Clinical and biomarker correlates of genetic risk factors for Alzheimer's disease

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Clinical and Biomarker Correlates of Genetic Risk Factors for Alzheimer's Disease

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Clinical and Biomarker Correlates of Genetic Risk Factors for Alzheimer's Disease

DISSERTATION

To obtain the degree of Doctor at Maastricht University,
on the authority of the Rectus Magnificus, Prof. dr. L.L.G. Soete
in accordance with the decision of the Board of Deans,
to be defended in public on
Wednesday, the 24th of April 2013 at 14:00 hrs

by

Lyzel Samaniego Elias-Sonnenschein

Supervisor:

Prof. dr. Frans RJ Verhey

Co-supervisor:

Dr. Pieter Jelle Visser

Assessment Committee:

Prof. dr. Marc De Baets, chair

Prof. dr. Christine EM de Die-Smulders

Prof. dr. Cornelia van Duijn-Marja (Erasmus Medical Center, Rotterdam)

Dr. Gunter Kenis

Dr. Bart Rutten

The research described in this dissertation was performed at the Alzheimer Centrum Limburg of the the Department of Psychiatry and Psychology, School for Mental Health and Neuroscience, Maastricht University, The Netherlands; and at the Institute of Clinical Medicine-Neurology, University of Eastern Finland, Kuopio, Finland.

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Alzheimer's disease (AD) is multifaceted and influenced by many factors. The goal of our research is to better understand how genetic risk factors may influence the pathophysiology and clinical presentation of AD.

CHAPTER 1

General introduction

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most common cause of dementia.¹ It is characterized by progressive neuronal death, memory loss, behavioral and affective changes, and impairments in activities of daily living. It culminates in dementia. The global prevalence of dementia in 2012 has been estimated at 35.6 million, which is expected to double by 2030 and more than triple by 2050² because of advancing age in the population. The global socio-economic costs of dementia are enormous, with estimates at US\$ 604 billion in 2010.²

There is currently no cure for AD-type dementia. Available treatments are aimed at slowing the progression of the disease and address its associated symptoms. Often, treatment is sought when the disease is already at an advanced phase because only then do clinical symptoms become evident. Early diagnosis of AD is important, as drugs with disease-delaying potentials are most effective at the predementia stage. Identifying those who are at risk is equally important for prognostic purposes. Individuals with mild cognitive impairment (MCI) have been reported to have an increased risk of developing AD. About 10-30% of MCI cases have been reported to progress to AD.^{3, 4}

Early detection of AD entails understanding the complex relationship between risk factors and AD pathophysiology. In order to further clarify this relationship, we investigated in the thesis the relation between genetic risk factors, and biomarkers and clinical correlates of AD. We focused on single nucleotide polymorphisms (SNPs), which are specific variations in the gene; and on sporadic AD with no obvious familial aggregation, which represents the majority of AD cases.

Genetic risk factors do not directly cause the disease but are associated with increased risk for developing the disease. Until recently, the only established genetic risk factor for sporadic AD has been the apolipoprotein E (*APOE*) ϵ_4 allele.⁵⁻⁷ With the development of high-throughput techniques in the past years, other variants that predispose individuals to develop AD have been identified in genome-wide association studies (GWAS) with clinical diagnosis of AD as outcome (for a review, see ⁸) and with biomarkers as outcome.⁹⁻¹¹ Candidate gene studies, in turn, have reported how other potential AD risk genes affect pathways or biomarkers in AD. Compared to *APOE*, these SNPs exert

only small risk effects but when taken in combination with other risk variants or non-genetic risk factors may have a considerable role in the development of AD.

Biomarkers are objectively quantifiable measures that could be used as indicators of pathogenic processes in AD. The pathological hallmarks of AD are neuritic plaques, which are mainly composed of aggregated amyloid- β (A β), and neurofibrillary tangles consisting of hyperphosphorylated tau (p-tau) proteins.¹ There are many isoforms of A β , but A β_{1-42} has been shown to be more prone to aggregate.¹² A β and tau concentrations can be measured *in vivo* in cerebrospinal fluid (CSF) and through positron emission tomography (PET) imaging. Changes in A β and tau levels years before the onset of dementia have been reported.^{13, 14} As neuritic plaques and tangles build up, the brain shrinks. The hippocampus in the medial temporal lobe is one of the earliest structures to be affected in AD.^{15, 16} Hippocampal volume loss has been reported in the early stage of the disease.¹⁷ In the thesis, we focused on the biomarkers CSF A β_{1-42} and tau, and hippocampal volume as measured by magnetic resonance imaging (MRI).

With respect to clinical correlates, cognitive decline is a key feature of AD and is already present in the predementia stage. Significant impairment in episodic memory is a core diagnostic criterion for probable AD.¹⁸⁻²⁰ Changes in episodic memory have been reported in nondemented elderly²¹ and in individuals with cognitive impairment.²² Meanwhile, neuropsychiatric symptoms are common in predementia.^{23, 24} Depression, apathy, agitation and aggression are prevalent symptoms that have been reported in both MCI and AD.²⁵⁻²⁷ We examined in the thesis the effects of AD risk genes on delayed memory and on 12 neuropsychiatric symptoms: delusions, hallucination, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor behavior, nighttime behavior, and appetite/eating abnormalities.

PURPOSE OF THE THESIS

The goal of our research is to gain better insight on how genetic risk factors influence the pathophysiology and clinical presentation of AD. We strived to attain our goal by investigating the influence of AD risk genes on the clinical and biomarker correlates of AD. Herewith we hope

to contribute to the development of early diagnostic markers for AD and eventually treatment strategies.

The thesis has three aims that serve as main research questions:

1. To evaluate the predictive and diagnostic value of AD risk genes for AD
2. To identify correlations between AD risk variants and clinical markers of AD
3. To investigate the relationship between AD risk variants and biomarkers of AD pathology

Related to these aims, we had the following objectives:

Objectives related to aim 1:

- 1.1 To test the predictive accuracy of the *APOE* ϵ_4 allele for progression from MCI to AD
- 1.2 To test the predictive accuracy of AlzGene top loci for progression to AD in nondemented individuals with cognitive impairments
- 1.3 To test the association of a selection of candidate genes with the diagnosis of clinical AD

Objectives related to aim 2:

- 2.1 To test the correlation between the *APOE* ϵ_4 allele and neuropsychiatric symptoms in individuals with MCI
- 2.2 To test the correlation between top AlzGene loci and memory function in MCI

Objectives related to aim 3:

- 3.1 To test the relation between genetic risk factors, and amyloid and tau markers through a literature review
- 3.2 To test the correlation between AlzGene top loci and CSF markers in MCI
- 3.3 To test the correlation between AlzGene top loci, and a selection of other GWAS and candidate gene loci, and CSF markers in AD
- 3.4 To test the correlation between a selection of AD risk genes and CSF markers in non-AD type dementia, AD, MCI and control subjects
- 3.5 To test the correlation between AlzGene top loci and hippocampal volume markers in MCI

APPROACH

Three main approaches were employed to respond to our aims and objectives. These are described below.

Gene selection

We first selected genes based on their reported association with AD or effect on A β and tau. Next, we classified the genes into one of the four categories below. A summary over the selected genes is shown in Table 1.

1. *AlzGene 10 'top results' loci*

AlzGene is an online database that provides meta-analyses of published genetic association studies for AD for all polymorphisms with a minor allele frequency of more than 1% in healthy controls and for which genotype data for the same polymorphism is available in at least four case-control studies.⁷ The ranking of 'top results' is according to the interim criteria of the Human Genome Epidemiology Network (HuGENet).²⁸ We selected SNPs from the 10 genes that had the strongest association with clinical AD.

2. *GWAS with clinical AD as outcome*

GWAS is a relatively hypothesis-free approach to determine genotypes of hundreds of thousands of SNPs on a genome-wide scale testing affected vs. controls. This is followed by statistical analyses to investigate differences in allele or genotype frequencies between affected and controls. Due to the multiple tests performed, a strict statistical level, usually $\sim P < 5 \times 10^{-8}$, is required to be declared as genome-wide significant.²⁹ The outcome is clinical diagnosis of AD. We selected a number of SNPs from genes that were identified in GWAS but were not included in the AlzGene top 10 results.

3. *GWAS with biomarkers as outcome*

The outcome we specifically looked for were A β and tau in CSF, plaques or tangles. The GWAS may be performed in the context of a population study with healthy controls, or in other settings with AD, MCI or controls.

4. *Candidate genes*

These are genes identified through preconceived hypotheses about the gene's functions. This candidate gene approach allows investigation of complex genetic traits and is a potent approach for direct gene discovery.³⁰ We selected SNPs that previously showed a relation with markers for amyloid or tau pathology.

Review and meta-analysis

Relation APOE ϵ_4 allele and progression to AD

We performed an exhaustive search of published literature of prospective cohort studies testing the *APOE* ϵ_4 allele as predictor for progression from MCI to AD-type dementia. The study was later on elaborated to determine the diagnostic test accuracy of *APOE*- ϵ_4 for AD and other dementia in the primary, secondary and community settings. Since outcomes may differ in different settings, we have written three parallel protocols. The protocol for the diagnostic test accuracy in the primary care setting is presented in the thesis as an addendum. The protocols for the secondary care and community settings are found in the Cochrane Collaboration Archie site.

Relation SNPs and biomarkers

We carried out a systematic review of published literature in which a number of high-risk genetic variants have been studied in relation to A β and tau.

Multicenter studies

We investigated the relation between different SNPs and cognition, neuropsychiatric symptoms, biomarkers and clinical diagnosis in a range of cohorts and diagnostic groups. We used datasets from three different multicenter European cohorts that had DNA for genetic analysis and one dataset from a US-based multicenter study.

DESCRIPA

The ‘Development of Screening Guidelines and Criteria for Predementia Alzheimer’s Disease’ (DESCRIPA) multicenter prospective cohort study is part of the European AD Consortium (a general description is found at www.descripa.eu). Its task is to develop screening guidelines and clinical criteria for the diagnosis of predementia AD. DESCRIPA included participants with MCI and subjective cognitive impairment (SCI), who were followed up for a period of five years. The participants were recruited from 20 memory clinics across Europe. MRI scans and CSF samples were available for a subset of participants.

FINN-AD

The Finnish-AD is a collaboration of research institutions and academic hospital in Kuopio, Oulu and Tampere in Finland. It is a multicenter case-control cohort comprised of 890 AD patients from a clinical setting and 701 age-matched healthy controls from a population setting. All patients were diagnosed with probable AD according to NINCDS-ADRDA¹⁸ criteria. Control subjects had no symptoms of cognitive impairment based on clinical interview and neuropsychological examination. CSF samples were available for a subset of the cohort.

