UNIVERSITY OF TURKU

EARLY DETECTION OF ALZHEIMER'S DISEASE

Twin Study on Episodic Memory and Imaging Biomarkers of Neuroinflammation and β-Amyloid

Noora Lindgrén

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To twins

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ABSTRACT

The disease process of Alzheimer's disease (AD) causes damage to the brain for several years leading to the development of mild cognitive impairment (MCI) and finally to dementia which interferes with independent living. The early detection of AD disease process is key for the prevention and treatment of disease. The aim of this thesis was to improve the assessment of episodic memory (EM) and cognitive performance with a telephone interview and neuroimaging of early AD. The study population belonged to the older Finnish Twin Cohort study. 2631 twins (856 pairs) participated in the telephone interview (TELE, TICS) during 1999–2007 and 1817 twins (559 pairs) participated in the interview (TELE, TICS, TICS-m) during 2013–2017. Cognitively discordant twin pairs were asked to participate in more detailed examinations. 11 twin pairs participated in [^{11}C]PBR28 positron emission tomography (PET) imaging measuring neuroinflammation during 2014–2017 and 45 twin pairs participated in [^{11}C]PiB PET imaging measuring β-amyloid (Aβ) deposits during 2005–2017.

Twins who had co-twins with dementia (n=101) performed poorer than average in a word list learning test. When using the telephone interview TICS-m, the education-adjusted classification resulted in a higher proportion of apolipoprotein (APOE) ε4 allele carriers among those identified as having MCI. Twins with poorer EM performance (n=10) had higher cortical [^{11}C]PBR28 uptake compared to their better-performing co-twins. In addition, higher cortical [^{11}C]PiB uptake was associated with poorer EM performance.

The results from the telephone interview studies indicate that poorer word list learning performance may be an early marker of dementia risk and that the use of education-adjustment may increase the accuracy of MCI classification. The twin pair setting controlling for genetic and environmental effects indicated that brain Aβ load and neuroinflammation have a negative association with EM performance.

KEYWORDS: dementia, mild cognitive impairment, Alzheimer's disease, twins, episodic memory, memory and learning tests, telephone screening, neuroinflammation, beta-amyloid, positron emission tomography, [^{11}C]PBR28, [^{11}C]PiB

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TIIVISTELMÄ

Alzheimerin taudin (AT) prosessi vaurioittaa aivoja vuosien ajan ja johtaa lievään kognitiiviseen heikentymiseen (MCI) ja lopulta itsenäistä selviytymistä häiritsevään dementiaan. AT:n dementiaan johtavan prosessin varhainen havaitseminen on avainasemassa ehkäisyn ja hoidon kannalta. Tämän väitöskirjatutkimuksen tavoitteena oli kehittää puhelinhaastattelun käyttöä episodisen muistin (EM) ja muiden tiedonkäsittely- eli kognitiivisten toimintojen arvioimisessa sekä AT:n varhaista kuvantamista. Tutkimusjoukko kuului vanhempaan suomalaisen kaksoskohorttitutkimukseen. 2631 kaksosta (856 paria) osallistui puhelinhaastatteluun (TELE, TICS) 1999–2007 aikana ja 1817 kaksosta (559 paria) osallistui haastatteluun (TELE, TICS, TICS-m) 2013–2017 aikana. Kognitiivisesti diskordantit kaksosparit kutsuttiin tarkempiin jatkotutkimuksiin. 11 kaksosparia osallistui neuroinflammaatiota mittaavaan [^{11}C]PBR28-merkkiaineen positroniemissiotomografia (PET) -kuvaukseen 2014–2017 aikana ja 45 kaksosparia osallistui aivojen β-amyloidikertymää mittaavaan [^{11}C]PiB-merkkiaineen PET-kuvaukseen 2005–2017 aikana.

Sellaisten kognitiivisesti normaalien ikääntyneiden kaksosten (n=101), joiden sisaruksella oli dementia, havaittiin suoriutuvan keskimääräistä heikommin sanalistan oppimista mittaavassa testissä. Käytettäessä TICS-m-puhelinhaastattelua koulutuskorjauksen käyttäminen johti siihen, että MCI:tä sairastavien joukossa oli suurempi osuus apolipoproteiini E:n (APOE) ε4-alleelin kantajia. Kaksosilla (n=10), jotka suoriutuivat heikommin EM-testeissä, oli suurempi aivokuoren [^{11}C]PBR28-kertymä verrattuna paremmin suoriutuviin sisaruksiinsa. Myös suurempi aivokuoren [^{11}C]PiB-kertymä oli yhteydessä heikompaan EM-suoritukseen.

Puhelinhaastattelujen tulokset viittaavat siihen, että sanalistan oppiminen voi olla dementiariskistä kertova varhainen merkki ja että koulutuskorjauksen käyttö voi lisätä MCI-luokittelun tarkkuutta. Kaksosasetelma, joka kontrolloi geneettisten ja ympäristötekijöiden vaikutusta, osoitti, että aivojen β-amyloidikertymä ja neuroinflammaatio ovat negatiivisessa yhteydessä EM:n toiminnan kanssa.

AVAINSANAT: dementia, lievä kognitiivinen heikentyminen, Alzheimerin tauti, kaksoset, episodinen muisti, muisti- ja oppimistestit, neuroinflammaatio, beeta-amyloidi, positroniemissiotomografia, [^{11}C]PBR28, [^{11}C]PIB

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Appendix 1: Questions and scoring of the telephone assessment for dementia (TELE), Telephone Interview for Cognitive Status (TICS) and the modified Telephone Interview for Cognitive Status (TICS-m) 119

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Abbreviations

[^{11}C]PBR28	*N*-acetyl-*N*-(2-[^{11}C]methoxybenzyl)-2-phenoxy-5-pyridinamine
[^{11}C]PiB	[^{11}C]Pittsburgh compound B
Aβ	β-amyloid
AD	Alzheimer's disease
ADAD	Autosomal dominant Alzheimer's disease
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive subscale
ADCS-ADL	Alzheimer's disease cooperative study - activities of daily living inventory
ADCS-PACC	ADCS Preclinical Alzheimer Cognitive Composite
aMCI	Amnestic mild cognitive impairment
API	Alzheimer's Prevention Initiative
APOE	Apolipoprotein E gene
APP	Amyloid precursor protein
AVLT	Rey Auditory Verbal Learning Test
B	Unstandardised regression coefficient β
BACE	β-secretase, β-site amyloid precursor protein cleaving enzyme
BP	Binding potential
CAA	Cerebral amyloid angiopathy
CD33	Siglec-3, sialic acid binding Ig-like lectin 3
CDR	Global assessment measures including the Clinical Dementia Rating
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CES-D	Center for Epidemiologic Studies Depression Scale
ChEI	Cholinesterase inhibitor
COX	Cyclo-oxygenase
CSF	Cerebrospinal fluid
CVD	Cerebrovascular disease
DLB	Dementia with Lewy bodies
DR	Delayed recall
DSM-5	Diagnostic and Statistical Manual of Mental Disorders
DZ	Dizygotic
EM	Episodic memory

EOAD	Early-onset Alzheimer's disease
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
FWE	Family-wise error
GDS/FAST	Global Deterioration Scale/Functional Assessment and Staging
GM	Grey matter
IQCODE	Informant Questionnaire for Cognitive Decline in the Elderly
IQR	Interquartile range
IR	Immediate recall
IWG	International working group
K_1	Delivery rate constant
LBD	Lewy body dementia
LOAD	Late-onset Alzheimer's disease
mAb	Monoclonal antibody
MAO-B	Monoamine oxidase-B
MCI	Mild cognitive impairment
MMSE	Mini-mental state examination
MRI	Magnetic resonance imaging
MZ	Monozygotic
NFT	Neurofibrillary tangle
NIA-AA	US National Institute on Aging−Alzheimer's Association
NSAID	Nonsteroidal anti-inflammatory drugs
PART	Primary age-related tauopathy
PET	Positron emission tomography
PDD	Parkinson's disease dementia
RBANS	Repeatable Battery for the Assessment of Neuropsychological Status
ROI	Region of interest
RRR	Relative risk ratio
SNP	Single nucleotide polymorphism
SUV	Standardised uptake value
SUVR	Standardised uptake value ratio
SVD	Small vessel disease
TAC	Time-activity curve
TDP-43	TAR DNA-binding protein 43
TELE	Telephone assessment for dementia
TICS	Telephone Interview for Cognitive Status
TICS-m	Telephone Interview for Cognitive Status-modified
TNF-α	Tumor necrosis factor α
TREM	Triggering receptors on myeloid cells
TSPO	Translocator protein 18 kilodaltons (kDa)

VaD	Vascular dementia
VCI	Vascular cognitive impairment
V_T	Distribution volume
XZ	Unknown zygosity
YKL-40	Chitinase 3-Like Protein 1
WMS-R	Wechsler Memory Scale-Revised

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Lindgren N, Kaprio J, Rinne JO, Vuoksimaa E. Immediate verbal recall and familial dementia risk: population-based study of over 4000 twins. *J Neurol Neurosurg Psychiatry.* 2019;90(1):90−97. doi: 10.1136/jnnp-2018-319122.

II Lindgren N, Rinne JO, Palviainen T, Kaprio J, Vuoksimaa E. Prevalence and correlates of dementia and mild cognitive impairment classified with different versions of modified Telephone Interview for Cognitive Status (TICS-m). *Int J Geriatr Psychiatry.* 2019;34:1883–1891. doi: 10.1002/gps.5205.

III Lindgren N, Tuisku J, Vuoksimaa E, Helin S, Karrasch M, Marjamäki P, Kaprio J, Rinne JO. Association of neuroinflammation with episodic memory: A [^{11}C]PBR28 PET study in cognitively discordant twin pairs. *Brain Communications.* 2020;2(1):fcaa024. doi: 10.1093/braincomms/fcaa024.

IV Lindgren N, Kaprio J, Karjalainen T, Ekblad L, Helin S, Karrasch M, Teuho J, Rinne JO*, Vuoksimaa E*. Episodic memory and cortical amyloid pathology: a PET study in cognitively discordant twin pairs. *Manuscript submitted for publication.*

1 Introduction

Progressive memory disorders, i.e., diseases that cause progressive loss of memory, other cognitive functions and functional abilities, are a growing global health problem in the ageing world population. Progressive memory disorders often lead to dementia (or major neurocognitive disorder according to the Diagnostic and Statistical Manual of Mental Disorders, DSM-5) which signifies the impairment of cognitive functions severely enough to interfere with independent living. Over 46 million people worldwide are estimated to have dementia, and the number is expected to increase to 131.5 million by 2050.[1] It is estimated that approximately every eighth individual aged over 65 years has dementia in Europe (12.4%).[2] The prevalence of dementia steeply increases with age.[2] Alzheimer's disease (AD) is the most common neurodegenerative disease and cause of dementia in the elderly, accounting for 60 to 70% of all dementia cases.[3] Other common causes of dementia include vascular dementia (VaD), mixed dementia, Lewy body dementia (LBD), and frontotemporal dementia (FTD). There is no available disease-modifying treatment for AD or other progressive memory disorders.

The most common dementia-causing diseases develop progressively over time. Once dementia is diagnosed, the underlying disease processes have already caused damage to the brain for several years. Before dementia occurs, brain changes cause mild symptoms in memory and other cognitive functions without having a significant effect on daily living. This stage is often called mild cognitive impairment (MCI). AD is now widely considered as a continuum consisting of a long-lasting preclinical stage, which may start even a decade or more before clinical symptoms occur, early and prodromal (i.e. MCI) stages with mild cognitive symptoms, and dementia stage with obvious cognitive and functional deficits.[4] Episodic memory (EM) is typically the most strikingly affected cognitive domain during the AD continuum.[4] When the first clinical symptoms of AD emerge, a profound loss of neurons has already occurred in critical memory circuits.[5] Therefore, treatment with disease-modifying drugs in predementia or even preclinical stages will likely be needed to prevent, slow down or even halt the progression of disease. Early and accurate detection of individuals who have brain changes leading to dementia is needed to enable early intervention with future disease-modifying drugs.

Even though the underlying causes for AD are unknown, multiple changes that typically occur during the disease processes are known. These include the deposition of β-amyloid (Aβ) peptides into extracellular plaques and cerebrovasculature, the aggregation of hyperphosphorylated tau protein into intracellular neurofibrillary tangles (NFTs), and neuroinflammatory changes. Eventually, loss of synapses and neurons occur. It is possible to detect these changes in living humans by using biomarkers. Biomarkers are defined as measurable *in vivo* indicators of specific disease-related pathologic processes. Positron emission tomography (PET) imaging enables the examination of brain functions such as energy metabolism, function of neurotransmitter receptors, and Aβ and tau pathologies. Individuals who have biomarker evidence of AD pathophysiology, but no or mild cognitive symptoms are believed to be in the preclinical and early stages of the AD continuum.[6] In addition to early detection, biomarkers are needed for the selection of individuals for clinical trials and for monitoring the efficacy and safety of new treatments. Sensitive cognitive tests are also potential early disease markers which can support the detection of high-risk individuals and the monitoring of drug effects and disease progression over time.

This thesis aims to develop the early detection of AD and dementia by studying the older Finnish Twin Cohort. Firstly, the thesis aims to improve the early detection of cognitive impairment with a telephone interview of cognition (**I-II**). Secondly, the neuroimaging biomarkers [^{11}C]PiB and [^{11}C]PBR28 PET, which measure fibrillar Aβ deposits and neuroinflammation, are investigated in twin pairs discordant for EM performance (**III-IV**). The overall description of studies **I-IV** is presented in Figure 1.

	Study I	Study II	Study III	Study IV
Population-based older Finnish Twin Cohort	4367 twins including 101 pairs discordant for dementia	1772 twins	22 twins (11 twin pairs)	90 twins (45 twin pairs)
Cognitive measures	Verbal immediate free recall, Telephone assessment for dementia (TELE)	Verbal immediate and delayed free recall, Telephone Interview for Cognitive Status (TICS-m)	Verbal immediate, verbal and visual delayed free recall, global cognition (CERAD-NB)	Verbal immediate verbal and visual delayed free recall, global cognition (CERAD-NB)
PET radioligands			[^{11}C]PBR28 (neuro-inflammation)	[^{11}C]PIB (β-amyloid)

Figure 1. Relationship of studies I–IV

2 Review of the Literature

2.1 Definitions along the continuum from healthy cognitive ageing to dementia

2.1.1 Cognition and episodic memory

Cognition is a complex system consisting of brain functions such as learning, memory, executive functions, language, and visuospatial abilities. Memory is classically categorised as declarative and non-declarative (implicit) memory.[7] Declarative memory is further divided into episodic memory (EM) and semantic memory. EM is the memory of events with a specific temporal and spatial context and semantic memory is the memory of general facts.[8] Executive functions include working memory, inhibitory control, cognitive flexibility, verbal fluency, processing speed, and planning. These functions are associated with the frontal cortex, particularly with the dorsolateral prefrontal cortex, and the networks associated with these regions.[9]

EM formation is composed of encoding, consolidation, and retrieval. In the encoding phase or learning, the brain forms a neural representation of an event or association.[10] Encoding can be intentional, i.e. there is a specific aim to learn and memorise information, or incidental, i.e. there is no specific aim to learn. Encoding is based on coordinated neural activity in a distributed neural network which includes regions such as the medial temporal lobe, prefrontal and parietal cortex.[10,11] The medial temporal lobe, including the hippocampus and the surrounding parahippocampal, entorhinal and perirhinal cortex, is considered as the key region in this network.[10] During encoding, information is processed in various neocortical areas including the sensory and association cortices and the prefrontal cortical executive system.[10] Neocortical neurons project to the medial temporal lobe and hippocampus in which a specific neural activation map is stored through activity-dependent changes in synaptic strength.[10,12] The neural map in the hippocampus is thought to associate with a specific set of neocortical neurons that were involved in forming the memory.[10] Long-term consolidation is based on the continued interaction between the hippocampus and neocortex and the occurrence of synaptic plasticity in the neocortical networks.[10,12] Rehearsal and sleep enhance memory

performance, most likely by affecting long-term consolidation.[13] In EM retrieval, the cortical activation pattern that was formed during encoding is thought to reactivate and to result in a conscious recollection of the memory. The function of the medial temporal lobe and many cortical areas, such as the medial prefrontal, posterior cingulate and posterior parietal cortex, is important for successful memory retrieval.[10] The important brain regions for EM functioning are illustrated in Figure 2.

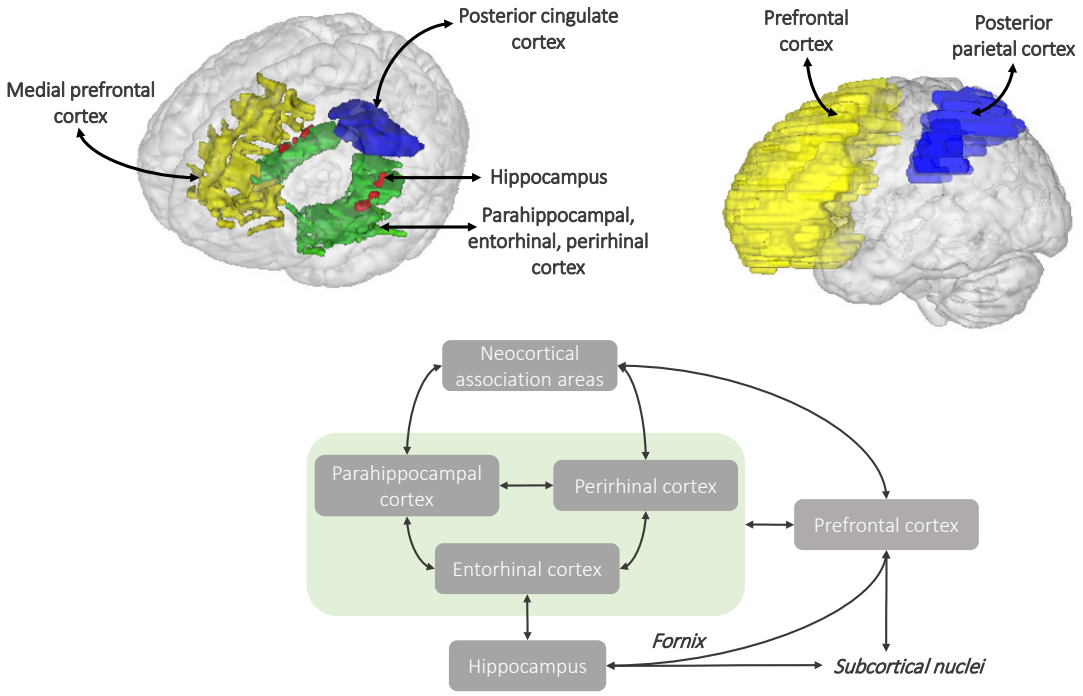

Figure 2. Anatomy and main connections of important brain areas for episodic memory function. The medial temporal lobe consists of the hippocampus, amygdala, entorhinal, perirhinal and parahippocampal cortex. The anatomical images were done using the Mango program and Talairach atlas. The image about connections was modified from Simons & Spiers 2003.[14]

2.1.2 Cognitive ageing, mild cognitive impairment, and dementia

Ageing, particularly after the age of 60, is associated with decreasing test performance in several cognitive domains, such as memory, processing speed and executive functions.[15] This age-related cognitive decline has been associated with lower synaptic density and white matter abnormalities particularly in the prefrontal cortex.[16] More severe cognitive decline is associated with age-related

neuropathologies, such as AD, Lewy body disease and cerebrovascular pathologies.[17] AD-related neuropathology, including Aβ and tau deposits, is the most common age-related neuropathology.[17,18] The cognitive impact of neuropathologies varies greatly at the individual level.[17]

The cognitive performance of some older individuals is equal or greater than that of middle-aged and younger adults.[19] In addition, some older individuals show a stable level of EM performance for up to 15 years.[20] The proportion of these successful agers has varied from 6% to over 40% across studies.[19] There are several theories aiming to explain individual differences in cognitive ageing. The most successful way to achieve healthy cognitive ageing is through the minimisation of age-related brain changes and absence of neuropathology. This preservation of brain integrity in old age is called brain maintenance.[21] On the other hand, the concept of reserve aims to explain why some older adults show little evidence of cognitive decline despite neuropathological changes. Brain reserve refers to individual differences in the brain, e.g. number of neurons and synapses, that allow some people to function better than others with a similar amount of neuropathology.[22] Cognitive reserve refers to individual differences in functional brain processes that allow some people to function better than others with similar amount of neuropathology.[22] Genetic and lifetime environmental factors, such as socially, mentally, and physically stimulating activities, may support the reserve and maintenance in old age.[21,22] Neuroimaging studies have reported that older adults often display overactivation of neural networks, e.g. in the prefrontal cortex. Several theories, such as the Hemispheric Asymmetry Reduction in Older Adults (HAROLD) model and the Compensation-Related Utilisation of Neural Circuits Hypothesis (CRUNCH), suggest that overactivation may reflect compensatory strategies against age-related decline.[23] Understanding individual variability in cognitive ageing and reserve is critical for the accurate detection and prediction of progression in the early stages of AD and other progressive memory disorders.

MCI is a heterogeneous condition between normal ageing and dementia characterised by impaired cognition without the loss of independent functioning. The prevalence of MCI is estimated to be 6.7% for ages 60–64, 8.4% for 65–69, 10.1% for 70–74, 14.8% for 75–79, and 25.2% for 80–84.[24] MCI can be caused by AD pathology or other disease pathology (i.e. neurodegenerative, neurologic, psychiatric or metabolic disorders). It is associated with an increased risk of dementia, hippocampal and entorhinal cortical atrophy, greater cognitive decline, and increased risk of death.[25,26] Individuals with MCI may progress to dementia (15% after a 2-year follow-up), revert to normal (14% to 38%) or remain stable.[24] MCI is often divided into four different subtypes: single-domain amnestic (isolated memory deficit), multiple-domain amnestic (memory and at least one other cognitive domain is affected), single-domain nonamnestic (isolated non-memory deficit) and multiple-

domain nonamnestic MCI. These subtypes are likely to have different outcomes (Table 1). Individuals with amnestic MCI (aMCI), both single- and multiple-domain, are more likely to progress to AD dementia compared to individuals with nonamnestic MCI.[27]

Table 1. Classification of mild cognitive impairment subtypes with presumed aetiology according to Petersen 2004[28]

Classification of MCI type		Aetiology		
		Degenerative	***Vascular***	***Psychiatric***
Single domain	Amnestic	AD		Depression
Single domain	Non-amnestic	FTD, LBD		
Multiple domain	Amnestic	AD	VaD	Depression
Multiple domain	Non-amnestic	LBD	VaD	

Abbreviations: AD, Alzheimer's disease; LBD, Lewy body dementia; FTD, Frontotemporal dementia; MCI, Mild cognitive impairment; VaD, Vascular dementia.

2.2 Alzheimer's disease

2.2.1 Overview, risk factors and current treatment

AD is often divided based on the age of onset into early-onset AD (EOAD) and late-onset AD (LOAD). EOAD typically begins at age 60 or before. Less than 10% of AD cases have early onset, and around 10% of EOAD cases are autosomal dominant AD (ADAD) that is due to autosomal dominant inheritance of mutations in the amyloid precursor protein (APP), presenilin 1 or presenilin 2 genes.[29] Even though the majority of EOAD cases are not due to dominant mutations, EOAD is almost an entirely genetically-based disease and may be due to autosomal recessive inheritance.[30] The most common form of AD is LOAD, a polygenic disease that typically begins after the age of 65.[30,31]

LOAD is thought to develop due to complex gene-environment interactions that occur with age. Age is the most important risk factor: the estimated prevalence of AD is 3% among those aged 65–74 years, 17% among those aged 75–84 years, and 32% among those aged 85 years or older.[32] LOAD has substantial genetic background with heritability around 60–80%.[30,33] After age, the greatest risk factors are carrying the apolipoprotein E (APOE) ε4 allele and having a family history of AD.[34] Carrying one APOE ε4 allele increases the lifetime risk of developing AD approximately 3-fold and carrying two APOE ε4 alleles increases the risk approximately 10-fold compared to non-carriers.[35] In addition to APOE, over 20 common genetic markers associated with the risk of LOAD have been identified with

genome-wide association studies.[31] However, the identified single nucleotide polymorphisms (SNPs) contribute to an individual's risk of developing AD substantially less than the APOE genotype. The identified genes are implicated in the function of the immune system, lipid metabolism, tau binding, and APP metabolism. The known SNPs explain only approximately 31% of the genetic variance of AD.[31]

AD is also classified into typical and atypical forms based on the clinical phenotype. Typical amnestic AD is characterised predominantly by memory problems and neurofibrillary tangle (NFT) pathology, and neurodegeneration in the medial temporal lobe. When the disease progresses, deficits in executive, language, and visuospatial functions occur. Atypical AD is usually early-onset and begins with visuospatial symptoms (posterior variant), or with symptoms characteristic for FTD in language (logopenic variant) or executive functions (frontal variant).[36]

Based on observational studies, there are many environmental and potentially modifiable risk factors for AD and dementia, including cardiovascular risk factors in midlife (e.g. hypertension, diabetes, obesity and smoking), low cognitive activity (e.g. low education attainment, occupational attainment and mentally stimulating activities), depression and low physical activity.[37] Stronger evidence of the effect of these risk factors on AD and dementia incidence and on the related brain changes is anticipated from randomised controlled trials, i.e. the World Wide FINGERS. Women are more often affected by AD and dementia than men (2:1 women:men ratio). This may be caused by the longer life expectancy of women and the survival of men with the best cardiovascular health into old age.[38] In addition, the prevalence of risk factors for AD, including cerebrovascular, metabolic, sociocultural factors, and depression, differ between aged men and women. These factors may also differentially affect the risk and development of AD in women and men.[39,40]

Current pharmacological AD treatment includes two groups of cognitive enhancing drugs: the cholinesterase inhibitors (ChEIs; donepezil, galantamine and rivastigmine), which enhance cholinergic function by inhibiting the hydrolysis of acetylcholine, and the *N*-methyl-D-aspartate receptor (NMDA) receptor antagonist, memantine. ChEIs are indicated in the symptomatic treatment of mild to severe AD dementia and memantine in moderate to severe AD dementia. Current treatment provides a small clinical benefit on cognitive, functional, and behavioural symptoms in AD.[41,42]

2.2.2 Diagnostic criteria: moving from clinical classification to biomarker-based classification

The first specific diagnostic criteria for AD were established in 1984 by the US National Institute on Aging−Alzheimer's Association (**NIA-AA**) workgroup.[43] The

NINCDS-ADRDA clinical criteria for **probable AD** included dementia established by a clinical and neuropsychological examination, insidious onset, progressive memory and other cognitive deficits, and lack of other diseases that could explain the deficits. A definite diagnosis of AD was only possible if there was also a histopathological confirmation of AD pathology. Validation studies of NINCDS-ADRDA criteria for probable AD against the neuropathological confirmation have reported a good sensitivity (average 81%, range 49–100%) but poorer specificity (average 70%, range 47–100%).[44]

In 1999, Petersen et al.[45] defined the clinical criteria for **mild cognitive impairment (MCI)** which was proposed as an intermediate condition between normal ageing and the diagnosis of probable AD. The criteria are as follows: "(1) memory complaint, (2) objective memory impairment for age, (3) relatively preserved general cognition, (4) largely intact activities of daily living, and (5) not demented." The International Working Group on MCI in 2004 further modified the criteria, resulting in the Petersen-Winblad criteria: (1) the person is neither normal or demented, (2) there is evidence of cognitive deterioration shown by either objectively measured decline over time and/or subjective report of decline by self and/or informant in conjunction with objective cognitive deficits, and (3) activities of daily living are preserved and complex instrumental functions are either intact or minimally impaired.[46]

In 2007, the International Working Group (**IWG**) revised the NINCDS-ADRDA clinical criteria due to the establishment of magnetic resonance imaging (MRI) and PET imaging, and cerebrospinal fluid (CSF) biomarkers for detecting pathophysiological changes of AD.[4] The objective of IWG criteria was to enable diagnosis of **probable AD** at an earlier stage (i.e. **prodromal AD**) before dementia. The core criterion of probable AD was the presence of early significant EM impairment with a gradual decrease over more than 6 months. In addition, one or more supportive features must be present: 1) presence of medial temporal lobe atrophy on MRI; 2) abnormal CSF biomarkers of β-amyloid or tau; 3) specific PET finding (glucose metabolism, amyloid); 4) proven AD autosomal dominant mutation in the family. The 2017 Finnish Current Care Guidelines for memory disorders recommend the 2007 IWG criteria as the diagnostic criteria of AD. The IWG criteria was later updated to include criteria for **atypical presentations of AD** (posterior variant, logopenic variant of primary progressive aphasia, frontal variant) and for mixed AD.[47] In addition, the following research criteria for **preclinical AD**, defined as asymptomatic at-risk state, was proposed: absence of clinical symptoms of AD and at least one biomarker evidence of AD pathophysiology or the presence of an AD autosomal dominant mutation.[47]

In 2011, **NIA-AA** published updated diagnostic criteria for **AD dementia**[48] and for **MCI due to AD**.[49] In addition, recommendations for defining **preclinical AD**

were proposed.[50] The criteria classified AD dementia to: 1) probable AD dementia, 2) possible AD dementia, and 3) probable or possible AD dementia with evidence of AD pathophysiological process (MRI, CSF or PET biomarker evidence). The first two classifications were intended for clinical criteria and the third for research criteria. The criteria for probable AD dementia were similar to the 1984 criteria, but non-amnestic (language, visuospatial or executive dysfunction) presentations of AD were also included. The criteria for possible AD dementia included atypical course or etiologically mixed presentation. **MCI due to AD** included a positive biomarker test of Aβ deposition and/or neuronal injury.[49] The proposed research criteria for **preclinical AD** included three stages: 1) asymptomatic with cerebral amyloidosis, 2) asymptomatic with cerebral amyloidosis, synaptic dysfunction and/or early neurodegeneration, 3) amyloid positivity, evidence of neurodegeneration and subtle cognitive decline.[50] IWG or NIA-AA criteria are not fully validated, and it is not established which criteria are the most sensitive and specific in the clinical setting.

