trajectories and used repeated measures linear regression (an exchangeable working within subject correlation model via a generalized estimating equation, GEE)[72] to estimate average rates of change in CSF and imaging biomarkers. Time-varying biomarkers were treated as the outcome and modeled by time and baseline age in the regression.

Longitudinal effect of reserve We used tertiles of reserve for primary analyses. To ensure that the longitudinal effect of reserve, if any, was not through baseline differences, we first examined the associations between reserve proxies and baseline biomarkers in multivariable linear regression models adjusting for age and sex. Each reserve proxy as well as its interaction with time (reserve proxy $\times$ time) was then entered into the GEE models of biomarkers in NC, MCI and AD. Coefficients of the interaction terms reflected the direction and magnitude of how cognitive reserve modified biomarker rates of change at different stages.

Longitudinal missing data Several baseline features have been identified as missing data predictors during follow-up.[113] To ensure that the effect of reserve on biomarker change was not confounded by missing data, we examined the associations between reserve proxies and missing data predictors using Pearson correlation coefficients. A correlation of 0.4 or less was considered insignificant.

Sensitivity analyses APOE 4 carriers are predisposed to develop AD and a previous study from ADNI also demonstrated that APOE 4 accelerated hippocampal atrophy in MCI and AD.[112] Therefore, we included APOE 4 carrier status in GEE models to test the robustness of any reserve effect. In addition to primary analyses using stratified reserve levels, we also used original continuous measures (e.g. years of education) to confirm that the statistical significance was not due to artificial stratification.

All statistical analyses and graphics were performed in R version 2.11.1. All tests of statistical significance were conducted at the two-tailed alpha level of 0.05.

Results

Trends of decreasing cognitive function, reserve states and AD biomarkers from NC to MCI to AD characterized the inter-group difference at baseline in ADNI (Table 4-1). After adjusting for age and sex, ICV correlated with MRI hippocampal volume in all diagnostic groups while ANART was associated with hippocampal volume only in AD; otherwise, reserve proxies were independent of CSF and imaging biomarkers at baseline. ICV was thus not included in the analysis of MRI hippocampal change. Except for a correlation ($r = 0.51$) between sex and ICV in FDG-PET follow-ups in the NC group, all other Pearson correlation coefficients for missing data predictors and reserve proxies were negligible.

Cognitive reserve indexed by education, occupation and ANART significantly modified the rates of CSF $A\beta_{42}$ change in cognitively normal participants (Table 4-2). People with higher levels of reserve had slower rates of CSF $A\beta_{42}$ decline (Table 4-3). CSF $A\beta_{42}$ trajectories of high, intermediate and low cognitive reserve levels were modeled for participants at age 75 (Figure 4-1A to C). Further analyses of the NC group with repeated CSF studies ($n = 35$) showed that their education levels correlated with occupation ($r = 0.56$) and ANART ($r = 0.63$).

AD participants with better ANART scores had slower progression of glucose hypometabolism (Table 4-2). This pattern also appeared in the NC group, but was not statistically significant (Table 4-4). The rates of hippocampal atrophy in MRI were, however, not modified by any of these reserve proxies in all three diagnostic groups (Table 4-5). Unlike NC and AD groups, there was no effect of cognitive reserve on AD biomarker changes in the MCI group.

The effects of reserve on AD biomarker rates remained unchanged after accounting for APOE 4 and longitudinal missing data. For those whose biomarker rates of change were modified by cognitive reserve, we further examined their cognitive performance at baseline and there was no apparent difference in MMSE, ADAS-cog and AVLT across each reserve stratum (Table 4-6 and 4-7).

Discussion

To the best of our knowledge, this is the first longitudinal study showing the protective effect of cognitive reserve against AD pathological progression. We found that higher levels of education, occupation and premorbid intelligence decelerated the decline of CSF $A\beta_{42}$ in participants with normal cognition and the effect of premorbid intelligence extended to FDG uptake in patients with AD. The longitudinal effect of cognitive reserve on CSF $A\beta_{42}$ is consistent across different proxies, regardless of whether in continuous or categorical variables, and was not confounded by baseline cognitive function, missing data or APOE 4. Our findings agree with a recent cross-sectional study, which reported that greater lifetime cognitive engagement was associated with reduced $A\beta$ deposition measured by Pittsburg Compound B uptake (PIB).[115] Therefore, cognitive reserve may not only modify the effect of AD pathology on cognitive performance but also exert direct biological influence to slow pathological progression.

This protective effect of cognitive reserve was mainly found among cognitively normal participants. $A\beta$ deposition is considered a pivotal event in the AD pathological cascade which precedes cognitive impairment and triggers subsequent changes in tau, glucose hypometabolism,

and hippocampal atrophy.[80] Therefore, if reserve has any direct influence on AD pathology, the effect may be more likely detected in the dynamics of CSF $A\beta_{42}$ before any cognitive deficit is manifested. We also observed that cognitive reserve indexed by ANART modulated the decline of FDG uptake in patients with AD. FDG-PET is thought to primarily reflect synaptic activity.[116] In a classic study of synapses in autopsied AD specimens, synapse loss was associated with enlargement of the remaining synapses, stabilizing total synaptic contact.[117] These ultrastructural findings provided evidence of neural plasticity at the synaptic level that may correspond to our FDG-PET results, supporting a role for cognitive reserve in this process.[43]

The underlying mechanism of how cognitive reserve might shape AD pathological change is intriguing. Transgenic mice studies have shown that exposure to environmental enrichment reduced cerebral $A\beta$ deposition,[118] suggesting that AD pathology can be modulated by environmental experience. Furthermore, as $A\beta$ release is synaptically regulated, greater synaptic activity increases the level of $A\beta$ in brain interstitial fluid and leads to region-specific $A\beta$ aggregation.[119,120] These vulnerable regions overlap with a set of highly interconnected networks, also known as cortical hubs, which include posterior cingulate, lateral temporal, lateral parietal, and medial/lateral prefrontal regions.[121] The spatial convergence of cortical amyloid and these metabolically active cortical hubs leads to a unifying framework recently proposed to explain the relationship between lifespan brain activity and AD.[122] Based on the framework, these interconnected networks are responsible for information processing and therefore synaptically active, which may in turn provoke regional deposition of $A\beta$. The role of cognitive reserve in this model is proposed to support neural efficiency and flexibility for cognitive function. Thus, individuals with higher cognitive reserve would utilize more efficient neural processes, require less synaptic activation and have slower cerebral deposition of $A\beta$ detected as slower CSF $A\beta_{42}$ decline.

Larger brain size was associated with a lower risk of AD in some[107] if not all studies.[123] In our study, the effect of large ICV also appeared to protect against CSF $A\beta_{42}$ and FDG uptake decline in the NC group, though not with statistical significance. Our brain size measure was approximated by combining the volume of CSF space, gray and white matter; however, how valid and precise this approach is for defining maximum synapse count in adulthood or the physical basis of reserve is unclear.

MCI participants were enrolled in ADNI based on clinical criteria, but their underlying pathological profile is likely to be heterogeneous.[124] Despite a larger sample size than NC and AD groups, the effect of reserve on AD biomarker changes did not appear in the MCI group, consistent with a higher degree of heterogeneity in MCI than in NC or AD.

Education might mediate microstructural changes in hippocampus,[125] but we did not observe any effect of cognitive reserve on the rates of MRI hippocampal atrophy. Hippocampal volume is a macrostructural measure and the effect of education or other proxies may be more difficult to detect by volumetric MRI. Most importantly, hippocampal atrophy probably represents a late change in the AD pathological cascade and cognitive reserve may be no longer protective once the pathological cascade becomes advanced.

One of the unique strengths in our study is that we have repeated measures of AD biomarkers to delineate AD pathological changes over time. Unlike cross-sectional approaches, longitudinal study does not require the assumption that an age effect is uniform across different individuals.

For instance, two different participants at age 60 and 70 in the same group are treated separately and we do not assume that the pathological burden of the individual at age 60 will develop into what the 70 year old expresses after 10 years. Second, we have multiple reserve proxies typically employed in past studies: education, occupation, ANART and even ICV, all within the same database; which is rarely available in a population study. These reserve proxies share some features but also capture different components of the reserve construct: cognitive experience early in life, cognitive activity during adulthood, and steady-state linguistic capacity for example. Cognitive reserve is a hypothetical construct and cannot be directly measured. It is therefore beneficial to address the effect of cognitive reserve from more than one perspective. Third, ADNI comprises a wealth of information, allowing us to assess the potential confounding from missing data, APOE 4 and baseline cognitive function. Since all participants were enrolled at different stages in the disease course, baseline evaluation did not reflect their pathological states when they first had cognitive change. We therefore carefully applied GEE to avoid the unverifiable assumption about the distribution of baseline AD pathology.[73] The longitudinal structure and comprehensiveness of ADNI data, together with our statistical approach, all strengthen our results to be less biased.

There are several limitations in our study. First, although over 800 people participated in ADNI, the robust longitudinal effect of cognitive reserve mainly came from 35 cognitively normal subjects with 3 repeated CSF studies. This small sample size may be a concern if we want to generalize our results to a larger population. Nevertheless, missing data and other potential confounders have been considered in our analyses, so that the internal validity for all 229 participants in the NC group is likely achieved. In addition, the statistical significance from a small sample suggests that the actual effect may be striking. Second, there are only up to 3 time points of CSF $A\beta_{42}$ for longitudinal analysis. Although we could evaluate both change and the variance of change based on 3 repeated measures, we might need more data points to sufficiently minimize the effect of "regression toward the mean". However, lumbar puncture is an invasive procedure; it is practically challenging to repeat CSF studies especially on normal participants. Amyloid PET imaging, a similar but non-invasive biomarker, may replace the use of CSF $A\beta_{42}$ to follow cerebral amyloid dynamics in the future. Third, only 55 participants (6.7%) had less than 12 years of education, and highly educated participants were over-represented in the ADNI population (Table 4-8). Therefore, the effects we detected were relatively confined to those with actually moderate to high levels of education. Nevertheless this relationship was also seen in our other measures of reserve. Fourth, ADNI participants were enrolled based on clinical criteria but not biomarker measurement. They likely had developed various degrees of AD pathology by the time of enrollment. Although we showed that baseline AD biomarkers were independent of cognitive reserve levels, how baseline differences in pathology would affect rates of change is not known. We therefore need to assume that baseline AD pathology for participants within the same cognitive group is homogeneous. Ideally, we would like to begin with a group of people with a similar amount of AD pathological burden and then follow them to assess the effect of cognitive reserve.