ADNI

The Alzheimer's Disease Neuroimaging Initiative is a longitudinal multicenter US-based study whose primary goal has been to test whether MRI, positron emission tomography (PET) and other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. ADNI recruited participants from 50 centers across the US and Canada. It included at baseline individuals aged 55 to 90 years clinically diagnosed with probable AD according to NINCDS-ADRDA¹⁸ criteria, MCI or normal cognition. The participants underwent routine physical, neurological and neuropsychological assessment. They were followed up for up to three years (for details, see adni.loni.ucla.edu and www.adni-info.org). ADNI has available clinical, imaging and biochemical biomarker data.

EDAR

The 'beta amyloid oligomers in the early diagnosis of AD and as marker for treatment response' (EDAR) is a European multicenter prospective cohort study (for general information, see www.edarstudy.eu) with 15 partners across Europe. Its goal is to develop new diagnostic markers for AD that can be used for the early diagnosis and for the monitoring of treatment response in drug trials. The project focuses on beta amyloid oligomers in CSF, serum and plasma, and the effect of genes involved in beta amyloid processing on these oligomers. EDAR recruited participants with MCI, AD-type dementia, non-AD dementia and cognitively healthy controls from memory centers. Participants

underwent clinical, neurological, neuropsychiatric, neuropsychological and functional assessments up to 18 months. EDAR has available clinical, neuropsychiatric, neuropsychological, imaging and CSF data.

THESIS OUTLINE

The main research questions, the chapters where we tackled these and the approach used are shown in Figure 1.

In *Chapter 2*, we performed a meta-analysis to assess the usefulness of *APOE* genotyping in predicting progression to AD among patients with MCI, and in selecting subjects for clinical trials aimed at preventing progression to dementia.

Chapter 3 is closely related to Chapter 2. It shows the protocol for the diagnostic test accuracy of *APOE* for dementia in the primary care setting. The protocol is written for the Cochrane Collaboration, is meant to serve as guide for similar reviews, and is a prerequisite for the conduct of full reviews.

Chapter 4 reports on the relation between neuropsychiatric symptoms and the *APOE* ϵ_4 allele in MCI in the DESCRIPA and ADNI cohorts. Analyses were first performed separately for each cohort, and afterwards in the pooled cohort.

Chapter 5 is a qualitative literature review of the possible role of AD genetic risk variants on A β and tau measured *in vivo* in CSF or by positron emission tomography, or upon neuropathological examination.

Chapter 6 is an exploratory study of the correlation between the top 10 AlzGene loci and key markers of AD pathology in CSF, MRI and cognition in nondemented individuals with cognitive impairment in the DESCRIPA study.

Chapter 7 presents the findings of a case-control genetic association study of 25 SNPs, and correlational study of 36 SNPs with CSF biomarkers in the FINN-AD cohort. The research was performed at the University of Eastern Finland in Kuopio.

Chapter 8 further investigates the effects of variants in the *MS4A* gene cluster on CSF A β , following findings in Chapter 7. The study was performed in a cohort consisting of patients with AD, MCI and cognitively healthy individuals from the EDAR study.

Finally, we present in *Chapter 9* an overall discussion of the main findings of the studies presented in thesis in the context of current knowledge, relate these to genes in AD pathophysiology, their implications and methodological considerations. We conclude by offering suggestions for future research.

Figure 1 Main research questions and approaches used

Table 1 List of genes in the thesis

Gene	Chap2	Chap3	Chap4	Chap5	Chap6	Chap7	Chap8
Top AlzGene							
<i>APOE</i>	√	√	√	√	√	√	√
<i>ABCA7</i>					√	√	
<i>BIN1</i>					√	√	
<i>CD2AP</i>					√	√	
<i>CD33</i>					√	√	
<i>CLU</i>					√	√	
<i>CR1</i>				√	√	√	
<i>MS4A4E</i>					√	√	√
<i>MS4A6A</i>					√	√	√
<i>PICALM</i>				√	√	√	
Selection GWAS not in top 10 AlzGene							
<i>EXOC3L2</i>						√	
<i>MS4A4A</i>						√	√
<i>MTHFD1L</i>						√	
GWAS with biomarkers as outcome							
<i>ADRA1</i>				√			
<i>ARL5B</i>				√			
<i>CDH4</i>				√			
<i>CHN2</i>				√			
<i>CYP19A</i>				√		√	
<i>C1QTNF1</i>				√			
<i>EPC2</i>						√	
<i>FLJ21511</i>				√			
<i>MSRA</i>				√			
<i>MTUS1</i>				√			
<i>NCAM2</i>				√			
<i>RBBP6</i>				√			
<i>RELN</i>				√		√	
<i>TMEM65</i>				√			
<i>TMPRSS2</i>				√			
<i>TOMM40</i>				√		√	
<i>UNC5C</i>				√			
<i>UPP2</i>				√			
Other candidate genes							
<i>ACE</i>				√		√	
<i>BDNF</i>						√	
<i>CYP46A1</i>						√	
<i>IDE</i>						√	
<i>MAPT</i>				√		√	
<i>MAPT H1/H2</i>				√			
<i>PPP3R1</i>						√	
<i>SORL1</i>				√		√	
<i>TF</i>						√	
<i>TOMM40^a</i>				√		√	

Abbreviations: Chap=chapter; GWAS=genome-wide association study

^a Different single nucleotide polymorphisms from those identified in GWAS with biomarker as outcome.

Gene names

<i>ABCA7</i>	ATP-binding cassette, subfamily A, member 7
<i>ACE</i>	angiotensin I converting enzyme 1
<i>ADRA1</i>	adrenoceptor alpha 1B
<i>ARL5B</i>	ADP-ribosylation factor-like 5B
<i>APOE</i>	apolipoprotein E
<i>BIN1</i>	bridging integrator 1
<i>BDNF</i>	brain-derived neurotrophic factor
<i>CD2AP</i>	CD2-associated protein
<i>CD33</i>	CD33 molecule
<i>CDH4</i>	cadherin 4, type 1, R-cadherin
<i>CHN2</i>	chimerin 2
<i>CLU</i>	clusterin
<i>CR1</i>	complement component receptor 1
<i>CYP19A</i>	cytochrome P450, family 19, subfamily a, polypeptide 1
<i>CYP46A</i>	cytochrome P450, family 46, subfamily A, polypeptide
<i>C1QTNF1</i>	C 1 q and tumor necrosis factor related protein 1
<i>EPC2</i>	enhancer of polycomb homolog 2
<i>EXOC3L2</i>	exocyst complex component 3-like 2
<i>FLJ21511</i>	similar to hypothetical protein FLJ21511
<i>IDE</i>	insulin-degrading enzyme
<i>MAPT</i>	microtubule-associated protein tau
<i>MS4A4A</i>	membrane-spanning 4-domains, subfamily A, member 4A
<i>MS4A4E</i>	membrane-spanning 4-domains, subfamily A, member 4E
<i>MS4A6A</i>	membrane-spanning 4-domains, subfamily A, member 6A
<i>MSRA</i>	methionine sulfoxide reductase A
<i>MTHFD1L</i>	methylenetetrahydrofolate dehydrogenase 1-like
<i>MTUS1</i>	microtubule associated tumor suppressor 1
<i>NCAM2</i>	neural cell adhesion molecule 2
<i>PICALM</i>	phosphatidylinositol binding clathrin assembly protein
<i>PPP3R1</i>	protein phosphatase 3, regulatory subunit B, alpha
<i>RELN</i>	reelin
<i>RBBP6</i>	retinoblastoma binding protein 6
<i>SORL1</i>	sortilin-related receptor
<i>TF</i>	transferrin
<i>TMEM65</i>	transmembrane protein 65
<i>TMPRSS2</i>	transmembrane protease, serine 2
<i>TOMM40</i>	translocase of outer mitochondrial membrane 40 homolog
<i>UNC5C</i>	unc-5 homolog C
<i>UPP2</i>	uridine phosphorylase 2

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APOE-ε₄ is by far the strongest known genetic risk factor for AD. By combining data from a large number of studies, we assessed the clinical utility of APOE-ε₄ for people with mild cognitive impairment.

CHAPTER 2

Predictive value of *APOE* ϵ_4 allele for
progression from MCI to AD-type dementia:
a meta-analysis

ABSTRACT

Background: The identification of subjects with mild cognitive impairment (MCI) at high risk for Alzheimer's disease (AD) is important for prognosis and early intervention. The *APOE* ϵ_4 allele is the strongest known genetic risk factor for AD. We performed a meta-analysis to establish the predictive accuracy of the *APOE* ϵ_4 allele for progression from MCI to AD-type dementia.

Methods: We included 35 prospective cohort studies of subjects with MCI, including 6095 subjects, of whom 1236 progressed to AD-type dementia after 2.9 years of follow-up. Pooled estimates of the odds ratio (OR), sensitivity, specificity, positive and negative predictive values (PPV and NPV), and positive and negative likelihood ratios (LR+ and LR–) were obtained using random-effects models.

Results: The OR for subjects with MCI who are carriers of the *APOE* ϵ_4 allele to progress to AD-type dementia was 2.29 (95% CI 1.88 to 2.80), the sensitivity was 0.53 (95% CI 0.46 to 0.61), the specificity was 0.67 (95% CI 0.62 to 0.71), the PPV was 0.57 (95% CI 0.48 to 0.66), the NPV was 0.75 (95% CI 0.70 to 0.80), the LR+ was 1.60 (95% CI 1.48 to 1.72), and the LR– was 0.75 (95% CI 0.67 to 0.82). Meta-regression showed that sensitivity, specificity, and NPV were dependent on age, *APOE* ϵ_4 allele background prevalence, or follow-up length.

Conclusions: The *APOE* ϵ_4 allele is associated with a moderately increased risk for progression from MCI to AD-type dementia. The low sensitivity and PPV makes genotyping of limited value for predicting AD-type dementia in clinical practice. For trials aiming to prevent progression from MCI to AD-type dementia, *APOE* genotyping may be of value as it helps to select subjects with a higher risk for progression to AD-type dementia.