In 2018, new **NIA-AA** guidelines were published.[6] Unlike the 2011 NIA-AA criteria, the new guidelines were intended to be used only as a research framework and not in clinical settings. The objective of updated, biomarker-based diagnostic criteria was to enable the definition of AD across its entire continuum. The greatest difference to the 2011 criteria was that the new guidelines defined AD solely based on biomarkers and not on clinical symptoms. According to the 2011 criteria, individuals having a typical dementia syndrome without biomarker evidence were classified as having possible or probable AD, whereas based on the 2018 criteria, they are considered to have **Alzheimer's clinical syndrome**. In more detail, biomarkers are grouped into those measuring Aβ (labelled "A"), pathologic tau ("T") and neurodegeneration or neuronal injury ("N") giving rise to the **AT(N) system** (Table 2). An individual's biomarker profile is formed based on binary classification (positive or negative) of each biomarker group. No cut-off scores were specified for different biomarkers. AD biomarkers will be discussed in more detail in chapter 2.4.

The decreasing emphasis on clinical symptoms may give the appearance of a radical shift in the research and diagnostic criteria for AD. However, the definite diagnosis of AD has persisted over decades of research: the neuropathologic confirmation of Aβ plaques and NFTs in the post-mortem brain. It is still uncertain if the accumulation of Aβ and phosphorylated tau causes progressive neurodegeneration associated with AD. In the 2018 NIA-AA guidelines, it is argued that Aβ and tau proteinopathies define AD as a unique disease even if they are not in the primary causal pathway.

Table 2. Biomarker profiles and biomarker categories combined with syndromal staging according to 2018 NIA-AA research framework guidelines

Biomarker profile	**Biomarker category**	**Syndromal stage**		
		CU	***MCI***	***Dementia***
A-T-(N)-	Normal AD biomarkers	Normal AD biomarkers, CU	Normal AD biomarkers with MCI	Normal AD biomarkers with dementia
A+T-(N)-	Alzheimer's pathologic change	Preclinical Alzheimer's pathologic change	Alzheimer's pathologic change with MCI	Alzheimer's pathologic change with dementia
A+T+(N)-	Alzheimer's disease	Preclinical Alzheimer's disease	Alzheimer's disease with MCI (prodromal AD)	Alzheimer's disease with dementia
A+T+(N)+	Alzheimer's disease			
A+T-(N)+	Alzheimer's and SNAP pathologic change	Alzheimer's and SNAP pathologic change, CU	Alzheimer's and SNAP pathologic change with MCI	Alzheimer's and SNAP pathologic change with dementia
A-T+(N)-	Non-AD pathologic change	non-AD change with CU	non-AD change with MCI	non-AD change with dementia
A-T-(N)+	Non-AD pathologic change			
A-T+(N)+	Non-AD pathologic change			

NOTE. A refers to amyloid biomarker evidence from amyloid PET or CSF measurement of $A\beta_{42}$, or $A\beta_{42}/A\beta_{40}$ ratio. T refers to biomarker evidence of tau pathology from tau PET or CSF measurement of phosphorylated tau. (N) refers to neurodegeneration and neuronal injury. A and T indicate specific neuropathologic features of AD, while (N) is not specific to AD. The box delineates the "Alzheimer's continuum". Amyloid biomarkers define if an individual is in the Alzheimer's continuum and tau biomarkers define if an individual in the continuum has AD. Neurodegeneration biomarkers and clinical symptoms are used to stage severity. SNAP is defined by the presence of normal amyloid biomarkers, but abnormal neurodegeneration biomarkers. Abbreviations: AD, Alzheimer's disease; CU, cognitively unimpaired; MCI, mild cognitive impairment; SNAP, suspected non-Alzheimer's pathophysiology.

2.2.3 Neuropathology

The gross pathology of AD includes hippocampal atrophy, thinning of cerebral cortex, and ventricular enlargement.[51] Classical neuropathological hallmarks of AD, extracellular amyloid plaques, and intraneuronal NFTs develop in the brain in different patterns starting decades before the onset of dementia. Amyloid plaques

consisting of Aβ peptide aggregates show considerable variation in size and shape. Diffuse plaques have an amorphous shape and do not contain large amounts of fibrillar Aβ, while cored plaques are spherical and have a dense core comprised of fibrillar Aβ. Neuritic plaques are a subset of cored plaques that are surrounded by dystrophic neurites, i.e. degenerating axons and dendrites that often contain hyperphosphorylated tau aggregates.[52] They are also characterised by glial activation and greater local synapse loss compared to other plaques.[52] **Thal staging** describes the sequence of Aβ deposition in the brain.[53] Initially, Aβ deposition occurs exclusively in the neocortex and is dominated by the deposition of diffuse plaques (**phase 1**). Aβ deposits expand to allocortical regions such as the entorhinal cortex and CA1 of the hippocampus (**phase 2**), to subcortical regions, e.g. in the striatum (**phase 3**), to brainstem (**phase 4**), and to the cerebellum (**phase 5**). During the progression of Aβ pathology, total density of plaques reaches a plateau, but there is a shift in the type of plaques as the proportion of neuritic plaques increases.[54]

Abnormally phosphorylated tau causes intraneuronal cytoskeletal changes − NFTs. The spread of these changes is not uniform but follows a hierarchical order. In contrast to Aβ deposition, NFT pathology begins in the allocortex before spreading to neocortical regions. In **Braak stages I and II (transentorhinal stages)**, NFTs are found in the transentorhinal region, i.e. the medial portion of the perirhinal cortex.[55] The presence of at least a few NFTs in the transentorhinal and entorhinal cortex may be almost inevitable at the latest by the sixth decade of life.[54] Abnormally phosphorylated, slightly aggregated tau, i.e., pretangle material is found in the nerve cells of brainstem (e.g. locus coeruleus) even earlier.[56] More severe NFT pathology (Braak stages III/IV) appears only after amyloid plaque deposition occurs.[54] In the **limbic stages III and IV**, NFT changes become more severe in the transentorhinal and entorhinal regions and the CA1 of the hippocampus becomes affected. Next, NFTs develop in the subiculum of the hippocampus, amygdala, thalamus and claustrum. In the **neocortical stages V and VI**, NFT changes extend into associative regions and finally to primary sensory, motor, and visual areas. The prevalence of abnormal tau and Aβ plaque deposits according to Braak stages and Thal phases is shown in Figure 3.

Clinicopathological studies show that NFTs, and to some degree neuritic plaques, correlate with the severity of cognitive impairment.[52] Progressive cognitive decline in AD is even more closely associated with synaptic and neuronal loss in the limbic system, basal forebrain, and neocortex.[51] In the preclinical stage, Aβ accumulation occurs in the neocortex and NFT pathology primarily affects the transentorhinal cortex (Braak I-II). Individuals in this stage often have no symptoms or mild EM decline. Early during the AD process, pyramidal neurons in layer II of the entorhinal cortex, the subiculum, the CA1 region, the cholinergic neurons in the basal forebrain and the noradrenergic neurons in the locus coeruleus, which are

vulnerable to the accumulation of hyperphosphorylated tau, are lost (Braak III–IV).[57] As a result MCI or mild dementia characterised by prominent EM impairment may be detected. As temporal, parietal and frontal association cortices become affected (Braak V–VI), executive functions, language, semantic memory, and visuospatial abilities typically decline.[51] 91% of individuals with Braak stage V–VI and frequent neuritic plaque density in the neocortex have moderate to severe dementia.[52]

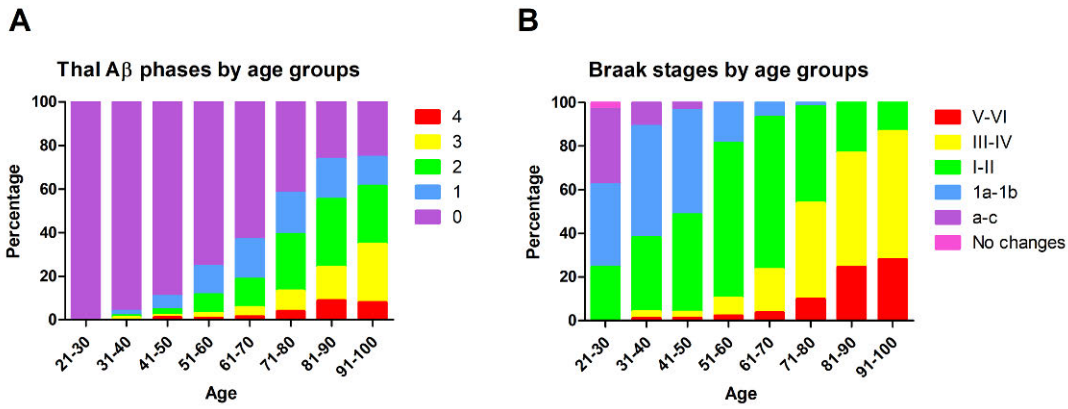

Figure 3. Prevalence of Thal β-amyloid (Aβ) phases (A) and Braak neurofibrillary tangle (NFT) pathology stages (B) by age groups in years. Figure is based on Braak et al. 2011.[56] Aβ pathology is divided according to Thal phases (0–5). NFT pathology is divided as follows: a-c, subtle subcortical pathology; 1a-1b, non-argyrophilic pathology in the cerebral cortex; I-VI Braak stages, argyrophilic NFT pathology.

The neuropathological diagnosis of AD is usually based on the Braak neurofibrillary staging, Consortium to Establish a Registry for AD (CERAD) ranking of neuritic plaque frequency in neocortical regions, and Thal amyloid staging.[58] Other neuropathological changes in AD include the deposition of Aβ in brain blood vessel walls, neuronal and synaptic loss, gliosis, degenerative changes in white matter, and often co-occurring protein aggregates like TDP-43 and Lewy bodies.[58]

2.2.4 Molecular pathogenesis

Multiple hypotheses have been proposed for the cause of LOAD. These include, for example, the amyloid-cascade hypothesis,[59] tau propagation hypothesis,[60] amyloid cascade-inflammatory hypothesis,[61] mitochondrial cascade hypothesis,[62] calcium homeostasis hypothesis,[63] and neurovascular hypothesis[64].

β-amyloid and tau proteinopathies

Aβ peptides are produced by neurons, microglia, and astrocytes in the brain and result from the β- and γ-secretase-mediated cleavage of the transmembrane protein APP. They are proteins with different lengths and post-translational modifications which assemble into monomers, oligomers, protofibrils and fibrils. The major peptide species in the brain are $A\beta_{40}$ and the highly self-aggregating $A\beta_{42}$. Aβ production, including $A\beta_{42}$, is a normal physiological process that is enhanced by synaptic activation and may be beneficial for synaptic plasticity at low physiological concentrations.[65] In addition, Aβ may protect the brain from infections.[66] Aβ is cleared from the brain by transportation to the periphery (across the blood-brain barrier and blood-CSF barrier, interstitial fluid bulk flow and CSF absorption into the lymphatic systems), proteolytic degradation and phagocytosis by various cells, including microglia, perivascular macrophages, and astrocytes.[67]

Tau is a microtubule-associated protein which is normally located in the axon and participates in the regulation of microtubular stability, dynamics, and transport. Like Aβ, tau exists as multiple isoforms and undergoes post-translational modification at many sites, mainly via phosphorylation. Under pathological conditions, tau is about 3-4-fold more phosphorylated than in normal physiological conditions.[68] Abnormally phosphorylated tau detaches from microtubules and accumulates in the somatodendritic compartment forming oligomers and eventually insoluble paired helical filaments which deposit as NFTs and neuropil threads.[69]

The amyloid-cascade hypothesis is the most dominant model of AD pathogenesis. According to the hypothesis, accumulation of Aβ is the causative agent of AD and leads to tau hyperphosphorylation, neurodegeneration, and cognitive impairment.[59] Different Aβ species and aggregation states probably have varying pathogenic effects. The focus of the amyloid hypothesis has shifted from insoluble Aβ pathology to soluble Aβ oligomers which accumulate around plaques and may be the most toxic form of Aβ.[70] The amyloid hypothesis is supported by genetic evidence. ADAD-causing mutations in the APP, presenilin 1 and 2 genes increase the production and aggregation of Aβ peptides.[70] In addition, an AD-protective mutation in the APP gene (A673T) decreases the cleavage of APP by β-secretase resulting in a lower lifelong risk of AD.[71] In LOAD, the accumulation of Aβ rather results from the impaired clearance of Aβ.[67] The increased AD risk conferred by ApoE ε4 seems to arise at least partly from its role in the regulation of Aβ clearance.[72]

The toxic effects of Aβ especially target synapses. Aβ oligomers impair synaptic plasticity, decrease synaptic density, and impair memory in rodents.[73] They also induce tau hyperphosphorylation and NFT formation in the rodent brain.[74,75] Oligomeric tau also causes synaptic dysfunction and impairs memory.[76] Both Aβ and tau oligomers disrupt mitochondrial function which in turn causes synaptic dysfunction, apoptosis and neurodegeneration.[69] Hyperphosphorylated tau also

destabilises microtubules, impairing axonal transport and causing axonal degeneration. As the size of NFTs increases, they interfere with cellular functions and cause neuronal injury.[69] Aβ and tau pathology may spread in distinctive patterns due to the successive involvement of neuronal subpopulations with varying vulnerabilities.[77] Another possibility is that tau and Aβ aggregates have prion-like properties and their release and uptake by recipient neurons (seeding and spreading) causes the progression.[78]

There are some contradictions to the amyloid-cascade hypothesis. Amyloid plaque load correlates less strongly with cognitive impairment than NFTs, and some clinically normal individuals can have substantial amyloid plaque load. The counterargument is that Aβ accumulation is a very early phenomenon and downstream changes correlate more strongly with cognitive impairment as they are more proximate to neuronal loss. In addition, clinically normal individuals with similar plaque levels as demented individuals may have substantially lower plaque-associated Aβ oligomer levels than demented individuals.[79] It is possible that plaques act as a reservoir of neurotoxic oligomers up to a point before releasing oligomers to the surroundings.

Findings suggesting that acute increases of Aβ levels may protect from brain injury and seal leaks in the blood-brain barrier, for example after transient acute brain injury and cerebrovascular insults, raise questions related to the amyloid cascade hypothesis.[66] Could Aβ accumulation be a compensatory response to upstream processes in AD? It cannot be ruled out that Aβ accumulation is induced by inflammation, abnormal microglial function, tau-associated network disruption and/or oxidative stress and is aimed initially at reducing or repairing neuronal injuries. In addition, the failure of multiple clinical trials targeting Aβ poses a great conflict to the amyloid-cascade hypothesis. Targeting patients with AD dementia, problems in the design of trials and properties of investigated drugs might explain the failures.

Another contradiction to the amyloid-cascade hypothesis is that neuropathological studies are somewhat in disagreement with the hypothesis. Based on neuropathological data, accumulation of tau occurs in the medial temporal lobe and brainstem before the deposition of diffuse amyloid plaques occur (Figure 3). One counterargument is that high levels of Aβ oligomers may be present in these very early stages. In addition, the accumulation of tau does not seem to lead to Aβ accumulation. Mutations in the gene encoding tau cause tau hyperphosphorylation and accumulation but do not lead to Aβ accumulation and AD dementia but to FTD. Another possibility to the sequential amyloid-cascade hypothesis is that tau hyperphosphorylation and Aβ accumulation are independent parallel processes which may have common upstream causes.[80,81] In conclusion, among the most important open questions related to the AD pathogenesis is whether Aβ

accumulation is the causative agent of AD and whether it causes the spread of medial temporal lobe tau pathology, and if so how. Recent evidence suggests that a defective inflammatory response may be a crucial factor in the interaction between Aβ and tau.

Neuroinflammation and its interplay with β-amyloid and tau

AD is associated with a chronic response of the innate immune system characterised by reactive glial cells and elevated levels of inflammatory mediators that is often referred to as neuroinflammation. Increasing evidence indicates that neuroinflammation is not merely a passive response to pathophysiological events, but a key player in the pathogenesis of AD from the very early stage. Genome-wide association studies have identified several novel risk genes for LOAD, such as TREM2[82–84] and CD33[85], which are important regulators of immune function, especially in microglial cells. Furthermore, APOE is primarily expressed by microglia and astrocytes in the brain, hence APOE ε4 expression in glial cells may contribute to the risk of AD.[86]

Microglia, the brain-resident macrophages, cover the whole human brain with a cell density ranging from 0.5% to 16.6% depending on the brain region.[87] They are embryonically derived, self-renewing brain-resident macrophages that are essential for maintaining CNS homeostasis from development to ageing.[88,89] Under homeostatic conditions, microglia with their numerous ramified processes are in constant motion and scan the entire brain once every few hours.[90] In addition to continuous surveillance, microglia phagocytose apoptotic debris, provide trophic support for neurons, and regulate the number of synapses.[91,92] In response to infectious pathogens and injurious self-proteins, microglia can become activated and initiate an innate immune response. Microglial activation can include morphological changes (hypertrophy of cell body and shortened ramifications), proliferation, phagocytosis, and secretion of cytokines and free radicals.[93] Activated phenotypes have been traditionally divided into proinflammatory, neurotoxic ‘M1’ type, and anti-inflammatory, neuroprotective ‘M2’ type. However, based on recent research, the activation is a far more dynamic process and microglia can acquire a wide range of phenotypes depending on the cellular surroundings and pathological conditions.[94]

In the human post-mortem AD brain, consistent increases of microglial activation-associated markers have been observed.[95] Activated microglia are located particularly in the proximity of fibrillar Aβ plaques, and, to a lesser but still significant degree, with paired helical filament tau, NFTs, and diffuse Aβ plaques.[96] Most of the knowledge of glial cell function in AD is based on animal and *in vitro* experiments. In the initial stages of amyloid plaque deposition, microglia may function as a barrier that protects neurons from the toxicity of ongoing aggregation of Aβ[97]. Neuroprotective properties of microglia also include the capability to

internalise soluble Aβ[98], phagocytose prefibrillar Aβ,[99] and degrade intracellularly and extracellularly Aβ with proteases such as neprilysin and insulin degrading enzyme[100]. On the other hand, microglia may have detrimental effects in the early AD process. Soluble Aβ oligomers may induce complement- and microglia-mediated early synapse loss.[101] The ongoing accumulation of Aβ may compromise protective functions of microglia and persistent activation of microglia may instead promote further Aβ deposition. The Aβ-induced activation of inflammasomes and secretion of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β, in microglia reduces the expression of microglial Aβ-phagocytic receptors and Aβ-degrading proteases, and therefore increases the formation and spreading of Aβ oligomers and aggregates.[102–104] Furthermore, proinflammatory microglia promote tau hyperphosphorylation and the spread of tau pathology *in vivo* and *in vitro* in the rodent brain.[105,106] A recent, large human post-mortem study also suggested that activated microglia induce accumulation of tau which in turn causes cognitive decline.[96] The possible role of microglia in the AD process is illustrated in Figure 4.

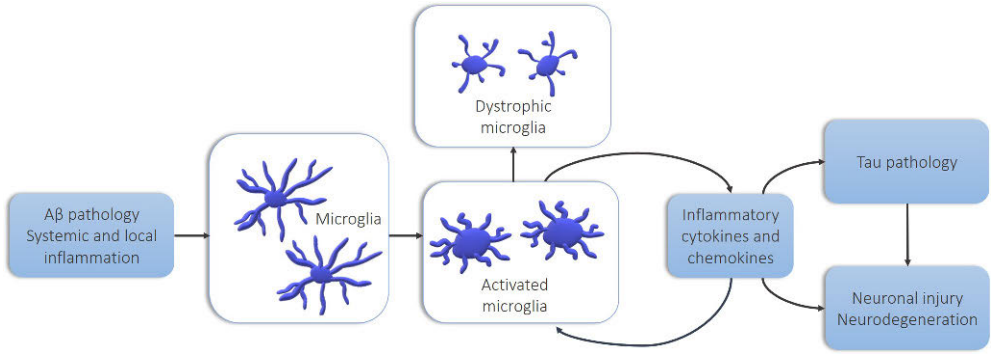

Figure 4. One hypothetical model of microglial function in the AD pathogenesis. Aβ pathology and other alterations in the CNS can activate, i.e. prime, microglia. Microglial activation can lead to various microglial phenotypes. Aβ sustains the activation of microglia resulting in the production of inflammatory cytokines which in turn aggravates glial activation. Ultimately, microglia may become "burnt out" dystrophic microglia. Microglial activation may also induce tau accumulation. The image is modified from Heppner et al. 2015.[107]

Astrocytes, the other main immune cell type in the CNS, are essential for the function of blood-brain barrier, brain energy metabolism, maintenance of extracellular ion balance, and modulation of synaptic plasticity.[108] Like activated microglia, reactive astrocytes are found in the proximity of fibrillar Aβ plaques and NFTs.[109] Aβ deposits, degenerative neurons, and microglia can activate astrocytes resulting in further release of cytokines and free radicals.[108,110] In addition, changes in the neuronal support by astrocytes can disrupt synaptic function and cause neuronal

injury.[110] On the other hand, astrocytes are able to degrade Aβ peptides and plaques. This process is ApoE-dependent and may be impaired in AD.[111] Besides microglia and astrocytes, other CNS-resident cells contribute to neuroinflammation. The inflammatory response and release of cytokines by vascular cells may be particularly important in the pathogenesis of AD.[112,113]

2.2.5 Mixed pathology and non-Alzheimer's pathology

The clinical phenotypes of progressive memory disorders are different due to the neuronal and synaptic loss in specific brain regions. The common feature of neurodegenerative diseases is the formation, aggregation, and propagation of pathologic proteins (a condition called proteinopathy). These include aggregates of Aβ peptide in AD, hyperphosphorylated tau protein in AD and other tauopathies, α-synuclein in synucleinopathies and TDP-43 in FTD. These proteins become pathologic through post-translational modifications, misfolding, oligomerisation, and fibrillisation. A neuroinflammatory reaction involving microglia is also considered increasingly critical in neurodegenerative diseases.