High levels of cognitive reserve may not only reduce the risk of AD or modify the effect of AD pathology on cognitive performance but also have direct biological influence to slow $A\beta$ deposition and compensate for synapse loss in the brain. The protective effect of reserve mainly occurs before cognitive impairment, implying that if cognitive intervention can be effective in preventing the occurrence of AD it should be initiated as early as possible.

Table 4-1 Baseline characteristics of 819 participants in ADNI

	ADNI diagnostic group		
	NC	MCI	AD
Sample size	229	397	193
Mean age, y (SD)	75.1 (5.0)	74.0 (7.5)	74.6 (7.5)
M : F, n	119 : 110	256 : 141	102 : 91
MMSE score, mean (SD)	29.1 (1.0)	27.0 (1.8)	23.3 (2.1)
ADAS-cog, mean (SD)	6.2 (2.9)	11.5 (4.4)	18.6 (6.3)
APOE 4 carrier, n (%)	61(26.6)	212 (53.4)	127 (65.8)
Reserve proxy			
Education, y (SD)	16.0 (2.9)	15.7 (3.0)	14.7 (3.1)
Occupation, n (%)			
I	138 (60.3)	190 (47.9)	75 (38.9)
II	54 (23.6)	115 (29.0)	59 (30.6)
III	37 (16.2)	92 (23.2)	59 (30.6)
ANART error, n (SD)	9.5 (8.8)	13.6 (9.9)	15.8 (10.0)
Intracranial volume, cm^3 (SD)	1540 (158)	1570 (169)	1550 (184)
Mean biomarker value			
CSF $A\beta_{42}$, pg/ml	209.6 (n=34)	159.9 (n=51)	139.6 (n=16)
FDG-PET ROIs, normalized intensity	1.28 (n=103)	1.20 (n=203)	1.08 (n=97)
MRI hippocampal volume, mm^3	3633 (n=228)	3233 (n=393)	2895 (n=193)

ADNI: Alzheimer's Disease Neuroimaging Initiative; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; Occupation: I: professional/managerial; II: skilled; III: partly skilled/unskilled. ANART: American national adult reading test; MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale- cognitive subscale; FDG-PET ROIs: fludeoxyglucose F18- PET region-of-interest.

Table 4-2 Coefficients of cognitive reserve and biomarker rate interactions

Reserve proxy	Biomarker	Coefficient of (reserve × time)		
		NC	MCI	AD
Education	CSF Aβ$_{42}$	0.37*	-5.0×10^{-3}	0.20†
	FDG-PET	1.83×10^{-4}	-3.48×10^{-5}	5.25×10^{-4}
	Volumetric MRI	-0.38	0.52†	0.25
Occupation	CSF Aβ$_{42}$	0.40*	-1.8×10^{-3}	-0.03
	FDG-PET	1.54×10^{-4}	-2.72×10^{-5}	2.84×10^{-4}
	Volumetric MRI	-0.29	0.11	0.28
ANART	CSF Aβ$_{42}$	0.51*	0.04	0.18
	FDG-PET	5.65×10^{-4}	-1.23×10^{-4}	1.71×10^{-3}*
	Volumetric MRI	-0.19	0.19	-0.11
ICV	CSF Aβ$_{42}$	0.23	-0.12	0.14
	FDG-PET	2.74×10^{-4}	4.97×10^{-4}	-1.29×10^{-4}

NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; FDG-PET: [18F] fluorodeoxyglucose PET; ANART: American national adult reading test; ICV: intracranial volume; *$p < 0.05$ or the rate of change is significantly modified by cognitive reserve; †$p < 0.05$ only appears when reserve is stratified into 3 levels but disappears when using continuous variables, e.g. years of education.

Positive coefficients indicate that the biomarker decline is slower in participants with higher cognitive reserve.

Table 4-3 CSF Aβ$_{42}$ rates of change stratified by cognitive reserve in GEE models

Reserve proxy	Reserve level	CSF Aβ$_{42}$ rates of change (pg/ml/month)		
		NC	MCI	AD
Education	Low	-0.89* (n = 7)	-0.38 (n = 11)	-0.52* (n = 7)
	Intermediate	-0.58* (n =12)	-0.14 (n = 21)	-0.13 (n = 3)
	High	-0.20 (n =16)	-0.32* (n = 20)	-0.12* (n = 6)
Occupation	Low	-0.83* (n = 6)	-0.27 (n = 14)	-0.21 (n = 5)
	Intermediate	-0.90* (n = 9)	-0.26* (n = 9)	-0.40* (n = 6)
	High	-0.17 (n = 20)	-0.26* (n = 29)	-0.26* (n = 5)
ANART	Low	-1.24* (n = 6)	-0.21 (n = 20)	-0.48* (n = 7)
	Intermediate	-0.47* (n = 13)	-0.49* (n = 13)	-0.18* (n = 4)
	High	-0.15 (n = 16)	-0.13 (n = 19)	-0.13 (n = 5)
ICV	Low	-0.72* (n = 11)	-0.08 (n = 11)	-0.40* (n = 8)
	Intermediate	-0.45 (n = 14)	-0.27 (n = 17)	-0.27 (n = 4)
	High	-0.26 (n = 9)	-0.34* (n = 23)	-0.11 (n = 4)

GEE: generalized estimating equations; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; ANART: American national adult reading test; ICV: intracranial volume. *$p < 0.05$ or the rate of change is significantly different from zero.

Table 4-4 FDG-PET ROIs rates of change stratified by cognitive reserve in GEE models

Reserve proxy	Reserve level	FDG-PET ROIs rates of change (10^{-3} normalized intensity/month)		
		NC	MCI	AD
Education	Low	-2.17* (n = 46)	-2.44* (n = 70)	-5.11* (n = 44)
	Intermediate	-1.18* (n = 41)	-2.50* (n = 72)	-1.35* (n = 30)
	High	-1.83* (n = 46)	-2.49* (n = 82)	-4.83* (n = 24)
Occupation	Low	-2.15* (n = 23)	-2.28* (n = 43)	-3.97* (n = 31)
	Intermediate	-1.65* (n = 32)	-2.60* (n = 70)	-4.70* (n = 29)
	High	-1.75* (n = 78)	-2.46* (n =111)	-3.37* (n = 38)
ANART	Low	-2.43* (n = 25)	-2.02* (n = 74)	-5.71* (n = 42)
	Intermediate	-2.13* (n = 44)	-3.64* (n = 72)	-3.32* (n = 28)
	High	-1.41* (n = 64)	-2.17* (n =78)	-2.21* (n = 27)
ICV	Low	-2.23* (n = 45)	-3.12* (n = 59)	-4.05* (n = 31)
	Intermediate	-1.91* (n = 41)	-2.36* (n = 77)	-3.77* (n = 28)
	High	-1.50* (n = 47)	-2.11* (n =87)	-4.25* (n = 38)

FDG-PET ROIs: fludeoxyglucose F18- PET region-of-interest; GEE: generalized estimating equations; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; ANART: American national adult reading test; ICV: intracranial volume. *$p < 0.05$ or the rate of change is significantly different from zero.

Table 4-5 MRI hippocampal volume rates of change stratified by cognitive reserve in GEE models

Reserve proxy	Reserve level	Hippocampal volume rates of change (mm^3/month)		
		NC	MCI	AD
Education	Low	-2.56* (n= 68)	-5.77* (n= 133)	-8.26* (n= 89)
	Intermediate	-2.84* (n= 79)	-5.80* (n=124)	-7.91* (n= 61)
	High	-3.29* (n= 81)	-4.78* (n=136)	-7.77* (n= 43)
Occupation	Low	-2.71* (n= 37)	-5.39* (n= 91)	-8.33* (n= 59)
	Intermediate	-2.54* (n= 54)	-5.77* (n=114)	-8.13* (n=59)
	High	-3.14* (n=137)	-5.28* (n=188)	-7.76* (n=75)
ANART	Low	-2.47* (n= 39)	-5.75* (n=136)	-7.94* (n=83)
	Intermediate	-3.10* (n= 74)	-5.23* (n=124)	-8.09* (n=61)
	High	-3.01* (n=115)	-5.36* (n=131)	-8.14* (n=47)

GEE: generalized estimating equations; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; ANART: American national adult reading test. *$p < 0.05$ or the rate of change is significantly different from zero.

Table 4-6 Baseline cognitive function and cognitive reserve levels in cognitively normal participants with repeated CSF studies, N = 35

Reserve proxy	Reserve level	Mean cognitive function			
		MMSE	ADAS-cog	IAVLT	DAVLT
Education	Low	29.4	4.7	42.0	8.2
	Intermediate	29.3	6.4	43.1	7.9
	High	29.6	6.0	47.8	8.1
Occupation	Low	29.5	5.7	44.8	7.5
	Intermediate	29.3	4.2	47.4	10.0
	High	29.5	6.9	44.1	7.3
ANART	Low	29.2	4.8	45.0	9.2
	Intermediate	29.1	7.1	44.9	7.5
	High	29.8	5.5	45.2	8.0

MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale-cognitive subscale; IAVLT: immediate auditory verbal learning test; DAVLT: delayed auditory verbal learning test; ANART: American national adult reading test.

Table 4-7 Baseline cognitive function and cognitive reserve levels in participants with mild AD with repeated FDG-PET studies, N = 97

Reserve proxy	Reserve level	Mean cognitive function			
		MMSE	ADAS-cog	IAVLT	DAVLT
ANART	Low	23.3	20.2	20.8	0.6
	Intermediate	23.2	18.8	23.3	0.3
	High	24.0	18.1	25.4	1.0

AD: Alzheimer's disease; FDG-PET: [18F] fluorodeoxyglucose PET; MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale- cognitive subscale; IAVLT: immediate auditory verbal learning test; DAVLT: delayed auditory verbal learning test; ANART: American national adult reading test.