Keywords: *APOE*, Alzheimer, mild cognitive impairment, meta-analysis, predictive value, sensitivity, specificity, meta-regression

INTRODUCTION

Subjects with mild cognitive impairment (MCI) have an increased risk for Alzheimer's disease (AD), but not all subjects will eventually become demented.¹ Identifying subjects with MCI at high risk for AD is important in order to give these subjects a prognosis. Drugs with the potential to modify the disease progression are suggested to be most effective in the prodementia stage of AD.

The apolipoprotein E (*APOE*) ϵ_4 allele is the strongest known genetic risk factor for AD. It is supposed to alter beta amyloid processing, a key pathological event in AD, or to modify the response to AD pathology.² Although *APOE* genotyping is not recommended for the diagnosis of AD,³ its role in predicting AD-type dementia in subjects with MCI is unclear. Previous studies yielded conflicting results, with odds ratios ranging from 0.58 to 16.7^{4,5} and with estimates for sensitivity, specificity, and positive predictive value often missing.

We used a meta-analysis approach to establish the predictive value of the *APOE* ϵ_4 allele in the progression from MCI to AD-type dementia. We also investigated study characteristics that may explain the variability of results between studies using meta-regression analysis. Study characteristics tested included age, background prevalence of the *APOE* ϵ_4 allele in the population from which subjects were selected, Mini-Mental State Examination (MMSE) score,⁶ length of follow-up, study setting, and type of MCI definition.

METHODS

Search strategy

We searched the PubMed database for publications from 1995 to 2008 on prospective cohort studies of subjects with MCI or related concepts in which the *APOE* ϵ_4 allele was tested as predictor of progression to AD-type dementia. The search terms used were "human" and "amnesic", "amnesic syndrome", "memory impairment", "mild cognitive decline", "mild cognitive impairment", "MCI", "age-associated cognitive decline", "AACD", "age-associated memory impairment", "AAMI", "cognitive impairment no dementia", "CIND", "memory clinic", "memory disorders clinic", or "dementia clinic". *APOE* ϵ_4 was not included in the search terms because this would exclude studies

that did not contain the term “*APOE* $\epsilon 4$ ” in their abstracts yet provided information on *APOE* genotyping in the main text. The search resulted in 17,233 hits. Two authors reviewed the titles and abstracts, and all relevant studies were retrieved. Additional studies were identified from the reference list of retrieved publications.

Study selection

Studies were included in the analyses if they had data on *APOE* genotype, defined MCI as impairment on a cognitive test or as decline in activities of daily function with an overall Clinical Dementia Rating scale score of 0.5 or based on an equivalent measure,⁷ and defined possible or probable AD at follow-up according to any of the following criteria: National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA),⁸ Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM IV),⁹ DSM 3rd edition,¹⁰ or the International Statistical Classification of Diseases and Related Health Problems 10th Revision.¹¹

Of the 17,233 abstracts reviewed, we retrieved 95 full articles in English that were relevant for our study. Of the 95 studies, 6 were excluded because the subjects were already demented at baseline, 3 did not distinguish MCI from AD, 8 had as outcome AD/dementia but not conversion from MCI to AD-type dementia, 6 had as outcome dementia instead of AD, 1 did not distinguish CIND from dementia, 7 were not cohort studies, 1 used weighted inclusion, 2 had unclear *APOE* genotyping data, 15 had no *APOE* data, and 11 referred to the same cohort. In the case wherein data from the same study had been reported in multiple papers, we used the paper in which the follow-up was longest, or if that was not different, in which the sample size was largest. Thirty-five studies with AD-type dementia as outcome were finally included in the meta-analysis (Figure 1).¹²

Data extraction

Three authors independently completed a data extraction form for each study. Studies were classified according to setting in (1) clinical studies, wherein only patients presenting with cognitive impairments

at a health care facility were included, (2) population-based studies, wherein a random sample of the general population was included, or (3) other studies, wherein subjects were recruited from both clinical and population settings, or through advertisements. MCI definitions were recorded as measured by (1) objective tests and (2) assessment of functional impairment. Definitions based on objective test performance were subdivided in studies that used amnesic MCI (aMCI) as inclusion criterion or studies that included both subjects with aMCI and non-amnesic MCI.

Data on the *APOE* ϵ_4 allele and outcome were summarized in a 2 x 3 table, which specified the number of subjects who were *APOE* ϵ_4 allele carriers and noncarriers who remained stable, progressed to AD-type dementia, or progressed to other types of dementia. We did not subdivide outcome according to number of *APOE* ϵ_4 alleles as only few studies reported data on all *APOE* genotypes.

Authors were contacted in case of incomplete data. For one study that included data from a drug trial, we included only data from placebo-treated subjects.¹³ For two trials that did not report separate data for placebo-controlled subjects, we included all subjects.^{14, 15} In both trials, the drug was not associated with progression to AD-type dementia.

Assessment of methodological quality

Two authors rated each study using 17 items for subject sampling, reference diagnosis, and test description selected from rating scales for methodological quality of diagnostic and prognostic studies (Supplementary tables 1, 2).^{16, 17} In case of discrepancies between scores, a third rater reviewed the paper and a consensus was reached.

Statistical analysis

Random-effects models were used for the meta-analysis. Egger's regression test was conducted to test for funnel plot asymmetry. An asymmetrical funnel plot and a significant *P*-value could indicate publication bias. The outcome measures were the pooled odds ratio (OR), sensitivity, specificity, positive and negative predictive values (PPV and NPV) and positive and negative likelihood ratios (LR+ and

LR-). The heterogeneity was assessed by a χ^2 test and is designated as Q . The amount of heterogeneity for each outcome measure was calculated using DerSimonian-Laird, with τ as an estimate for the standard deviation of the underlying true outcomes across studies. The proportion of variation due to heterogeneity was likewise computed and expressed as I^2 .

Mixed-effects meta-regression analyses using restricted maximum likelihood (REML) estimation were conducted to examine the influence of average age and MMSE score of the sample, the setting, definition of MCI, length of follow-up, and background *APOE* ϵ_4 allele frequency in the population in which the study was performed. Information on the background *APOE* ϵ_4 allele prevalence in the population from which subjects were recruited was obtained from published data on the prevalence of the *APOE* ϵ_4 allele in the general population.^{18,19} The Hardy-Weinberg equilibrium was tested using χ^2 test in studies that reported the *APOE* alleles and frequencies. The *APOE* alleles are assumed to be in Hardy-Weinberg equilibrium if the P -value obtained is greater than 0.05. Average age and MMSE score, follow-up length, and background *APOE* ϵ_4 allele prevalence were included as continuous variables.

Analyses were performed using Stata/SE 9.2 for Mac OS X (Stata Corporation, Texas, USA). R was used to create the figures.²⁰ In the main analyses, the outcome was dichotomized as nondemented versus AD-type dementia at follow-up because not all studies provided data on non-AD dementia at follow-up. In secondary analyses, which included all studies that provided outcome data on non-AD dementia, the outcome was dichotomized as no AD (combining subjects without dementia and subjects with non-AD type dementia) and AD-type dementia. One study presented data on subjects without dementia and subjects with non-AD type dementia as one group.¹³ We included these subjects in the non-dementia group in the main analysis. Analyses were first performed for all studies combined, and then separately for clinical, population-based, and other studies.

FINDINGS

Study characteristics

We included 21 clinical, 6 population, and 8 other studies (Table 1). The studies represented 7322 cases with MCI, of whom 56% ($n=4092$) were females. The majority of the subjects were from population studies (53%, $n=3914$); 23% ($n=1668$) were from clinical studies, and 24% ($n=1740$), from other studies. The pooled average baseline characteristics were as follows: age 72.1 (range 61–87) years, follow-up period 2.9 (1–8) years, MMSE score 27 (24–29), and education 11.6 (5–16) years.

Subjects with MCI from the population studies were older and were followed up for a longer period compared with those in the clinical and other studies (age 77.6 versus 70.1 and 73.4; follow-up length 4.7 versus 2.4 and 3.1) but these differences did not reach statistical significance ($P>0.05$).

APOE genotyping was available for 6095 subjects (Table 2). Of these, 4170 were *APOE* ϵ_4 allele noncarriers and 1925 (32%) were carriers of at least one *APOE* ϵ_4 allele. Among *APOE* ϵ_4 allele carriers, 658 (34%) progressed to AD-type dementia, 32 (1.7%) developed non-AD type dementias, and the rest were not demented. Of the noncarriers, 578 (14%) developed AD-type dementia and 56 (1.3%) developed non-AD type dementias.

Data on zygosity were available for nine studies, representing 794 subjects with MCI of whom 256 (32%) progressed to AD-type dementia. The *APOE* allele distribution was in Hardy-Weinberg equilibrium in the six studies ($P>0.05$; $N=762$ cases of MCI) that provided the data to allow the calculation of this equilibrium.

Meta-analysis

Visual inspection of the funnel plot for the OR did not reveal any marked asymmetry and Egger's regression test was not significant ($P=0.94$; Supplementary figure 1). This indicates that the obtained results are not likely to be owing to publication bias. A significant overall outcome was obtained for all measures of predictive accuracy (Supplementary table 3). In the presence of at least 1 *APOE* ϵ_4 allele, the pooled OR for AD-type dementia in all studies was 2.29 (95% CI 1.88 to 2.80; Figure

2), the sensitivity 0.53 (95% CI 0.46 to 0.61), the specificity 0.67 (95% CI 0.62 to 0.71), the PPV 0.43 (95% CI 0.48 to 0.66), the NPV 0.75 (95% CI 0.70 to 0.80), the LR+ 1.60 (95% CI 1.48 to 1.72) and the LR– 0.74 (95% CI 0.67 to 0.82; Supplementary figures 2a to 2f, Table 3). The OR (3.94, 95% CI 2.09 to 7.33), specificity (0.93, 95% CI 0.90 to 0.97) and LR+ (3.01, 95% CI 2.11 to 4.30) were higher, and the sensitivity was lower (0.21, 95% CI 0.10 to 0.33) among *APOE*- ϵ_4 homozygotes (Table 3). Overall effect and heterogeneity across studies (Q , I^2 and τ values) are shown in Supplementary table 3.

There were some differences in measures of predictive accuracy between settings, but these differences were not statistically significant ($P>0.10$). Likewise, the type of MCI definition was not associated with predictive accuracy ($P>0.20$).

Assessment of methodological quality

Use of blinding procedures was the only item that could not be rated in more than 70% of the studies. Based on items that could be scored, 76% of the clinical studies and 50% of population-based and other studies had a positive score on at least 80% of the items.

Meta-regression results showed that studies with a score below 80% of the items did not differ in any outcome measure from studies that scored positive on at least 80% of the items. The results were similar when items that could not be scored were rated as a negative score.