The differential diagnosis of progressive memory disorders is based on the patient's medical history, clinical evaluation, laboratory tests, cognitive tests, and brain imaging. The differential diagnosis is complicated by the fact that many patients with LOAD, especially in old age, have concomitant neuropathology which contributes to cognitive impairment and modifies the clinical phenotype.[17]

Cerebrovascular disease

Vascular cognitive impairment (VCI) and vascular dementia (VaD) describe the cognitive impairment from MCI to dementia resulting from cerebrovascular lesions. Major types of VCI/VaD include AD with cerebrovascular disease (CVD), small vessel disease (SVD), cerebral amyloid angiopathy (CAA), large vessel dementia, and strategic infarct disease. Coexistence of CVD with AD is very common and increases with age. CVD and AD share many risk factors such as age, midlife hypertension, obesity and hyperlipidaemia, diabetes, and the APOE ε4 allele.[114]

SVD is the most important vascular contributor of dementia and it is present to some extent in almost all individuals aged 60 years or older. SVD affects small cerebral blood vessels causing white matter hyperintensities, lacunar infarcts, and microbleeds. These pathological changes may affect frontal-subcortical networks, cause a decrease of processing speed and executive function and affect motor performance and mood regulation. In addition, deficits in memory, language, attention, and visuospatial abilities may occur. The variation in clinical symptoms is large.[115] CAA is characterised by the deposition of Aβ in the walls of cerebral and

leptomeningeal blood vessels. CAA can cause haemorrhage, cerebral ischaemia and inflammatory changes in the occipital cortex and other neocortical regions. These changes may contribute to cognitive decline. CAA is present in more than half of dementia cases and more than 80% of AD patients.[116] Large vessel dementia is characterised by multiple large and small infarcts in the major cerebral arteries due to atherosclerosis of extra- and intracranial blood vessels. Clinical symptoms include cognitive symptoms and motor and sensory deficits. The clinical symptoms of strategic infarct disease vary depending on the location of the lesion.[117]

Synucleinopathies

LBDs, dementia with Lewy bodies (DLB) and Parkinson's disease-dementia (PDD), are characterised by the deposition of α-synuclein into Lewy bodies. DLB and PDD are sporadic diseases but genetic factors may be involved. The clinical symptoms of both disorders include cognitive impairment, including prominent executive dysfunction, visuospatial deficits, cognitive fluctuations and variable memory impairments, parkinsonism, hallucinations, REM sleep behaviour disorder, autonomic dysfunction, and mood disturbances. DLB is diagnosed when cognitive impairment occurs prior to parkinsonism and PDD when cognitive impairment develops after parkinsonism.[118] AD-related pathology is very common in DLB and PDD as over 80% of cases have concomitant Aβ pathology and over 50% have NFT pathology.[119]

Primary tauopathies

Tauopathies are characterised by intra-cellular neuronal and/or glial inclusions of tau. Secondary tauopathies are associated with other aetiologies, such as AD, whereas in primary tauopathies, the tau pathology is predominant. Primary tauopathies are associated with the atrophy and gliosis of frontal and temporal lobes resulting in frontotemporal lobar degeneration (FTLD). The clinical phenotypes of FTLD-tau are disorders with movement, behaviour and cognitive symptoms, and include several forms of FTD (i.e. behavioural variant of FTD, progressive non-fluent aphasia, and semantic dementia), progressive supranuclear palsy syndrome, and corticobasal syndrome.[120] Even though genetic factors are important for the risk of FTLD-tau and mutations in the microtubule-associated tau gene cause autosomal dominant FTLD, most cases are sporadic.[120] Primary age-related tauopathy (PART) refers to the presence of NFT pathology in the medial temporal lobe across the continuum from normal cognition to dementia (NFT-predominant dementia).[121] The concept of PART is controversial and differences between normal ageing, AD, and PART are unclear.

TDP-43 proteinopathies

Another common molecular pathology underlying FTLD is TDP-43 proteinopathy. Most semantic dementia cases and about half of cases with behavioural variant of FTD are associated with FTLD-TDP pathology.[120] Hippocampal sclerosis of ageing/cerebral age-related TDP-43 and arteriosclerosis is a condition with neuronal loss and gliosis in the hippocampus and is strongly associated with TDP-43 pathology, brain arteriosclerosis and EM impairment.[122]

2.3 Measures of cognition and episodic memory in Alzheimer's disease

Multiple neuropsychological tests and test batteries have been developed to assess and quantify performance in different cognitive domains, such as memory, executive functions, attention, language, and visuospatial abilities. In research and clinical settings, global cognition can be evaluated with a comprehensive neuropsychological evaluation which includes several tests assessing major cognitive domains and usually lasts two to four hours.

Information about the neuropsychological test performance of healthy aged individuals is needed to determine if a studied individual has cognitive impairment. The aim is to determine if the individual's performance has declined from their baseline level and to avoid misclassifying healthy individuals with low premorbid cognitive abilities as cognitively impaired or impaired individuals with high premorbid levels as cognitively unimpaired. Because age and education are known to affect performance in most tests, age- and education-based normative data of test scores is useful for drawing correct conclusions.[123] Gender-based normative data may also be useful, especially for EM test scores.[124] In addition, information about the individual's occupational background and measures assessing premorbid cognitive abilities can assist in determining the presence or absence of cognitive impairment.

Brief cognitive screening tests, such as the Mini-Mental State Examination (MMSE)[125], and concise neuropsychological test batteries, such as the Consortium to Establish a Registry for Alzheimer's disease Neuropsychological Battery (CERAD-NB)[126], provide a quicker and more easily administered assessment of global cognitive function. However, they are not equivalent to comprehensive neuropsychological evaluation. In clinical practice, cognitive screening tests together with questionnaire-based assessment for dementia, such as the IQCODE (Informant Questionnaire for Cognitive Decline in the Elderly), are commonly used first-line screening tools for detecting cognitive impairment. In addition to traditional face-to-face evaluation, multiple telephone-based cognitive screening tests have been developed and used in research settings (for a review, see [127]). The

telephone interview for Cognitive Status (TICS) and its modified version (TICS-m) are the most frequently used telephone-based cognitive screening tests.[128]

Cognitive changes in neurodegenerative diseases affect the functional abilities and independence. Instrumental activities of daily living questionnaires, such as the Alzheimer's disease cooperative study – activities of daily living inventory (ADCS-ADL), measure the competence of patients in basic (e.g. dressing, bathing, feeding) and instrumental activities of daily living (e.g. housekeeping, financial management, shopping). Global assessment measures including the Clinical Dementia Rating (CDR)[129] scale and Global Deterioration Scale/Functional Assessment and Staging (GDS/FAST)[130] are used to stage dementia severity based on the semi-structured interview of patient and informant.

Neuropsychological tests developed to measure EM performance assess the encoding and recall of verbal material (word lists, word pairs, paragraphs, and stories) or visual material (pictures, objects etc.). Tests often measure the free recall, cued recall, and recognition of these materials. In these tests, items are presented, usually multiple times, to an examinee who is asked after each trial to recall the items in any order without any cues (immediate free recall). Immediate free recall is thought to measure encoding that requires attentional abilities and short-term memory capacity.[131] In the immediate recall of a list, individuals tend to recall more items in the beginning (primacy effect) and end (recency effect) than in the middle of a list, which produces an U-shaped serial position curve.[132]

After a delay of several minutes, the persistence of encoding and retrieval of information is measured with a delayed free recall trial. Typically, the main outcomes are the number of items recalled in immediate recall trials, number of items recalled in the delayed recall trial and the percent savings, i.e. the number of items recalled after a delay divided by the number of learnt items. In addition, a test can include a recognition test (i.e. "was the word rabbit in the list?"). Tests using cued recall or selective reminding (e.g. Free and Cued Selective Reminding test) measure encoding and recall of items belonging to a few different semantic categories and use these category cues to help the retrieval of items that were not retrieved by free recall. Well-established word list learning and memory tests include the CERAD 10-item word list learning, recall and recognition test, 16-item California Verbal Learning Test, 15-item Rey Auditory Verbal Learning Test, and 12-item Hopkins Verbal Learning Test. In the Logical Memory subtest of the Wechsler Memory Scale-Revised, two brief stories are read to the examinee and recall is assessed immediately and after a 30-minute delay.

Cognitive tests are non-specific. For example, the performance in verbal EM tests also depends on auditory or visual attention, visuospatial processing, and executive functions. The delayed recall performance is dependent on the successfulness of the initial encoding.

2.3.1 Cognition and episodic memory in mild cognitive impairment and dementia

Cognitive screening measures

The MMSE is one of the most widely used cognitive screening methods for dementia due to its easy and short administration. It includes tests of orientation, attention, memory, language, and visuospatial skills. Based on a meta-analysis of 15 community-based studies, the sensitivity was 85% and specificity was 90% for detecting all-cause dementia.[133] The CERAD-NB, consisting of the verbal fluency test, 15-item Boston naming test, MMSE, 10-item word list learning, recall and recognition test, and constructional praxis and recall test, is a relatively short battery developed to measure early cognitive impairment in AD.[126] The CERAD total score has been calculated as the sum of six or seven subtests excluding the MMSE and sometimes followed by a correction for age, sex, and years of education.[134,135] The CERAD total scores are suitable for screening AD and non-AD dementia, even in mild stages.[134–136] The discrimination ability of CERAD total scores for MCI, particularly for aMCI or progressive-MCI to AD, is superior compared to the MMSE.[134,135,137]

Episodic memory measures

The core criterion of typical AD is the presence of amnestic syndrome of hippocampal type which can be identified using list-learning and other EM tests.[4] EM impairment in AD is thought to result from the diminished ability to encode new information into long-term memory which is seen as impaired recall and recognition of verbal and visual information. Individuals with AD typically have impaired free recall performance, do not benefit from cueing in recall, show pronounced forgetting, intrusion errors (i.e. respond with semantically related but incorrect words), and recency effects (i.e. recall only the last few presented items).[138] In addition to EM, early deficits are often detected in semantic memory, such as category fluency, and in visuospatial skills, such as figure copying and clock drawing.[138] It should be noted that EM may be affected in progressive memory disorders other than AD, but the memory impairment is typically less striking and the profile of memory impairment is different.[138]

Based on a meta-analysis of 47 studies, memory measures have excellent diagnostic accuracy for identifying AD dementia from healthy controls.[139] Delayed memory measures, including verbal list free recall, verbal list cued or selective reminding, story recall, and visual free recall, had an overall sensitivity and specificity of 89%. Likewise, immediate memory measures, including verbal list free

recall, verbal list cued or selective reminding, and visual free recall, had good diagnostic accuracy (sensitivity = 86%, specificity = 88%) for discriminating AD dementia from healthy controls. The data did not demonstrate the superiority of any single free or cued recall test over other tests to identify AD dementia.

The main diagnostic criterion of MCI is the impairment of one or more cognitive domains without the significant impairment of functional independence. MCI with EM impairment in isolation or in combination with other impaired cognitive domains is considered to be an early cognitive phenotype of AD.[27,28] In research settings, the operationalised criteria of MCI has often included, in addition to the presence of cognitive impairment, the presence of cognitive complaint, CDR score of 0.5, MMSE score of 24 or more, and/or intact ADL.[24] The criteria for memory impairment has varied a great deal and different memory tests with varying properties, norms and cut-offs have been used. The most typical criterion has been performance falling 1.5 SDs or more below the age- and/or education-appropriate norm on a single verbal EM measure (typically the delayed recall of Logical Memory test).[140] On the contrary, some have emphasised the use of clinical judgment not based on a specific test score.[24] Variation in the operationalisation of MCI criteria has led to variable prevalence rates of MCI and to even more variable annual conversion rates from MCI to dementia. In addition, classifying MCI based on a single test score seems to result in a sizeable portion of false positives, as 14% to 56% of MCI individuals revert to normal cognition at follow-up.[24] This is not surprising as a quarter of healthy older adults score 1.5 SDs or more below the age-adjusted norm in a single memory test.[141] Recent research indicates that the reliability of MCI diagnosis increases if objective memory impairment is defined with a more comprehensive approach, i.e. as a performance below 1 SD from the normative mean on two EM measures.[140]

EM measures are good predictors for future progression to AD dementia in MCI. Verbal EM tests (including free immediate, cued immediate, free delayed and cued delayed recall measures) had adequate sensitivity and specificity values (≥70%) based on a meta-analysis consisting of over 2000 MCI individuals who were followed for 31 months on average.[142] No major difference was detected between the ability of different verbal EM measures to predict AD dementia. Studies that used a combination of memory, executive, and language measures had the highest predictive accuracy values for AD dementia.

2.3.2 Cognition and episodic memory in preclinical stage

Recent research indicates that cognitive changes may be detected years before the diagnosis of MCI. EM has been the cognitive domain that has most consistently predicted future AD in cognitively normal individuals. EM functioning has predicted

future progression to AD 7 to 15 years before the diagnosis.[143] Both immediate and delayed verbal recall measures have been found to predict future AD.[143] Studies that have examined the individual trajectories of immediate and delayed recall performance have typically found that the delayed recall is a more sensitive early marker of AD than immediate recall.[144–146] In a more recent study, it was found that the decline of immediate word list recall was detected before that of delayed recall, whereas the delayed recall scores showed a faster rate of decline compared to immediate recall.[147] In other words, immediate recall was the best predictor of future dementia during the earliest preclinical stages, but delayed recall became a better predictor during later stages. In addition to EM, early decline in semantic memory, visuospatial processing, and executive functions in clinically normal individuals has been detected up to 12 years before the diagnosis of AD.[143]

There are inconsistencies between studies concerning which cognitive domain shows decline first and what is the rate of decline compared to normal ageing. One cause for the variability of results is likely due to the different properties of tests, including reliability, retest effects, and ceiling effects. Cognitive deficits are more likely to be detected with tests that have high reliability without prominent ceiling effects compared to less sensitive and reliable tests. Future longitudinal studies that follow individuals from a younger age and include more sensitive neuropsychological tests targeting more finely the neural networks that are first affected by AD pathology will likely clarify the temporal trajectories of decline in different cognitive subsystems.

Cognitive composite scores, such as the Alzheimer's Prevention Initiative (API),[148] ADCS Preclinical Alzheimer Cognitive Composite (ADCS-PACC),[149] and Repeatable Battery for the Assessment of Neuropsychological Status (RBANS),[150] have been proposed for measuring subtle cognitive decline in preclinical AD. These global cognitive scores combine test scores measuring several cognitive domains, such as the immediate and delayed EM, language, executive functions, visuospatial abilities, and global cognition. Cognitive composite scores are being used as endpoints in large preclinical prevention trials for which the traditional outcomes, including cognitive and functional outcomes developed for MCI and AD, may not be well-suited.

In addition to individuals who subsequently develop AD, preclinical/ asymptomatic AD may be investigated with individuals who are at high risk of developing AD due to genetic reasons, such as carriers of the APOE ε4 allele, carriers of rare ADAD mutations and clinically normal individuals with a first-degree relative with AD, or due to high levels of AD biomarkers, such as individuals with cerebral amyloidosis. The limitation of these studies is that an unknown portion of at-risk individuals will ultimately progress to AD. Clinically normal older APOE ε4 carriers have been shown to have poorer cognitive performance at baseline and

greater cognitive decline, most consistently in EM, compared to non-carriers.[143,151] In ADAD carriers, cognitive decline has already been detected 12 years before the estimated MCI diagnosis and 17 years before the estimated dementia diagnosis, with EM declining first.[152] There is also some evidence that family history of AD and dementia can have subtle effects on EM, executive functions, and visuospatial performance in clinically normal older and middle-aged individuals.[153–157]

2.4 Alzheimer's disease biomarkers: emphasis on β-amyloid and neuroinflammation PET imaging

Molecular imaging techniques and analyses of CSF have enabled researchers to examine pathological indicators of AD and other neurodegenerative diseases in the living brain. These measurable *in vivo* indicators of specific disease-related changes are called biomarkers. Biomarkers may be used to confirm the presence of a disease (i.e. diagnostic biomarkers), assess disease progression or treatment effect (i.e. monitoring, pharmacodynamic/response biomarkers) and predict future disease progression (prognostic biomarkers).[158] The ability to visualise and quantify biomarkers has improved the understanding of AD pathogenesis and differential diagnostics of atypical conditions. In addition, biomarkers have become increasingly important in clinical trials for AD-modifying drugs. The use of AD biomarkers for participant inclusion and monitoring of treatment effect may lower the cost and duration of clinical trials. The most recent diagnostic criteria of AD include the presence of one or more biomarkers as a required or supportive evidence for the diagnosis.[4,48] The included biomarkers indicate the neuronal loss particularly in the medial temporal lobe, intraneuronal NFTs composed of hyperphosphorylated tau protein, and extracellular amyloid plaques consisting of aggregated Aβ peptides.

The most well-established biomarkers of neuronal loss and injury are structural MRI, [^{18}F]2-fluoro-2-deoxy-D-glucose (FDG) PET and CSF total tau. AD is associated with hippocampal atrophy and cortical thinning in typical AD signature regions, including the temporal, parietal, and precuneus-posterior cingulate cortex, which can be measured with structural MRI.[159] Hypometabolism measured with the glucose analogue [^{18}F]FDG PET also predominantly affects the temporoparietal and precuneus-posterior cingulate regions.[160] Although, the AD signature of atrophy and hypometabolism are not entirely specific for AD, they can be useful in distinguishing AD from FTD and DLB.[161]

Biomarkers of tau include the CSF phospho-tau and recently developed tau PET tracers which bind to paired-helical filament tau. A high level of CSF phospho-tau is specific for AD.[162] Tau PET tracer retention has been found to be higher in individuals with AD compared to controls, correlate with biomarkers of neuronal injury, and with post-mortem Braak tangle stage.[163,164] However, the use of tau PET

tracers is limited by the off-target binding of many tau tracers and possibly by the limited ability to detect subtle early tau deposition.[163,164]

Biomarkers of Aβ will be discussed in more detail in chapter 2.4.2. Aβ and tau pathologies coexist with other neuropathologies, including neuroinflammation and non-AD pathologies, such as TDP-43 and α-synuclein proteinopathies. The development of biomarkers for neuroinflammation is an active research area, but the clinical usefulness of these biomarkers is currently unclear (discussed in chapter 2.4.3). There are currently no reliable imaging or fluid biomarkers for TDP-43 or α-synuclein pathology. There are promising new PET and fluid biomarkers for synaptic loss and fluid biomarkers for axonal injury.[165,166]

Abnormal levels of AD biomarkers are not detected simultaneously during the AD disease process but in a sequential overlapping order.[160] In the dynamic biomarker model of AD, biomarkers of Aβ deposition become abnormal first, and after a lag phase, biomarkers of neuronal loss and injury become abnormal (Figure 5). Aggregation of Aβ is thought to promote medial temporal tauopathy and its spreading to the neocortex that is followed by neuronal loss. Biomarkers of Aβ reach a plateau before the clinical symptoms of AD appear, whereas biomarkers of neuronal loss and injury correlate strongly with clinical symptoms. Therefore, Aβ biomarkers are good biomarkers for the preclinical and prodromal stages of AD, whereas biomarkers of neuronal loss are useful biomarkers for disease progression.

The dynamic biomarker model is supported by the evidence that FDG PET, Aβ PET, CSF levels of Aβ and tau, structural imaging and cognitive markers are good diagnostic and prognostic biomarkers for AD, especially when used in combination. The combination of abnormal levels of neuronal loss together with Aβ and/or EM performance are associated with a high probability of progression from MCI to AD dementia within a 2- to 3-year follow-up.[167–169] In cognitively normal individuals, abnormal levels of both Aβ and neuronal loss are associated with greater cognitive decline and progression to AD compared to individuals not positive for both biomarkers.[170,171] The progression rate to AD is even greater in cognitively normal individuals with abnormal levels of Aβ, neuronal loss, and subtle EM impairment.[171]

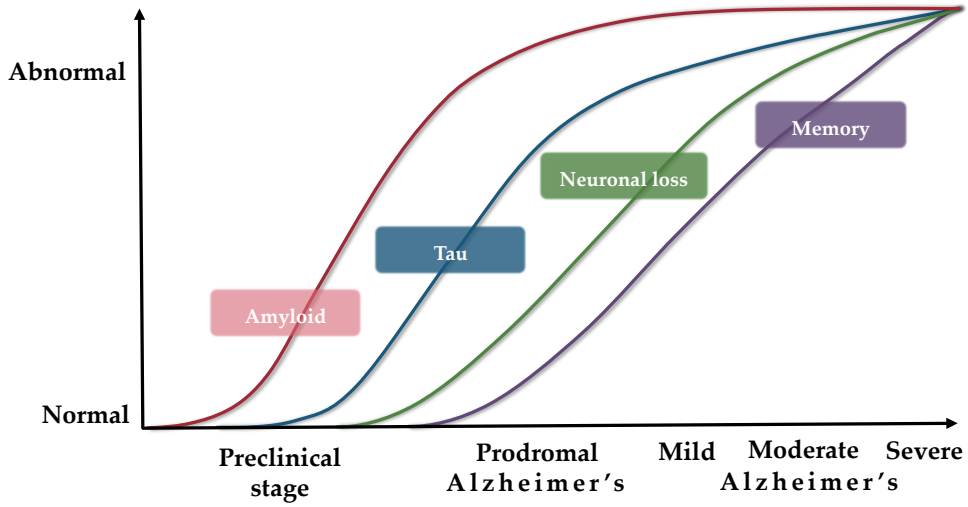

Figure 5. Dynamic biomarker model of AD. The figure is modified from Jack & Holtzman 2013.[160] The y axis represents the magnitude of biomarker abnormality starting at the detection threshold. The model is thought to best represent the temporal order of biomarker changes in "pure" AD, i.e. EOAD. In LOAD, age-related changes and comorbid non-AD pathologies are very common, and they may advance the detection of abnormal biomarker levels of neuronal loss and injury.

2.4.1 Basics of PET imaging

PET is a non-invasive imaging technique which uses short-lived positron-emitting radiopharmaceuticals (radiotracers, radioligands) to image and quantify biological processes in living subjects. Radiopharmaceuticals consist of a carrier molecule designed for a specific molecular target and a positron-emitting radioactive atom (radionuclide). The radiopharmaceutical is typically given intravenously in a tracer quantity which has a negligible pharmacological effect on the body. After the administration, tracer molecules start to localise more to areas containing a greater amount of target molecules, which is monitored with a PET scanner. The aim is to obtain an index of abundance of target binding sites.

Over a thousand different molecules have been labelled with radionuclides, many of which are used especially in brain studies. Such radiotracers can be used to study the distribution, density, and activity of receptors, transporters, enzymes, and other molecular targets. The design of brain PET radiotracers is challenging. An ideal brain PET radiotracer: 1) has high affinity, 2) high selectivity for the target, 3) capacity to penetrate blood-brain barrier through passive transfer or active transport, 4) is not highly susceptible as a substrate of efflux transporters, such as P-glycoprotein, 5) has a negligible amount of blood-brain barrier penetrating radiometabolites and formation of radiometabolites in the brain, 6) has low non-specific binding, 7) has suitable brain pharmacokinetics, 8) can be labelled with a

radionuclide at high specific radioactivity, and 9) is safe for administration in tracer quantities.[172] Most common PET radionuclides are ^{11}C (half-life 20 min), ^{18}F (half-life 110 min), ^{13}N (half-life 10 min), and ^{15}O (half-life 2 min). The short half-life of these radionuclides apart from ^{18}F-labelled tracers means that the tracers must be produced and used in the same location. The production of the most often used radionuclides requires a cyclotron. In addition, a specialised radiochemistry production laboratory is needed for the synthesis of tracers.

A PET scanner measures annihilation radiation using a coincidence detecting technique. The unstable neutron-deficient nucleus of radionuclide undergoes nuclear decay with the emission of a positron, the antimatter particle of an electron. The emitted positron rapidly combines with an electron, resulting in the annihilation event which causes the simultaneous emission of two 511 keV photos traveling in opposite directions from each other. During a PET scan, the ring of detectors around a scanned subject detects millions of coincidence events along the line between the two parallel opposite detectors. The information about the number and localisation of coincidence events during each predefined time frame forms the PET raw data, i.e. sinograms. The actual 3D PET image representing the distribution and concentration of radioactivity is formed after application of an image reconstruction algorithm and several corrections, including attenuation, scatter, physical decay, and motion correction.

Radiotracer concentration in tissue is not only dependent on the density of targets but also on several confounding factors, such as the tracer's availability and kinetic behaviour (e.g. tissue extraction, retention, and clearance). Furthermore, all tracer molecules are not specifically bound to the target molecules (specific binding), but some are non-specifically bound to other molecules than the target or are free in the tissue water. In order to achieve a fully quantitative measurement of tracer concentration *in vivo*, dynamic PET scanning, pharmacokinetic modelling, and information about the concentration of radiotracer in arterial blood or in a tissue that contains a negligible number of targets (i.e. reference region) are needed. Dynamic PET acquisition is started upon the tracer injection and continued throughout the scan. This generates time-activity curves (TACs) of the tissue concentration of radioactivity over time. Kinetic modelling based on the TACs produces a model describing the dynamic behaviour of a tracer. Common quantitative outcome measures include the total volume of distribution (V_T) and binding potential (BP).

A static PET image is a single time frame comprising the average amount of radioactivity during a certain time interval, usually scanned a certain time after the tracer administration. Only semiquantitative information can be attained with a static acquisition. The most common semiquantitative outcome measure is the standardised uptake value (SUV). It is the activity concentration in the tissue divided by the injected activity normalised to body weight. SUV is easy to calculate and does

not require arterial blood sampling. However, it is an oversimplification of the dynamic behaviour of a tracer. Another way to normalise SUV is to calculate a standardised uptake value ratio (SUVR) which is the ratio of a tissue SUV to a reference region SUV.

Brain PET data is often analysed using an anatomically defined region of interest (ROI) method. Typically, anatomic ROIs are defined on the subject's MRI image manually or automatically and then transferred to the PET data. The average TAC of a ROI is then used to obtain the estimate of SUV, V_T, BP etc. Alternatively, a quantitative value can be generated for each voxel. Voxel-based analysis has the advantage of sampling the entire brain, while the data has more noise compared to ROI-level data.