Table 4-8 Average proxy measurement at each reserve level in participants with normal cognition and Alzheimer's disease

NC (N=229)	Cognitive reserve level		
	Low	Intermediate	High
Education, yr	12.5 (30%)	16.0 (34%)	18.9 (36%)
Occupation	N=37 (16%)	N=54 (24%)	N=138 (60%)
ANART error, n	24.9 (17%)	11.1 (33%)	3.3 (50%)
AD (N=193)			
Education, yr	11.9 (46%)	16.0 (32%)	18.7 (22%)
Occupation	N=59 (31%)	N=59 (31%)	N=75 (39%)
ANART error, n	25.1 (43%)	12.2 (32%)	4.0 (25%)

NC: normal cognition; AD: Alzheimer's disease; ANART: American national adult reading test.
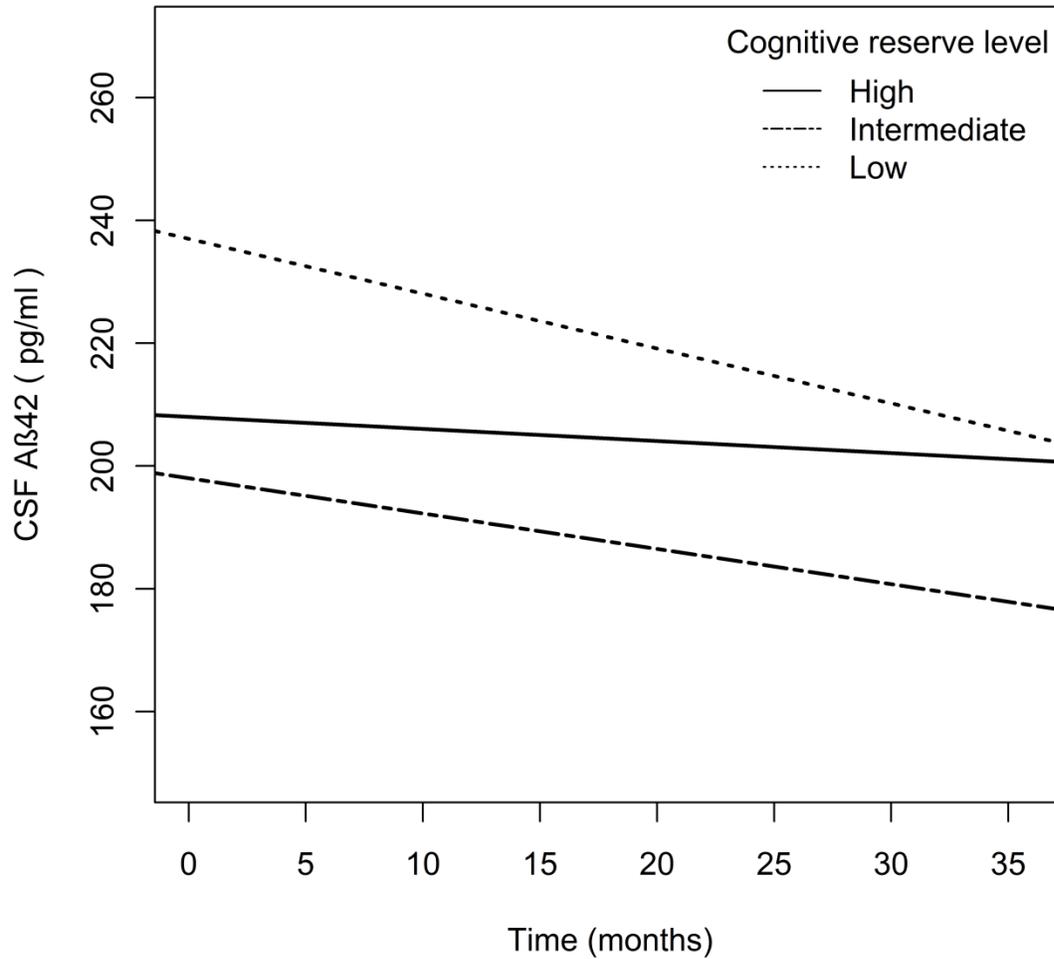
A. Education

Figure 4-1A The effect of education on CSF $A\beta_{42}$ rates of change modeled for participants with normal cognition at age 75.
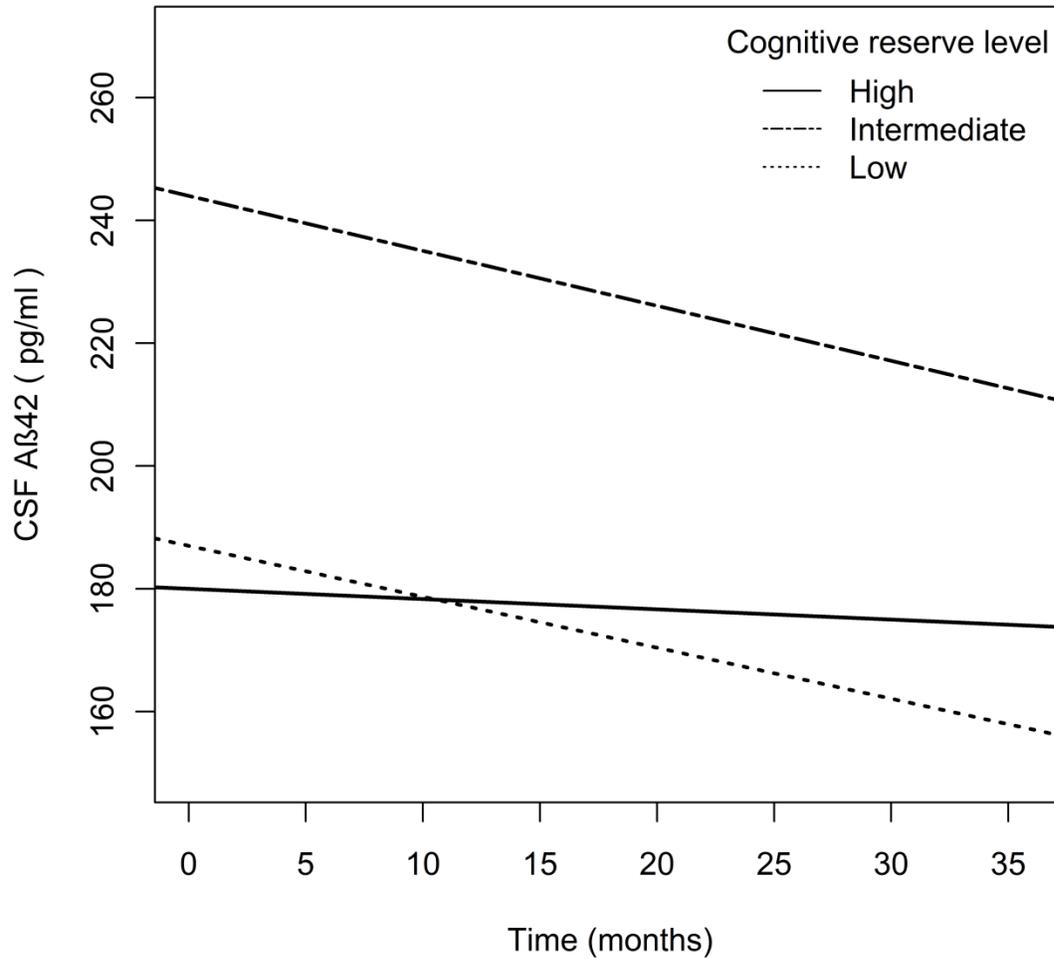
B. Occupation

Figure 4-1B The effect of occupation on CSF Aβ_{42} rates of change modeled for participants with normal cognition at age 75.
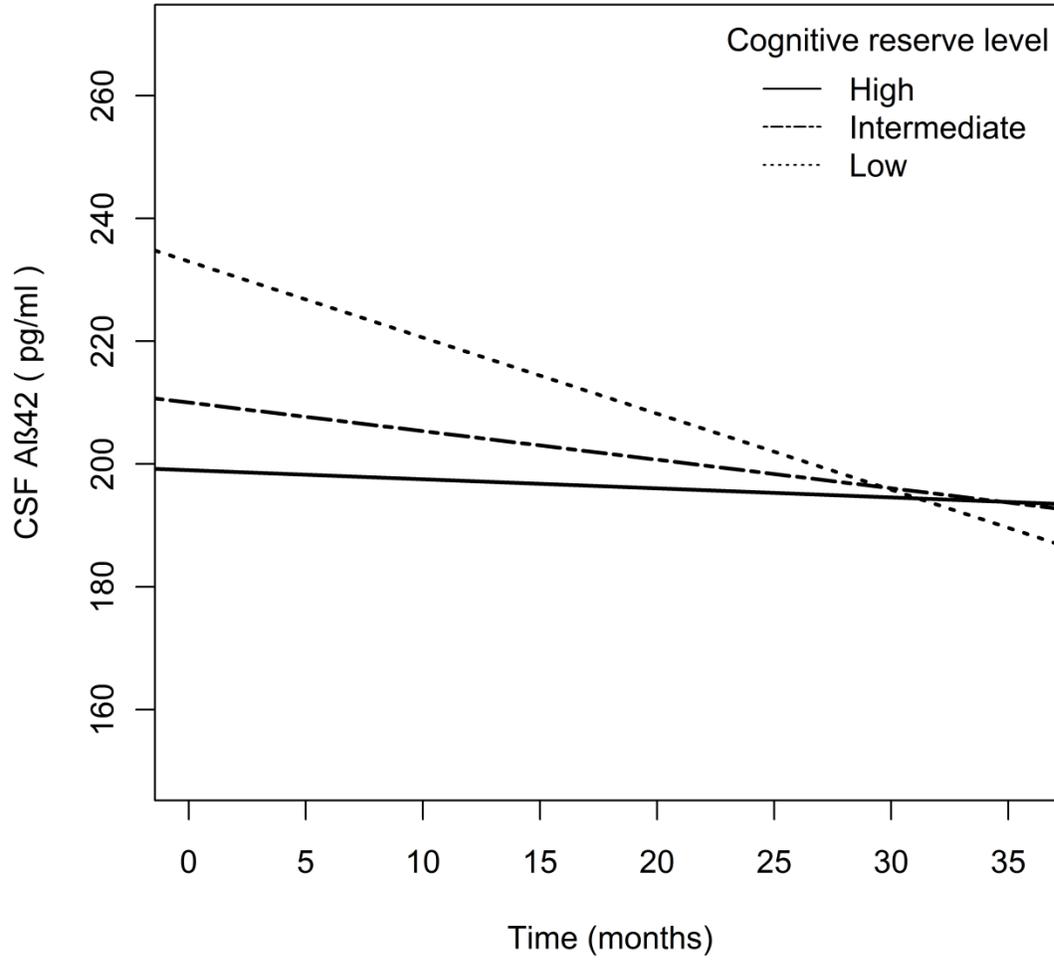
C. Premorbid intelligence

Figure 4-1C The effect of premorbid intelligence on CSF $A\beta_{42}$ rates of change modeled for participants with normal cognition at age 75.