Effect of age, background *APOE* ϵ_4 allele prevalence, MMSE score, and length of follow-up on the predictive accuracy

Increasing average study age was associated with decreased sensitivity ($P=0.001$), increased specificity ($P=0.017$), and decreased NPV ($P=0.003$) (Figure 3a). Increasing background *APOE* ϵ_4 allele prevalence in the population from which subjects were selected significantly increased sensitivity ($P=0.014$) and decreased specificity ($P=0.004$, Figure 3b). An increase in average length of follow-up was associated with decreased sensitivity ($P=0.025$) and NPV ($P=0.031$, Figure 3c). The average MMSE score was not associated with any outcome measure. The strength of the associations was similar when analyses were performed in

clinical, population-based, and other studies separately, although some associations were no longer statistically significant (data not shown).

Non-AD type dementia

Twenty-six studies presented *APOE* ϵ_4 allele data on subjects who progressed to non-AD type dementia. Analyses with outcome AD ($n=771$) versus no AD ($n=4049$, including 3961 subjects without dementia and 88 subjects with non-AD type dementia at follow-up), yielded similar measures of predictive accuracy compared to analyses with outcome AD versus no dementia (Supplementary table 4).

DISCUSSION

Meta-analysis

Our results showed that the presence of at least one *APOE* ϵ_4 allele is associated with a moderately high increased risk of progression from MCI to AD-type dementia. The estimates for sensitivity, specificity, PPV, NPV, LR+, and LR- were low to moderately high. Meta-regression showed that part of the variability between studies could be partly explained by age, background *APOE* ϵ_4 allele prevalence, and length of follow-up.

APOE- ϵ_4 homozygotes had a four-fold risk of progression to AD-type dementia. The specificity and LR+ were higher and the LR- lower compared to *APOE* ϵ_4 heterozygotes. However, the sensitivity was lower which could result in a high number of false negatives.

The OR we obtained in the presence of at least 1 *APOE* ϵ_4 allele and in the presence of two *APOE* ϵ_4 alleles were lower than the OR reported in a meta-analysis of cross-sectional studies that compared the prevalence of the *APOE* ϵ_4 allele in subjects with AD-type dementia relative to healthy controls.^{52, 53} A possible explanation for the lower OR in our meta-analysis is that not all subjects with MCI and an *APOE* ϵ_4 allele who are destined to progress to AD-type dementia would develop AD during an average follow-up of 3 years. This could have resulted in a higher number of false positives compared to studies that included healthy controls and subjects with AD-type dementia.

It is also worth noting that although we only included subjects from whom *APOE* data had been collected, a substantial number of

subjects within these studies did not have *APOE* data available. This may have caused a bias, but this could not be ascertained.

Meta-regression

We noted a number of associations between study characteristics and predictive accuracy. With increasing age, the *APOE* ϵ_4 allele became less sensitive and more specific for the prediction of AD. This suggests that with increasing age, the number of *APOE* ϵ_4 allele carriers decreases, which was indeed observed in our data. This could mean that *APOE* ϵ_4 allele carriers may either progress to AD-type dementia at a younger age, or die earlier.⁵⁴ The decrease in sensitivity and negative predictive value with age suggests that with increasing age, subjects with MCI who progress to AD-type dementia are more likely to be noncarriers of the *APOE* ϵ_4 allele, which is consistent with previous studies.⁵³

As would be expected, the NPV decreased with follow-up length. The decrease in sensitivity with follow-up length suggests that subjects without the *APOE* ϵ_4 allele progress slower to AD-type dementia than subjects with the *APOE* ϵ_4 allele.

A higher background prevalence of the *APOE* ϵ_4 allele increased sensitivity and decreased specificity, which would be expected. Remarkably, neither the PPV nor the OR depended on the background prevalence, which suggests that the risk of progression to AD-type dementia is similar in populations with different *APOE* ϵ_4 allele background prevalence.

A number of study characteristics were not associated with predictive accuracy. Setting was not associated with any outcome measure, although previous studies have shown that progression rates from MCI to AD-type dementia was higher in clinical settings than in the general population.⁵⁵ Still, we observed relatively large differences in sensitivity and specificity between population-based studies and clinical studies, which may not have reached statistical significance as only six population-based studies were included in the meta-analysis.

Although MMSE score is associated with risk for AD, the average study MMSE score did not modify the relation between the *APOE* ϵ_4 allele and AD-type dementia. It is possible that the spread of MMSE scores in the studies was too small to detect an effect.

The relation between *APOE* genotype and risk for AD-type dementia was also not dependent on the definition of MCI used. This could be attributed to overlap in MCI definitions. Definitions based on objective tests could include subjects with mild functional impairment and vice versa.^{56, 57}

Methodological quality of studies

The scores of methodological quality were moderate. We may have underestimated these scores as they were only based on information provided in the paper. Information on whether clinicians were blinded to the *APOE* genotype when the follow-up diagnosis was made was often missing. This incomplete reporting was probably because the *APOE* genotype was not the main predictor in most studies. Still, we cannot exclude the possibility that the diagnostic accuracy may have been overestimated because clinicians who made the follow-up diagnosis had access to the *APOE* genotype. However, it should be noted that criteria for the diagnosis of AD refer to clinical information only, not to any biomarker. Our meta-regression analyses showed that methodological quality had no significant effect on measures of predictive accuracy.

Limitations

Our study has several limitations. First is the significant heterogeneity between studies. We modeled the heterogeneity by means of a random-effects model. With meta-regression, we found possible explanations for this heterogeneity but other factors that contribute to the heterogeneity remain to be identified. Second, the definition of MCI varied between studies but this effect may have been limited as discussed above. Third, meta-regression was performed with average study scores, which may lead to an under- or overestimation of the relation between the variables tested in the meta-regression and measures of predictive accuracy.

Implications

The *APOE* ϵ_4 allele is a moderately strong predictor of progression from MCI to AD-type dementia. The risk is twice as high for *APOE*- ϵ_4 heterozygotes and four times as high for *APOE*- ϵ_4 homozygotes

compared with noncarriers. The effect of the *APOE* ϵ_4 allele is consistent. In spite of this, the low sensitivity and PPV makes genotyping of limited value for predicting AD-type dementia in clinical practice since half of the subjects who progressed to AD-type dementia had no *APOE* ϵ_4 allele while 40% of those with an *APOE* ϵ_4 allele did not progress to AD-type dementia over a 3-year period. The background prevalence of the *APOE* ϵ_4 allele in the population should be taken into account when interpreting the sensitivity and specificity of the *APOE* ϵ_4 allele for the prediction of AD-type dementia. *APOE* genotyping may be of value in selecting subjects for trials that aim to prevent progression to dementia, as it helps select subjects with a higher risk for progression to AD-type dementia. For example, with an annual progression rate of 15% in subjects with MCI and 19% of subjects with MCI and at least one *APOE* ϵ_4 allele (conversion rate based on the meta-analysis of clinical studies), the number of subjects needed in a 3-year placebo-controlled trial with 50% on active treatment designed to detect a relative decrease in conversion rate to AD-type dementia of 20% (assuming a power of 90%, a 2-sided alpha of 5%, and a drop-out rate of 30%) would be 1720 if subjects with MCI were included, and 1113 if subjects with MCI and at least one *APOE* ϵ_4 allele were included. However, in order to select 1113 MCI *APOE* ϵ_4 allele carriers, 2572 subjects with MCI would be needed for screening, which could be a limitation.

FUTURE PERSPECTIVES

The *APOE* ϵ_4 allele is thus far the strongest known genetic risk factor for AD.⁵⁸ Interaction with other genes may increase the predictive accuracy. Notably the translocase of outer mitochondrial membrane 40 homolog (*TOMM40*) could interact with the effect of the *APOE* ϵ_4 allele on the development of AD.^{58, 59} *TOMM40* and the apoE protein are both located on chromosome 19, and are both associated with an increased risk for AD.⁵⁹ How strong this interaction will be requires further investigation. In addition, the *APOE* genotype may interact with the predictive accuracy of other markers for AD, typically markers related to beta amyloid metabolism, such as Pittsburgh Compound-B (PIB) and beta amyloid in cerebrospinal fluid (CSF).⁶⁰ Apart from selecting subjects at an increased risk for AD, *APOE* genotyping might be useful to select or

stratify subjects for trials with drugs for which the mode of action of profile of side effects may be dependent on the *APOE* genotype.⁶¹

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Table 1 Characteristics of studies included in the meta-analysis

Study		MCI defini- tion	Exclusion criteria	Sample size, base- line (% FU)	
Clinical studies					
1995	Coria [5]	aMCI	DCCD	56 (100)	
1996	Tierney [21]	MFI	DCCD	178 (80)	
2002	Visser [22]	aMCI	DCCD, age <40 y	74 (85)	
2004	Amieva [14]	MCI	DCCD, age 50-85 y	104 (87)	
	Korf [23]	MCI	DCCD, age <40 y, FU<6 mo	75 (100) ^b	
	Maruyama [24]	aMCI	Depression	57 (100)	
	Mosconi [25]	aMCI	DCCD	37 (100)	
2005	Drzezga [26]	aMCI	DCCD	30 (100)	
2006	Hansson [27]	aMCI	DCCD	137 (98)	
	Wang [28]	aMCI	DCCD	58 (100)	
2007	Barabash [29]	aMCI	DCCD, MMSE <25	89 (100)	
	Bouwman [30]	MCI	DCCD	64 (92)	
	Devanand [31]	MCI/MFI	DCCD	139 (100)	
	Ewers [32]	MCI	DCCD	88 (100) ^b	
	Gabryelewicz [33]	aMCI	DCCD	105 (100) ^b	
	Liu [34]	MFI	DCCD, <65 y	124 (90)	
	Rozzini [35]	aMCI	-	119 (100)	
	Caroli [36]	aMCI	DCCD, MMSE <24, age > 90 y	28 (86)	
2008	Gavrilova [37]	MCI	DCCD, <50 y	40 (90)	
	Forsberg [38]	aMCI	-	21(100)	
	Nobili [39]	aMCI	DCCD	36 (92)	
Population-based studies					
1999	Touchon [40]	MFI	age <60 y	397 (50)	
2004	Hsiung [41]	MCI/MFI	age < 65 y	296 (88)	
2005	Aggarwal [42]	MCI	DCCD, age < 65 y	242 (75)	
2006	Erten-Lyons [4]	MFI	DCCD, age < 65 y	37 (100) ^b	
2008	Tapiola [43]	MCI	-	60 (100)	
	Artero [44]	MCI	age < 65 y	2882 (100) ^b	
Other studies					
1999	Jack [45]	aMCI	DCCD, age <60 y and >90 y	80 (96)	
2001	Albert [46]	MFI	DCCD, age <65 y	123 (99)	