2.4.2 Biomarkers of β-amyloid

In vivo human PET β-amyloid imaging

^{11}C-labelled radioligand PiB (Pittsburgh Compound B, N-methyl-[11]C-2-4'-methylaminophenyl-6-hydroxybenzathiazole) is the most widely used PET marker of Aβ-related cerebral amyloidosis. It has high affinity for insoluble, fibrillar Aβ. At PET tracer concentrations, PiB retention primarily reflects cerebral amyloidosis (cored plaques, CAA, and diffuse plaques) and not Lewy body or NFT pathology that have similar β-sheet structure as fibrillar Aβ.[173] However, it is possible that the amyloid radioligands have differential binding to the polymorphic forms of Aβ fibrils.[174] Second-generation Aβ tracers labelled with ^{18}F include florbetaben, flutemetamol, and florbetapir that have been approved in the US, Europe, and Japan for the visual detection of significant β-amyloid plaque density in adult patients with cognitive impairment. The cortical retention of these ^{18}F-labelled radioligands has high correlation with PiB.[175,176] The *in vivo* Aβ tracer retention has shown high correlation with the density of Aβ deposits in the human post-mortem brain tissue.[177–179]

In clinical practice, amyloid imaging results are typically analysed by visual inspection as amyloid-positive or negative. In a positive [^{11}C]PiB scan, cortical retention is higher compared to that of subjacent white matter. Florbetaben, flutemetamol, and florbetapir have higher non-specific binding to white matter than PiB and a scan is interpreted as positive when there is a loss of normal grey-white matter distinction in tracer retention.[180] Aβ tracer binding is often quantified as the ratio of cortical binding to that of cerebellum (most often cerebellar grey), pons, brainstem, or white matter. Typically, either a short late scan is used to calculate a composite neocortical SUV ratio, or a dynamic scan is used to calculate a distribution volume ratio (DVR) using the Logan graphical method. The neocortical ratio usually

includes the amyloid-accumulating regions, frontal, lateral parietal, medial parietal, lateral temporal, and anterior and posterior cingulate cortex. The quantitative outcome is examined as a continuous variable or participants may be dichotomised into amyloid-positive and negative categories. Several thresholds for amyloid positivity and methods for defining the threshold have been suggested. The suggested thresholds for PiB-positivity range from cortical to cerebellar SUVR of 1.21 (DVR 1.08),[181] SUVR 1.4 (DVR 1.2),[182] to SUVR 1.5 (DVR 1.26).[183] The threshold of SUVR 1.4 is approximately equivalent to the transition to Thal phase 2.[182] The challenge is that a threshold is dependent on the method used to define it and on the imaging methodology, including the reference region and time period of image acquisition etc.[181,184]

β-amyloid PET imaging in the clinical stages of Alzheimer's disease

AD patients show approximately 1.5 to 2-fold higher Aβ tracer retention particularly in the frontal, cingulate, parietal, lateral temporal cortex, precuneus, and striatum compared to age-matched cognitively normal controls.[183,185–187] The differences of tracer retention in the occipital, sensorimotor and medial temporal cortex are smaller between AD and healthy controls. In clinical practice, Aβ PET imaging is appropriate when the diagnosis of AD is uncertain, i.e. in patients with persistent or progressive unexplained MCI, patients with progressive atypical or mixed presentation of dementia, and dementia patients with an early age of onset.[188] Aβ imaging may especially assist in the differential diagnosis of AD and FTD as there is no cortical Aβ tracer retention in FTD.[189] However, DLB patients have often increased Aβ tracer retention.[189]

MCI individuals have, on average, higher cortical Aβ tracer retention compared to cognitively normal controls but lower than individuals with AD dementia. Approximately half of individuals with MCI have high cortical Aβ tracer retention with a similar regional pattern as in AD dementia.[190,191] Individuals who have MCI and high cortical Aβ burden are often considered to have MCI due to AD or prodromal AD, while MCI individuals with low Aβ tracer retention are suspected to have non-AD pathophysiology. Amyloid-positive MCI individuals are more likely to have future cognitive decline,[192] and to progress to AD dementia compared to amyloid-negative MCI individuals (60% vs 7% within an average of 2-year follow-up).[193] The sensitivity and specificity of PiB imaging for detecting individuals with MCI who will progress to AD dementia has been between 83% and 100% and 46% and 88%, respectively.[194]

A significant proportion of older individuals with normal cognition have high cortical Aβ tracer retention. The prevalence of amyloid-positive PET scans increases from 3-10% in the age of 50 to over 40% in the age of 90.[195,196] These individuals

are thought to have preclinical AD. The findings of amyloid-positive cognitively normal individuals having a greater risk of future cognitive decline and progression to MCI and dementia compared to amyloid-negative individuals support the concept of preclinical AD.[196,197] During a four-year follow-up, 32% of amyloid-positive cognitively normal older individuals progressed to prodromal AD compared to 15% of amyloid-negative individuals.[197]

Based on 200 participants who were followed every 18 months for 4 years, Aβ deposition developed on average in 12 years from the level of healthy controls with low PiB tracer retention to the threshold of amyloid-positivity (1.5 SUVR) and in 19 years from the threshold of positivity to the levels observed in AD dementia (2.3 SUVR).[198] The earliest Aβ accumulation occurs in the precuneus, medial orbitofrontal and posterior cingulate cortex.[199] As AD progresses, the almost linear increase of Aβ deposition starts to reach a plateau.[198] The distribution of Aβ deposition overlaps with regions of high connectivity and energy demand, including the default-mode network, frontoparietal control network, and dorsal attention network, and Aβ-associated disruption of connectivity within these networks has already been detected in cognitively normal individuals.[199,200]

Relationship of β-amyloid with genetic and environmental factors

The most important risk factors of LOAD, age and APOE ε4 allele, are associated with cortical Aβ tracer retention. The prevalence of amyloid-positivity is increased in APOE ε4 carriers with normal cognition or MCI compared to non-carriers.[183,195] The age at which 15% of cognitively normal APOE ε4ε4 carriers are amyloid positive is 40 years, 50 years for ε2ε4 carriers, 55 years for ε3ε4 carriers, 65 years for ε3ε3 carriers, and 95 years for ε2ε3 carriers.[195] Family history of AD, especially maternal family history, is associated with higher Aβ tracer retention of AD-vulnerable regions in cognitively normal individuals, even after controlling for APOE genotype.[201,202]

AD and MCI individuals who have high cognitive reserve, which is identified using a proxy measure such as education, can display similar cognitive performance with higher levels of Aβ compared to AD and MCI individuals with low cognitive reserve.[203,204] Some studies have reported that greater cognitive activity or physical activity may prevent or slow down Aβ deposition in cognitively normal individuals,[205–207] or particularly in cognitively normal APOE ε4 carriers.[208–210] In contrast, several others have not found that cognitive or physical activity affect Aβ levels.[211–214] In addition, lower sleep quality has been associated with greater Aβ levels.[215]

Relationship of β-amyloid with other Alzheimer's disease markers

In AD dementia, Aβ tracer binding and cognition have typically only shown a weak relationship.[216] In contrast, Aβ deposition has shown a stronger independent effect on cognition and rate of cognitive decline in healthy individuals and in MCI.[216–220] Based on a meta-analysis of 30 cross-sectional (N=5005) and 14 longitudinal (N=2584) studies including cognitively normal individuals, Aβ levels had a small association with multiple cognitive domains cross-sectionally (global cognition, Cohen's *d*=0.32; visuospatial function, *d*=0.25; processing speed, *d*=0.18; EM and executive function, *d*'s=0.15), and with cognitive decline (global cognition, *d*=0.30; semantic memory, *d*=0.28; visuospatial function, *d*=0.25; EM, *d*=0.24).[221] Although, Aβ deposition may have an independent effect on cognition at early AD stages, as the disease progresses, the Aβ-related cognitive impairment becomes mediated by hippocampal atrophy.[222,223]

Cross-sectional studies examining regional Aβ-cognition correlations have found that Aβ deposition in the posterior cingulum/precuneus,[224–226] frontal cortex,[224,226] and temporal cortex,[224,226] is especially negatively associated with EM performance. In addition, there have been findings of a negative correlation between Aβ deposition in the precuneus and working memory, semantic processing, language and visuospatial perception performance,[225] and between parietal Aβ deposition and language, executive functioning and visuoconstructive praxis performance.[226] In a longitudinal study including initially amyloid-negative healthy adults (N=126, age 30-89 y), increase of Aβ burden in the posterior cingulate, precuneus, and lateral parietal cortex correlated with EM decline.[219] The effect was stronger within the middle-aged subsample.

Several studies have found that Aβ deposition is associated with cortical thickness and atrophy rates.[227–229] Amyloid-positive healthy individuals show a higher atrophy rate in the hippocampus, posterior cingulate, precuneus and temporal cortex compared to amyloid-negative healthy individuals.[230] It is possible that Aβ deposition and neurodegeneration might arise via independent pathways and aggravate the development of each other, thus resulting in the observed association between Aβ and neurodegeneration. On the other hand, neurodegeneration may be a downstream consequence of Aβ deposition for which the interaction between Aβ and tau is probably critical. Baseline Aβ tracer retention and rate of Aβ accumulation have been associated with subsequent tau PET tracer retention in the Braak regions in cognitively normal older adults.[231–233] The association between initial Aβ level and final cognition seems to be mediated by tau burden changes.[231] These findings are consistent with the idea that Aβ increases tau accumulation and pathogenicity which then drives neurodegeneration and cognitive decline.

Fluid biomarkers of β-amyloid

The 42 amino acid form of Aβ (Aβ42) and the Aβ42/40 ratio measured in CSF are well-established biomarkers of cerebral Aβ deposition. The CSF AD signature consisting of high phospho-tau together with low Aβ42 and high total tau has high sensitivity and specificity for the diagnosis of AD and are highly predictive for the progression from MCI to AD dementia.[162] CSF and PET measures of Aβ are inversely correlated with each other such that AD dementia is associated with the decrease of CSF Aβ42. The PET measure is thought to reflect the accumulation of fibrillar Aβ over many years and the CSF measure reflects the current status of Aβ production versus clearance. Thus, CSF and PET measures of Aβ may provide complementary information about Aβ pathology.[234] Aβ peptides measured in plasma have shown absent or weak correlation with cerebral Aβ deposition except the most recent studies using ultrasensitive technology.[234]

Conclusions

Aβ PET imaging and CSF levels of Aβ42 are well-established biomarkers of Aβ accumulation. Higher Aβ burden is consistently detected in AD dementia and in individuals with MCI or normal cognition who are more likely to show future cognitive decline and progression to AD dementia. At an individual level, it is not possible to accurately predict the progression to AD. However, the likelihood for progressing to AD is very low for individuals with normal cognition or MCI who have low amyloid load. Abnormal levels of Aβ may be detected up to 20 years before AD dementia is diagnosed. The presence of Aβ deposition in cognitively normal individuals indicates that Aβ deposition is an early and necessary event in the development of AD, but alone is not enough to cause AD. The non-benign nature of Aβ deposition is supported by the negative association between Aβ and both cognition and neurodegeneration at the very early AD stages. At severe disease stages, these associations are negligible. This is probably due to other critical factors that may arise downstream of Aβ deposition or may be independent or partly independent of Aβ, i.e. spreading of tau aggregation, neurodegeneration, and cerebrovascular burden.

2.4.3 Biomarkers of neuroinflammation

In vivo human neuroinflammation PET imaging

PET radioligands that bind to the translocator protein (18 kDa) (TSPO), formerly known as the peripheral benzodiazepine receptor, are potential biomarkers of

neuroinflammation in the living brain. TSPO is upregulated in response to injury and neurodegenerative and neuroinflammatory diseases.[235] In the healthy human brain, there is low-level constitutive TSPO protein expression that is located to vascular endothelial cells, smooth muscle cells, glial cells, macrophages, intravascular monocytes, ependymal cells of ventricles, and to choroid plexus.[235] TSPO protein is also widely expressed in the periphery with the highest expression in tissues participating in steroid synthesis[236], and in hematopoietic cells such as monocytes, neutrophils, and lymphocytes.[237] The subcellular location of TSPO is primarily the outer mitochondrial membrane.[236] The function of TSPO is unclear but it may participate in cell proliferation, secretion of cytokines, and regulation of mitochondrial functions.[238]

In autoradiography examinations, increased TSPO ligand binding has been detected in the human post-mortem AD brain, including the temporal, parietal and frontal cortex, and hippocampus.[239,240] TSPO PET imaging is often described as a biomarker of microglial activation even though the cellular origin of TSPO PET signal is not well described. Immunohistochemistry studies of human post-mortem brain tissue have described that the increased TSPO ligand binding and TSPO expression in AD originates mainly from microglia, but astrocytes may also contribute.[235,240] On the contrary, in a recent post-mortem study of AD and normal brains, cortical TSPO protein level had a substantial overlap between AD and control brains and TSPO expression did not correlate with the amount of activated microglia or astrocytes.[241] In rodents, increase of TSPO expression seems to be associated selectively with proinflammatory activation of microglia and astrocytes and not with anti-inflammatory activation.[242,243] On the contrary, human cultured microglia did not show an increase of TSPO expression with proinflammatory or anti-inflammatory stimulation.[243] In addition to microglia and astrocytes, TSPO binding sites are present in CNS vascular cells, including endothelial cells, smooth muscle cells, and perivascular macrophages,[241,244] as well as intravascular blood cells,[245] which may also contribute to the TSPO PET signal in the brain.

TSPO PET radioligands include the prototype ligand [^{11}C](*R*)PK11195 and newer second-generation ligands, such as [^{11}C]PBR28, [^{18}F]DPA714 and [^{11}C]ER176, which have higher signal-to-noise ratio and binding affinity than [^{11}C](*R*)PK11195.[246] The binding affinity of newer tracers has large inter-individual variability due to the rs6971 (Ala147Thr) single-nucleotide polymorphism (SNP) in the TSPO binding site. In the European population, 49% of individuals are homozygous for the high-affinity binding site, 42% are heterozygotes for the high- and low-affinity binding site, and 9% are homozygous for the low-affinity binding site.[247]

Quantification of TSPO PET signal is a major challenge. Due to the TSPO expression throughout the brain and blood vessels, there is no brain region devoid of

specific TSPO binding sites that would be needed for quantifying the specific binding of TSPO tracers. The volume of distribution (V_T) estimated based on compartmental modelling and measurements of metabolite-corrected arterial input function is considered as the gold standard for measuring brain TSPO binding.[248,249] It has been proposed that the kinetic modelling of TSPO tracers should also include a vascular component to correct for endothelial TSPO binding.[250] There have been efforts to validate reference tissue methods that use the cerebellar grey as a pseudo-reference region[251,252] or a data-driven approach to define a cluster of reference voxels that have kinetic behaviour resembling that of normal grey matter.[253] Overall, the optimal TSPO analysis method or tracer cannot be concluded without further validation studies (for a more thorough review, see[254]).

TSPO PET imaging in the clinical stages of Alzheimer's disease

Despite the limitations of the currently available TSPO radioligands and their quantification, several clinical studies have used TSPO PET imaging to examine differences in TSPO binding between individuals with probable AD dementia, individuals with MCI or prodromal AD (i.e. MCI with biomarker evidence of AD) and healthy control individuals.

In most studies using the [^{11}C](*R*)PK11195 ligand, higher binding was detected in AD dementia, especially in the temporal, parietal, cingulate and frontal cortices, compared to healthy controls.[255–260] On the contrary, in two studies,[261,262] one being the only study that included biomarker evidence of AD pathophysiology while also including the highest number of AD individuals,[262] no significant difference, except small clusters of increased [^{11}C](*R*)PK11195 binding, was detected between AD and healthy controls. All [^{11}C](*R*)PK11195 studies have used a simplified reference tissue model with a pseudo-reference region. In addition to AD dementia, findings of higher [^{11}C](*R*)PK11195 binding have been reported in other neurodegenerative diseases, including DLB and PDD.[259,263–265]

In most studies using second-generation TSPO ligands,[266–271] higher cortical binding was detected in AD dementia, especially in the temporal and parietal regions, compared to healthy controls. These studies mainly used the diagnostic criteria of probable AD dementia with biomarker evidence of AD pathophysiology. However, there were considerable methodological differences in the quantification of TSPO binding. In studies using absolute quantification, higher V_T values were detected in AD using [^{18}F]FEMPA or [^{18}F]FEPPA tracer,[270,271] or detected only if [^{11}C]PBR28 V_T values were corrected for the free fraction of radioligand in plasma,[252,266] whereas in some cases, there was no statistically significant group difference in [^{11}C]PBR28 V_T between AD and healthy individuals.[272,273] Other studies detected higher binding in AD using the SUVR method with cerebellar grey

matter as a pseudo-reference region and the [^{11}C]PBR28 or [^{18}F]DPA714 ligand.[267–269]

The results of binding differences between MCI and healthy controls are conflicting. In a number of studies, there was no statistically significant difference in TSPO binding between MCI and healthy controls[261,262,266,274,275], even when a more restricted definition of MCI, prodromal AD, was used[262,266,274]. In contrast, in about half of the studies, individuals with MCI[276] or with prodromal AD[268,269,277–279] had higher TSPO binding in cortical areas, especially in the parietal, temporal, cingulate and frontal cortex, compared to healthy controls. A possible reason for these conflicting findings is the high heterogeneity of TSPO binding at the individual level during the AD continuum. Two studies have reported that only around 35% of MCI individuals (both amyloid-positive and amyloid-negative) have higher TSPO binding compared to healthy controls.[275,277] On the contrary, one study found higher TSPO binding in 85% of amyloid-positive MCI and 25% of amyloid-negative MCI individuals.[278]

There is an interesting preliminary finding concerning TSPO binding in preclinical AD: 6 control individuals with a positive amyloid PET scan had higher [^{18}F]DPA714 SUVR values in the grey matter, especially in the cingulum and precuneus, compared to 20 amyloid-negative healthy controls.[268]

Relationship of TSPO PET imaging with other Alzheimer's disease markers

Results on the association between TSPO binding and disease severity are conflicting. Cross-sectionally, higher TSPO binding in multiple cortical areas has correlated with poorer cognitive performance in the prodromal and dementia stages of AD,[256,257,259,260,266,271,280] but some studies have not found a statistically significant correlation.[262,270,274,281] In addition, a negative correlation between [^{11}C]PBR28 SUVR and grey matter volume has been detected in the prodromal and dementia stages of AD,[266,267] and between [^{11}C](*R*)PK11195 BP and hippocampal volume in AD dementia.[259] On the contrary, in the two largest TSPO PET studies (n=58 and n=52 for individuals with AD), higher [^{18}F]DPA714 SUVR values in cortical grey matter correlated with both better cognitive performance and larger grey matter volume in the prodromal and dementia stages of AD.[268,269] There is also a finding of a positive correlation between cortical [^{11}C]PBR28 V_T and grey matter volume in both amyloid-negative and amyloid-positive MCI.[275]

In the majority of studies that examined the association between TSPO PET and [^{11}C]PiB PET, positive correlations between cortical TSPO and PiB binding were observed in the prodromal and dementia stages of AD.[258,268,269,272,274–276,278,280,281] In the largest study, the correlation between [^{11}C]PiB and [^{18}F]DPA714 binding

remained significant after adjusting for age, disease severity, APOE, and TSPO genotypes.[268]

There are only a few studies that have included both TSPO and tau PET imaging. In one study, no voxel-wise or regional correlation was observed between binding of [^{11}C](*R*)PK11195 and [^{18}F]flortaucipir in 16 individuals with AD in the prodromal or dementia stage.[281] On the contrary, positive voxel-wise correlations between [^{11}C]PBR28 and [^{18}F]AV1451 binding were detected in multiple cortical regions in 9 amyloid-positive MCI, 7 amyloid-negative MCI and 16 individuals with AD.[272]

Longitudinal TSPO PET studies

To date, there are only a few longitudinal TSPO PET imaging studies. Fan et al.[258,276] detected higher [^{11}C](*R*)PK11195 binding both in AD and MCI compared to healthy controls at baseline and after a 2-year follow-up. Longitudinally, an increase of global [^{11}C](*R*)PK11195 binding was detected in AD, but a decrease of binding in MCI.[258,276] However, these studies had very limited sample sizes (AD dementia, n=8; MCI, n=8). Based on the results, Fan et al. proposed a dual peak hypothesis of neuroinflammation in AD. They hypothesised that there is an early peak of microglial activation in MCI that is protective and aims to remove Aβ. As the disease progresses, anti-inflammatory microglia become ineffective and a second peak of activation occurs which is characterised by the detrimental actions of proinflammatory microglia.

Kreisl et al.[282] observed an increase of [^{11}C]PBR28 SUVR in multiple cortical areas and hippocampus of individuals in prodromal or dementia stages of AD compared to controls with mean annual increases of 2.5−7.7% vs -2.2−0.4%, respectively. In addition, they found that the increase in cortical binding correlated with functional decline and grey matter volume decrease. Instead, no correlation was observed between changes in [^{11}C]PBR28 and [^{11}C]PiB binding.

In line with Kreisl et al.[282], Hamelin et al.[269] detected that cortical [^{18}F]DPA714 SUVR values increased in AD individuals compared to controls over time (mean annual increase of 8.3% in the dementia stage, 15.8% in the prodromal stage, and 4.2% in controls). They also observed that the increase of TSPO binding in multiple cortical areas correlated with cognitive and functional decline and decrease in grey matter volume in AD (prodromal and dementia combined).[268,269] Interestingly, higher TSPO binding at baseline was associated with a better clinical prognosis after a 2-year follow-up in 52 individuals with AD (prodromal or dementia). It seemed that AD individuals with the lowest TSPO binding at baseline had larger increases in TSPO binding and worse clinical prognosis, whereas individuals with the highest TSPO binding at baseline had lower increase in TSPO binding and better prognosis.

Fluid biomarkers of neuroinflammation

Based on a meta-analysis of 170 studies, several CSF and blood inflammatory biomarkers are altered in AD and MCI compared to healthy controls.[283] For example, the CSF levels of glial cell-derived inflammatory mediator YKL-40 (Chitinase 3-Like Protein 1) and sTREM2 (Soluble triggering receptor expressed on myeloid cells 2) are already increased in preclinical AD and are associated with higher CSF levels of tau, smaller grey matter volume, and for YKL-40, with future cognitive decline.[284,285]

Conclusions

There were several discrepancies between the results of TSPO PET studies in AD that are likely due to the methodological differences (different TSPO ligands, quantification methods, statistical approaches, and scanners) and differences between study populations. In addition, the limited sample sizes of most TSPO PET studies probably contributes to the discrepant results, as there is relatively high inter-individual and intra-individual variability in TSPO PET tracer binding.[286,287]

Most cross-sectional studies detected around 30% higher TSPO binding in AD dementia compared to healthy controls. However, the group difference was not observed consistently in all studies and the difference was modest. The results were more conflicting for MCI, probably because the condition is even more heterogeneous. Studies also suggest that TSPO binding increases in AD over time and that binding is correlated with fibrillar Aβ load. These results are consistent with the examinations of human post-mortem AD brains that show increased immunostaining of microglia with progression of AD and a positive correlation with Aβ load.[288] Results on the association between TSPO binding and cognitive function and disease prognosis are versatile suggesting that TSPO binding may reflect both protective and detrimental neuroinflammatory processes depending on the patient and disease stage.

In order to better understand the role and temporal dynamics of glial cells in AD, development and validation of ligands that bind more selectively to (M1 and/or M2-type) activated microglia or to reactive astrocytes would be a great advancement. Several targets have been proposed that may be more selective for microglia than TSPO, such as the triggering receptors on myeloid cells (TREM), cyclooxygenase 1 (COX-1) and purinergic receptor $P2Y_{12}$, while fewer targets that would allow more selective imaging of astrocytes have been suggested (monoamine oxidase-B (MAO-B) and type-2 imidazoline receptor).[289] However, none of the novel targets provide true target specificity as they are not only expressed by microglia or astrocytes. PET imaging of MAO-B, which is located in astrocytes and serotonergic neurons and upregulated in reactive astrocytes, is in a relatively preliminary clinical development

stage. The preliminary findings suggest that astrocytosis may be an early phenomenon in AD. The binding of MAO-B tracer [^{11}C]-deuterium-L-deprenyl ([^{11}C]DED) has been higher in AD, particularly in prodromal AD, compared to healthy controls.[290,291] In addition, presymptomatic ADAD carriers have shown increased [^{11}C]DED binding which showed longitudinal decrease with disease progression.[291]

2.5 Therapeutic targeting of β-amyloid and neuroinflammation

In February 2019, there were 132 AD drugs in the clinical drug development process.[292] Most drug candidates (73%) were disease-modifying. A disease-modifying drug for AD would intervene with the underlying pathophysiological mechanisms leading to neuronal death and this would delay the onset or progression of symptoms.[293] Most disease-modifying drugs were anti-amyloid drugs (40%) followed by anti-tau drugs (18%).[292] AD drugs can also be categorised into primary, secondary and tertiary preventive treatments. Primary prevention refers to interventions in individuals who do not have AD pathophysiology or cognitive symptoms. Secondary prevention targets individuals with AD pathophysiology and no cognitive symptoms and tertiary prevention is directed at symptomatic AD patients.

The development of an AD drug requires on average 13 years.[293] The typical drug development pipeline progresses from target identification, lead identification and optimisation to preclinical studies which evaluate the pharmacokinetics, toxicity, and efficacy of lead candidates. The clinical drug development stages are divided into phases I, II and III. In phase I, the safety and tolerability of a drug candidate is evaluated in healthy human volunteers, or in the case of biological drugs, in individuals with AD. The goal of phase II is to gain proof of concept for the drug's efficacy and target engagement and to determine appropriate dosing for phase III studies. Phase III studies aim to demonstrate a drug-placebo difference with a co-primary endpoint consisting of cognitive and functional or global outcome measures. Clinical outcomes of AD dementia trials have typically included the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog) screening tool,[294] the Clinical Dementia Rating - Sum of Boxes (CDR-SB) global assessment tool and the ADCS-ADL scale. Prodromal trials often use a composite endpoint comprised of cognitive and functional measures from several scales, such as the CDR-SB and the ADCS-ADL. The outcomes of preclinical trials include more sensitive cognitive composite tests, such as the ADCS-PACC. The cognitive and functional outcome measures require a long trial duration and studies are increasingly using biomarker outcomes to obtain signals of drug efficacy and target engagement. Biomarkers are

chosen based on the drug's mechanism of action. Most common biomarkers are MRI volumetrics, amyloid PET, CSF Aβ, total tau and phospho-tau. However, no biomarker at present can be considered as a surrogate outcome because there is not strong enough evidence that a biomarker change would predict clinical benefit.[295] Biomarkers are also typically used to confirm the presence of AD pathophysiology.

Anti-amyloid drugs that are currently in phase III include β-secretase (BACE) inhibitors and passive and active anti-Aβ immunotherapies. At the moment, there are only a few BACE1 inhibitors in phase III trials. The clinical development of several BACE1 inhibitors has been discontinued previously due to toxicity and lack of efficacy.[296] Passive anti-Aβ immunotherapies consist mainly of monoclonal antibodies (mAb) which interact with a specific epitope in the Aβ molecule. The mAbs may limit the oligomerisation of Aβ, increase the microglial-mediated removal of Aβ, or increase the removal of Aβ by binding to peripheral Aβ to form a peripheral sink.[293] There are five mAbs, solanezumab, gantenerumab, crenezumab, aducanumab and BAN2401, in the late clinical development stages. Phase III studies of solanezumab,[297,298] crenezumab (NCT02670083, NCT03114657) and gantenerumab[299] have failed to demonstrate an effect on cognitive decline in prodromal or mild to moderate AD. Aducanumab was the first drug to show a reduced brain Aβ load and at the same time a positive effect on cognition and global function in patients with prodromal or mild AD.[300] After these encouraging phase Ib results, two phase III studies (NCT02484547, NCT02477800) in individuals with prodromal AD were initiated but terminated after interim analyses showed a lack of efficacy. However, later Biogen announced that the results were wrong and that one of the phase III studies reached its primary endpoint according to the analysis of a larger data set.[301] Solanezumab, gantenerumab and crenezumab are undergoing phase II and III trials in very early AD stages, for example in cognitively normal or mildly symptomatic older individuals with biomarker evidence of cerebral amyloidosis (Anti-Amyloid Treatment in Asymptomatic Alzheimer's disease (A4) study (NCT02008357)), and in cognitively normal or mildly symptomatic ADAD carriers (DIAN Trials Unit study (NCT01760005) and the Alzheimer's Prevention Initiative (NCT01998841)). There is only one active anti-Aβ immunotherapy in phase III, the anti-Aβ vaccine CAD106. The vaccine is an Aβ antigen coupled to an adjuvant carrier which produces an immunological response. CAD106 is tested in the phase II/III in cognitively normal homozygous APOE e4 carriers (Generation S1 study (NCT02565511)). Some previous anti-Aβ vaccines have reduced Aβ deposits but without producing clinical benefits in mild to moderate AD.[296] The main safety concern of drugs targeted to remove Aβ is amyloid-related imaging abnormalities (ARIA).