Chapter 5

Vascular Burden and Alzheimer Disease Biomarker Dynamics

Introduction

Both Alzheimer's disease (AD) and vascular pathology are common in the elderly population, and multiple brain pathologies account for most patients with dementia.[44] Many cardiovascular risk factors including midlife hypertension, diabetes, dyslipidemia and smoking seem to increase the risk of AD, suggesting a vascular contribution to the etiology of AD.[126,127] The concept of the neurovascular unit has been proposed to link vascular dysfunction to neuronal injury and cognitive impairment.[128] Within this framework, vascular dysfunction not only gives rise to neuronal damage but also may reduce the clearance of Aβ via the blood-brain-barrier or indirectly increases Aβ deposition. Amyloid deposition is considered the pivotal event in the AD pathological cascade,[80] but whether the accumulation is accelerated by vascular risks remains unclear.

   White matter hyperintensities (WMH) on brain MRI reflect cardiovascular risk profiles, even for people without stroke and dementia.[129] Greater WMH volume is associated with cognitive decline, brain atrophy and reduced cerebral metabolism.[130,131] Underlying microinfarcts may account for these changes via brain atrophy or reduction of brain reserve but the mechanism appears to be independent of typical Alzheimer lesions.[132] A recent study from the Alzheimer's Disease Neuroimaging Initiative (ADNI) has shown that the longitudinal changes of CSF $A\beta_{42}$, [^{18}F] fluorodeoxyglucose PET (FDG-PET) uptake and MRI hippocampal volume are reflective of AD progression following cerebral amyloid deposition.[112] Vascular effects on brain reserve may also lower the clinical threshold for cognitive impairment without influencing the rates of AD pathological progression. In this chapter I aim to assess the vascular effect on the longitudinal change of AD biomarkers in ADNI using cardiovascular risk profile and WMH as vascular burden surrogates.

Methods

Study Population The ADNI study population has been defined in previous chapters. All participants were recruited between the ages of 55 and 90, and had at least 6 years of education and a study partner able to provide an independent evaluation of functioning. Use of specific psychoactive medications and a Hachinski Ischemic Scale score of 4 or greater were excluded. Follow-up time was extended to 5 years. We used the data from ADNI up to the date 11/01/11. The study procedures were approved by institutional review boards of all participating institutions.

Cognitive function assessment In addition to MMSE, the Alzheimer's Disease Assessment Scale- Cognitive Subscale (ADAS-cog) was used as a dependent measure to examine relationships between vascular burden and cognitive change. This test contains 11 items covering language, memory, praxis and comprehension function. The total score ranges from 0 to 70 and higher scores indicate poorer cognitive function. Baseline and multiple follow-up MMSE and ADAS-cog assessments were available for all participants.

Biomarkers of AD Pathology Protocols and acquisitions of CSF, FDG-PET and MRI biomarkers can be referenced from previous chapters.

Surrogate markers of vascular burden

Cardiovascular risk profile Cardiovascular risk score was calculated using the office-based cardiovascular risk profile prediction function from the Framingham Heart Study, which took age, gender, body mass index, blood pressure, smoking and diabetes into account;[93] higher scores indicated higher risks of cardiovascular events. The cardiovascular risk score was normally distributed and treated as a continuous but time-fixed variable in the analysis.

White matter hyperintensity volume The automated imaging procedure to estimate WMH volume has been detailed in an earlier ADNI publication.[131] WMH volume was not normally distributed and therefore was log-transformed for analysis. All participants had at least one MRI WMH measurement at baseline and 38% (310/819) had repeated measures for three years.

Statistical Analyses

AD Biomarker trajectories Participants with repeated measures were entered into analyses. We used repeated measures linear regression (an exchangeable working within subject correlation model via a generalized estimating equation, GEE)[72] to estimate average rates of change in cognitive function and AD biomarkers. The primary GEE model of biomarker trajectory treated time-varying biomarkers as the outcome with covariates of time and baseline age in the regression.

Longitudinal effect of vascular burden Cardiovascular risk score and WMH were proxy measures of vascular burden. We first examined the inter-relationship among WMH, age, *APOE* 4 and cardiovascular risk score at baseline in multivariable linear regression models. For participants with repeated WMH, we delineated WMH changes over time in NC, MCI and AD groups. Each vascular burden proxy as well as its interaction with time (vascular burden proxy × time) was then entered into the GEE models of biomarkers in NC, MCI and AD (model 1: CV risk score as vascular proxy; model 2: baseline WMH as vascular proxy). Coefficients of the interaction terms reflected the direction and magnitude of how vascular risks modified biomarker rates of change at different stages. For a subgroup of participants with repeated measures of WMH, time-varying WMH was taken into the GEE models to evaluate how AD biomarkers varied with WMH over time (model 3).

Secondary analyses Although our focus was the association between vascular burden and AD biomarkers, we employed similar GEE models to evaluate vascular effects on cognitive decline indexed by time-varying MMSE and ADAS-cog scores.

Sensitivity analyses APOE 4 carriers are predisposed to develop AD and a previous study from ADNI also demonstrated that APOE 4 accelerated hippocampal atrophy in MCI and AD.[112] Therefore, we included APOE 4 carrier status in GEE models to test the robustness of any vascular effect.

All statistical analyses and graphics were performed in R version 2.11.1. All tests of statistical significance were conducted at the two-tailed alpha level of 0.05.

Results

Baseline cognitive function, AD biomarkers, cardiovascular risk score, WMH volume and other demographic features in NC, MCI and AD groups are shown in Table 5-1. The effect of age seemed stronger than cardiovascular risk score on baseline WMH; while APOE 4 did not appear to affect WMH (Table 5-2). WMH volume significantly increased over time and the average rate of change (rate: 10^{-3} log-transformed volume/ month) was faster in MCI (7.6) and AD (7.4) than in NC (4.9) after adjusting for age.

Vascular contribution to AD biomarker changes are summarized in Table 5-3 to 5-5. Cardiovascular risk score was not associated with baseline AD biomarker values and it did not affect AD biomarker rates of change either. CSF $A\beta_{42}$ declined over time in NC and MCI groups but there was no association between vascular burden and CSF $A\beta_{42}$ cross-sectionally and longitudinally. Greater baseline WMH was associated with lower FDG uptake in AD and the dynamic change of WMH was inversely associated with FDG uptake in cognitively normal participants. Additionally, increased baseline WMH volume was associated with faster MRI hippocampal atrophy also in cognitively normal participants; but this finding was not replicated when using time-varying WMH for analysis. These results remained unchanged after accounting for APOE 4.

For the relationship between cognitive function and vascular risks, cardiovascular risk score was not predictive of cognitive function in all three groups; greater baseline WMH volume was associated with lower MMSE score in the AD group and higher ADAS-cog score in both NC and AD groups. Neither cardiovascular risk score nor WMH volume was associated with rates of cognitive decline indexed by MMSE and ADAS-cog scores (Table 5-6 and 5-7).

Discussion

In this longitudinal study we found no evidence of vascular effect on the longitudinal change of CSF $A\beta_{42}$, FDG uptake in typical AD-related ROIs, and MRI hippocampal atrophy during cognitive decline. CSF $A\beta_{42}$ is an amyloid specific marker for AD and its decline appears to be faster early in the disease course;[112] however, none of these vascular proxy measures was associated with CSF $A\beta_{42}$ either cross-sectionally or longitudinally. Likewise, MRI hippocampal volume serves as a sensitive surrogate marker for AD pathology,[133] but neither cardiovascular risk profile nor WMH was clearly associated with hippocampal volume or its rate of atrophy; although there was a suggestion of a relationship between WMH and longitudinal hippocampal atrophy in the NC group, the lack of a relationship between WMH change and hippocampal change argues against its significance. Our findings are consistent with previous studies that cerebrovascular burden and Alzheimer pathology may be two independent factors contributing to dementia.[132,134-136]

Vascular factors not only increase the risk of AD but also predict dementia progression in AD.[137] Their contribution is considered additive to augment the expression of AD.[138] Although the neurovascular hypotheses concerning $A\beta$ clearance seem plausible,[139] little evidence from human studies suggests that vascular risks are also amyloidogenic.[140] Even though amyloid deposition can be enhanced by circulatory defects, vascular effects may be easily overwhelmed by once AD pathology becomes advanced.[135] However, it is possible that vascular risks play an

initiating role and therefore, observation of their influence on amyloid accumulation may require longer study periods.

The significant inverse association between baseline WMH volume and FDG uptake in PET in the AD group may reflect that the vascular burden contributes to reduced synaptic activity in patients with AD; however, the rate of glucose hypometabolism was not influenced by vascular risks in MCI or AD, arguing against the interaction between vascular risks and AD pathologic progression. We used the average normalized intensity from five FDG-ROIs including bilateral temporal and angular gyri and posterior cingulate gyrus, which were commonly affected in AD. Although an earlier study demonstrated that WMH was associated with frontoparietal metabolism in FDG-PET rather than our ROIs,[141] the underlying neuropathology for these regions is still undetermined. FDG-PET generally represents synaptic activity[116] and ROIs may be sensitive to AD pathological changes; but FDG-ROIs may be not specific enough to exclude vascular or other pathological contribution. Our finding in the NC group that greater amount of WMH was associated with faster decline of FDG-ROIs uptake is in line with this notion. These cognitively normal participants are not necessarily going to develop AD and they may be considerably different from MCI and AD participants in ADNI. Therefore, the significant associations between FDG uptake in PET and WMH volume in our study may reflect the general effect of vascular burden on synaptic activity rather than Alzheimer specific pathology.

Age has long been considered a prominent predictor of WMH severity,[142] and here with repeated measures we have further shown that after adjusting for age, WMH volume increased significantly over time and the rate of increase appeared to be faster for participants with cognitive impairment. Although vascular risks such as hypertension are important factors for WMH severity,[143] we cannot exclude the contribution from other pathologies. A voxel-based morphometric MRI study has shown that gray matter reduction is correlated with the increase of WMH volume;[144] however, the temporal relationship between these findings is unknown. People with MCI or AD may have accelerated gray matter or cortical atrophy and thus develop faster WMH progression. Without further investigation of the underlying neuropathology of WMH, we cannot be sure that WMH change simply represents the progression of vascular burden.

APOE 4 is a strong genetic risk factor for AD and predictive of cognitive decline.[75] As a key component in the transport of cholesterol and lipid, APOE 4 plays a role in both coronary risk and cerebral amyloid deposition.[79,145] We did not find any association between APOE 4 and cardiovascular risk score or WMH. The relationship between AD biomarkers and vascular burden was not affected by the presence of APOE 4 either. On the other hand, APOE 4 has been shown to be associated with lower baseline CSF $A\beta_{42}$, FDG uptake and accelerated hippocampal atrophy in an earlier ADNI study.[112] Therefore, APOE 4 seems to contribute to AD via amyloid pathway more than through increasing vascular burden. However, people with Hachinski Ischemic Scale scores of 4 or greater are not included in the study. We do not know whether the vascular influence of APOE 4 would become more pronounced when people with a wider range of vascular burden are also enrolled.