	Mean age, y	Mean education, y	MMSE score	Follow-up, y	Converters to dementia (% AD)
	71.2	-	-	3	30 (100)
	72.3	13.9	27.6	2	29 (97) ^a
	61.1	10.5	27.7	3.7	19 (100)
	70.2	-	27.7	2	29 (100)
	62.9	10.2	26.9	2.8	37 (92)
	73.9	11.6	25.6	2	17 (100)
	67.1	9.6	28.0	1	8 (100)
	70.0	11.6	26.9	1.3	12 (100)
	71.0	-	27.0	5.2	78 (73)
	76.3	11.8	25.9	3	19 (100)
	75.0	-	-	4.1	62 (100) ^a
	69.6	-	25.9	1.6	33 (91)
	66.8	15.2	27.5	4.2	37 (100)
	70.9	-	27.0	1.8	43 (100)
	69.3	-	27.2	3.1	23 (87)
	74.7	10.3	25.2	1.7	42 (100)
	70.6	7.8	26.9	1	40 (100)
	70.2	9.7	26.9	1.6	10 (90) ^a
	69.3	-	27.5	2	5 (100)
	63.6	12.7	28.2	0.7	7 (100)
	75.5	8.7	27.5	1.1	12 (92)
	74.7	-	26.9	3	48 (46)
	77.8	9.2	-	5	147 (48) ^a
	78.7	17.8	27.4	5.7	82 (96) ^a
	86.9	13.8	27.3	7.6	23 (96)
	72.7	6.7	23.9	2.8	13 (77)
	74.6	-	27.2C	4	145 (77)
	77.7	13.1	26.4	2.7	27 (100)
	72.2	14.9	29.1	3	26 (88) ^a

Table 1 Characteristics of studies included in the meta-analysis *Continued*

Study		MCI defini- tion	Exclusion criteria	Sample size, base- line (% FU)	
2005	Stoub [47]	aMCI	DCCD, age <65 y	23 (100) ^b	
2006	Griffith [48]	aMCI	DCCD	49 (100) ^b	
	Lee [49]	MFI	DCCD, age <60 y and >90 y	106 (68)	
2007	Feldman [13]	aMCI	DCCD, age <55 y and >85 y	510 (68)	
	Fleisher [15]	aMCI	DCCD, MMSE<24, age <55 y and >90 y	769 (70)	
	Herukka [50]	MCI	DCCD	79(100)	

Abbreviations: MCI=mild cognitive impairment, y=years; FU=follow-up length; MMSE=Mini-Mental State Examination; AD=Alzheimer-type dementia; aMCI=amnesic mild cognitive impairment; MFI=mild functional impairment that interferes with daily living; DCCD=disorders causing cognitive decline; - = not specified/no data

^a Study did not provide *APOE* data on subjects with non-AD type dementia.

^b Only completed cases reported.

^c Obtained from The 3C Study Group (2003).[51]

Table 2 Number of subjects with at least 1 *APOE* ϵ_4 allele according to outcome and setting

	Baseline sample	Outcome at follow-up		
		Not demented	AD-type dementia	Non-AD type dementia
All studies				
ε4+	1925	1235	658	32
ε4−	4170	3536	578	56
Clinical studies				
ε4+	618	314	293	11
ε4−	862	615	228	19
Population-based studies				
ε4+	731	601	110	20
ε4−	2702	2436	229	37
Other studies				
ε4+	576	320	255	1
ε4−	606	485	121	0

Abbreviations: ϵ_4+ = *APOE* ϵ_4 allele carriers; ϵ_4- =*APOE* ϵ_4 allele noncarriers

	Mean age, y	Mean education, y	MMSE score	Follow-up, y	Converters to dementia (% AD)
	82.1	16	27	3.6	11 (100)
	68.5	13.4	28.4	1.3	13 (100)
	73.1	5.2	-	3	17 (82) ^a
	70.6	11.1	26.9	4	109 (100)
	72.8	14.8	-	3	212 (99) ^a
	70.4	-	24.0	4.1	33 (97)

Table 3 Measures of predictive accuracy

Cat-egory	OR (95% CI)	Sensi-tivity (95% CI)	Speci-ficity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)
At least 1 ϵ_4 allele versus no ϵ_4 allele							
All studies (N=35)	2.29 (1.88-2.80)	0.53 (0.46-0.61)	0.67 (0.62-0.71)	0.43 (0.34-0.52)	0.75 (0.70-0.80)	1.60 (1.48-1.78)	0.74 (0.67-0.82)
Clinical studies (n=21)	2.36 (1.81-3.07)	0.58 (0.49-0.66)	0.65 (0.60-0.70)	0.46 (0.38-0.54)	0.74 (0.69-0.80)	1.56 (1.39-1.76)	0.73 (0.63-0.83)
Popula-tion-based studies (n=6)	1.83 (1.39-2.40)	0.31 (0.23-0.39)	0.78 (0.72-0.84)	0.34 (0.12-0.55)	0.73 (0.56-0.89)	1.54 (1.28-1.86)	0.86 (0.80-0.94)
Other studies (n=8)	2.64 (1.58-4.42)	0.60 (0.49-0.71)	0.64 (0.57-0.70)	0.42 (0.29-0.54)	0.81 (0.75-0.86)	1.61 (1.29-2.02)	0.63 (0.47-0.85)
APOE-ϵ_4 homozygotes versus no ϵ_4 allele							
All studies (N=9)	3.94 (2.09-7.33)	0.21 (0.10-0.33)	0.93 (0.90-0.97)	0.59 (0.50-0.68)	0.69 (0.60-0.79)	3.01 (2.11-4.30)	0.89 (0.79-0.99)

Abbreviations: OR=odds ratio; PPV=positive predictive value; NPV=negative predictive value; LR+=positive likelihood ratio; LR-=negative likelihood ratio

Figure 1 Flow diagram of study selection^a

^a Flow diagram adapted from Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). [12]

Figure 2 Forest plot odds ratio

AD—no dementia; AD+=with Alzheimer-type dementia; ϵ_4 —=*APOE*- ϵ_4 allele noncarriers; ϵ_4 +=*APOE*- ϵ_4 allele carriers; OR=odds ratio; CI=confidence interval; RE=random effects. The squares represent the weight of the particular study in the overall analysis. The horizontal line across the square shows the confidence interval. The diamond represents the overall effect.

Figure 3 Meta-regression results

Shown are variables that have a statistically significant association with outcome in the meta-regression analysis. The size of the data markers indicates weight of the study.

Supplementary Table 1 Methodological quality rating*

Item	Criterion
1. Sampling of subjects	
1.1 Random or consecutive selection	Indication that consecutive subjects or randomly selected subjects were included
1.2 Recruited as single cohort unclassified by disease state	Indication that all patient were included as a single cohort at the same time in the diagnostic phase

Supplementary Table 1 Methodological quality rating* *Continued*

Item	Criterion
1.3 Recruited from a clinical setting where test would be used	Study performed in a first-line, second-line or third-line health care facility. Only scored for clinical studies
1.4 Recruited at the time point in the diagnostic process where the test would be used	Subjects enrolled at time baseline visit. Only scored for clinical studies
1.5 Selection and referral processes fully described	Description of sampling method and the inclusion and exclusion criteria; and (for clinical studies only), full description of referral pattern
1.6 Clinical and demographic characteristics fully described	Data on age, gender, educational level, and score for the MMSE or equivalent cognitive screening test
1.7 Complete	<i>APOE</i> genotype available for all eligible subjects; if available for subgroup only description of differences in baseline characteristics between subjects with and without data on the <i>APOE</i> genotype.
1.8 Large sample size	Baseline sample of at least 50 subjects
2. Reference diagnosis	
2.1 Methods and tests described in detail	Description of person who made the diagnosis and of data used for diagnosis
2.2 Positive and negative diagnoses clearly described	Description of dementia criteria used
2.3 Diagnosis likely to be close to truth	This item was scored present for all studies
2.4 Available for all subjects	Follow-up rate of at least 85%
2.5 Based on the same tests and same information in all subjects	Description that diagnostic procedure at follow-up did not differ for subjects
2.6 Blinding procedures used to prevent knowledge of <i>APOE</i> genotype when follow-up diagnosis was made (i.e. verification bias)	Indication that the person who made the diagnosis at follow-up was blinded for the <i>APOE</i> genotype
3. Experimental test	
3.1 Description of test described in detail	Description of method of <i>APOE</i> genotyping such that it could be reproduced
3.2 Positive and negative test results described	This item was scored present for all studies
3.3 All results described	Although none of the studies reported data on the failure rate of the <i>APOE</i> test, this item was scored present for all studies

Abbreviations: MMSE= Mini-Mental State examination; *APOE*= Apolipoprotein E

*Items were selected from rating scales of methodological quality of diagnostic and prognostic studies as described in Altman DG, Systematic reviews of evaluations of prognostic variables, *BMJ* 2001;**323**:224–8; and Deeks JJ, Systematic reviews in health care: Systematic reviews of evaluations of diagnostic and screening tests. *BMJ* 2001;**323**:157–62.

Supplementary Table 2 Meta-analysis of observational studies in epidemiology (MOOSE) statement

Checklist item		Brief description of how the criteria were handled in the review
Reporting of background		
1	Problem definition	To establish the predictive accuracy of the <i>APOE-ε4</i> allele for conversion from mild cognitive impairment (MCI) to Alzheimer’s disease (AD)
2	Hypothesis statement	The presence of the <i>APOE-ε4</i> allele increases the risk for AD
3	Description of study outcomes	The outcome is conversion from MCI to AD. The measures of predictive accuracy are the pooled odds ratio, sensitivity, specificity, positive and negative predictive value, and positive and negative likelihood ratio.
4	Type of exposure	Carrier or non-carrier of <i>APOE-ε4</i> allele
5	Type of study designs used	Prospective cohort studies of subjects with MCI or related concepts in which the <i>APOE-ε4</i> allele was tested as predictor of conversion to AD
6	Study population	Subjects with MCI
Reporting of search strategy should include		
7	Qualifications of searchers	LS Elias-Sonnenschein, MA, MSc; PJ Visser, MD, PhD
8	Search strategy, including time period included in the synthesis and keywords	Time period: January 1995 to December 2008 Keywords: “human” and “amnestic”, “amnestic syndrome”, “memory impairment”, “mild cognitive decline”, “mild cognitive impairment”, “MCI”, “age-associated cognitive decline”, “AACD”, “age-associated memory impairment”, “AAMI”, “cognitive impairment no dementia”, “CIND”, “memory clinic”, “memory disorders clinic”, or “dementia clinic
9	Effort to include all available studies, including contact with authors	Efforts were made to include all available studies. Authors of studies with unclear outcome measures were contacted.
10	Databases and registries searched	PubMed
	(Supplementary Table 2 continued)	
11	Search software used, name and version, including special features	ExLibris MetaLib was used to search PubMed.
12	Use of hand searching	Additional studies were identified from the reference list of retrieved publications.