The reasons for high failure rate of AD trials are the lack of efficacy of drugs, inappropriately low dosing, excessive toxicity, recruitment of non-AD patients, and

excessive measurement variability of global trials.[293] In addition, incomplete knowledge of AD pathophysiology has likely contributed to failures. The ongoing and planned clinical trials aim to overcome the failures of previous trials by incorporating biomarkers and targeting AD at an earlier stage. However, some anti-amyloid drugs, including the BACE1 inhibitors atabecestat and verubecestat, and mAbs solanezumab and gantenerumab, have now been investigated in biomarker-confirmed preclinical and prodromal AD stages without showing significant clinical benefit.[296]

There is a larger range of targets and mechanisms of actions among the AD drugs in earlier development stages. Neuroinflammation is one promising target for intervention. Epidemiological studies have suggested that the use of nonsteroidal anti-inflammatory drugs (NSAIDs), which are inhibitors of COX enzymes mediating the synthesis of prostaglandins, may prevent AD. The largest epidemiological study to date found that the risk of incident AD decreased in relation to the length of classical NSAID (nonselective inhibitors of COX) treatment.[302] In contrast to encouraging findings from observational studies, NSAIDs have shown no efficacy in the treatment of symptomatic AD,[303] or in the primary prevention of AD[304,305] in randomised controlled trials.

There are interesting immune targets in preclinical and phase I studies (reviewed in [107]). For example, mAbs targeting the microglial receptors TREM2 and CD33/SIGLEC-3, which interacts with TREM2, are undergoing phase I trials (NCT03635047, NCT03822208). The complexity and changing nature of neuroinflammatory pathways during the AD process is a great challenge for the development of drugs targeting neuroinflammation. For example, it is unclear whether TREM2 should be upregulated, inhibited, or modulated in a more specific way to achieve beneficial effects on the microglial-mediated Aβ phagocytosis and cytokine release. It may be that a combination therapy including drugs targeting Aβ, tau, and neuroinflammation is required to achieve a significant modulation of disease progression.

2.6 Twin studies and Alzheimer's disease

2.6.1 Basic principles of twin studies

Twin studies can be used to unravel environmental and genetic factors in the aetiology of diseases, such as AD. The basis of twin studies is that monozygotic (MZ) twin pairs are genetically identical, while dizygotic (DZ) twins share, on average, 50% of their segregating genes. In addition, MZ and DZ twins growing up in the same family share many environmental factors that may affect cognitive ability even late in life. The most basic information in a twin study is the number of

concordant pairs (both twins have the disease), the number of discordant pairs (only one twin has the disease) and the concordance rate (the conditional probability of being affected, given that a twin sibling is affected). If twin concordance rates exceed the prevalence rate, this is an indication that familial factors play a role. If in addition the concordance rate of MZ twins is higher than that of DZ twins, this suggests that genetic factors play a role.[306]

The classical twin model estimates the relative contribution of genetic and environmental factors on a disease or trait by comparing the phenotypic resemblance of MZ and DZ twins. The overall phenotypic variance in a certain population at a particular time is comprised of genetic and environmental variance. The part of phenotypic variance that is attributed to the genetic differences between individuals is called heritability. The genetic variance is further divided into additive (combined effects of alleles in different loci that are equal to the sum of their individual effects, denoted by A) and dominant (interactions between alleles at the same locus, denoted by D) genetic effects. The environmental variance is divided into common environmental influences (environmental effects that make co-twins similar, denoted by C) and unique (environmental effects that make co-twins dissimilar, denoted by E) environmental effects. The E effects also include the measurement error. A, D, and C effects cannot all be estimated simultaneously in the classical twin model and this would require additional information from e.g. the family members of twins.[307] MZ twins differ only due to unique environmental effects as they share 100% of genetic and common environmental effects, while DZ twins share 100% of common environmental effects, 50% of additive genetic effects and 25% of dominant genetic effects. From this follows that if a trait is more correlated between MZ twins compared to DZ twins, genetic effects explain some of the variance in the trait, and if the MZ correlation is more than twice as large as the DZ correlation, dominant genetic effects may play a role. In modern twin research, the A, C/D, and E parameters are modelled with maximum-likelihood-based structural equation modelling. In addition to examining the relative contribution of genetic and environmental factors on the variance of trait, modern methods allow to examine if two or more traits correlate due to shared genetic or environmental effects or due to a causal relationship between them. In addition, it can be studied if the genetic effects on a trait are moderated by an environmental factor (gene x environment interaction).[308]

Another approach is the co-twin control study which uses co-twins who are discordant for a disease, endophenotype of a disease, trait, or exposure. The co-twin control design is a version of the matched case-control study. The design matches controls and cases on a wide range of measured but also unmeasured variables, such as cultural and family background, and for MZ twins also on genes. Co-twin control studies can investigate whether associations between exposures and outcomes or

traits are causal or due to shared genetic and/or environmental factors.[308] Twin pair discordance can be treated as a continuous outcome or dichotomised into categories by using different cutoffs or diagnostic criteria. Conditional logistic regression analysis can be used for binary outcomes and conditional linear regression analysis for continuous outcomes. The analyses need to take into account the dependency between twin siblings. If the association is similarly strong in within-pair (co-twin) analyses as in unpaired analyses, it suggests a causal effect of the exposure on the outcome. If the estimate of effect (e.g. odds ratio or regression coefficient) is greatly reduced in within-pair analyses in all twins, then shared environmental factors and/or genetic factors may be responsible for the observed association in unpaired analyses. If the exposure and outcome have shared environmental background, the estimate of effect would be significantly larger in MZ twins compared to DZ twins because the effect of genetic factors is controlled in MZ pairs. If shared genetic factors are involved, significantly higher estimate of effect would be expected in DZ twins compared to MZ twins.[309,310] In addition, discordant MZ twins are valuable for studying gene expression differences.[308]

Individuals who have an identical twin affected by a disease may be in a higher risk of developing the disease due to shared familial (genetic and environmental) risk factors. Investigating how unaffected twins who have affected co-twins compare to unaffected twin pairs or to the general population may help to identify indicators of preclinical disease or susceptibility to disease. In genetics, these quantitative, subclinical traits, which can be e.g. neuropsychological, biochemical, or neuroanatomical, are called endophenotypes. Endophenotypes are associated with the disease, are heritable and are found in non-affected family members at a higher rate than in the general population. Quantitative endophenotypes may better identify disease-related genes than the presence of disease alone.[311]

2.6.2 Twin studies in Alzheimer's disease and dementia

Concordance for AD has been found higher among MZ twin pairs compared to DZ twin pairs (concordance rates from 45% to 61% in MZ twins and from 19% to 41 % in DZ twins),[33] reflecting the importance of genetics also in LOAD. However, the existence of discordance between MZ twins indicates that environmental factors also contribute to the development of AD. The concordance rate for all-cause dementia in the same study was from 44% to 58% for MZ twins and from 25% to 45% for DZ twins. Based on a large twin study, the heritability (A effects) for AD was estimated to be 79% (95% CI 67 to 88) and E effects 21% (95% CI 12 to 33).[33]

The co-twin design allows to assess which specific risk factors may have resulted in the occurrence of AD in one twin but not in the other. Twin studies have indicated that midlife obesity, hypertension, low leisure time physical activity, moderate-to-

heavy alcohol use, and diabetes increase the risk of cognitive impairment and that greater midlife cognitive activity is protective.[312–315] Swedish twin pairs have been followed longitudinally to identify pairs in which one twin develops dementia and the other one does not and to study what preclinical differences predict which twin develops dementia. It was shown that poorer lipid values and grip strength predicted which twin would develop dementia.[316]

Discordant twin pairs can also be used to study what biological AD markers differentiate the affected twin from the unaffected co-twin. A study including Finnish cognitively discordant twin pairs detected peripheral blood DNA methylation differences between the affected and unaffected twins.[317] In the Danish twin cohort, circulating micro-RNA and cytokine levels were investigated in MZ twin pairs discordant for dementia and differences in the expression levels of micro-RNA targeting genes regulating inflammation, lipoprotein transportation, and APP were found between the affected and unaffected twins.[318]

Previous studies conducted in the Turku PET Centre showed that unaffected older MZ twins from cognitively discordant twin pairs have higher cortical PiB uptake and reduced cerebral glucose metabolism as measured with FDG PET compared to cognitively normal non-twin controls, while there was no significant difference in hippocampal volumes.[319–321] There were no statistically significant differences in the amyloid load or glucose metabolism between the unaffected DZ co-twins of probands and healthy controls. The results suggest that genetic factors are important in the development of Aβ deposits and reduced glucose metabolism. However, environmental factors seem to affect the relationship between these AD biomarkers and cognition.

3 Aims

I To examine if familial risk for dementia could be detected with a word list recall test by comparing the performance of cognitively normal twins who had demented co-twins to the performance of cognitively normal twins who had cognitively normal co-twins.

II To evaluate the utility of telephone-based interview (TICS-m) to measure cognitive functioning, verbal episodic learning, and memory performance, and to distinguish MCI and dementia in a population-based twin sample. First, to examine the prevalence of MCI and dementia using the commonly used classification methods. Second, to examine the associations of demographic factors, APOE ε4 carrier status, and depressive symptoms on cognitive performance measures.

III To investigate the uptake of TSPO PET tracer [^{11}C]PBR28 in a discordant twin pair setting which controls for genetic and environmental effects. First, to determine if the uptake of [^{11}C]PBR28 is higher in twins with worse EM performance as compared to their better-performing co-twins. Second, to investigate the relationship between continuous measures of [^{11}C]PBR28 binding and EM performance within twin pairs.

IV To investigate the uptake of Aβ PET tracer [^{11}C]PiB in a discordant twin pair setting. First, to determine if the uptake of Aβ PET tracer [^{11}C]PiB is higher in MZ and DZ twins with worse EM performance as compared to their better-performing co-twins. Second, to examine the relationship between continuous measures of [^{11}C]PiB retention and EM performance within MZ and DZ twin pairs. Third, to determine if the uptake of [^{11}C]PiB is higher in cognitively normal twins with EM impaired co-twins compared to cognitively normal non-twin controls.

4 Participants and Methods

4.1 Participants and study design

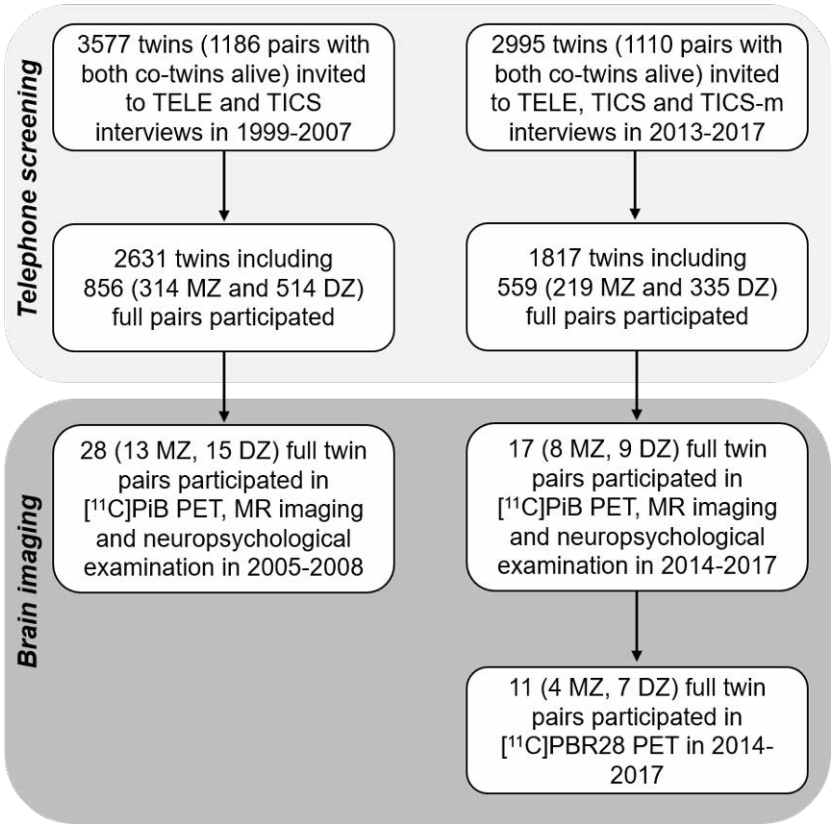

Figure 6. Flow diagram of study design

The participants belonged to the older Finnish Twin Cohort (FTC) which was established in 1975 and consisted of 13888 same-sex twin pairs born before 1958.[322,323] Postal questionnaire data collections on twins' health and lifestyle were conducted in 1975 and 1981 (participation rates 89% and 84%). Twin individuals who were 65 years old or older were asked to participate in a telephone interview:

those born before 1938 were interviewed during 1999–2007 (participation rate 73%) and those born in 1938–1944 were interviewed during 2013–2017 (participation rate 61%). During 2013–2017, individuals were also asked to participate in a collection of saliva samples for DNA extraction and genotyping. Based on the telephone interviews, cognitively discordant twin pairs without neurological or psychiatric disorders other than AD or MCI were asked to participate in more detailed examinations including brain PET and MR imaging, neuropsychological testing, and blood sampling at the Turku PET Centre in 2005–2008 and 2014–2017. The flow diagram of studies is shown in Figure 6. In addition, healthy non-twin controls were recruited to participate in the brain imaging studies.

The study population **I** consisted of individuals interviewed between 1999–2017 (n=4367 twins), and the study population **II** consisted of individuals interviewed during 2013–2017 (n=1772). The participant flow diagrams are shown in Figure 1 of original publication **I** and in Supplementary figure 1 of original publication **II**. Telephone interview consisted of cognitive evaluation questions, questions about memory problems, independent living, general health, diseases, and medications. In the beginning of the interview, individuals were asked not to use any external aids, to find a quiet place and to turn off television and radio. They were also asked if they used a hearing aid and to confirm that they could hear the interviewer well.

The study population **III** consisted of 11 twin pairs (4 MZ and 7 DZ pairs) who participated in [^{11}C]PBR28 PET imaging in 2014–2017. Participants were genotyped for the rs6971 (C/T) polymorphism in the TSPO gene and individuals with TT genotype (low-affinity binders) were excluded from [^{11}C]PBR28 imaging. The twin pairs also participated in [^{11}C]PiB PET and MR imaging.

The study population **IV** consisted of 45 twin pairs (22 MZ and 25 DZ pairs) in which both co-twins had available [^{11}C]PiB, MRI, and neuropsychological data (2 MZ pairs were excluded due to image quality issues and 1 MZ pair due to incomplete neuropsychological data).

The telephone interview and clinical studies were approved by the Ethics Committee of the Hospital District of Southwest Finland. Informed consent was obtained from all participants.

4.2 Cognitive measures

The telephone interview protocol consisted of the telephone assessment for dementia (TELE)[324] and the Telephone Interview for Cognitive Status (TICS)[325] during 1999–2017 and 2013–2017. The instruments had been previously validated in Finnish for the detection of dementia.[326] In 2013-2017, a few questions, including a delayed recall of 10-item word list, were added to the original interview protocol to form the

modified Telephone Interview for Cognitive Status (TICS-m). The questions of TELE, TICS, TICS-m and their scoring are shown in Appendix 1.

In study **I**, TELE score (range 0-20) was used to define cognitive status of participants: cut-off score >17.5 was used for healthy cognition and cut-off score <16.0 for dementia. Verbal episodic learning was measured with the free recall of a 10-item word list after a single administration. The serial position effect was studied by measuring primacy effect with the number of recalled words among the first three words and recency effect with the number of recalled words among the last three words.

In study **II**, TICS-m total scores were used as continuous and categorical variables after using previously published scoring protocols. We followed the procedure published by Knopman et al.[327] in which total scores of original TICS-m instrument (0–50 points) were adjusted for education and the cut-off score ≤27 was used for classifying dementia, scores 28–31 for MCI, and ≥32 for healthy cognition. The scores were adjusted for education in the following way: 5 points were added to the score of individuals with less than 8 years of education, 2 points were added to individuals with 8 to 10 years of education, no points were added to individuals with 11 to 15 years of education, and 2 points were subtracted from individuals with 16 or more years of education. We also used the 27-point version of TICS-m developed by Langa & Weir[328] as a continuous and categorical variable. TICS-m scores from this abbreviated instrument version were not adjusted for education and the cut-off score ≤6 was used to classify dementia, 7–11 to classify cognitive impairment not dementia (CIND) and ≥12 to classify healthy cognition. The definition of CIND corresponded very closely to MCI. Immediate (IR) and delayed recall (DR) of the 10-item word list was used to measure verbal EM performance.

In studies **III–IV**, participants were administered a neuropsychological test battery consisting of multiple cognitive measures. The primary measures of EM were the delayed word list recall from the CERAD-NB and Logical Memory delayed recall from the Wechsler Memory Scale-Revised (WMS-R). The test performances were transformed into SD units based on age-appropriate Finnish norms.[329,330] The mean of the two SDs constituted a continuous verbal DR score. The presence of aMCI was defined according to the Jak/Bondi actuarial neuropsychological criteria as a performance of -1 SD or poorer in both of the two tests.[140] In addition, the mean of SDs of the delayed visual reproductions test from the WMS-R and the delayed constructional praxis savings from the CERAD-NB was used as a measure of visual DR performance. The verbal IR performance was assessed with the mean of SDs of the immediate word list recall from the CERAD-NB and Logical Memory immediate recall from the WMS-R. Global cognitive performance was measured with the CERAD total score.[134] In study **III**, the free recall score of memo-BNT was used to measure incidental memory.

4.3 Brain imaging

In study **III**, the participants underwent a 70-minute dynamic PET scan using a High Resolution Research Tomograph (HRRT) (Siemens/CTI, Knoxville, TN, USA) after receiving the mean injection of 492 (SD 21) MBq of [^{11}C]PBR28 with >99.9% radiochemical purity and mean molar activity of 293 (SD 104) MBq/nmol. Twin siblings had PET scans within one week from each other. The details of [^{11}C]PBR28 synthesis, PET acquisition and preprocessing have been described elsewhere.[331] An automated ROI generation was performed with FreeSurfer software (version 6.0.0). For all participants, a grey matter (GM) composite SUV (the ratio of tissue radioactivity concentration [kBq/mL] and administered dose [MBq] divided by body weight [kg]) from 30 to 70 minutes after injection was calculated as the volume-weighted average SUV across six ROIs (prefrontal, parietal, lateral temporal, precuneus, posterior cingulate, and medial temporal cortex). For 18 twins (7 full pairs) with available metabolite-corrected arterial input function, a GM distribution volume (V_T) and a delivery rate constant (K_1) were estimated with the two-tissue-compartment model by using an in-house created software (http://www.turkupetcentre.net/petanalysis/tpcclib/doc/fitk4.html). The metabolite and delay-corrected arterial plasma curve was used as an input function, blood volume fraction was fixed to 5% and the estimation was weighted by using the frame lengths. T1-weighted MRI and [^{11}C]PiB scans were acquired from the participants using a 3T PET-MRI scanner (Philips Ingenuity TF PET/MR, Philips Medical Systems, Cleveland, OH, USA). Here, to examine the relationship of [^{11}C]PBR28 and [^{11}C]PiB uptake, [^{11}C]PiB SUVRs were calculated using the automatic analysis pipeline Magia,[332] FreeSurfer-generated ROIs, 60 to 90-minute scan duration and cerebellar cortex as the reference region.

Study **IV** consisted of [^{11}C]PiB scans carried out in 2005–2008 and 2014–2017. In 2005–2008, participants underwent a 90-minute dynamic [^{11}C]PiB PET scan with an ECAT EXACT HR+ scanner (CTI, Knoxville, TN) after receiving the mean injection of 469 (SD 63) MBq of [11C]PiB with mean molar activity of 35 (SD 10) MBq/nmol. In 2014–2017, participants underwent a [^{11}C]PiB PET scan from 40 to 90 minutes after the mean injection of 490 (SD 39) MBq of [11C]PiB with mean molar activity of 615 (SD 399) MBq/nmol. The mean radiochemical purity of [^{11}C]PiB injections was 99% (SD 1). In addition, participants had T1-weighted MRI scans (1.5T Intera scanner/Philips, Best, the Netherlands in 2005–2008 or 3T scanner/Philips Ingenuity TF PET/MR, Philips Medical Systems, Cleveland, OH, USA in 2014–2017). The T1-weighted single subject image was coregistered with the single subject [11C]PiB PET image and normalized to the Montreal Neurological Institute (MNI) space. Automated ROI analysis was applied using the anatomic labelling (AAL) atlas[333] to generate cortical gray matter and cerebellar cortex ROIs. Region to cerebellar cortex standardized uptake value ratios (SUVRs) were

generated over the 60 to 90-minute scan duration. A cortical composite PiB SUVR was formed as the average of prefrontal, parietal, lateral temporal, anterior cingulate, posterior cingulate, and precuneus ROI SUVRs.

4.4 Other measures

In studies **I** and **II**, information on education was obtained through an eight-category question from self-report questionnaires in 1975 and 1981. Answers were transformed into years of education and treated as a continuous variable. The higher level of education was used if a person had given contradicting information in the two questionnaires.

In study **II**, depressive symptoms were evaluated with the 20-item Center for Epidemiologic Studies Depression Scale (CES-D)[334] at the same time as cognition. CES-D scores range from 0 to 60 with higher scores indicating more depressive symptoms. APOE genotypes were determined from DNA samples extracted from collected saliva samples and genotyped on Illumina HumanCoreExome array. The two SNPs (rs429358 and rs7412) were not directly available on the array. Therefore, APOE genotype was determined with imputation using a validated method and Haplotype Reference Consortium release 1.1 reference panel, which has a 99.88% correspondence with directly genotyped APOE genotype.

In study **III**, TSPO genotype was determined by genotyping the SNP rs6971. In study **IV**, APOE genotype was determined by genotyping the SNPs rs7412 and rs429358. Zygosity was determined by genotyping multiple polymorphic markers for twins with available DNA samples, for others, zygosity was determined with a validated questionnaire.[335]

4.5 Statistical analyses

In discordant twin pair analyses of study **I**, a linear regression model was used to compare number or immediate recalled words between the cognitively normal twins with demented co-twins and twins who were concordant for normal cognition. Ordered logistic regression was used to compare the number of immediately recalled primacy words or recency words between the mentioned groups. Differences in delayed word list recall scores were analysed with negative binomial regression because the assumptions of linear regression were not met. All analyses were controlled for age, sex, years of education, and family relatedness. Results were reported as unstandardised coefficients with 95% confidence intervals (CI) and *P* values. The heritability of IR was evaluated. First, MZ and DZ twin correlations of age-adjusted standardised residuals of IR scores were calculated in men and women. Next, MZ and DZ twin correlations were calculated using age and sex-adjusted

standardised residuals of IR scores. Finally, a maximum likelihood based structural equation modelling approach was used to estimate the relative proportion of A (additive genetic effects), C (common environmental), and E (individual environmental variance) effects.[336]

In study **II**, multinomial logistic regression was used to examine the association between cognitive status (healthy, MCI/CIND, dementia) and sex, age, APOE ε4 carrier status, CES-D score, and education. The results were reported as relative risk ratios (RRR) with 95% CIs and *P* values. Linear regression was used to study associations of sex, age, education, CES-D score, APOE ε4 carrier status and the interactions of both age, APOE ε4 carrier status and CES-D score with sex on the continuous total TICS-m score or on the immediate word list recall score. In a similar manner, associations of variables with the delayed word list recall score were studied using negative binomial regression. The results were reported as unstandardised regression coefficients with 95% CIs and *P* values. In these analyses, individuals with APOE ε2/ε4 genotype (n=39) or unknown genotype (n=164) formed their own category, as did 476 APOE ε4 carriers (genotypes ε3/ε4 and ε4/ε4) and 1093 APOE ε4 non-carriers (genotypes ε3/ε3, ε2/ε3 and ε2/ε2). Years of age and CES-D score were centred at their mean values. Family structure of the data was considered in all analyses by using robust standard errors adjusted for family relatedness. When group differences were compared without adjusting for other variables, design-based F-test corrected for family structure was used.

In study **III**, paired t-test was used to estimate the differences in [^{11}C]PBR28 GM SUV between twins discordant for EM or aMCI status and with the same TSPO genotype (similar results with Wilcoxon signed-rank tests not shown). The results were reported with mean SUVs and mean intrapair difference as percentage and SUV with 95% CIs and *P* value. When additional twin pairs with differing TSPO genotypes were included in the analyses, the linear conditional fixed effects regression with TSPO genotype as a covariate was used (mean intrapair differences as percentage of difference and SUV with 95% CIs and *P* value reported). The associations of continuous memory scores and CERAD total score with the [^{11}C]PBR28 GM SUV were tested using linear conditional fixed effects regression with TSPO genotype as a covariate (unstandardised regression coefficients with 95% CIs and *P* values reported).

In study **IV**, paired t-test was used to test the differences in [^{11}C]PiB cortical SUVRs between pairs discordant for EM performance or aMCI status (mean SUVRs and mean intrapair difference as percentage and SUVR with 95% CIs and *P* value reported). The pairwise differences were also tested using the linear conditional fixed effects regression that included the APOE ε4 carrier status (categorised into APOE ε3/ε4, APOE ε4/ε4 and APOE ε4 non-carriers (APOE ε3/ε3 and ε2/ε3 genotypes). Mean intrapair differences as percentage and SUVR with 95% CIs and *P* value were

reported. Linear regression with APOE ε4 carrier status, project (i.e., different scanners in 2005-2008 and 2013-2017), sex, and age as covariates was used to compare the differences in [^{11}C]PiB cortical SUVRs between the cognitively normal twins who had co-twins with aMCI and the cognitively normal non-twin controls (adjusted group means and difference between groups as percentage and SUVR with 95% CIs and *P* value reported). Pearson's correlation was used to test the correlation of within-twin pair differences of [^{11}C]PiB SUVR with within-twin pair differences of continuous memory scores and CERAD total scores in all participated twin pairs. The within-twin pair associations of continuous memory scores and CERAD total score with the [^{11}C]PiB cortical SUVR were also tested using the linear conditional fixed effects regression with APOE ε4 carrier status as a covariate (unstandardised regression coefficients with 95% CIs and *P* values reported).