Greater WMH volume was as expected associated with worse cognitive performance in the study. Despite a larger sample size than NC and AD groups, this association did not appear in the MCI group. MCI participants were enrolled in ADNI based on clinical criteria, but their underlying pathological profile is likely to be heterogeneous.[124] In addition to heterogeneity in MCI, previous studies showed that WMH was more associated with psychomotor speed or

executive function.[141,146] We assessed global cognitive function by MMSE and ADAS-cog, which might not be sensitive enough to track vascular-related cognitive decline.

There are several limitations in our study. First, vascular burden in the study was generally low since enrollment criteria excluded people with severe vascular insults or significant stroke. As a result, the range of vascular burden in ADNI was narrow and our findings cannot be generalized to populations with more than mild vascular risks. Furthermore, our length of observation ranged from 2 to 5 years. The mild degree of vascular may not be detectable within the period of observation. Nevertheless, this limitation provides us a unique opportunity to evaluate mild vascular effects that are uncomplicated by comorbidities, motor and sensory alternations and other signs and symptoms that might increase measurement error, especially for cognition. Another limitation is that we do not have pathological data showing the concordance between these Alzheimer type biomarker changes and the severity of amyloid deposition. How specific these AD biomarkers are to detect the amyloid pathological cascade at each cognitive stage in contrast with vascular progression is not known. Cardiovascular risk profile was derived from hypertension, diabetes and other factors measured only once at baseline and many of them may not have been quantified with adequate precision. Although the cardiovascular risk score was normally distributed, it predicts future cardiovascular events but is not necessarily reflective of the underlying vascular pathology. Outcomes could also have been affected by treatment of hypertension or other vascular risk factors, and the degree of medical control was not considered in our analyses. These treatments presumably change over time, depending upon the previous outcome and also affecting the follow-up predictor as well as outcome. Handling these time-varying confounders is beyond the scope of ADNI data and we are not sure how the lack of handling of these confounders would affect our results.

The unique strength of the study is its longitudinal setting and repeated measurement to capture the dynamics of AD biomarkers and WMH. Time-varying predictors and outcome are rarely available in population studies; therefore, the assumption that an age effect is uniform across different participants is not as necessary as in cross-sectional studies. In addition, the majority of participants had 3 or more repeated measures, allowing us to evaluate not only the single difference between two time points but also the variance of change. With more than 3 or 4 repeated measures, the "regression towards the mean" effect can be further minimized.

Within a defined range of WMH or vascular burden, the longitudinal changes of AD biomarkers were not modified by vascular risks during cognitive decline in ADNI. There is no evidence that cerebral amyloid deposition is affected by vascular burden. Vascular contribution to Alzheimer dementia thus appears to be independent of amyloid pathway.

Table 5-1 Baseline characteristics of 819 participants in ADNI

	ADNI diagnostic group		
	NC	MCI	AD
Sample size	229	397	193
Mean age, y (SD)	75.1 (5.0)	74.0 (7.5)	74.6 (7.5)
M : F, n	119 : 110	256 : 141	102 : 91
MMSE score, mean (SD)	29.1 (1.0)	27.0 (1.8)	23.3 (2.1)
ADAS-cog, mean (SD)	6.2 (2.9)	11.5 (4.4)	18.6 (6.3)
APOE 4 carrier, n (%)	61(26.6)	212 (53.4)	127 (65.8)
Education, y (SD)	16.0 (2.9)	15.7 (3.0)	14.7 (3.1)
CV risk score, mean (SD)	18.9 (3.6)	18.4 (3.9)	18.7 (4.1)
Log-transformed WMH volume, mean (SD)	-1.58 (1.61)	-1.39 (1.66)	-0.90 (1.60)
Mean biomarker value			
CSF $A\beta_{42}$, pg/ml	222.8 (n=50)	172.2 (n=72)	143.3 (n=17)
CSF Tau, pg/ml	73.1 (n=50)	98.2 (n=72)	144.9 (n=15)
FDG-PET ROIs, normalized intensity	1.28 (n=103)	1.20 (n=203)	1.08 (n=97)
MRI hippocampal volume, mm^3	3633 (n=228)	3233 (n=393)	2895 (n=193)

ADNI: Alzheimer's Disease Neuroimaging Initiative; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; MMSE: mini-mental state examination; ADAS-cog: Alzheimer's disease assessment scale- cognitive subscale; CV: cardiovascular; WMH: white matter hyperintensities; FDG-PET ROIs: fludeoxyglucose F18- PET region-of-interest.

Table 5-2 Association of baseline WMH with age, cardiovascular risk score and APOE 4

	Age	CV risk score	*APOE* 4
NC (n=226)	0.05*	0.03	0.27
MCI (n=396)	0.04*	0.08*	-0.29
AD (n=192)	0.06*	0.01	0.21

WMH: white matter hyperintensities; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; CV: cardiovascular. *$p < 0.05$. Entries show regression coefficients in the multivariable linear models with baseline WMH as the outcome and age, CV risk score and APOE 4 as predictors.

Table 5-3 Regression coefficients in GEE models for CSF $A\beta_{42}$ biomarker

Model 1	Age	Time	CV risk score	(CV risk×Time)
NC (n=50)	0.67	-0.47	-0.21	0.01
MCI (n=74)	1.72	0.27	1.76	-0.03
AD (n=18)	1.25	0.13	-2.31	-0.01
Model 2	Age	Time	WMH_{t0}	(WMH_{t0}×Time)
NC (n=50)	0.61	-0.38*	-4.41	-0.04
MCI (n=74)	2.22	-0.20*	-2.99	-0.003
AD (n=18)	0.60	0.03	-1.11	0.02
Model 3	Age	Time	WMH_{t0}	(WMH_t-WMH_{t0})
NC (n=50)	0.69	-0.31*	-4.86	0.65
MCI (n=72)	2.36	-0.23*	-2.56	-0.91
AD (n=17)	0.67	-0.20	-1.20	-5.04

GEE: generalized estimating equations; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; CV: cardiovascular; WMH: white matter hyperintensities; WMH_{t0} is the baseline WMH; WMH_t is the time-varying WMH. *$p < 0.05$.

Entries show regression coefficients in the GEE models with time-varying CSF biomarker as the outcome of interest (unit: pg/ml); and age, time, vascular proxy measures and their interaction with time or time-varying WMH as predictors.

Table 5-4 Regression coefficients in GEE models for FDG-PET biomarker

Model 1	Age	Time	CV risk score	(CV risk×Time)
NC (n=130)	-4.50*	-1.76	-4.90	7.29×10^{-4}
MCI (n=223)	-1.36	-3.17*	-4.99†	3.64×10^{-2}
AD (n=98)	7.24*	-6.59*	3.05	0.14
Model 2	Age	Time	WMH_{t0}	$(WMH_{t0}\times Time)$
NC (n=130)	-5.77*	-1.63*	7.42	6.95×10^{-2}
MCI (n=223)	-2.15	-2.59*	-3.01	6.00×10^{-2}
AD (n=98)	9.41*	-4.12*	-15.5*	-0.18
Model 3	Age	Time	WMH_{t0}	(WMH_t-WMH_{t0})
NC (n=103)	-8.08*	-0.60*	-2.34	-6.74*
MCI (n=202)	-2.25	-1.74*	-9.12	-1.50
AD (n=97)	8.88*	-4.23*	-16.3*	1.66

GEE: generalized estimating equations; FDG-PET: fludeoxyglucose F18- PET; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; CV: cardiovascular; WMH: white matter hyperintensities; WMH_{t0} is the baseline WMH; WMH_t is the time-varying WMH. *$p < 0.05$; † statistical significance disappears after accounting for APOE 4.

Entries show regression coefficients in the GEE models with time-varying PET biomarker as the outcome of interest (unit: 10^{-3} normalized intensity); and age, time, vascular proxy measures and their interaction with time or time-varying WMH as predictors.

Table 5-5 Regression coefficients in GEE models for MRI hippocampal atrophy

Model 1	Age	Time	CV risk score	(CV risk×Time)
NC (n=225)	-31.0*	-2.89*	4.78	-1.24×10^{-3}
MCI (n=389)	-24.0*	-3.97*	-6.67	-7.98×10^{-2}
AD (n=190)	-26.7*	-8.49*	7.11	2.56×10^{-2}
Model 2	Age	Time	WMH_{t0}	($WMH_{t0}\times$Time)
NC (n=225)	-31.2*	-3.37*	17.3	-0.27*
MCI (n=389)	-23.9*	-5.77*	-25.5	-0.21
AD (n=190)	-23.4*	-8.04*	-22.4	-0.03
Model 3	Age	Time	WMH_{t0}	(WMH_t-WMH_{t0})
NC (n=226)	-31.2*	-2.79*	7.82	-0.71
MCI (n=389)	-23.8*	-5.60*	-27.7	-0.48
AD (n=190)	-23.4*	-8.55*	-21.9	1.04

GEE: generalized estimating equations; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; CV: cardiovascular; WMH: white matter hyperintensities; WMH_{t0} is the baseline WMH; WMH_t is the time-varying WMH. *$p < 0.05$

Entries show regression coefficients in the GEE models with time-varying MRI biomarker as the outcome of interest (unit: mm^3); and age, time, vascular proxy measures and their interaction with time or time-varying WMH as predictors.

Table 5-6 Regression coefficients in GEE models for MMSE

Model 1	Age	Time	CV risk score	(CV risk×Time)
NC (n=228)	-2.30*	1.35	-2.41	-9.1×10^{-2}
MCI (n=397)	0.67	-5.63	-5.56	-0.11
AD (n=193)	6.54	-32.8*	-5.64	0.75
Model 2	Age	Time	WMH_{t0}	(WMH_{t0}×Time)
NC (n=226)	-2.32*	-0.63	8.59×10^{-2}	-0.13
MCI (n=396)	-5.2×10^{-2}	-8.59*	-4.57	-0.58
AD (n=192)	8.66*	-20.2*	-23.3*	-1.34
Model 3	Age	Time	WMH_{t0}	(WMH_t-WMH_{t0})
NC (n=225)	-2.37*	-0.60	-1.73	-0.57
MCI (n=396)	-0.57	-7.43*	-11.8	-8.08
AD (n=192)	5.12	-19.0*	-24.5	-0.46

GEE: generalized estimating equations; MMSE: Mini-Mental State Examination; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; CV: cardiovascular; WMH: white matter hyperintensities; WMH_{t0} is the baseline WMH; WMH_t is the time-varying WMH. *$p < 0.05$

Entries show regression coefficients in the GEE models with time-varying MMSE score as the outcome of interest (unit: 10^{-2} point); and age, time, vascular proxy measures and their interaction with time or time-varying WMH as predictors.