Supplementary Table 2 Continued

Checklist item		Brief description of how the criteria were handled in the review
13	List of citations located and those excluded, including justifications	Of the 17,233 abstracts reviewed, we retrieved 95 full articles in English that were relevant for our study. Of the 95 studies, 6 were excluded because the subjects were already demented at baseline, 3 did not distinguish MCI from AD, 11 referred to the same cohort, 8 had as outcome AD/dementia but not conversion from MCI to AD, 6 had as outcome dementia instead of AD, 1 did not distinguish CIND from dementia, 7 were not cohort studies, 1 used weighted inclusion, 2 had unclear <i>APOE</i> genotyping data, and 15 had no <i>APOE</i> data (Figure 1).
14	Method of addressing articles published in languages other than English	Only publications in English were included.
15	Method of handling abstracts and unpublished studies	No searching of the grey literature was performed.
16	Description of any contact with authors	Authors were contacted in case of incomplete data.
Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Table of included studies (Table 1)
18	Rationale for the selection and coding of data	To assess the effect of a priori variables on the measures of predictive accuracy.
19	Documentation of how data were classified and coded	Three authors independently completed a data extraction form for each study. Data on the <i>APOE</i> - ϵ_4 allele and outcome were summarized in a 2 x 3 table, which specified the number of subjects who were <i>APOE</i> ϵ_4 allele carriers and noncarriers who remained stable, progressed to AD, or progressed to other types of dementia.
20	Assessment of confounding	Meta-regression was conducted to examine the influence of average age and MMSE score of the sample, the setting, definition of MCI, length of follow-up, and background <i>APOE</i> ϵ_4 allele frequency in the population in which the study was performed.

Supplementary Table 2 *Continued*

Checklist item		Brief description of how the criteria were handled in the review
21	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Two authors rated each study using 17 items for subject sampling, reference diagnosis, and test description selected from rating scales for methodological quality of diagnostic and prognostic studies (Supplementary table 1). In case of discrepancies between scores, a third rater reviewed the paper and a consensus was reached.
22	Assessment of heterogeneity	Heterogeneity was assessed by a chi-square test, Q. The amount of heterogeneity for each outcome measure was calculated using DerSimonian-Laird, with t as an estimate for the standard deviation of the underlying true outcomes across studies. The proportion of variation due to heterogeneity, was likewise computed and expressed as I ² .
23	Description of statistical methods in sufficient detail to be replicated	We used random-effects models for the meta-analysis and performed mixed-effects meta-regression analyses using restricted maximum likelihood estimation. Analyses were performed using Stata/SE 9.2 for Mac OS X.
24	Provision of appropriate tables and graphics	Appropriate tables and figures are presented. R was used to create the figures.
Reporting of results should include		
25	Graph summarizing individual study estimates and overall estimate	A forest plot for the odds ratio is presented as figure 2. The forest plots for sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios are presented in the supplementary data (Supplementary figures 2a to 2f).
26	Table giving descriptive information for each study included	The study characteristics are shown in Table 1.
27	Results of sensitivity testing	Use of blinding procedures was the only item that could not be rated in more than 70% of the studies. Based on items that could be scored, 76% of the clinical studies and 50% of population-based and other studies had a positive score on at least 80% of the items.
28	Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary estimates (table 3), t values (table 3), results of methodological quality assessment, and result of funnel plot asymmetry test.

Supplementary Table 2 *Continued*

Checklist item		Brief description of how the criteria were handled in the review
Reporting of discussion should include		
29	Quantitative assessment of bias	We addressed the heterogeneity between studies by using a random-effects model.
30	Justification for exclusion	All studies were excluded based on the pre-defined inclusion criteria.
31	Assessment of quality of included studies	The scores of methodological quality were relatively low. We may have underestimated these scores as they were only based on information provided in the paper. Our meta-regression analyses showed that methodological quality had no significant effect on measures of predictive accuracy.
32	Consideration of alternative explanations for observed results	The meta-regression was performed with average study scores, which may lead to an under- or overestimation of the relation between the variables tested in the meta-regression and measures of predictive accuracy. Alternative explanations are further discussed in the manuscript.
33	Generalization of the conclusions	Discussed in the context of the results of the cohort study.
34	Guidelines for future research	Interaction with other genes and biomarkers may increase the predictive accuracy of the APOE- ϵ_4 allele for the conversion from MCI to AD. This is further discussed in the manuscript.
35	Disclosure of funding source	We have nothing to disclose.

Adapted from: Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thaker SB.

Meta-analysis of observational studies in epidemiology: a proposal for reporting. *JAMA* 2000;283:2008–2012.

Supplementary Table 3 Measures of overall effect and heterogeneity

Category	OR	Sensitivity	Specificity	
At least 1 $\epsilon 4$ allele versus no $\epsilon 4$ allele				
All studies (N=35)				
Effect (95% CI)	2.29 (1.88 - 2.80)	0.53 (0.46 - 0.61)	0.67 (0.62 - 0.71)	
Test for overall effect	$z=8.11$ $P<0.001$	$z=14.08$ $P<0.001$	$z=29.72$ $P<0.001$	
Test for heterogeneity	$Q=49.31$ $P=0.043$ $t=0.31$ $I^2=31.04\%$	$Q=252.29$ $P<0.001$ $t=0.20$ $I^2=86.52\%$	$Q=237.36$ $P<0.001$ $t=0.11$ $I^2=85.68\%$	
Clinical studies (n=21)				
Effect (95% CI)	2.36 (1.81 - 3.07)	0.58 (0.49 - 0.66)	0.65 (0.60 - 0.70)	
Test for overall effect	$z=6.32$ $P<0.001$	$z=13.70$ $P<0.001$	$z=24.88$ $P<0.001$	
Test for heterogeneity	$Q=23.56$ $P=0.262$ $t=0.24$ $I^2=15.10\%$	$Q=79.09$ $P<0.001$ $t=0.16$ $I^2=74.71\%$	$Q=54.10$ $P<0.001$ $t=0.09$ $I^2=63.03\%$	
Population-based studies (n=6)				
Effect (95% CI)	1.83 (1.39 - 2.40)	0.31 (0.23 - 0.39)	0.78 (0.72 - 0.84)	
Test for overall effect	$z=4.33$ $P<0.001$	$z=7.22$ $P<0.001$	$z=26.94$ $P<0.001$	
Test for heterogeneity	$Q=1.78$ $P=0.879$ $t=0$ $I^2=0\%$	$Q=11.85$ $P=0.04$ $t=0.08$ $I^2=57.80\%$	$Q=16.46$ $P=0.006$ $t=0.05$ $I^2=69.63\%$	
Other studies (n=8)				
Effect (95% CI)	2.64 (1.58 - 4.42)	0.60 (0.49 - 0.71)	0.64 (0.57 - 0.70)	
Test for overall effect	$z=3.70$ $P<0.001$	$z=10.37$ $P<0.001$	$z=18.28$ $P<0.001$	
Test for heterogeneity	$Q=17.76$ $P=0.013$ $t=0.54$ $I^2=60.59\%$	$Q=26.18$ $P<0.001$ $t=0.13$ $I^2=73.26\%$	$Q=22.69$ $P=0.002$ $t=0.08$ $I^2=69.15\%$	

	PPV	NPV	LR+	LR-
	0.43 (0.34 - 0.52)	0.75 (0.70 - 0.80)	1.60 (1.48 - 1.78)	0.74 (0.67 - 0.82)
	Z=9.30 P<0.001	Z=27.49 P<0.001	Z=12.23 P<0.001	Z=-5.85 P<0.001
	Q=625.81 P<0.001 t=0.26 I ² =94.57%	Q=461.22 P<0.001 t=0.14 I ² =92.63%	Q=32.73 P=0.530 t=0 I ² = 0%	Q=70.90 P<0.001 t=0.19 I ² =52.04%
	0.46 (0.38 - 0.54)	0.74 (0.69 - 0.80)	1.56 (1.39 - 1.76)	0.73 (0.63 - 0.83)
	Z=11.43 P<0.001	Z=27.31 P<0.001	Z=7.39 P<0.001	Z=-4.67 P<0.001
	Q=86.64 P<0.001 t=0.16 I ² =76.37%	Q=65.50 P<0.001 t=0.10 I ² =69.47%	Q=16.20 P=0.704 t=0 I ² = 0%	Q=29.454 P=0.079 t=0.17 I ² =32.10%
	0.34 (0.12 - 0.55)	0.73 (0.56 - 0.89)	1.54 (1.28 - 1.86)	0.86 (0.80 - 0.94)
	Z=3.05 P=0.002	Z=8.76 P<0.001	Z=4.56 P<0.001	Z=-3.58 P<0.001
	Q=83.38 P<0.001 t=0.25 I ² =94.00%	Q=164.82 P<0.001 t=0.20 I ² =96.97%	Q=1.846 P=0.870 t=0 I ² = 0%	Q=2.87 P=0.721 t=0 I ² = 0%
	0.42 (0.29 - 0.54)	0.81 (0.75 - 0.86)	1.61 (1.29 - 2.02)	0.63 (0.47 - 0.85)
	Z=6.62 P<0.001	Z=31.00 P<0.001	Z=4.24 P<0.001	Z=-3.01 P=0.003
	Q=49.56 P<0.001 t=0.15 I ² =85.88%	Q=14.98 P=0.036 t=0.04 I ² =53.28%	Q=15.06 P=0.050 t=0.21 I ² =50.22%	Q=22.35 P=0.002 t=0.34 I ² =68.68%