In all the previously described analyses of studies **I–IV**, two-tailed *P* values <.05 indicated statistical significance. The analyses were conducted using Stata 14.2 (Stata Corp., College Station, TX).

In study **III**, voxel-wise comparisons in 8mm FWHM smoothed [^{11}C]PBR28 SUV images between EM discordant twins with the same TSPO genotype were done using Statistical NonParametric Mapping (version 13) with a paired t-test design and a cluster defining threshold of *P*<.01 (corrected for family-wise error (FWE) at the significance level of *P*<.05).

5 Results

5.1 Results of telephone interviews (I–II)

5.1.1 Characteristics of telephone interview study participants (I–II)

The whole study population of study **I** included 4367 twins (mean age 74.1 y, SD=4.1 y, range 65–97 y; mean education 7.9 y, SD=2.9 y, range 5−16 y; 48.6% females) who were interviewed between 1999-2017, participated in TELE and TICS interviews and had no missing information in TELE, immediate word list recall measure of TICS or education. There were 1375 (533 MZ, 823 DZ and 19 unknown (XZ) pairs) complete twin pairs among the study population. Out of them, 101 pairs (31 MZ, 66 DZ and 4 XZ pairs) were discordant for dementia and 770 pairs (328 MZ, 432 DZ and 10 XZ pairs) were concordant for normal cognition based on TELE (for their characteristics, see I: table 1).

In study **II**, the study population consisted of twins (n=1772; mean age 73.8 y, SD=1.5 y, range 71–78 y; mean education 8.5 y, SD=3.2 y, range 5–16 y; females 49.7%) who were interviewed during 2013–2017 and had no missing information in TICS-m, CES-D or education. 1608 individuals had available APOE genotype information: the percentage of APOE ε4/ε4 genotype was 3.3%, APOE ε3/ε4 26.3%, APOE ε3/ε3 60.0%, APOE ε2/ε4 2.4%, APOE ε2/ε3 7.6%, and APOE ε2/ε2 0.4%. The frequency of APOE ε4 carriers did not differ between men (28.9%; 226/781) and women (31.7%; 250/788). Women scored higher on the CES-D scale than men but did not differ in age or education (for the characteristics according to sex, see II: Table 1).

In comparison to non-participants, the interviewed individuals were more often men (1999-2007 interviews: 51.4% vs 43.8%, F(1,4275)=25.82, *P*<.001; 2013–2017 interviews: 50.3% vs 44.6%, F(1,1884)=7.31, *P*=.007) and more educated (1999−2007: 7.9 y vs 7.2 y, F(1,4250)=94.90, *P*<.001; 2013–2017: 8.5 y vs 7.4 y, F(1,1880)=103.65, *P*<.001). Individuals from twin pairs with only one twin interviewed had lower cognitive performance compared to individuals from pairs with both twins interviewed (mean TICS: 27.76 vs 28.62, F(1,2991)=33.63, *P*<.001).

5.1.2 Discordant twin pair analyses of word list recall (I)

In study population **I**, there were 101 pairs (31 MZ, 66 DZ and 4 XZ twins) who were discordant for dementia and 770 pairs (328 MZ, 432 DZ and 10 XZ pairs) who were concordant for normal cognition based on TELE. The main interest was to examine if IR performance differed between cognitively normal twins with demented co-twins and twins who were concordant for normal cognition. The unadjusted mean IR score was 3.52 words (SD=1.52) for 101 cognitively normal twins with demented co-twins, 2.70 words (SD=1.67) for 101 demented co-twins and 4.27 words (SD=1.67) for 1540 cognitively normal twins from concordant pairs.

Cognitively normal twins with demented co-twins were found to have poorer total IR score than twins who were concordant for normal cognition after adjusting for age, sex, and education (B=-0.44, 95% CI -0.73 to -0.14, *P*=.003) (Figure 7). The magnitude of difference was similar in MZ and DZ twins, however, the difference was not statistically significant in MZ twins (MZ: B=-0.41, 95% CI -0.96 to 0.13, *P*=.13; DZ: B=-0.47, 95% CI -0.84 to -0.10, *P*=.01). The magnitude of difference between groups had a Cohen's d of 0.3.

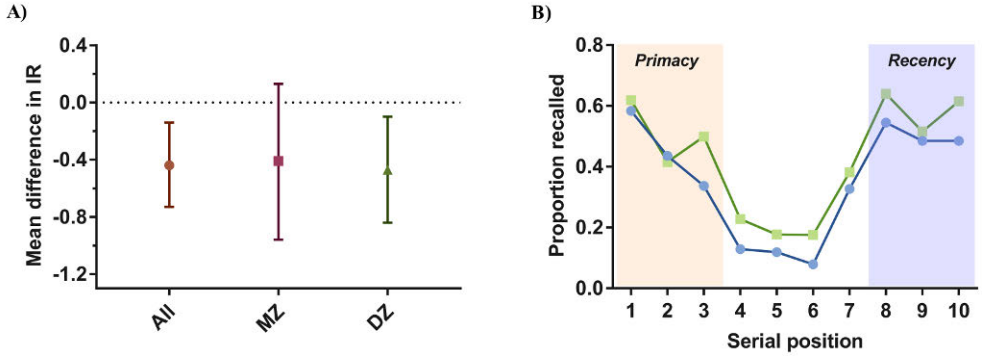

Figure 7. Difference in immediate recall (IR) performance of cognitively normal twins who had demented co-twins compared to twins who were concordant for normal cognition. (A) Mean differences in total number of immediately recalled words in a single free recall trial of a 10-word list between all/monozygotic (MZ)/dizygotic (DZ) cognitively normal twins with demented co-twins compared to twins with cognitively normal co-twins. (B) Proportion of correctly recalled words according to the position of the word in the list. The green line represents the twins who are concordant for normal cognition and the blue line represents the cognitively normal twins with demented co-twins. There was no statistically significant difference specifically in primacy words (B=-0.11, 95% CI -0.50 to 0.27, *P*=.56) or recency words (B=-0.25, 95% CI -0.59 to 0.10, *P*=.16) between the two groups.

At the time of the publication, APOE genotype information was not available for participants interviewed during 2013–2017. Now, the APOE ε4 carrier status was available for 72% of demented twins, 79% of their cognitively normal co-twins, and

81% of controls. The proportion of APOE ε4 carriers was 39% among demented twins, 44% among their cognitively healthy co-twins, and 28% among controls (F(1.45,1080.14)=5.48, *P*=.01). When the analysis was repeated after including only individuals with available APOE ε4 carrier status information (n=1397) and APOE ε4 carrier status as a covariate, the difference between cognitively normal twins with demented co-twins remained statistically significant (B=-0.47, 95% CI -0.80 to -0.14, *P*=.006).

Heritability of immediate word list recall (I)

Within-twin pair correlations of IR performance were higher in MZ than DZ twins for both sexes (rMZ=0.38, rDZ=0.18). The univariate biometrical model controlled for age and sex indicated a moderate heritability for IR performance (0.37, 95% CI 0.21 to 0.43).

Discordant twin pair analyses of delayed word list recall (unpublished)

A subset of twin pairs included 28 twin pairs discordant for dementia and 396 pairs concordant for normal cognition who were interviewed during 2013-2017 and had available DR scores. The unadjusted mean DR score was 1.54 words (SD=1.57, median=1, interquartile range (IQR)=0-2.5) for cognitively normal twins who had demented co-twins, 0.57 words (SD=1.17, median=0, IQR 0–0.5) for demented co-twins, and 2.46 words (SD=1.93, median=2, IQR 1–3) for cognitively normal twins from concordant pairs. The difference between cognitively normal twins with demented co-twins and cognitively normal twins with normal co-twins was tested with negative binomial regression adjusted for age, sex, education, and family relatedness because the assumptions for linear regression were not met. The difference was not found as statistically significant (B=-0.36, 95% CI -0.73 to 0.01, *P*=.06).

5.1.3 Overall characteristics of telephone interview of cognition (II)

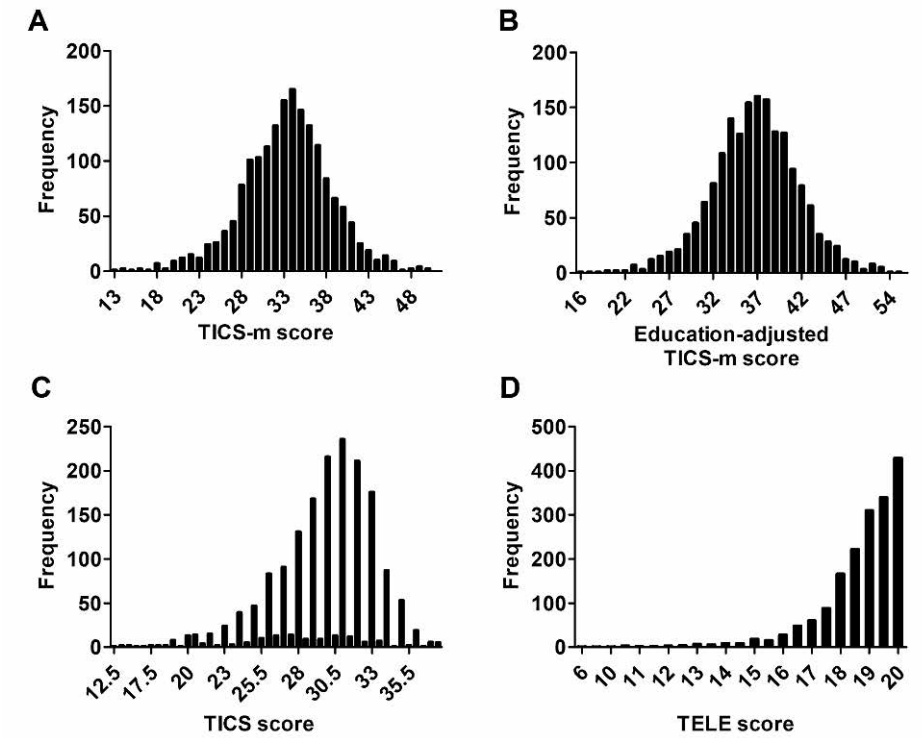

Figure 8. Distribution of total scores from telephone-based cognitive screening instruments. (A) The mean of unadjusted TICS-m scores from the instrument version with a maximum score of 50 was 33.4 (SD=5.2, range 13-50, skewness=-0.2, kurtosis=3.7). The total unadjusted scores of the abbreviated TICS-m versions with maximum scores of 35 and 27 also followed an approximately normal distribution. (B) The mean education-adjusted TICS-m score was 36.7 (SD=4.9, range 16-55, skewness=-0.1, kurtosis 3.7). (C) The mean unadjusted TICS score was 29.7 (SD 3.5, range 12.5-38, skewness -0.9, kurtosis 4.5). (D) The mean unadjusted TELE score was 18.7 (SD=1.5, range 6–20, skewness -2.4, kurtosis 12.2).

The properties of TELE, TICS and TICS-m instruments were examined in study population **II** in which the individuals had information from all three instruments. In addition, the commonly used abbreviated versions of TICS-m were examined. The individual items of instruments, their scoring and the percentage of individuals with correct answer to items are shown in Appendix 1. The mean overall duration of interviews was 37 minutes (SD=10, IQR 30-42). The mean duration of all cognitive questions was 11 minutes (SD=3, IQR 9-12). The distribution of total scores from TELE, TICS and TICS-m instruments is shown in Figure 8. Correlation between TICS and TICS-m and between different TICS-m versions were very high

(Spearman's rho from 0.89 to 0.98, *P*<.001). Correlation of TELE with both TICS and TICS-m instruments was moderate (Spearman's rho from 0.47 to 0.59, *P*<.001).

Immediate recall (IR) scores were approximately normally distributed but delayed recall (DR) scores had a positively skewed distribution. In the total sample, the mean of IR score was 4.2 words (SD=1.7, median=4, range 0–10, IQR 3–5, skewness=0.4, kurtosis=3.6) and the mean of DR score was 2.1 words (SD=2.0, median=2, range 0–10, IQR 1–3, skewness=1.1, kurtosis=4.2).

The distribution of DR measure indicated the presence of a floor effect because zero was the most frequent (24%) score. The floor effect was not as strong in individuals with high education (13 years or more) (Figure 9). Every tenth individual with high education had a score of zero, while one third of individuals with low education (6 years or less) recalled zero words (F(2.00, 2473.80)=35.82, *P*<.001). There was a linear correlation between IR and DR scores (r=0.72, *P*<.001, n=1772).

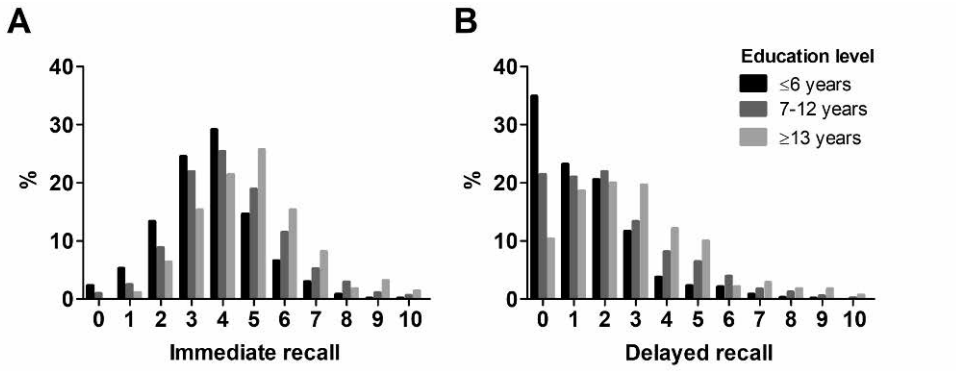

Figure 9. The percentage of free immediate and delayed word list recall scores (0–10) from the modified Telephone Interview for Cognitive Status (TICS-m) within education levels (n=594 for 6 or less years of education, n=900 for 7–12 years of education and n=278 for 13 or more years of education). (A) The distribution of immediate word list recall scores. (B) The distribution of delayed word list recall scores.

Effect of hearing loss (unpublished)

99% of individuals answered "yes" to the interviewer's question "can you hear me well". 170 individuals reported that they used a hearing aid (not necessarily on the telephone) and 1592 individuals reported no use of a hearing aid. 98% of those needing a hearing aid answered "yes" to the question "can you hear me well" in the beginning of the interview. The performance in TELE was similar between those who needed a hearing aid and those who did not (mean TELE: 18.7 vs 18.7). The overall performance of TICS and TICS-m were not statistically significantly different between the two groups (hearing aid vs no aid: mean TICS 29.3 vs 29.8, F(1,1229)=2.15, *P*=.14; mean TICS-m 32.7 vs 33.4, F(1,1230)=2.46, *P*=.12). The

performance in immediate word list recall and repetition of sentences were examined more closely because they were considered as measures most likely affected by hearing problems. In the immediate word list recall, only the recall performance of the first word in the list was poorer in those using a hearing aid (45% of those with a hearing aid vs 59% of those without one recalled the first word, F(1,1230)=13.33, *P*<.001). The repetition of the first sentence was poorer in those using a hearing aid (78% of those with a hearing aid vs 86% of those without were able to repeat the sentence correctly, F(1,1230)=8.26, *P*=.004).

5.1.4 Classification of cognitive status and association with demographic factors (II)

The prevalence of dementia and MCI/CIND in the population-based sample was compared across the previously published classification methods by Knopman et al. and Langa & Weir using the TICS-m. The prevalence of dementia ranged from 3.7% to 11% and the prevalence of MCI/CIND from 9.3% to 41.3% (Figure 10). The lowest prevalence values resulted after following the published methodology by Knopman et al.[327] which included an education adjustment of TICS-m scores.

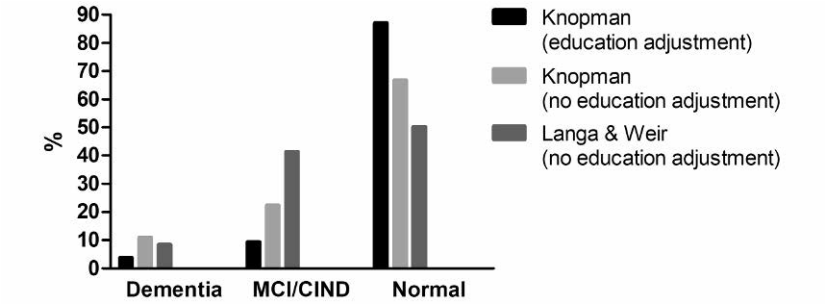

Figure 10. The prevalence of dementia, mild cognitive impairment (MCI)/cognitive impairment no dementia (CIND) and normal cognition according to different modified Telephone Interview for Cognitive Status (TICS-m) classification methods.

The association of sex, education, CES-D score, age, and APOE ε4 carrier status with cognitive status classified using the TICS-m and methods published by Knopman or Langa & Weir was examined with multinomial regression models (for detailed results see II: Table 2). APOE ε4 carriers were more likely to be demented relative to non-carriers according to both classification methods (Knopman: RRR=1.95, 95% CI 1.08 to 3.52; Langa: RRR=2.13, 95% CI 1.41 to 3.21). APOE ε4 carrier status was associated with MCI/CIND only when the education-adjusted classification by Knopman was used (RRR=1.78, 95% CI 1.23 to 2.56). APOE ε4 carrier status was not associated with MCI/CIND when using the method by Langa

& Weir (RRR=1.23, 95% CI 0.96 to 1.56) or when using the cut-off values of Knopman classification but without adjusting them for education (RRR=1.21, 95% CI 0.91 to 1.61). Table 3 demonstrates that the proportion of APOE ε4 carriers is higher in the MCI/CIND group when the education-adjusted classification method was used.

Table 3. The number and percentage of APOE ε4/ε4, APOE ε4/ε3, and APOE ε4 non-carriers among cognitive status groups classified according to Knopman or Langa & Weir

	Knopman						**Langa & Weir**		
	education adjustment			*no education adjustment*			*no education adjustment*		
	Dementia	MCI	Normal	Dementia	MCI	Normal	Dementia	CIND	Normal
APOE ε4/ε4 carriers	3 (6%)	7 (5%)	43 (3%)	6 (4%)	14 (4%)	33 (3%)	3 (3%)	29 (4%)	21 (3%)
APOE ε4/ε3 carriers	18 (38%)	53 (37%)	352 (26%)	57 (37%)	96 (27%)	270 (25%)	47 (40%)	176 (27%)	200 (25%)
APOE ε4 non-carriers	27 (56%)	84 (58%)	982 (71%)	92 (59%)	241 (69%)	760 (72%)	67 (57%)	452 (69%)	574 (72%)
Total (100%)	48	144	1377	155	351	1063	117	657	795

Note. APOE ε4 non-carriers include genotypes ε3/ε3, ε2/ε3, and ε2/ε2. Abbreviations: APOE, apolipoprotein E; CIND, cognitive impairment no dementia; MCI, mild cognitive impairment.

Dementia and MCI/CIND were associated with higher age according to both classification methods. Dementia, but not MCI/CIND, was associated with higher CES-D scores. The relative risk ratio for dementia did not differ statistically significantly between men and women. The relative risk ratio for MCI/CIND was lower in women relative to men when the method by Langa & Weir was used (RRR=0.74, 95% CI 0.60 to 0.91), while there was no statistically significant difference when using the Knopman method.

5.1.5 Association of TICS-m total scores and word list recall scores with demographic factors (II)

TICS-m total scores

As expected, age (r[1722]=-0.20, *P*<.001), education (r[1722]=0.34, *P*<.001), and CES-D score (r[1772]=-0.07, *P*=.005) correlated with the total TICS-m score (50-point scale). The linear regression model including age, education, sex, CES-D

score, and APOE ε4 carrier status (F[6,1237]=57.89, *P*<.001) indicated that 17% of the variance of the total score was accounted for by the linear combination of these variables. Specifically, higher age (B=-0.60 per year, 95% CI -0.75 to -0.45), lower education (B=0.53 per year, 95% CI 0.46 to 0.60), male sex (B=0.98, 95% CI 0.51 to 1.45), more depressive symptoms (B=-0.06 per unit, 95% CI -0.10 to -0.03), and APOE ε4 carrier status (B=-0.79, 95% CI -1.34 to -0.25) were associated with poorer total TICS-m performance.

A sex difference in the TICS-m total score seemed to be present only in individuals classified as cognitively normal (unpublished result; education-adjusted Knopman classification: mean TICS-m 34.0 in men vs 35.0 in women, F(1,112)=17.55, *P*<.001; Langa & Weir: 36.6 in men vs 37.7 in women, F(1,715)=22.03, *P*<.001). No sex difference was detected in individuals classified with MCI/CIND (education-adjusted Knopman: 27.1 in men vs 27.4 in women, F(1,155)=0.45, *P*=.50; Langa & Weir: 30.6 in men vs 30.6 in women, F(1,628)=0.04, *P*=.84), or with dementia (education-adjusted Knopman: 21.9 in men vs 20.3 in women, F(1,62)=3.65, *P*=.06; Langa & Weir: 24.1 in men vs 24.0 in women, F(1,147)=0.03, *P*=.86).

The dose-dependent effect of APOE ε4 allele was examined by dividing APOE ε4 carriers into two groups. Individuals with two APOE ε4 alleles had on average 1.59 (95% CI -2.81 to -0.37, n=53) and those with one ε4 allele 0.69 (95% CI -1.27 to -0.12, n=423) poorer TICS-m scores compared to APOE ε4 non-carriers (unpublished result).

The interaction effects between sex and age, sex and APOE ε4 carrier status, and sex and CES-D score were added to the linear model (F[10,1237]=34.84, *P*<.001, R^2=0.18). Age had a statistically significant interaction with sex (*P*=.007): the effect size of age was twice as large for females (B=-0.83, 95% CI -1.08 to -0.58) compared to males (B=-0.42, 95% CI -0.60 to -0.24). No interaction of sex with APOE ε4 carrier status or CES-D score was detected (both *P*>0.7).

The results were similar for total scores from abbreviated TICS-m instrument versions (II: Supplementary table 4).

Word list recall scores

Age (r[1722]=-0.16, *P*<.001) and education (r[1722]=0.23, *P*<.001) correlated statistically significantly with the IR score. The linear regression model including age, education, sex, CES-D score, and APOE ε4 carrier status (F[6,1237]=28.24, *P*<.001) indicated that 10% of the variance of total score was accounted for by the linear combination of these variables. Poorer IR score was associated with higher age (B=-0.16, 95% CI -0.21 to -0.11), lower education (B=0.12, 95% CI 0.09 to 0.15), male sex (B=0.52, 95% CI 0.35 to 0.68), higher CES-D score (B=-0.01, 95%

CI -0.02 to -0.001), but not with APOE ε4 carrier status (B=-0.11, 95% CI -0.30 to 0.07). The observed sex difference in the total TICS-m score was caused by better word list recall performance of women (II: Supplementary figure 2).

The interaction effects between sex and age, APOE ε4 carrier status and CES-D score were added to the linear model (F[10,1237)=17.28, *P*<.001, R^2=0.11). The interaction term between age and sex was statistically significant (p=0.01) with age having twice as large effect for women (B=-0.23, 95% CI -0.31 to -0.15) as compared to men (-0.10, 95% CI -0.16 to -0.04). The interaction effect between sex and APOE ε4 carrier status and between sex and CES-D were non-significant (both *P*>.4).

Age (r[1722]=-0.16, *P*<.001) and education (r[1722]=0.24, *P*<.001) correlated statistically significantly with the DR score. Poorer DR score was associated with higher age (B=-0.09, 95% CI -0.12 to -0.06), lower education (B=0.06, 95% CI 0.05 to 0.07), male sex (B=0.29, 95% CI 0.20 to 0.38), CES-D score (B=-0.01, 95% CI -0.02 to -0.004), and being an APOE ε4 carrier (B=-0.12, 95% CI -0.23 to -0.01). All interaction effects with sex were non-significant (all *P*>0.3).

5.2 PET imaging studies (III–IV)

5.2.1 Characteristics of PET imaging study participants (III–IV)

Eleven (4 MZ, 7DZ) twin pairs participated in [^{11}C]PBR28 imaging in study **III**. They were, on average, 74 years old (range 72–77 y) and had 7 years of education (range 6–13 y). Eight twin pairs were concordant for the duration of education. Six twin pairs were female-female and five were male-male. In three twin pairs, one sibling had a previous diagnosis of AD. Ten (4 MZ, 6 DZ) twin pairs were discordant for the primary measure of EM performance. Eight (2 MZ, 6 DZ) twin pairs were discordant according to the more stringent criterion with one twin affected by at least an MCI-level impairment and the other twin being cognitively normal. The characteristics of participants are shown in Table 4 (for more detailed characteristics, see III: Table 1).

Table 4. Characteristics of study population III

		Discordant for EM performance	Discordant for at least aMCI	Zyg	TSPO	APOE	Verbal DR	PBR28 GM SUV	PiB GM SUVR
Pair 1	Twin 1	control	no	MZ	HAB	ε3ε3	1.2	1.01	1.58
	Twin 2	case		MZ	HAB	ε3ε3	-0.1	1.06	1.44
Pair 2	Twin 1	control	control	MZ	MAB	ε4ε4	-0.7	0.78	1.85
	Twin 2	case	case	MZ	MAB	ε4ε4	-1.2	0.94	2.08
Pair 3	Twin 1	control	control	MZ	HAB	ε3ε4	-1.0	1.37	1.68
	Twin 2	case	case	MZ	HAB	ε3ε4	-1.2	1.62	1.42
Pair 4	Twin 1	control	no	MZ	MAB	ε3ε4	1.2	1.07	1.52
	Twin 2	case		MZ	MAB	ε3ε4	-0.4	1.11	2.58
Pair 5	Twin 1	control	control	DZ	HAB	ε3ε4	0.3	1.34	1.61
	Twin 2	case	case	DZ	MAB	ε3ε4	-3.1	0.71	2.91
Pair 6	Twin 1	not discordant		DZ	HAB	ε3ε3	-0.5	1.08	1.48
	Twin 2			DZ	HAB	ε3ε3	-0.5	1.21	1.44
Pair 7	Twin 1	control	control	DZ	HAB	ε3ε3	2.4	1.14	1.58
	Twin 2	case	case	DZ	HAB	ε3ε3	-1.6	1.34	1.39
Pair 8	Twin 1	control	control	DZ	HAB	ε3ε3	0.6	0.94	1.46
	Twin 2	case	case	DZ	MAB	ε3ε3	-1.2	1.02	2.07
Pair 9	Twin 1	control	control	DZ	MAB	ε3ε3	0.6	0.84	1.31
	Twin 2	case	case	DZ	MAB	ε3ε4	-2.8	1.22	2.94
Pair 10	Twin 1	control	control	DZ	HAB	ε3ε4	1.1	0.85	1.43
	Twin 2	case	case	DZ	HAB	ε4ε4	-2.1	1.41	2.47
Pair 11	Twin 1	control	control	DZ	HAB	ε4ε4	-0.9	1.24	1.93
	Twin 2	case	case	DZ	HAB	ε3ε4	-1.3	1.26	1.35

Note. Case refers to the twin with poorer EM performance as compared to the control co-twin. Verbal DR is the average of SD units from the delayed word list recall from the CERAD-NB and the delayed Logical Memory recall from the WMS-R. aMCI is defined by -1 SD or poorer performance in both verbal DR tests. PBR28 GM consists of prefrontal cortex, parietal cortex, lateral temporal cortex, precuneus, posterior cingulate, and mesial temporal cortex. PiB GM consists of prefrontal, parietal, lateral temporal, anterior cingulate, posterior cingulate, and precuneus cortex. Abbreviations: aMCI, amnestic mild cognitive impairment; APOE, Apolipoprotein E; DR, delayed recall; DZ, dizygotic; EM, episodic memory; GM, grey matter; HAB, high-affinity binder (CC genotype); MAB, mixed-affinity binder (CT genotype); MZ, monozygotic; SUV(R), standardised uptake value (ratio); TSPO, translocator protein; Zyg, zygosity.