Table 5-7 Regression coefficients in GEE models for ADAS-cog

Model 1	Age	Time	CV risk score	(CV risk×Time)
NC (n=190)	7.66*	-0.12	-2.76	1.5×10^{-2}
MCI (n=311)	-3.31	10.9	3.78	0.13
AD (n=151)	-9.72	56.7*	24.3	-1.00
Model 2	Age	Time	WMH_{t0}	$(WMH_{t0} \times Time)$
NC (n=226)	6.41*	-0.20	22.8*	2.5×10^{-2}
MCI (n=396)	2.79	14.6*	12.5	1.17
AD (n=190)	-17.0*	40.9*	80.7*	1.59
Model 3	Age	Time	WMH_{t0}	$(WMH_t - WMH_{t0})$
NC (n=226)	6.65*	-1.02	22.3*	5.95
MCI (n=396)	3.00	13.0*	21.1	4.68
AD (n=190)	-10.2	33.8*	81.6*	3.51

GEE: generalized estimating equations; ADAS-cog: Alzheimer's Disease Assessment Scale-Cognitive Subscale; NC: normal cognition; MCI: mild cognitive impairment; AD: Alzheimer's disease; CV: cardiovascular; WMH: white matter hyperintensities; WMH_{t0} is the baseline WMH; WMH_t is the time-varying WMH. $*p < 0.05$

Entries show regression coefficients in the GEE models with time-varying ADAS-cog score as the outcome of interest (unit: 10^{-2} point); and age, time, vascular proxy measures and their interaction with time or time-varying WMH as predictors.

Chapter 6

Conclusion

Trajectories of CSF Aβ_{42}, FDG uptake and hippocampal volume vary across different cognitive stages and support a hypothetical sequence of AD pathology in which amyloid deposition is an early event followed by glucose hypometabolism and hippocampal atrophy. Biomarker based prediction of AD is therefore stage dependent. Early in the course of AD before cognitive impairment, the change of CSF Aβ_{42} may be more informative; when cognitive function begins to decline, imaging markers may be better to capture the pathological progression. Temporality should be critically considered when combining multiple biomarkers for AD prediction.

Ideally, using multiple biomarkers to follow up individuals would allow us to observe the longitudinal change rather than cross sectional difference, especially for outcome measures in clinical trials. However, missing biomarker data seem to be inevitable during follow-ups. The missing data structure is found not completely at random. Repeated measures of CSF biomarkers likely retain AD like participants. Poor cognitive function is predictive of longitudinal missingness but less critical for those who already have overt dementia. Depression is a major factor associated with missing imaging data. Patterns of missingness are not always the same for MCI and AD, suggesting that surrogate decision-making may play a role. Biomarker, cognitive stage, and certain clinical features are all important to determine missing data.

Education, occupation, premorbid intelligence and brain volume are common proxy measures of cognitive reserve. They are considered to compensate or resist the deleterious effect of AD pathology on cognitive performance but our findings also support that high reserve may slow AD pathological progression, particularly among cognitively intact participants. These results are compatible with a newly proposed framework that people with higher education or increased lifetime cognitive activity would have developed an efficient way to process information with less synaptic activity. Within the framework, synaptic activity is associated with amyloid deposition, and it is therefore plausible to observe that people with higher cognitive reserve have slower rates of amyloid accumulation.

Many cardiovascular risks including diabetes, smoking and hypertension are also known to increase the risk of AD. Multiple strokes reduce brain capacity to cope with cognitive challenges and in addition, vascular dysfunction may reduce cerebral amyloid clearance and indirectly increase the risk of AD. However, there is no evidence that AD type pathological progression or rates of CSF Aβ_{42} decrement, FDG-PET hypometabolism and MRI hippocampal atrophy are altered by WMH or cardiovascular risk profile. The vascular contribution to AD appears to be independent of the amyloid pathway.

Lifetime cognitive experience may have direct effects on AD pathology and the greater implication is that the course of AD can be modified if cognitive intervention is implemented early in life. On the contrary, vascular burden leads to cognitive decline via its own pathway independent of amyloid deposition. Controlling these vascular risks may preserve the threshold of cognitive impairment if not halt amyloid deposition. The dissertation provides the basis of AD prediction with biomarkers and supports the notion that AD can be a preventable disease.

<center>**References**</center>

1. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol.* Mar 1999;56(3):303-308.

2. Mulnard RA, Cotman CW, Kawas C, et al. Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: a randomized controlled trial. Alzheimer's Disease Cooperative Study. *Jama.* Feb 23 2000;283(8):1007-1015.

3. Rapp SR, Espeland MA, Shumaker SA, et al. Effect of estrogen plus progestin on global cognitive function in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *Jama.* May 28 2003;289(20):2663-2672.

4. Shumaker SA, Legault C, Rapp SR, et al. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *Jama.* May 28 2003;289(20):2651-2662.

5. Lu PH, Masterman DA, Mulnard R, et al. Effects of testosterone on cognition and mood in male patients with mild Alzheimer disease and healthy elderly men. *Arch Neurol.* Feb 2006;63(2):177-185.

6. Bentham P, Gray R, Sellwood E, Hills R, Crome P, Raftery J. Aspirin in Alzheimer's disease (AD2000): a randomised open-label trial. *Lancet Neurol.* Jan 2008;7(1):41-49.

7. Aisen PS, Schafer KA, Grundman M, et al. Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. *Jama.* Jun 4 2003;289(21):2819-2826.

8. Reines SA, Block GA, Morris JC, et al. Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. *Neurology.* Jan 13 2004;62(1):66-71.

9. Aisen PS, Davis KL, Berg JD, et al. A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study. *Neurology.* Feb 8 2000;54(3):588-593.

10. Freund-Levi Y, Eriksdotter-Jonhagen M, Cederholm T, et al. Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. *Arch Neurol.* Oct 2006;63(10):1402-1408.

11. Wolkowitz OM, Kramer JH, Reus VI, et al. DHEA treatment of Alzheimer's disease: a randomized, double-blind, placebo-controlled study. *Neurology.* Apr 8 2003;60(7):1071-1076.

12. Kral VA. Senescent forgetfulness: benign and malignant. *Can Med Assoc J.* Feb 10 1962;86:257-260.

13. Crook T, Bahar H, Sudilovsky A. Age-associated memory impairment: diagnostic criteria and treatment strategies. *Int J Neurol.* 1987;21-22:73-82.

14. Levy R. Aging-associated cognitive decline. Working Party of the International Psychogeriatric Association in collaboration with the World Health Organization. *Int Psychogeriatr.* Spring 1994;6(1):63-68.

15. Graham JE, Rockwood K, Beattie BL, et al. Prevalence and severity of cognitive impairment with and without dementia in an elderly population. *Lancet.* Jun 21 1997;349(9068):1793-1796.

16. Barker A, Jones R, Jennison C. A prevalence study of age-associated memory impairment. *Br J Psychiatry.* Nov 1995;167(5):642-648.

17. Coria F, Gomez de Caso JA, Minguez L, Rodriguez-Artalejo F, Claveria LE. Prevalence of age-associated memory impairment and dementia in a rural community. *J Neurol Neurosurg Psychiatry.* Sep 1993;56(9):973-976.

18. Larrabee GJ, Crook TH, 3rd. Estimated prevalence of age-associated memory impairment derived from standardized tests of memory function. *Int Psychogeriatr.* Spring 1994;6(1):95-104.

19. Smith G, Ivnik RJ, Petersen RC, Malec JF, Kokmen E, Tangalos E. Age-associated memory impairment diagnoses: problems of reliability and concerns for terminology. *Psychol Aging.* Dec 1991;6(4):551-558.

20. Bowen J, Teri L, Kukull W, McCormick W, McCurry SM, Larson EB. Progression to dementia in patients with isolated memory loss. *Lancet.* Mar 15 1997;349(9054):763-765.

21. Petersen RC, Doody R, Kurz A, et al. Current concepts in mild cognitive impairment. *Arch Neurol.* Dec 2001;58(12):1985-1992.

22. Palmer K, Backman L, Winblad B, Fratiglioni L. Mild cognitive impairment in the general population: occurrence and progression to Alzheimer disease. *Am J Geriatr Psychiatry.* Jul 2008;16(7):603-611.

23. Palmer K, Backman L, Winblad B, Fratiglioni L. Early symptoms and signs of cognitive deficits might not always be detectable in persons who develop Alzheimer's disease. *Int Psychogeriatr.* Apr 2008;20(2):252-258.

24. Ram CV. The evolving definition of systemic hypertension. *Am J Cardiol.* Apr 15 2007;99(8):1168-1170.

25.   Cohen L. *No aging in India: Alzheimer's, the bad family, and other modern things.* University of California Press; 1998.

26.   Erkinjuntti T, Ostbye T, Steenhuis R, Hachinski V. The effect of different diagnostic criteria on the prevalence of dementia. *N Engl J Med.* Dec 4 1997;337(23):1667-1674.

27.   . *Diagnostic and statistical manual of mental disorders*. 4th ed. Washington D.C.: American Psychiatric Association; 1994.

28.   McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology.* Jul 1984;34(7):939-944.

29.   Kukull WA, Larson EB, Reifler BV, Lampe TH, Yerby M, Hughes J. Interrater reliability of Alzheimer's disease diagnosis. *Neurology.* Feb 1990;40(2):257-260.

30.   Kukull WA, Larson EB, Reifler BV, Lampe TH, Yerby MS, Hughes JP. The validity of 3 clinical diagnostic criteria for Alzheimer's disease. *Neurology.* Sep 1990;40(9):1364-1369.

31.   Blacker D, Albert MS, Bassett SS, Go RC, Harrell LE, Folstein MF. Reliability and validity of NINCDS-ADRDA criteria for Alzheimer's disease. The National Institute of Mental Health Genetics Initiative. *Arch Neurol.* Dec 1994;51(12):1198-1204.

32.   Stern Y, Gurland B, Tatemichi TK, Tang MX, Wilder D, Mayeux R. Influence of education and occupation on the incidence of Alzheimer's disease. *JAMA.* Apr 6 1994;271(13):1004-1010.