Supplementary Table 3 Measures of overall effect and heterogeneity *Continued*

Category	OR	Sensitivity	Specificity	
APOE-ε4 homozygotes versus no ε4 allele				
All studies (N=9)				
Effect (95% CI)	3.94 (2.09 - 7.33)	0.21 (0.10 - 0.33)	0.93 (0.90 - 0.97)	
Test for overall effect	z=4.28 P<0.001	z=3.79 P<0.001	z=50.16 P<0.001	
Test for heterogeneity	Q=10.83 P=0.21 τ=0.47 I ² =26.12%	Q=66.94 P<0.001 τ=0.15 I ² =88.05%	Q=23.11 P=0.003 τ=0.045 I ² =65.38%	

Abbreviations: OR=odds ratio; PPV=positive predictive value; NPV=negative predictive value; LR+=positive likelihood ratio; LR-=negative likelihood ratio; z=standardized distribution; Q=chi-square test for heterogeneity; τ=estimated standard deviation of between study variance; I²=proportion of variation due to heterogeneity

Supplementary Table 4 Measures of predictive accuracy for AD in studies that provided APOE data for subjects progressing to non-AD type dementia

Category	OR	Sensitivity	Specificity	
AD versus no dementia				
Effect (95% CI)	2.20 (1.72 - 2.80)	0.54 (0.45 - 0.63)	0.66 (0.60 - 0.71)	
AD versus no AD				
Effect (95% CI)	2.27 (1.74 - 2.85)	0.54 (0.45 - 0.63)	0.66 (0.61 - 0.71)	

Abbreviations: AD=Alzheimer-type dementia; OR=odds ratio; PPV=positive predictive value; NPV=negative predictive value; LR+=positive likelihood ratio; LR-=negative likelihood ratio; CI=confidence interval

Note: Included were 4818 subjects from 26 studies with AD at follow-up (n=801), no dementia (n=3873), and other types of dementia (n=144). The analysis AD versus no dementia is similar to that reported in the main paper but now for the selection of studies that provided data on the APOE genotype in subjects with non-AD dementia at follow-up. In the analysis AD versus no AD, subjects without dementia and subjects with non-AD type dementia at follow-up are included in the no AD group.

	PPV	NPV	LR+	LR-
	0.59 (0.50 - 0.68)	0.69 (0.60 - 0.79)	3.01 (2.11 - 4.30)	0.89 (0.79 - 0.99)
	z=12.72 P<0.001	z=14.31 P<0.001	z=6.06 P<0.001	z=-2.00 P=0.045
	Q=6.56 P=0.585 t=0.16 I ² = 0%	Q=61.98 P<0.001 t=0.13 I ² =44.11%	Q=8.10 P=0.424 t=0.71 I ² = 1.17%	Q=24.57 P=0.002 t=0.13 I ² =67.43%

	PPV	NPV	LR+	LR-
	0.42 (0.31 - 0.52)	0.76 (0.70 - 0.82)	1.53 (1.39 - 1.69)	0.77 (0.69 - 0.86)
	0.41 (0.31 - 0.51)	0.76 (0.71 - 0.82)	1.55 (1.40 - 1.71)	0.76 (0.68 - 0.85)

Supplementary Figure 1 Funnel plot for visual assessment of publication bias

The dots represent the studies included in the meta-analysis

Supplementary Figure 2a Forest plot sensitivity

AD—no dementia; AD+=with Alzheimer-type dementia; $\epsilon 4^-$ —=APOE- $\epsilon 4$ allele noncarriers; $\epsilon 4^+$ —=APOE- $\epsilon 4$ allele carriers; OR=odds ratio; CI=confidence interval; RE=random effects.

The squares represent the weight of the particular study in the overall analysis. The horizontal line across the square shows the confidence interval. The diamond represents the overall effect.

Supplementary Figure 2b Forest plot specificity

AD—=no dementia; AD+=with Alzheimer-type dementia; ϵ_4- =*APOE- ϵ_4* allele noncarriers; ϵ_4+ =*APOE- ϵ_4* allele carriers; OR=odds ratio; CI=confidence interval; RE=random effects. The squares represent the weight of the particular study in the overall analysis. The horizontal line across the square shows the confidence interval. The diamond represents the overall effect.

Supplementary Figure 2c Forest plot positive predictive value

AD—no dementia; AD+=with Alzheimer-type dementia; $\epsilon 4^-$ —=APOE- $\epsilon 4$ allele noncarriers; $\epsilon 4^+$ —=APOE- $\epsilon 4$ allele carriers; OR=odds ratio; CI=confidence interval; RE=random effects.

The squares represent the weight of the particular study in the overall analysis. The horizontal line across the square shows the confidence interval. The diamond represents the overall effect.

Supplementary Figure 2d Forest plot negative predictive value

AD—=no dementia; AD+=with Alzheimer-type dementia; $\epsilon 4-$ =*APOE- $\epsilon 4$* allele noncarriers; $\epsilon 4+$ =*APOE- $\epsilon 4$* allele carriers; OR=odds ratio; CI=confidence interval; RE=random effects. The squares represent the weight of the particular study in the overall analysis. The horizontal line across the square shows the confidence interval. The diamond represents the overall effect.

Supplementary Figure 2e Forest plot positive likelihood ratio

AD—no dementia; AD+=with Alzheimer-type dementia; $\epsilon 4^-$ —=APOE- $\epsilon 4$ allele noncarriers; $\epsilon 4^+$ —=APOE- $\epsilon 4$ allele carriers; OR=odds ratio; CI=confidence interval; RE=random effects.

The squares represent the weight of the particular study in the overall analysis. The horizontal line across the square shows the confidence interval. The diamond represents the overall effect.

Supplementary Figure 2f Forest plot negative likelihood ratio

AD—=no dementia; AD+=with Alzheimer-type dementia; $\epsilon 4-$ —=*APOE*- $\epsilon 4$ allele noncarriers; $\epsilon 4+$ =*APOE*- $\epsilon 4$ allele carriers; OR=odds ratio; CI=confidence interval; RE=random effects. The squares represent the weight of the particular study in the overall analysis. The horizontal line across the square shows the confidence interval. The diamond represents the overall effect.

The review protocol for APOE- ϵ_4 that we drafted in this chapter is meant to ensure that subsequent reviews are performed in a consistent and systematic manner.

CHAPTER 3

APOE ϵ_4 allele for the diagnosis of Alzheimer's
and other dementia disorders in people with MCI
in a primary care setting

BACKGROUND

Mild cognitive impairment (MCI) refers to cognitive impairment without dementia (Morris 2001). It is a heterogeneous condition that has been operationalised in several ways. Matthews (Matthews 2008) has categorised MCI into age-related cognitive change, pathological decline, category systems, and the Mayo Clinic criteria. Under age-related cognitive change are age-consistent memory impairment (ACMI) and age-related cognitive decline (ARCD). Pathological decline encompasses the conditions of cognitive impairment no dementia (CIND), age-associated memory impairment (AAMI), age-associated cognitive decline (AACD), mild cognitive disorder (MCD), questionable dementia (QD), minimal dementia (MD), mild neurocognitive disorder (MNCd), limited cognitive disturbance (LCD), and benign senescent forgetfulness (BSF). The category systems cover subjective memory complaint (SMC) and MCI defined as a score of 22 to 26 on the 30-item Mini-Mental State Examination. The Mayo Clinic criteria differentiate MCI into three subtypes: non-amnestic MCI (N-MCI), amnestic MCI (A-MCI), multiple MCI (M-MCI). A-MCI refers to objective memory impairment with generally preserved cognitive functioning, or subjective memory complaints corroborated by an informant (Petersen 2004).

Throughout this review, we will use the term “MCI” to refer to any of the abovementioned conditions. In addition, we will be including in our definition of MCI impairment on a cognitive test with an overall Clinical Dementia Rating (CDR) score of 0.5 or based on an equivalent measure (Morris 2001).

People with MCI are at an increased risk for Alzheimer’s disease (AD). About 35 percent of those with MCI have been reported to progress to AD over a follow-up period of 4.5 years, with a rate of progression that was thrice faster compared to cognitively healthy individuals (Bennett 2002). AD is a neurodegenerative disorder characterized pathologically by neuritic plaques and neurofibrillary tangles; and clinically by progressive memory loss, impairments in activities of daily living, and behavioral and neuropsychiatric symptoms. AD is the most common cause of dementia (Blennow 2006) and accounts for the majority of dementia cases worldwide (World Health Organization 2012).

There is currently no cure for AD dementia, although previous studies have shown some benefits of drug treatments in delaying cognitive decline and slowing disease progression (Rountree 2012; Peters 2012). Identifying those who are at an increased risk of developing AD dementia is therefore important for prognosis purposes and early intervention, when and if a treatment for reducing incident AD dementia is found.

Among the known risk factors for AD dementia is the ϵ_4 allele of the apolipoprotein gene. The *APOE* gene transports cholesterol and lipids in the brain, and is involved in neuronal repair (Bu 2009). *APOE* has three polymorphic alleles (ϵ_2 , ϵ_3 and ϵ_4), the combinations of which result in six genotypes: $\epsilon_2\epsilon_2$, $\epsilon_2\epsilon_3$, $\epsilon_2\epsilon_4$, $\epsilon_3\epsilon_3$, $\epsilon_3\epsilon_4$ and $\epsilon_4\epsilon_4$ (Leoni 2011). The *APOE* ϵ_4 allele has been shown to have high-avidity binding to amyloid beta (Strittmatter 1993), which is the main constituent of neuritic plaques.

About 15% of the general population has been reported to be *APOE* ϵ_4 allele carriers (Bu 2009). In Europe, *APOE*- ϵ_4 prevalence has been reported to follow a north-south gradient, which was highest in northern Europe (~60%), followed by middle Europe (~40%) and southern Europe (~30%) (Norberg 2011). An estimated 40% of those with AD dementia in the general population carry the ϵ_4 allele (Bu 2009). *APOE*- ϵ_4 has been associated with a ~3.7-fold increased risk for AD dementia in case-control studies (Bertram 2007), while carriership of two ϵ_4 alleles has been reported to increase the risk to as high as ~15-fold (Farrer 1997). Although other genetic risk factors for AD dementia have been identified in genome-wide association studies in recent years, these genes usually show a much weaker effect (odds ratio of <1.5) compared to *APOE*- ϵ_4 . In a meta-analysis, we found the *APOE* ϵ_4 allele to be a moderately strong predictor of progression from MCI to AD dementia (odds ratio of ~2.3) (Elias-Sonnenschein 2011).