45 (21 MZ, 24 DZ) twin pairs had available PET and neuropsychological data and were included in study **IV** (mean age 73, range 57 to 83 years; 40% were women). 43 twin pairs (20 MZ, 23 DZ) had available APOE genotype information with 36% of twins having at least one APOE ε4 allele. In addition, 15 healthy non-twin control

individuals were included (mean age 72, range 67 to 77 years; 67% were women) with 20% having at least one APOE ε4 allele. 42 (19 MZ, 23 DZ) pairs were discordant for the primary measure of EM (IV: Table 1). 15 (5 MZ, 10 DZ) twin pairs were also discordant for at least an aMCI-level impairment.

5.2.2 Discordant twin pair analyses of [^{11}C]PBR28 PET study (III)

Pairs discordant for episodic memory performance

Ten pairs had differing EM test performance and eight pairs were identical for TSPO genotype (5 CC; 3 CT). Eight twins with poorer EM (mean=1.25 SUV) as compared to their co-twins (mean=1.04 SUV) had on average 20% higher [^{11}C]PBR28 GM binding (intra-pair difference 0.21 SUV, 95% CI 0.05 to 0.37, *P*=.02) (Figure 11). The ROI-level results were supported by the voxel-level result which detected higher cortical TSPO binding especially in the posterior cingulate/precuneus, parietal and temporal cortex in twins with poorer EM (III: Figure 1). When including ten pairs and controlling for TSPO genotype, the result remained the same. In the ten discordant twin pairs, poorer-performing twin (mean=2.06 SUVR) also had on average 29% (0.47 SUVR, 95% -0.08 to 1.02, *P*=.08) higher cortical [^{11}C]PiB retention compared to their better-performing twin (mean=1.60 SUVR) (unpublished) (Figure 11). When controlling for APOE genotype, poorer-performing twins had on average 20% (0.34 SUVR, 95% CI -0.11 to 0.79, *P*=.12) higher [^{11}C]PiB SUVR compared to their co-twins (unpublished).

Six twin pairs identical for TSPO genotype had available [^{11}C]PBR28 V_T results. Twins with poorer EM had on average 16% higher [^{11}C]PBR28 GM V_T compared to their better-performing co-twins but the result did not reach statistical significance (intra-pair difference 0.58 mL/cm3, 95% CI -0.74 to 1.90, *P*=.31). The K_1 of cortical GM was similar between twins with poorer EM compared to their better-performing twins with mean K_1 values of 0.194 (SD 0.064) and 0.199 (SD 0.037) mL/min/mL, respectively (intra-pair difference 0.00, 95% -0.05 to 0.04, *P*=.80). Twins with poorer EM performance also had higher cerebellar binding of [^{11}C]PBR28 (intra-pair difference 0.21 SUV, 95% CI 0.11 to 0.32, *P*=.002, n=8 pairs; 0.60 mL/cm3, 95% CI -0.51 to 1.71, *P*=.22, n=6 pairs).

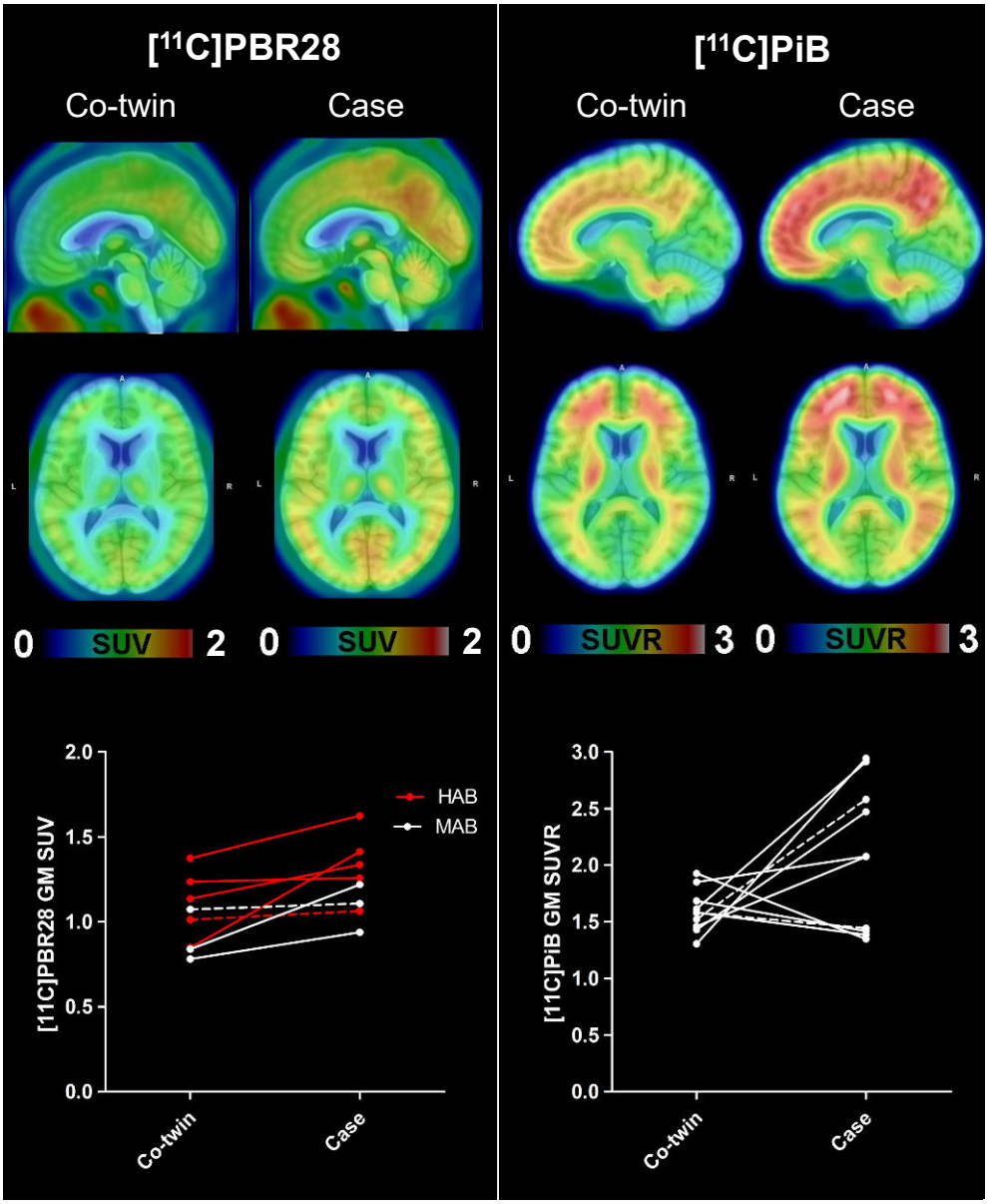

Figure 11. Mean parametric [^{11}C]PBR28 and [^{11}C]PiB images (above) and ROI results (below) of twin pairs discordant for EM performance. Case refers to the twin with poorer EM. Below, full lines represent the pairs that are also discordant for aMCI. High-affinity binders (HAB) represented with red lines and mixed-affinity binders (MAB) with white lines for the [^{11}C]PBR28 ROI results.

Pairs discordant for episodic memory impairment

Eight twin pairs were discordant for EM impairment. In the six pairs with the same genotype (4 CC; 2 CT), twins with at least an aMCI-level impairment (mean=1.30 SUV) had on average 25% higher [^{11}C]PBR28 GM binding compared to their cognitively normal co-twins (mean=1.04 SUV) (intra-pair difference 0.26 SUV, 95% CI 0.06 to 0.46, *P*=.02). When including eight pairs and controlling for the TSPO genotype, the result remained the same. In the four pairs with the same TSPO genotype and available [^{11}C]PBR28 V_T values, impaired twins had on average 11% higher [^{11}C]PBR28 GM V_T compared to their better-performing co-twins, but the difference was not statistically significant (intra-pair difference 0.5 mL/cm^3, 95% CI -1.9 to 2.9, *P*=.53). In the eight discordant pairs, impaired twins (mean=2.08 SUVR) also had on average 29% (0.47 SUVR, 95% CI -0.20 to 1.15, *P*=.14) higher cortical [^{11}C]PiB SUVR compared to their cognitively normal co-twins (mean=1.61 SUVR), but the result was statistically nonsignificant (unpublished). When controlling for the APOE genotype, the impaired twins had on average 18% (0.31 SUVR, 95% CI -0.22 to 0.83, *P*=.21) statistically nonsignificantly higher [^{11}C]PiB SUVR compared to their co-twins (unpublished).

5.2.3 Discordant twin pair analyses of [^{11}C]PiB PET study (IV)

Pairs discordant for episodic memory performance

42 (19 MZ, 23 DZ) twins with poorer EM had slightly higher cortical [^{11}C]PiB SUVR (mean=1.44) compared to their co-twins (mean=1.36), but the difference was not statistically significant (Table 5). 15 (10 DZ, 5 MZ) twins who had at least an aMCI-level impairment had nonstatistically significantly higher [^{11}C]PiB SUVR (mean=1.62) than their cognitively normal co-twins (mean=1.45). The results are also shown by zygosity in Table 5. When the APOE ε4 carrier status was controlled for, the results remained unchanged.

Table 5. Pairwise differences of cortical [^{11}C]PiB uptake in discordant twin pairs

	All twins		MZs		DZs	
	SUVR difference	95% CIs	SUVR difference	95% CIs	SUVR difference	95% CIs
Twin pairs discordant for EM performance						
Better-performing twins	Reference					
Poorer-performing co-twins	0.08 (6%)	-0.05 to 0.20	0.04 (3%)	-0.08 to 0.17	0.10 (7%)	-0.11 to 0.32
Twin pairs discordant for EM impairment						
Cognitively normal twins	Reference					
Impaired co-twins	0.17 (12%)	-0.13 to 0.47	-0.03 (-2%)	-0.24 to 0.17	0.27 (19%)	-0.18 to 0.73

Note. [^{11}C]PiB uptake was measured as the average of prefrontal, parietal, lateral temporal, anterior cingulate, posterior cingulate, and precuneus ROI SUVRs. EM performance was evaluated as the average of SD units from the delayed word list recall from the CERAD-NB and the delayed Logical Memory recall from the WMS-R. EM impairment was defined by -1 SD or poorer performance in both delayed EM tests. Abbreviations: DZ, dizygotic; EM, episodic memory; MZ, monozygotic; SUVR, standardised uptake value ratio.

5.2.4 Associations between continuous episodic memory performance, [^{11}C]PBR28 and [^{11}C]PiB binding (III–IV)

In study **III**, higher cortical [^{11}C]PBR28 SUV was related to poorer verbal DR, visual DR and incidental memory performance within 11 twin pairs (Table 6). On the contrary, statistically significant within-pair associations were not detected between [^{11}C]PBR28 binding and verbal IR and CERAD total score (Table 6).

In study **IV**, greater within-twin pair difference in continuous [^{11}C]PiB SUVR correlated with greater within-twin pair difference in EM test scores and total CERAD score in 45 (21 MZ, 24 DZ) twin pairs (Figure 12). Correlations were statistically significant in DZ pairs, whereas in MZ pairs, correlations were statistically nonsignificant (Figure 12). However, there were no significant zygosity-EM interactions (*P*s>.05) on SUVR indicating that within-pair EM differences were similarly related to within-pair SUVR difference in DZ and MZ pairs. Based on the linear conditional fixed effects regression models including the APOE ε4 carrier status as a covariate, greater [^{11}C]PiB was related to poorer EM test scores and CERAD total score within twin pairs (Table 6). When MZ and DZ twins were examined separately, statistical significance was not reached (Table 6).

The [^{11}C]PBR28 binding adjusted for the TSPO genotype and [^{11}C]PiB binding in the prefrontal, lateral temporal, parietal, precuneus, posterior cingulate or anterior cingulate cortex had positive, but statistically nonsignificant, correlations within 11 twin pairs (r=0.23 to 0.45, all *P*s>0.17, uncorrected for multiple comparisons) and

between individuals (between-family) (r=0.13 to 0.27, all *P*s>0.22, uncorrected for multiple comparisons, n=22) (unpublished).

Table 6. Association of cortical [^{11}C]PiB SUVR and [^{11}C]PBR28 SUV with episodic memory, global cognition and incidental memory performance

	All twins			MZs			DZs		
	B	**95% CIs**	***P***	**B**	**95% CIs**	***P***	**B**	**95% CIs**	***P***
[^{11}C]PiB (SUVR)									
Verbal DR	-0.08	-0.17 to 0.02	.10	-0.09	-0.36 to 0.17	0.47	-0.07	-0.23 to 0.09	.35
Visual DR	-0.14	-0.27 to -0.01	**.03**	-0.09	-0.26 to 0.08	0.27	-0.16	-0.42 to 0.10	.22
Verbal IR	-0.10	-0.20 to -0.01	**.04**	-0.10	-0.33 to 0.13	0.39	-0.10	-0.29 to 0.08	.24
CERAD total	-0.01	-0.02 to -0.002	**.02**	-0.01	-0.03 to 0.02	0.51	-0.01	-0.03 to 0.004	.15
[^{11}C]PBR28 (SUV)									
Verbal DR	-0.08	-0.15 to -0.01	**.03**						
Visual DR	-0.13	-0.26 to -0.005	**.04**						
Verbal IR	-0.03	-0.12 to 0.06	.44						
CERAD total	0.00	-0.02 to 0.01	.56						
memo-BNT	-0.05	-0.09 to -0.0004	**.05**						

Note. Statistically significant *P* values are in bold. The verbal DR is the average of SD units from the delayed word list recall from the CERAD-NB and the delayed Logical Memory recall from the WMS-R. The visual DR is the average of SD units from the delayed visual reproductions test from WMS-R and the delayed constructional praxis savings from the CERAD-NB. The verbal IR is the average of SD units from the immediate word list recall from the CERAD-NB and the immediate Logical Memory recall from the WMS-R. The free recall score of memo-BNT was used to measure incidental memory performance. Cortical [^{11}C]PBR28 consists of prefrontal cortex, parietal cortex, lateral temporal cortex, precuneus, posterior cingulate, and mesial temporal cortex. Cortical [^{11}C]PiB consists of prefrontal, parietal, lateral temporal, anterior cingulate, posterior cingulate, and precuneus cortex. Abbreviations: DR, delayed recall; DZ, dizygotic; IR, immediate recall; MZ, monozygotic; SUV(R), standardised uptake value (ratio).

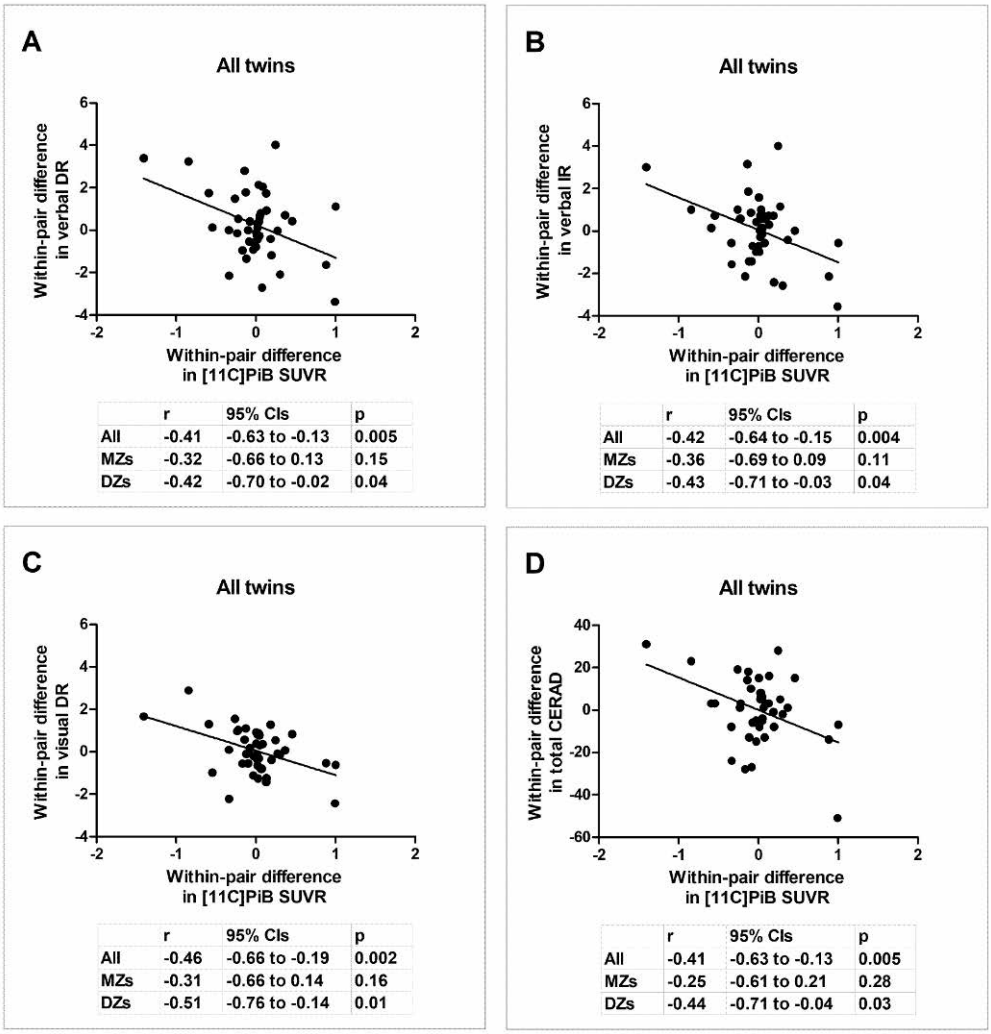

Figure 12. Correlations between within-pair difference in cortical [^{11}C]PiB SUVR and within-pair difference in episodic memory and global cognition performance in 45 (21 monozygotic (MZ), 24 dizygotic (DZ)) twin pairs. (A) Verbal delayed recall (DR), (B) verbal immediate recall (IR), (C) and visual DR were used to measure episodic memory performance. The total score from the CERAD-NB was used to measure global cognition performance (D).

6 Discussion

6.1 Telephone interview of cognition and episodic memory

6.1.1 Word list recall performance in dementia discordant twin pairs

The main result of study **I** was that cognitively normal twins who had a demented twin sibling had poorer IR performance than controls who were cognitively normal twins whose twin sibling was not demented. The analysis was controlled for age, sex, and education. Even when the analysis was further controlled for APOE ε4 carrier status, the difference in IR performance remained. The difference was subtle but statistically significant with a modest effect size. The 10-item word list of TICS-m is only repeated once. It is possible that a larger effect size would have been detected by using multiple trials. Other commonly used word list learning tests measure episodic encoding performance over three or five trials. Poorer IR performance in the cognitively healthy twins with demented co-twins seemed to be associated with familial (genetic and shared environmental) risk for dementia because the direction and magnitude of difference was similar in both MZ and DZ twins. However, the sample size in MZ pairs was less than half of that in DZ twin pairs, and a larger number of discordant MZ pairs would have been needed for more definite conclusions.

The finding of an association between familial risk of dementia and poorer baseline verbal EM encoding is supported by previous studies. Family history of AD has been associated with poorer baseline verbal EM encoding, delayed recall and recognition, executive functioning, and processing speed, but not with greater cognitive decline, compared with those without a family history.[154,155,337] The effect was not explained by APOE ε4 carrier status in this study or in the previous studies.[154,155] The association between family history of AD and poorer cognitive performance has been detected in individuals who were on average 65 years old. The effect of family history of AD and dementia on cognition is stronger at older age close to the hypothesised age of dementia onset, and often no difference in baseline

cognitive performance has been detected between middle-aged individuals with familial history of dementia or AD and controls.[156,337,338]

This study did not detect statistically significantly poorer performance in other word list test measures than IR total score in individuals with increased familial risk of dementia. However, the test properties may have limited the ability to detect subtle differences. Cognitively healthy twins with demented co-twins were expected to have particularly poorer immediate primacy effect (i.e. remembering less words from the beginning of a word list) compared to control subjects based on previous findings of an association between diminished primacy effect and progressive MCI,[339] and with the family history of AD in cognitively healthy middle-aged individuals.[153] However, there was no statistically significant difference in primacy words. It is possible that the use of a single-trial test and the quite short 10-item word list in this study decreased the sensitivity of detecting differences in the recall of serial position regions. The DR performance in a subset of twins interviewed during 2013-2017 was also examined. The DR performance difference between cognitively healthy twins with demented co-twins and controls was not statistically significant (B=-0.36, 95% CI -0.73 to 0.01, *P*=.06). The floor effect in the DR test of TICS-m probably affects its sensitivity. The number of discordant twin pairs who had available DR scores was also smaller than the number of discordant pairs with available IR scores. However, the magnitude of difference was very similar to IR performance difference suggesting that IR and DR tests may detect equally well early cognitive changes in older individuals.

This study supports poorer word list learning as an early marker of dementia risk and that the familial risk of dementia is reflected as a poorer verbal EM encoding performance regardless of whether an individual is an APOE ε4 carrier. The familial risk of dementia probably reflects a great number of different genetic and environmental risk factors with complicated interactions with each other. In addition to known and unknown genetic factors, lifelong environmental and early-life developmental factors, such as socioeconomic status, nutrition, learnt dietary and health behaviours, and intellectual stimulation, may have an influence on the overall cognitive abilities and reserve and/or on the neurodegenerative, vascular, and inflammatory processes affecting the development of dementia and AD.[340–342]

The poorer IR performance may reflect a certain cognitive phenotype that could increase the risk of clinical presentation of dementia. It is also possible that the detected subtle alteration in IR is an early sign of underlying neuropathology. Other studies have seen differences in AD biomarkers, such as Aβ deposition, atrophy and glucose metabolism, in cognitively normal individuals with a family history of AD compared to individuals without a family history.[202,343] Poorer IR performance could reflect pathological changes in the neural networks mediating IR performance which involves the lateral temporoparietal, posterior cingulate, precuneus, and frontal

regions.[344] The parietal and frontal brain regions are associated with the dorsal attention network,[11] and the posterior cingulate and precuneus are particularly associated with the default mode network which is affected very early during the AD process.[345–347] However, the absence of longitudinal follow-up and biomarker information limits the ability to draw these conclusions from this study.

The immediate recall of the 10-item word list showed substantial heritability as 37% of the variance was explained by genetic effects. Previously, it has been reported that the first trial of the 16-item word list shows zero heritability indicating that a single-trial administration of a word list may not be reliable enough to show consistent heritability.[348] This study demonstrates that the single-trial administration of a shorter word list may be more reliable compared to the presentation of a longer supraspan list (i.e., exceeding typical working memory capacity). The heritability of the used IR measure and the result that poorer IR performance was found at a higher rate in non-affected family members compared to the general population suggest that this measure is an appropriate endophenotype for genetic association studies.

6.1.2 Overall performance and classification of cognitive status with telephone screening

The main result of study **II** was that the prevalence of dementia and MCI varied considerably when using different TICS-m classification methods with and without education adjustment. APOE ε4 carrier status was associated with MCI only when education adjustment was applied.

The telephone screening of cognition consisting of TELE, TICS and TICS-m interviews provided a fast and convenient way to assess cognitive performance in a geographically dispersed population of older Finnish twins. The TELE, TICS and TICS-m interviews have previously been validated for the detection of dementia.[324,325,349] Particularly, the TICS-m has also been suggested to be useful in the detection of MCI.[350–352] Properties underlying the usefulness of TICS-m have been proposed to be the inclusion of delayed free recall of word list test,[353] and the approximately normal-shaped distribution of TICS-m total scores suggesting that the TICS-m is less limited by a ceiling effect usually limiting the utility of screening tests to detect MCI.[351] In this study, it was observed that the total scores of TICS-m better followed a normal distribution compared to TICS and TELE scores. The normal distribution of total TICS-m scores was due to the normal distribution of IR scores and the positively skewed distribution of DR scores as all the other measures had a negatively skewed distribution.

However, several others have argued that the TICS-m, like other cognitive screening instruments, performs only fairly in detecting MCI.[327,353,354] This study indicates that the properties of delayed word list recall test are a likely reason for the

discrepant reports on the utility of TICS-m for detecting MCI. The delayed recall performance had a notable floor effect in low-educated individuals with over a third of those with less than 6 years of education recalling no words at all after a delay. Hence, the utility of TICS-m to detect MCI is likely to vary in populations with a different educational background. Another reason for the variable utility of TICS-m is the existence of different instrument versions and approaches used to classify cognitive status. The inclusion (or exclusion) of education adjustment in the classification of cognitive status is a particularly important difference. In this study, the utility of different approaches was compared in the same population for the first time. There were considerable differences in the prevalence estimates of cognitive impairment after applying different approaches to classify cognitive status. The prevalence of dementia and MCI/CIND were 3.7% and 9.5% according to the classification by Knopman et al.[327] which includes an education adjustment. The prevalence of dementia and MCI/CIND were 8.5% and 41.4% according to the classification by Langa & Weir[328] which does not include an education adjustment of scores. The estimated frequency of MCI/CIND according to the Knopman classification is better supported by the previously reported MCI prevalence estimates in this age group.[24]

Demographic factors and depressive symptoms were associated with dementia and MCI/CIND in a fairly similar manner irrespective of the classification approach. Even though APOE ε4 status was associated with dementia irrespective of the classification method, it was associated with MCI/CIND only when education adjustment was applied. The association between the most important AD risk gene and education-adjusted MCI classification suggests that education adjustment increases the accuracy of detecting increased risk of future dementia. The results of this study supporting the use of education adjustment are in line with earlier findings. Adjusting neuropsychological test scores for premorbid cognitive ability has been seen to improve the precision to classify MCI.[355]

Individuals with more years of education are expected to have better cognitive test performance due to better premorbid cognitive abilities compared to individuals with fewer years of education. Adjusting cut-off values for education (or other proxy of premorbid cognitive ability) aims to identify the highly educated individuals who still show a good test performance despite having declined from their own baseline level. On the other hand, the aim is also to identify fewer individuals who have low educational level and baseline performance without any significant cognitive decline. Even though education is associated with decreased risk of dementia, it does not seem to protect individuals from neurodegenerative and cerebrovascular pathology.[356] Studies suggest that AD neuropathology must be more severe in high-educated individuals before clinical symptoms of dementia are detected.[204,356] Once AD is diagnosed, the cognitive decline is faster compared to patients with less

education.[357] Therefore, high-educated individuals with AD must be detected at an earlier stage to enable early intervention.