33.   Lyman KA. Bringing the social back in: a critique of the biomedicalization of dementia. *Gerontologist.* Oct 1989;29(5):597-605.

34.   Katzman R, Terry R, DeTeresa R, et al. Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Ann Neurol.* Feb 1988;23(2):138-144.

35.   Bennett DA, Schneider JA, Arvanitakis Z, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology.* Jun 27 2006;66(12):1837-1844.

36.   de la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. *Lancet Neurol.* Mar 2004;3(3):184-190.

37.   Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA. The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Ann Neurol.* Aug 2009;66(2):200-208.

38.   Kendell RE. Clinical validity. *Psychol Med.* Feb 1989;19(1):45-55.

39.   Manly JJ, Tang MX, Schupf N, Stern Y, Vonsattel JP, Mayeux R. Frequency and course of mild cognitive impairment in a multiethnic community. *Ann Neurol.* Apr 2008;63(4):494-506.

40.   Kryscio RJ, Schmitt FA, Salazar JC, Mendiondo MS, Markesbery WR. Risk factors for transitions from normal to mild cognitive impairment and dementia. *Neurology.* Mar 28 2006;66(6):828-832.

41.   Rosch EL, Barbara *Cognition and Categorization.* 1st ed. New York: Lawrence Erlbaum; 1978.

42.   Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol.* Aug 2007;6(8):734-746.

43.   Stern Y. What is cognitive reserve? Theory and research application of the reserve concept. *J Int Neuropsychol Soc.* Mar 2002;8(3):448-460.

44.   Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology.* Dec 11 2007;69(24):2197-2204.

45.   Sunderland T, Linker G, Mirza N, et al. Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. *JAMA.* Apr 23-30 2003;289(16):2094-2103.

46.   Silverman DH, Small GW, Chang CY, et al. Positron emission tomography in evaluation of dementia: Regional brain metabolism and long-term outcome. *JAMA.* Nov 7 2001;286(17):2120-2127.

47.   Jack CR, Jr., Petersen RC, O'Brien PC, Tangalos EG. MR-based hippocampal volumetry in the diagnosis of Alzheimer's disease. *Neurology.* Jan 1992;42(1):183-188.

48.   Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology.* Feb 25 2003;60(4):652-656.

49.   Mosconi L, Mistur R, Switalski R, et al. FDG-PET changes in brain glucose metabolism from normal cognition to pathologically verified Alzheimer's disease. *Eur J Nucl Med Mol Imaging.* May 2009;36(5):811-822.

50.   Whitwell JL, Josephs KA, Murray ME, et al. MRI correlates of neurofibrillary tangle pathology at autopsy: a voxel-based morphometry study. *Neurology.* Sep 2 2008;71(10):743-749.

51. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol.* Mar 2006;5(3):228-234.

52. Blennow K, Zetterberg H, Minthon L, et al. Longitudinal stability of CSF biomarkers in Alzheimer's disease. *Neurosci Lett.* May 23 2007;419(1):18-22.

53. Zetterberg H, Pedersen M, Lind K, et al. Intra-individual stability of CSF biomarkers for Alzheimer's disease over two years. *J Alzheimers Dis.* Nov 2007;12(3):255-260.

54. Jack CR, Jr., Shiung MM, Gunter JL, et al. Comparison of different MRI brain atrophy rate measures with clinical disease progression in AD. *Neurology.* Feb 24 2004;62(4):591-600.

55. Chetelat G, Landeau B, Eustache F, et al. Using voxel-based morphometry to map the structural changes associated with rapid conversion in MCI: a longitudinal MRI study. *Neuroimage.* Oct 1 2005;27(4):934-946.

56. Mungas D, Harvey D, Reed BR, et al. Longitudinal volumetric MRI change and rate of cognitive decline. *Neurology.* Aug 23 2005;65(4):565-571.

57. Jack CR, Jr., Weigand SD, Shiung MM, et al. Atrophy rates accelerate in amnestic mild cognitive impairment. *Neurology.* May 6 2008;70(19 Pt 2):1740-1752.

58. Alexander GE, Chen K, Pietrini P, Rapoport SI, Reiman EM. Longitudinal PET Evaluation of Cerebral Metabolic Decline in Dementia: A Potential Outcome Measure in Alzheimer's Disease Treatment Studies. *Am J Psychiatry.* May 2002;159(5):738-745.

59. Drzezga A, Lautenschlager N, Siebner H, et al. Cerebral metabolic changes accompanying conversion of mild cognitive impairment into Alzheimer's disease: a PET follow-up study. *Eur J Nucl Med Mol Imaging.* Aug 2003;30(8):1104-1113.

60. Mosconi L, De Santi S, Li J, et al. Hippocampal hypometabolism predicts cognitive decline from normal aging. *Neurobiol Aging.* May 2008;29(5):676-692.

61. Fouquet M, Desgranges B, Landeau B, et al. Longitudinal brain metabolic changes from amnestic mild cognitive impairment to Alzheimer's disease. *Brain.* Aug 2009;132(Pt 8):2058-2067.

62. Schuff N, Woerner N, Boreta L, et al. MRI of hippocampal volume loss in early Alzheimer's disease in relation to ApoE genotype and biomarkers. *Brain.* Apr 2009;132(Pt 4):1067-1077.

63. Fagan AM, Head D, Shah AR, et al. Decreased cerebrospinal fluid Abeta(42) correlates with brain atrophy in cognitively normal elderly. *Ann Neurol.* Feb 2009;65(2):176-183.

64.   Tosun D, Schuff N, Truran-Sacrey D, et al. Relations between brain tissue loss, CSF biomarkers, and the ApoE genetic profile: a longitudinal MRI study. *Neurobiol Aging.* Aug 2010;31(8):1340-1354.

65.   Petrie EC, Cross DJ, Galasko D, et al. Preclinical evidence of Alzheimer changes: convergent cerebrospinal fluid biomarker and fluorodeoxyglucose positron emission tomography findings. *Arch Neurol.* May 2009;66(5):632-637.

66.   de Leon MJ, DeSanti S, Zinkowski R, et al. Longitudinal CSF and MRI biomarkers improve the diagnosis of mild cognitive impairment. *Neurobiol Aging.* Mar 2006;27(3):394-401.

67.   Sluimer JD, Bouwman FH, Vrenken H, et al. Whole-brain atrophy rate and CSF biomarker levels in MCI and AD: a longitudinal study. *Neurobiol Aging.* May 2010;31(5):758-764.

68.   Petersen RC, Aisen PS, Beckett LA, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. *Neurology.* Jan 19 2010;74(3):201-209.

69.   Shaw LM. PENN biomarker core of the Alzheimer's disease Neuroimaging Initiative. *Neurosignals.* 2008;16(1):19-23.

70.   Landau SM, Harvey D, Madison CM, et al. Associations between cognitive, functional, and FDG-PET measures of decline in AD and MCI. *Neurobiol Aging.* Aug 4 2009.

71.   Jack CR, Jr., Bernstein MA, Fox NC, et al. The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. *J Magn Reson Imaging.* Apr 2008;27(4):685-691.

72.   Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. *Biometrics.* Dec 1988;44(4):1049-1060.

73.   Hubbard AE, Ahern J, Fleischer NL, et al. To GEE or not to GEE: comparing population average and mixed models for estimating the associations between neighborhood risk factors and health. *Epidemiology.* Jul 2010;21(4):467-474.

74.   Beckett LA, Harvey DJ, Gamst A, et al. The Alzheimer's Disease Neuroimaging Initiative: Annual change in biomarkers and clinical outcomes. *Alzheimers Dement.* May 2010;6(3):257-264.

75.   Caselli RJ, Dueck AC, Osborne D, et al. Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. *N Engl J Med.* Jul 16 2009;361(3):255-263.

76.   Moffat SD, Szekely CA, Zonderman AB, Kabani NJ, Resnick SM. Longitudinal change in hippocampal volume as a function of apolipoprotein E genotype. *Neurology.* Jul 12 2000;55(1):134-136.

77. Reiman EM, Caselli RJ, Chen K, Alexander GE, Bandy D, Frost J. Declining brain activity in cognitively normal apolipoprotein E epsilon 4 heterozygotes: A foundation for using positron emission tomography to efficiently test treatments to prevent Alzheimer's disease. *Proc Natl Acad Sci U S A.* Mar 13 2001;98(6):3334-3339.

78. Polvikoski T, Sulkava R, Haltia M, et al. Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. *N Engl J Med.* Nov 9 1995;333(19):1242-1247.

79. Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A.* Apr 21 2009;106(16):6820-6825.

80. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* Jan 2010;9(1):119-128.

81. Caroli A, Frisoni GB. The dynamics of Alzheimer's disease biomarkers in the Alzheimer's Disease Neuroimaging Initiative cohort. *Neurobiol Aging.* Aug 2010;31(8):1263-1274.

82. Stomrud E, Hansson O, Zetterberg H, Blennow K, Minthon L, Londos E. Correlation of longitudinal cerebrospinal fluid biomarkers with cognitive decline in healthy older adults. *Arch Neurol.* Feb 2010;67(2):217-223.

83. Tapiola T, Pirttila T, Mikkonen M, et al. Three-year follow-up of cerebrospinal fluid tau, beta-amyloid 42 and 40 concentrations in Alzheimer's disease. *Neurosci Lett.* Feb 18 2000;280(2):119-122.

84. Buchhave P, Blennow K, Zetterberg H, et al. Longitudinal study of CSF biomarkers in patients with Alzheimer's disease. *PLoS One.* 2009;4(7):e6294.

85. Hardy SE, Allore H, Studenski SA. Missing data: a special challenge in aging research. *J Am Geriatr Soc.* Apr 2009;57(4):722-729.

86. Atkinson HH, Rosano C, Simonsick EM, et al. Cognitive function, gait speed decline, and comorbidities: the health, aging and body composition study. *J Gerontol A Biol Sci Med Sci.* Aug 2007;62(8):844-850.

87. Greenland S, Finkle WD. A critical look at methods for handling missing covariates in epidemiologic regression analyses. *Am J Epidemiol.* Dec 15 1995;142(12):1255-1264.

88. Little RJ. Modeling the drop-out mechanism in repeated-measures studies. *J Am Stat Assoc.* 1995;90(431):1112-1121.

89. Twisk J, de Vente W. Attrition in longitudinal studies. How to deal with missing data. *J Clin Epidemiol.* Apr 2002;55(4):329-337.