6.1.3 Association of TICS-m total scores and word list recall scores with demographic factors

Poorer TICS-m score was associated with higher age, lower education, male sex, more depressive symptoms, and APOE ε4 carrier status. The associations were similar with different TICS-m versions. Previous studies support the negative association of TICS-m with age,[351,358–361] and depressive symptoms.[358,361] Most previous studies support the positive association between education and TICS-m,[358–361] except for one contradictory result.[351] The cross-sectional association between TICS-m and APOE ε4 has not been previously investigated, but APOE ε4 has been associated with faster cognitive decline as measured with the TICS-m.[362] The most important measures of TICS-m, the immediate and delayed 10-item list recall, were also negatively associated with age, CES-D, male sex, and positively associated with education. Poorer DR score was associated with the APOE ε4 carrier status, but no statistically significant association between the IR score and APOE ε4 carrier status was observed.

Men had on average poorer TICS-m scores compared to women, while there was no sex difference in education, age, or APOE ε4 status. In the few studies which have investigated sex differences in the TICS-m, poorer TICS-m performance has been detected in men by large studies including over 500 individuals,[359,360] but not by smaller studies.[351,361] The poorer mean TICS-m score in men was due to poorer IR and DR performance of men compared to women, which is consistent with the fact that women typically perform better than men in verbal EM tests.[363] Interestingly, the negative association between age and both TICS-m and IR scores was twice as strong in women than in men during their 70s. In other words, the magnitude of sex difference seemed to diminish with age. Another large population-based study has observed that the sex difference in TICS-m total scores was less clear with increasing age.[359] There was no statistically significant interaction between sex and DR score. This was consistent with an earlier report of a diminishing sex difference with increasing age for IR but not for DR scores of TICS-m.[364] The interaction between sex and either APOE ε4 status or depressive symptoms was not statistically significant for the TICS-m or word list recall performance.

The detected interaction between sex and age could be explained by the sex difference in survival and selective survival of men with the best cardiovascular health into old age.[38] Hence, the age-related increase in the neuropathological burden could be less pronounced among men compared to women. Another important contributing factor to the age-sex interaction may be the elimination of the female

advantage in verbal EM performance with pathological ageing.[365] In this study, the female advantage in TICS-m and word list recall performance was seen in individuals classified as cognitively normal, but not in those who were classified with MCI/CIND or dementia. It is possible that the female advantage in verbal EM performance leads to the delayed detection of MCI among women. A recent study demonstrated that the use of sex-adjusted verbal EM cut-off values may improve the diagnostic accuracy of aMCI in such a way that 10% of women who were classified as cognitively normal using the unadjusted cut-off value were correctly reclassified as having aMCI and 10% of men who were classified as having aMCI were correctly reclassified as cognitively normal.[124] Early detection of MCI in women is critical, because once MCI is diagnosed, women tend to show greater cognitive and clinical decline and atrophy rates than men.[40]

6.1.4 Limitations of telephone interview studies

Telephone interview is limited by the restricted control over external distractors. The participants were inquired about their hearing problems and were asked to confirm that they were in a quiet place and had no external memory aids at the beginning of the interview. Hearing impairment did not appear to be a major problem in this study. For example, the overall performance of individuals who had a hearing aid was not significantly different compared to those who did not have a hearing aid. Although individuals with severe hearing problems were not able to participate in orally presented cognitive tests, individuals with mild to moderate hearing impairment often stated that they hear better in the telephone than face-to-face. Another limitation is that the assessment of some cognitive domains, such as the visuospatial function, is not possible via the telephone.

It is possible that cognitively impaired individuals were underrepresented because they, especially the most severely impaired, may be more often not willing or able to participate. This is supported by the fact that the twins from pairs with only one twin interviewed in study I were more often classified as having dementia compared to twins from pairs with both twins interviewed (16.5% vs 9.4%). Non-participants were less educated and more often women, but these differences were not prominent.

The major limitation of study **I** is that the definition of dementia is not based on clinical diagnostic criteria but on the validated telephone interview TELE. The use of a brief cognitive screening test likely leads to false positive and negative dementia cases. Because the used TELE cut-off score for healthy cognition (>17.5) has the sensitivity of 97% for identifying dementia,[326] the twins who are defined as cognitively normal should include very few demented individuals. The cut-off score for dementia (<16.0) provides the specificity of 90% to 100% for dementia.[324,326]

Consequently, the demented twins in discordant pairs may include an unknown proportion of false-positive dementia cases. Most false-positive cases are likely cases that suffer from cognitive impairment and who subsequently develop dementia, but in some cases, a low test score may be due to a mental disorder, impaired hearing, low education or low intelligence.[324] The possible inclusion of false-positive cases may have decreased the effect size of IR difference between the cognitively healthy co-twins from discordant pairs and controls. On the other hand, it is possible that the risk of a false-positive result might be associated with common familial factors. The used definition of dementia in this study did not allow to study the effect of familial risk by dementia subtypes. However, the assessment of all-cause dementia risk, not only disease-specific is valuable because AD and non-AD type pathologies coexist very frequently in older adults.[17]

The major limitation of study **II** is the lack of comparison of cognitive status classifications to clinical diagnostic criteria. Due to the cross-sectional nature, it was not possible to evaluate if the individuals classified as having MCI based on the education-adjusted classification show future cognitive decline.

6.2 Imaging biomarkers

6.2.1 [^{11}C]PBR28 PET imaging

Twin pairs who were discordant for EM performance provided a unique matched case-control study design that controlled for genetic and environmental effects. The main result was that twins with poorer EM had approximately 20% higher [^{11}C]PBR28 uptake in cortical GM regions, especially in the posterior cingulate/precuneus, parietal cortex, temporal cortex and anterior frontal lobe, compared to their better-performing co-twins. When only twin pairs who were discordant for memory impairment were examined, twins with at least an aMCI-level impairment had approximately 25% higher [^{11}C]PBR28 uptake compared to their intact co-twins. Despite the small sample size, the differences in [^{11}C]PBR28 SUV values were statistically significant and showed consistency in ROI and voxel-level analyses. In addition, continuous EM score showed a negative association with [^{11}C]PBR28 SUV in the within twin-pair analysis. The arterial input and therefore [^{11}C]PBR28 V_T values were not available for all twins. Twins with poorer EM, who had available [^{11}C]PBR28 V_T, had higher V_T values but the difference was not statistically significant, probably due to the limited sample size.

Previous studies in MCI and AD using [^{11}C]PBR28 have indicated conflicting results. Higher [^{11}C]PBR28 binding has been detected in AD dementia and MCI in some studies,[252,267,273,279] while others have not found a statistically significant group difference compared to healthy controls.[272,275] Studies using other second-generation

TSPO ligands than [^{11}C]PBR28 show similar results: most studies have found higher cortical binding in AD dementia but results on the group differences between individuals with MCI and healthy controls are more conflicting (III: supplementary table 1). The high interindividual variance in [^{11}C]PBR28 binding,[286,331] and the heterogeneity of quantification methods and study populations may contribute to the conflicting results.

In this study, there were statistically significant associations between the continuous measures of delayed verbal EM, delayed visual EM and incidental memory performance, and [^{11}C]PBR28 binding. On the contrary, there were no statistically significant associations between the immediate verbal EM or global cognitive performance with [^{11}C]PBR28 binding. Previous results on the relationship of TSPO binding with cognition are incoherent. Several cross-sectional studies have detected an association between higher TSPO binding and worse cognitive performance, but almost as many have not (III: Supplementary table 1). Furthermore, in the largest study, higher cortical TSPO binding was cross-sectionally associated with better cognitive performance.[268,269] In addition, longitudinal increase in cortical TSPO PET signal has been associated with decline in cognition, function, and grey matter volume in AD.[269,282] Fewer studies have examined the association of TSPO binding particularly with EM performance. One study found that poorer delayed word list recall score was associated with higher TSPO binding in the precuneus in AD,[260] while two studies did not detect a correlation between TSPO binding and verbal EM test scores.[267,274]

Twins with poorer EM and greater cortical [^{11}C]PBR28 binding also had 20-30% greater cortical [^{11}C]PiB binding compared to their better-performing co-twins, although the difference was not statistically significant (Figure 11). The trend-level results suggest that greater TSPO signal and greater amyloid load may be associated temporally. [^{11}C]PBR28 and [^{11}C]PiB binding were positively, but statistically nonsignificantly, correlated in the prefrontal, lateral temporal, parietal, precuneus, posterior cingulate, and anterior cingulate cortex. The absence of significant regional correlation is in disagreement with the majority of previous studies investigating the association between TSPO and amyloid PET that have detected positive correlations between cortical TSPO and amyloid tracer binding (III: Supplementary table 1).[258,268,269,272,274–276,278,280,281] The relationship between TSPO and amyloid PET would also be expected based on the observations of activated microglia being located close to fibrillar Aβ plaques in the human post-mortem AD brain.[96] The limited sample size may have limited the detection of significant regional correlation between [^{11}C]PBR28 and [^{11}C]PiB binding.

The results of this study supported the presence of negative relationships between TSPO binding and multiple memory tests, specifically the delayed EM recall measures. Both twin discordance and within-twin pair differences in the

delayed verbal free recall performance were associated with TSPO binding. Within-twin pair differences in delayed visual recall and incidental memory performance were also negatively associated with TSPO binding. Immediate and delayed recall measures have at least partly different neural correlates and genetic influences.[344,348] Immediate verbal free recall is associated with multiple cortical networks, including the default mode, dorsal attention, frontoparietal and language networks, and with the hippocampus.[11] Delayed verbal free recall is most strongly associated with the hippocampus but also with the frontoparietal and default mode networks.[11] Delayed recall measures are typically suggested to be more sensitive than immediate recall measures in early AD stages,[144–146] although not always.[147] EM measures are also more sensitive in the early stages of AD than global cognitive screening measures that typically detect impairment later during the AD continuum.[144,147] This may explain why the association of delayed EM recall measures with neuroinflammation was detected more readily compared to immediate EM recall and global cognitive measures.

6.2.2 [^{11}C]PiB PET imaging

There was a negative association between cortical fibrillar Aβ pathology and EM performance within twin pairs. Twins with poorer delayed verbal EM performance as compared to their co-twins had nonstatistically significantly higher cortical [^{11}C]PiB uptake than their better-performing co-twins (Table 5). Within-twin pair analyses of continuous measures of EM and [^{11}C]PiB uptake indicated that worse EM performance was statistically significantly associated with greater amyloid load. Statistically significant moderate within-twin pair correlations were detected between [^{11}C]PiB uptake and multiple EM measures (verbal delayed, verbal immediate, and visual delayed free recall) (Figure 12). The within-twin pair association of [^{11}C]PiB uptake and visual DR and verbal IR were also detected using linear conditional regression models controlling for the APOE ε4 status, but the association with verbal DR was statistically nonsignificant (Table 6). There were no statistically significant differences between MZ and DZ pair correlations.

As the negative EM-Aβ association was not significantly different between MZ and DZ twins, this suggest that genetic factors do not contribute to the negative EM-Aβ association. A previous study including 96 cognitively normal MZ twin pairs did not find a significant association between within-twin pair differences in amyloid load and visuospatial memory performance.[366] The study neither found statistically significant differences between 14 amyloid-PET positive and negative co-twins in four memory tests, apart from a trend-level effect in the visuospatial memory performance. Our results are to some extent in disagreement with this previous study. In our study, the within-pair correlations in MZ twins, while not statistically

significant, were still close to the magnitude of those of DZ twins. The twins of our study had a wider range of memory performance scores and more frequently had high amyloid load compared to the previous twin study. This may explain differences between the results.

The aim was also to replicate the results of a previous study conducted in the Turku PET Centre in which nine unaffected MZ twins from cognitively discordant twin pairs were found to have higher cortical [^{11}C]PiB uptake compared to nine cognitively normal non-twin controls.[319] In the current study, unaffected MZ or DZ twins did not have greater [^{11}C]PiB uptake compared to non-twin controls. An important difference between the studies are differences in the definition of cognitive discordance. In the current study, delayed verbal EM performance and the Jak/Bondi actuarial neuropsychological criteria for aMCI[140] were used that resulted in a small number of pairs discordant for EM impairment. In the current study, the non-twin healthy controls were better matched for age with the twins than in the previous study in which MZ twins were older compared to non-twin controls. Older age is known to be associated with greater amyloid load.[196] The non-twin controls who were recruited through open invitation did not represent the general Finnish population as well as the twins. The non-twin controls were more educated than the twins. Higher education may result in a better EM performance even in the presence of Aβ pathology.[203]

6.2.3 Limitations of PET studies

In studies **III-IV**, EM performance and amnestic impairment were used to investigate the early stages of AD. EM performance is a good predictor of progression to AD in individuals with MCI and in healthy individuals.[27,142,143] However, the limitation is that an unknown portion of individuals with poorer or impaired EM do not progress to AD. Studies **III** and **IV** were cross-sectional and therefore it is not possible to conclude whether neuroinflammation or amyloid accumulation is a primary pathology that leads to poorer memory performance or a secondary response to other pathologies. In study **III**, the smaller number of twin pairs than planned is a limitation and did not allow to study the differences by zygosity. Particularly, the identification of discordant MZ pairs was a challenge in studies **III-IV**. In study **IV**, DZ co-twins were on average more discordant for EM performance than MZ co-twins that may limit the ability to detect within-pair differences in MZ twins. The lack of available education- and sex-adjusted normative data for neuropsychological measures is another possible limitation. In study **IV**, data from participants belonging to two different projects was combined. The study was conducted in a similar manner (e.g. the same scanner was used) for

both co-twins and therefore within-twin pair results should not be biased by differences between projects.

A major limitation of study **III** is that the primary outcome measure of [^{11}C]PBR28 binding was not obtained using the gold-standard quantification method: compartmental analysis relative to the metabolite-corrected arterial input function. We used SUV as the primary outcome measure of [^{11}C]PBR28 binding because arterial input function was not available for all twins. [^{11}C]PBR28 SUV is moderately associated with V_T and has high test-retest reliability.[367,368] However, the SUV differences may potentially be biased by differential peripheral TSPO expression, tracer metabolism or tracer delivery to tissue. At least intra-pair differences in cerebral perfusion did not account for those in [^{11}C]PBR28 SUV because there was no difference in the available K_1 values. In addition, intra-pair differences in V_T, which is independent of perfusion, even though statistically nonsignificant, seemed to support the SUV results.

The accurate quantification of specific binding of TSPO tracers is problematic. There is no brain region devoid of specific TSPO binding sites enabling the use of reference tissue models. This problem has been bypassed in the non-invasive quantification of [^{11}C] (*R*)PK11195 tracer by using a supervised clustering algorithm which identifies a cluster of reference voxels that have kinetic behaviour resembling that of normal grey matter. This procedure was not applied in this study because its suitability for the high-affinity TSPO tracers like [^{11}C]PBR28 is debatable.[369,370] Some studies have used the cerebellum as a pseudo-reference region to quantify cortical-to-cerebellum [^{11}C]PBR28 SUVR values and have detected higher binding in individuals with AD compared to healthy controls.[252,267] The detection of no difference in cerebellar binding of [^{11}C]PBR28 between individuals with AD and healthy controls has been used to reason the use of cerebellum as a reference tissue.[252,273] In this study, the SUVR approach was not applied because twins with poorer EM performance also had higher [^{11}C]PBR28 cerebellar SUVs compared to their co-twins. Some previous studies have also detected higher cerebellar TSPO PET binding in individuals with AD compared to healthy controls.[279,371] Most studies have not detected differences in microglial markers between the post-mortem AD and healthy cerebellum.[95] In addition to microglia, TSPO PET signal may originate from astrocytes and vascular cells.[240,244] Thus, higher TSPO PET signal in the cortex and cerebellum of EM impaired twins may not only be explained by increased microglial activation, but vascular inflammation and reactive astrocytes may contribute to the detected difference.

There are difficulties even with the gold-standard quantification method of [^{11}C]PBR28 binding (reviewed in [254]). For example, it is unclear if [^{11}C]PBR28 V_T values should be corrected for the free fraction of tracer in plasma. Previous studies have only reported differences in [^{11}C]PBR28 binding between individuals with AD

and healthy controls after correcting the values for the plasma free fraction,[252,267] and statistically significant group-wise differences in the uncorrected [^{11}C]PBR28 V_T between AD and healthy individuals have not been detected.[252,267,272,273,275] However, we found that the measurement of plasma free fraction of [^{11}C]PBR28 is too low for reliable measurements. Furthermore, it is unclear if the modified compartmental model with an additional parameter for the binding of [^{11}C]PBR28 to the endothelium[250] provides a more accurate estimate of TSPO binding sites in the brain or results in an over-specified model.

6.3 Future directions

6.3.1 Telephone interview of cognition and episodic memory

The results of studies **I** and **II** add support to the usefulness of cognitive markers in the early detection of AD and dementia. The 2018 NIA-AA guidelines define AD strictly based on biomarkers without including cognition. However, the results of this thesis and several other studies discussed in this thesis indicate that subtle cognitive deficits are detected in the preclinical and prodromal disease stages before dementia occurs. It is likely that a combination of cognitive tests, especially in combination with pathophysiological biomarkers, will sustain as the most practicable foundation for the diagnosis of AD and other progressive memory disorders. The use of non-invasive and affordable markers, such as cognitive markers, is necessary in research and clinical settings for defining a target population which undergoes more invasive and expensive assessments, such as PET and CSF measurements.

However, as this thesis highlights, the choice of tests and their properties (e.g. floor and ceiling effects, sensitivity and specificity for pathological changes) is critical for the accurate detection of individuals at risk for developing AD and other progressive memory disorders. The detection of subtle early cognitive changes, which show high individual variation, also poses demands on the normative test data. Establishing norms that consider age, education, sex, and cohort effects and exclude individuals in the preclinical and prodromal disease stages (i.e. individuals who progress to dementia during the following years or have neuropathological changes) will likely increase the usefulness of cognitive markers.

More specifically, when using the telephone interview for cognition in future studies, it is recommended to use multiple learning trials of the 10-word list instead of a single trial in order to improve the properties of delayed EM measure. The addition of more sensitive tests measuring EM and other cognitive domains would also increase the capability for detecting early cognitive changes. If cognitive screening of twin populations is carried out in the future with the aim to identify twin

pairs discordant for memory impairment, more sensitive tests would be needed to increase the number of identified pairs. The possibility to carry out a new validation study in an unselected population-based sample should be considered in order to establish normative data that takes into account demographic factors. Including a measure of premorbid cognitive abilities, such as a vocabulary test, and an objective hearing test could also be valuable additions to the interview. In addition, an informant-rated cognition could be useful in decreasing the false-positive rate at least in the screening of dementia.[372]

There are large unanswered questions as to what the temporal trajectories of decline in different cognitive systems during the preclinical and prodromal disease stages are and how age, sex, education, APOE ε4 allele, and other risk factors modulate these trajectories. The results from future longitudinal studies that include sensitive neuropsychological tests targeting the neural networks first affected by AD pathologies will be of great interest.

6.3.2 Imaging biomarkers

Imaging biomarkers have become increasingly important in AD research and clinical trials. Imaging findings have led to the development of an AD biomarker model which states that AD pathogenesis with Aβ deposition as the initiating event starts decades before clinical symptoms arise.[160] Research is now focusing on the preclinical and prodromal stages of AD because it is likely that future disease-modifying treatments are more effective in these early stages. Large longitudinal imaging studies using multiple tracers aim to increase the understanding of early AD by disentangling the spatiotemporal relationships between early AD pathologies and their relationships with cognitive outcome. The development of disease pathology and pathology-cognition relationships are likely modified by genetic and environmental factors. As this thesis demonstrates, twin studies may also be useful for shedding light on the complex development of AD.

Although, the understanding of AD pathology has increased significantly, it is still far from complete. This is demonstrated by the numerous failed Aβ-targeted clinical drug trials. It is becoming clear that other pathological changes than Aβ pathology are critical for the development of AD. The results of this thesis support that neuroinflammation is a critical player in the early AD process and that TSPO PET can be used as an indicator of the early AD process. However, as this thesis has pointed out, there are some shortcomings related to TSPO PET imaging. The quantitation is a great challenge and TSPO genotype affects the binding of the majority of TSPO tracers. In addition, the biological knowledge needed to fully interpret TSPO PET studies is insufficient. Further understanding of TSPO biology and the development of TSPO PET tracers and their quantitation or tracer

development for other neuroinflammatory targets is needed before neuroinflammation imaging could be reliably used in clinical trials or possibly in clinical practice.

At present, we can detect early pathological changes related to AD already in healthy individuals or individuals with only mild symptoms. As the predictive value of biomarkers at the individual level is still unclear and there are no available disease-modifying drugs, we should be cautious when disclosing biomarker information to individuals. It is possible that a "diagnosis" of preclinical AD or high-risk state causes unnecessary anxiety to individuals without clear benefits.[373] The current concept of AD drug development is based on the early detection of AD and its continued improvement. The future holds promise that the value of early detection will actualise with the emergence of new treatments.

7 Conclusions

The studies in this thesis demonstrate that both cognition and biomarkers are key markers of the early AD process. This thesis also demonstrates that twin pair studies can be useful in obtaining knowledge from AD pathology and its cognitive correlates.

The main conclusions of this thesis are:

1. Familial risk of cognitive impairment is reflected in the IR performance of older persons who are cognitively normal based on a cognitive status screening instrument. Poorer IR performance may be an early indicator for increased risk of dementia. Simple word list learning task administered via telephone may be a useful phenotype in genome-wide association studies of AD and dementia.
2. Education-adjusted classification of TICS-m resulted in a lower prevalence of dementia and MCI and in a higher proportion of APOE ε4 allele carriers among those identified as having MCI. The results support the use of education-adjusted scoring for more accurate classification of MCI. The delayed word list recall test, which is often considered as the most important measure of TICS-m, had a floor effect in low-educated individuals and its properties may be improved with multiple learning trials.
3. Increased cortical neuroinflammation measured with [^{11}C]PBR28 PET was associated with poorer EM performance when genetic and environmental effects were controlled with a discordant twin pair setting. The findings support the use of TSPO PET as an indicator of AD process.
4. Within-twin pair analyses indicated that cortical Aβ pathology measured with [^{11}C]PiB PET is negatively related to EM when genetic and environmental effects were controlled with a twin pair setting. The uptake of [^{11}C]PiB was not found to be higher in cognitively normal twins with EM impaired co-twins compared to cognitively normal non-twin controls.

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Appendices

Appendix 1: Questions and scoring of the telephone assessment for dementia (TELE), Telephone Interview for Cognitive Status (TICS) and the modified Telephone Interview for Cognitive Status (TICS-m)

Item	TELE points (max 20)	TICS points (max 38)	TICS-m points (max 50)	TICS-m points (max 35)	TICS-m points (max 27)	% of individuals with correct answer
1. Name	1	1	2			100
2. Age	1		1			97
3. Year of birth	1					100
4. Day and month of birth	1					100
5. Telephone number			1			87
6. Date						
day	1	1	1	1		89
month	1	1	1	1		99
year	1	1	1	1		98
week day	1	1	1	1		98
season	1	1	1			99
7. Repeating 'rose, ball, key'	1					100
8. Counting backwards from 20 by threes						
17	0.5					95
14	0.5					92
11	0.5					93
8	0.5					87
5	0.5					91
2	0.5					88

Item	TELE points (max 20)	TICS points (max 38)	TICS-m points (max 50)	TICS-m points (max 35)	TICS-m points (max 27)	% of individuals with correct answer
9. Current president of Finland	1.0	1.0	2	1		90
10. Previous president of Finland	1.0		2	1		81
11. Recalling 'rose, ball, key' (or recognition [0.5])						
rose	1.0					90
ball	1.0					88
key	1.0					67
12. Similarities between pair of nouns						
orange and banana	1.0					95
table and chair	1.0					65
13. Counting backwards from 20 to 0		2	2	2	2	91
14. Immediate word list recall						
cabin		1	1	1	1	58
pipe		1	1	1	1	38
elephant		1	1	1	1	49
chest		1	1	1	1	23
silk		1	1	1	1	18
theatre		1	1	1	1	19
watch		1	1	1	1	36
whip		1	1	1	1	62
pillow		1	1	1	1	54
giant		1	1	1	1	59
15. Counting backwards from 100 by sevens						44 (all correct)
93		1	1	1	1	92
86		1	1	1	1	69
79		1	1	1	1	70
72		1	1	1	1	68
65		1	1	1	1	70

Item	TELE points (max 20)	TICS points (max 38)	TICS-m points (max 50)	TICS-m points (max 35)	TICS-m points (max 27)	% of individuals with correct answer
16. Responsive naming						
"What do people usually use to cut paper?"		1	1	1		99
"How many things are in a dozen?"		1	1			99
"What do you call a prickly green plant living in the desert?"		1	1	1		91
"What animal does wool come from?"		1	1			100
17. Repetition of sentences						
"The pupil solved a complicated task"		1	1			85
"No ifs, ands or buts"		1	1			84
18. Tapping 5 times with finger		2	2			94
19. Word opposites						
The opposite of "west"		1	1			92
The opposite of "generous"		1	1			84
20. Delayed word list recall						
cabin			1	1	1	31
pipe			1	1	1	24
elephant			1	1	1	37
chest			1	1	1	18
silk			1	1	1	15
theatre			1	1	1	15
watch			1	1	1	18
whip			1	1	1	21
pillow			1	1	1	20
giant			1	1	1	11
21. "Where are you right now?" (street, city, zip code, county)		4				94

NOTE. The percentage of individuals with correct answer is calculated based on study population II (n=1772; mean age 73.8 y, SD=1.5 y, range 71−78 y; mean education 8.5 y, SD=3.2 y, range 5−16 y; females 49.7%).

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