90.   Little RJA, Rubin DB. *Statistical analysis with missing data.* 2nd ed. New Jersey: Wiley; 2002.

91.   Chandola T, Jenkinson C. The new UK National Statistics Socio-Economic Classification (NS-SEC); investigating social class differences in self-reported health status. *J Public Health Med.* Jun 2000;22(2):182-190.

92.   Grober E, Sliwinski M. Development and validation of a model for estimating premorbid verbal intelligence in the elderly. *J Clin Exp Neuropsychol.* Nov 1991;13(6):933-949.

93.   D'Agostino RB, Sr., Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation.* Feb 12 2008;117(6):743-753.

94.   Pfeffer RI, Kurosaki TT, Harrah CH, Jr., Chance JM, Filos S. Measurement of functional activities in older adults in the community. *J Gerontol.* May 1982;37(3):323-329.

95.   Yesavage JA. Geriatric Depression Scale. *Psychopharmacol Bull.* 1988;24(4):709-711.

96.   Kaufer DI, Cummings JL, Ketchel P, et al. Validation of the NPI-Q, a brief clinical form of the Neuropsychiatric Inventory. *J Neuropsychiatry Clin Neurosci.* Spring 2000;12(2):233-239.

97.   Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology.* Nov 1993;43(11):2412-2414.

98.   Kim SY, Cox C, Caine ED. Impaired decision-making ability in subjects with Alzheimer's disease and willingness to participate in research. *Am J Psychiatry.* 2002;159(5):797-802.

99.   Kim SY, Kim HM, McCallum C, Tariot PN. What do people at risk for Alzheimer disease think about surrogate consent for research? *Neurology.* Nov 8 2005;65(9):1395-1401.

100.   Beck C, Shue V. Surrogate decision-making and related issues. *Alzheimer Dis Assoc Disord.* Apr-Jun 2003;17 Suppl. 1:S12-S16.

101.   Rubin DB, Schenker N. Multiple imputation in health-care databases: an overview and some applications. *Stat Med.* Apr 1991;10(4):585-598.

102.   Evans DA, Hebert LE, Beckett LA, et al. Education and other measures of socioeconomic status and risk of incident Alzheimer disease in a defined population of older persons. *Arch Neurol.* Nov 1997;54(11):1399-1405.

103.   Karp A, Kareholt I, Qiu C, Bellander T, Winblad B, Fratiglioni L. Relation of education and occupation-based socioeconomic status to incident Alzheimer's disease. *Am J Epidemiol.* Jan 15 2004;159(2):175-183.

104.   Qiu C, Karp A, von Strauss E, Winblad B, Fratiglioni L, Bellander T. Lifetime principal occupation and risk of Alzheimer's disease in the Kungsholmen project. *Am J Ind Med.* Feb 2003;43(2):204-211.

105.   Fratiglioni L, Ahlbom A, Viitanen M, Winblad B. Risk factors for late-onset Alzheimer's disease: a population-based, case-control study. *Ann Neurol.* Mar 1993;33(3):258-266.

106.   Schmand B, Smit JH, Geerlings MI, Lindeboom J. The effects of intelligence and education on the development of dementia. A test of the brain reserve hypothesis. *Psychol Med.* Nov 1997;27(6):1337-1344.

107.   Schofield PW, Logroscino G, Andrews HF, Albert S, Stern Y. An association between head circumference and Alzheimer's disease in a population-based study of aging and dementia. *Neurology.* Jul 1997;49(1):30-37.

108.   Unverzagt FW, Hui SL, Farlow MR, Hall KS, Hendrie HC. Cognitive decline and education in mild dementia. *Neurology.* Jan 1998;50(1):181-185.

109.   Wilson RS, Li Y, Aggarwal NT, et al. Education and the course of cognitive decline in Alzheimer disease. *Neurology.* Oct 12 2004;63(7):1198-1202.

110.   Scarmeas N, Albert SM, Manly JJ, Stern Y. Education and rates of cognitive decline in incident Alzheimer's disease. *J Neurol Neurosurg Psychiatry.* Mar 2006;77(3):308-316.

111.   Del Ser T, Hachinski V, Merskey H, Munoz DG. An autopsy-verified study of the effect of education on degenerative dementia. *Brain.* Dec 1999;122 (Pt 12):2309-2319.

112.   Lo RY, Hubbard AE, Shaw LM, et al. Longitudinal change of biomarkers in cognitive decline. *Arch Neurol.* Oct 2011;68(10):1257-1266.

113.   Lo RY, Jagust WJ. Predicting missing biomarker data in a longitudinal study of Alzheimer disease. *Neurology.* in press.

114.   McGurn B, Starr JM, Topfer JA, et al. Pronunciation of irregular words is preserved in dementia, validating premorbid IQ estimation. *Neurology.* Apr 13 2004;62(7):1184-1186.

115.   Landau SM, Marks SM, Mormino EC, et al. Lifetime cognitive engagement is associated with low beta-amyloid deposition. *Arch Neurol.* in press.

116.   Rocher AB, Chapon F, Blaizot X, Baron JC, Chavoix C. Resting-state brain glucose utilization as measured by PET is directly related to regional synaptophysin levels: a study in baboons. *Neuroimage.* Nov 2003;20(3):1894-1898.

117. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann Neurol.* May 1990;27(5):457-464.

118. Lazarov O, Robinson J, Tang YP, et al. Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell.* Mar 11 2005;120(5):701-713.

119. Cirrito JR, Yamada KA, Finn MB, et al. Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron.* Dec 22 2005;48(6):913-922.

120. Bero AW, Yan P, Roh JH, et al. Neuronal activity regulates the regional vulnerability to amyloid-beta deposition. *Nat Neurosci.* Jun 2011;14(6):750-756.

121. Buckner RL, Sepulcre J, Talukdar T, et al. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *J Neurosci.* Feb 11 2009;29(6):1860-1873.

122. Jagust WJ, Mormino EC. Lifespan brain activity, beta-amyloid, and Alzheimer's disease. *Trends Cogn Sci.* Nov 2011;15(11):520-526.

123. Edland SD, Xu Y, Plevak M, et al. Total intracranial volume: normative values and lack of association with Alzheimer's disease. *Neurology.* Jul 23 2002;59(2):272-274.

124. Stephan BC, Matthews FE, Hunter S, et al. Neuropathological Profile of Mild Cognitive Impairment from a Population Perspective. *Alzheimer Dis Assoc Disord.* Sep 22 2011.

125. Piras F, Cherubini A, Caltagirone C, Spalletta G. Education mediates microstructural changes in bilateral hippocampus. *Hum Brain Mapp.* Mar 24 2010.

126. Breteler MM. Vascular risk factors for Alzheimer's disease: an epidemiologic perspective. *Neurobiol Aging.* Mar-Apr 2000;21(2):153-160.

127. Purnell C, Gao S, Callahan CM, Hendrie HC. Cardiovascular risk factors and incident Alzheimer disease: a systematic review of the literature. *Alzheimer Dis Assoc Disord.* Jan-Mar 2009;23(1):1-10.

128. Zlokovic BV. Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci.* Apr 2005;28(4):202-208.

129. Jeerakathil T, Wolf PA, Beiser A, et al. Stroke risk profile predicts white matter hyperintensity volume: the Framingham Study. *Stroke.* Aug 2004;35(8):1857-1861.

130. DeCarli C, Murphy DG, Tranh M, et al. The effect of white matter hyperintensity volume on brain structure, cognitive performance, and cerebral metabolism of glucose in 51 healthy adults. *Neurology.* Nov 1995;45(11):2077-2084.

131. Carmichael O, Schwarz C, Drucker D, et al. Longitudinal changes in white matter disease and cognition in the first year of the Alzheimer disease neuroimaging initiative. *Arch Neurol.* Nov 2010;67(11):1370-1378.

132. Launer LJ, Hughes TM, White LR. Microinfarcts, brain atrophy, and cognitive function: The Honolulu Asia Aging Study Autopsy Study. *Ann Neurol.* Nov 2011;70(5):774-780.

133. Gosche KM, Mortimer JA, Smith CD, Markesbery WR, Snowdon DA. Hippocampal volume as an index of Alzheimer neuropathology: findings from the Nun Study. *Neurology.* May 28 2002;58(10):1476-1482.

134. Schneider JA, Wilson RS, Bienias JL, Evans DA, Bennett DA. Cerebral infarctions and the likelihood of dementia from Alzheimer disease pathology. *Neurology.* Apr 13 2004;62(7):1148-1155.

135. Chui HC, Zarow C, Mack WJ, et al. Cognitive impact of subcortical vascular and Alzheimer's disease pathology. *Ann Neurol.* Dec 2006;60(6):677-687.

136. Marchant NL, Reed BR, Decarli CS, et al. Cerebrovascular disease, beta-amyloid, and cognition in aging. *Neurobiol Aging.* Oct 31 2011.

137. Mielke MM, Rosenberg PB, Tschanz J, et al. Vascular factors predict rate of progression in Alzheimer disease. *Neurology.* Nov 6 2007;69(19):1850-1858.

138. Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. *JAMA.* Mar 12 1997;277(10):813-817.

139. Bell RD, Zlokovic BV. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. *Acta Neuropathol.* Jul 2009;118(1):103-113.

140. Reed BR, Marchant NL, Jagust WJ, Decarli CC, Mack W, Chui HC. Coronary risk correlates with cerebral amyloid deposition. *Neurobiol Aging.* Nov 9 2011.

141. Kuczynski B, Reed B, Mungas D, Weiner M, Chui HC, Jagust W. Cognitive and anatomic contributions of metabolic decline in Alzheimer disease and cerebrovascular disease. *Arch Neurol.* May 2008;65(5):650-655.

142. Jernigan TL, Archibald SL, Berhow MT, Sowell ER, Foster DS, Hesselink JR. Cerebral structure on MRI, Part I: Localization of age-related changes. *Biol Psychiatry.* Jan 1 1991;29(1):55-67.

143. Dufouil C, de Kersaint-Gilly A, Besancon V, et al. Longitudinal study of blood pressure and white matter hyperintensities: the EVA MRI Cohort. *Neurology.* Apr 10 2001;56(7):921-926.

144. Wen W, Sachdev PS, Chen X, Anstey K. Gray matter reduction is correlated with white matter hyperintensity volume: a voxel-based morphometric study in a large epidemiological sample. *Neuroimage.* Feb 15 2006;29(4):1031-1039.

145. Bennet AM, Di Angelantonio E, Ye Z, et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. *JAMA.* Sep 19 2007;298(11):1300-1311.

146. de Groot JC, de Leeuw FE, Oudkerk M, et al. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Ann Neurol.* Feb 2000;47(2):145-151.