The purpose of the current review is to (1) assess through meta-analysis the diagnostic accuracy of the *APOE* ϵ_4 allele for identifying AD and predicting progression to AD dementia and other dementia among people with MCI; and (2) update and expand our previous meta-analysis. It differs from our earlier study in the following aspects. First, the period that will be covered is from 1993, when the

first publication on the relationship between the *APOE* ϵ_4 allele and AD appeared (Strittmatter 1993), to 2012. Second, the definition of MCI will include the concepts of Matthews (Matthews 2008). Third, the methodological quality of the studies that will be included in the review will be assessed using the revised quality assessment of diagnostic accuracy studies (QUADAS-2)(Whiting 2011). Fourth, in the statistical analyses, we will be taking into account correlations between outcome measures, which is ideally assessed using a bivariate model. Fifth, no language restriction will be imposed on the search for relevant literature for the review. And sixth, as the diagnostic accuracy of the *APOE* ϵ_4 allele in one setting may be different from other settings, we will be including in this review only studies in a primary care setting. We have prepared a similar review for studies in secondary care and in community settings.

We will be evaluating the accuracy of the *APOE* ϵ_4 allele at baseline in relation to (1) progression from MCI to AD dementia; and (2) progression from MCI to other forms of dementia.

Target condition being diagnosed

There are two target conditions in this review: (1) AD dementia and (2) other forms of dementia, which will be assessed at follow-up.

We will be comparing the results of the index test obtained at baseline with the results of the reference standards at follow-up (delayed verification).

Index test(s)

The index test is the *APOE* genotype, in particular, carriership of the *APOE* ϵ_4 allele as assessed by genotyping.

Clinical pathway

Dementia develops over several years. There is a presumed period when people are asymptomatic, and when pathology is accumulating. Individuals or their relatives may then notice subtle impairments of recent memory. Gradually, more cognitive domains become involved, and difficulty in planning complex tasks becomes increasingly apparent. In the Netherlands, people usually present to their general practitioner,

who may administer a cognitive screening test and refer the person to a hospital memory clinic. However, many people with dementia do not present until much later in the disorder and will follow a different pathway to diagnosis, for example being identified during an admission to general hospital for a physical illness. Thus, the pathway influences the accuracy of the diagnostic test. The accuracy of the test will vary with the experience of the administrator, and the accuracy of the subsequent diagnosis will vary with the history of referrals to the particular healthcare setting. Diagnostic assessment pathways may vary in other countries and diagnoses may be made by a variety of specialists including neurologists and geriatricians.

Standard assessment of dementia includes history taking, clinical examination (including neurological, mental state, and cognitive examinations), and informant interview. Prior to diagnosis of dementia, the clinician normally rules out, and if possible treats, other physical or mental conditions that may be causing the cognitive impairment. Neuroimaging (computed tomography or magnetic resonance imaging) is recommended in most recent guidelines (McKhann 2011; NICE 2006). Most neuroimaging tests, and also recently cerebrospinal fluid sampling, are performed after a cognitive deficit is noted. However, individuals with abnormalities on brain imaging, which may be performed for any number of reasons, are more likely to be tested subsequently for cognitive impairment. *APOE* is currently not part of the standard assessment procedure for dementia.

Dementia as diagnosed is defined by a deficit in more than two cognitive domains of sufficient degree to impair functional activities of daily living. The different diagnostic criteria for dementia are presented hereunder in the 'Reference standards' section and in Appendix 1.

Rationale

An increasing number of studies suggest genetic susceptibility to AD dementia (Hollingworth 2011; Naj 2011; Seshadri 2010; Lambert 2009). The *APOE* ϵ_4 allele is the strongest known genetic risk factor for AD dementia. As a diagnostic tool, *APOE* genotyping might aid in:

- identifying individuals with MCI at high risk of developing AD dementia
- increasing the accuracy of dementia diagnostics in addition to the reference standards
- selecting candidates for trials with drugs that aim to slow down the progression of AD dementia

Particular advantages of *APOE* genotyping is that it is safe and relatively inexpensive to perform.

Objectives

To determine the diagnostic accuracy of the *APOE* ϵ_4 allele in detecting AD dementia and other forms of dementia in people with MCI in a primary care setting.

Secondary objectives

To investigate heterogeneity of test accuracy in the included studies.

To highlight the quality and quantity of research evidence available about the effectiveness of *APOE* as an index test in people with MCI in a primary care setting.

METHODS

Criteria for considering studies for this review

Types of studies

We will be including longitudinal cohort studies in a primary care setting of people with MCI in which *APOE* was genotyped at baseline and the reference standard results were obtained at follow-up. These studies necessarily employed delayed verification of progression to dementia and are sometimes labelled as 'delayed verification cross-sectional studies' (Bossuyt 2008).

Case-control studies will be included if they incorporate a delayed verification design. As these can only occur in the context of a cohort study, these studies are invariably diagnostic nested case-control studies.

Participants

Participants with MCI will be included in this review. MCI will be defined according to the criteria of Matthews, Petersen or Morris (Matthews

2008; Petersen 2004; Morris 2001), as described above. These criteria include a CDR score of 0.5, memory impairment confirmed by neuropsychological assessment, subjective memory complaints corroborated by an informant, generally preserved cognitive functions, no or minimal impairment of activities of daily living, absence of dementia.

The participants for this review are those in a primary care setting. By primary care, we mean all generalist office-based practices, including general practice and family physician. An office-based neurology, psychiatry, gerontology or other specialist memory service will not be included. Subjects in this category may have presented with subjective memory problems or be part of a "screening" program.

We will exclude participants with cognitive impairment due to alcohol or substance abuse, head injury, major depression or other neuropsychiatric disorders, Parkinson's disease or other neurological disorders, somatic disorders, or endocrine disorders.

Studies that have more than 20% drop-out will be considered separately. The causes for the drop-out will be noted down. If this information is missing, the reliability of the conclusions must be questioned.

Index tests

Participants genotyped for *APOE* will be classified as ϵ_4 allele carriers or noncarriers; heterozygote carriers or homozygote carriers. We will record the genotypes for the other *APOE* alleles, which may then be the subject of subgroup analyses.

Target conditions

There are two target conditions in this review:

1. AD dementia (conversion from MCI to AD dementia)
2. Any other forms of dementia (conversion from MCI to any other forms of dementia)

Reference standards

Several definitions of AD dementia are acceptable for the purposes of this review.

Post-mortem diagnosis is the 'gold standard'. Pathological confirmation of AD according to the Braak, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD), or the National Institute for Aging and Ronald and Nancy Reagan Institute for the Alzheimer's Association (NIA-RIA) criteria are based on the density and distribution of neurofibrillary tangles (NFTs) or neuritic plaques (NP) (Murayama 2004; Appendix 1).

The acceptable clinical reference standards for diagnosing AD dementia include that of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann 1984); the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) (World Health Organization 1993); the Diagnostic and Statistical Manual of Mental Disorders 4th edition text revised (DSM-IV-TR) (American Psychiatric Association 2000); DSM 4th edition (DSM-IV) (American Psychiatric Association 1994); and DSM 3rd revised edition (DSM-III-R) (American Psychiatric Association 1987).

NINCDS-ADRDA differentiates between probable and possible AD. A diagnosis of probable AD is supported by abnormal biomarkers, whereas possible AD mainly has a clinical presentation. Details of the criteria are shown in Appendix 1. If sufficient data is available, subgroup analyses for probable and for possible AD will be performed.

Recently, the NINCDS-ADRDA criteria has been revised to include as key diagnostic feature early episodic memory impairment, the presence of abnormal biomarkers, namely medial temporal lobe atrophy, abnormal cerebrospinal fluid markers, specific metabolic pattern on neuroimaging methods, and familial genetic mutations (Dubois 2007). Studies that used this revised criteria will be included in the review.

The key distinction in all clinical criteria is extent of social and occupational functional impairment. The different iterations of these clinical criteria may not be directly comparable over time, and the validity of the diagnoses may vary depending on how they were operationalised. We will take these issues into consideration in interpreting results, assessing sources of heterogeneity and performing

sensitivity analyses, as appropriate.

With regard to other dementia, the reference standard for Lewy Body Dementia is the McKeith criteria (McKeith 2005); for fronto-temporal dementia, the Lund criteria (The Lund and Manchester Groups 1994), Boxer criteria (Boxer 2005), DSM and ICD; and for vascular dementia, the National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria (Roman 1993), DSM and ICD.

In this review, we will perform separate analyses for AD dementia diagnosis based on pathological criteria and on clinical criteria. The reference standards used will be included in the investigation of sources of heterogeneity.

Search methods for identification of studies

Electronic searches

We will search ALOIS, the Cochrane Dementia and Cognitive Improvement Group's specialised register, MEDLINE (Ovid SP), EMBASE (Ovid SP), BIOSIS Previews (Ovid SP), Science Citation Index (ISI Web of Knowledge), PsycINFO (Ovid SP), and LILACS (Bireme). See Appendix 2 for a proposed draft strategy to be run in MEDLINE (Ovid SP) plus additional narrative on the search process. Similarly structured search strategies will be designed using search terms appropriate for each database listed above. We will also request a search of the Cochrane Register of Diagnostic Test Accuracy Studies (maintained by the Cochrane Renal Group).

There will be no restriction based on language of the study report. Translation services will be used, as necessary.

Initial searches will be performed by a single researcher with extensive experience of systematic review.

Searching other resources

- Grey literature: Chosen electronic databases will include assessments of conference proceedings.
- Handsearching: We will not perform handsearching as there is little published evidence of the benefits of handsearching for reports of DTA studies (Glanville 2010).

- Reference lists: Reference lists of all eligible studies and reviews in the field will be scanned for further possible titles and the process repeated until no new titles are found (Horsley 2011; Greenhalgh 2005). The citation tracking and “related articles” will be checked to identify studies that could be included in the review.

Data collection and analysis

Selection of studies

One researcher will screen all titles generated by electronic database searches for relevance. The results of these electronic searches will receive an initial screen, including de-duplication, by a team of experienced assessors (predominantly senior medical students). This group has previously been shown to have good inter-rater reliability results.

Two review authors with content expertise will perform a second level of screening and identify studies for inclusion in the review. In case of differences in opinion on whether or not to include a particular study, a third author will serve as arbiter. When multiple publications are based on the same cohort, the study with the largest sample will be selected. The PRISMA flowchart will be used to illustrate the selection process.

Data extraction and management

Two review authors will independently complete a data extraction form for each study (see Appendix 3). The data extraction form includes information on the baseline characteristics of the population, *APOE* genotyping, outcomes at follow-up, MCI definition of the study, study duration, and inclusion and exclusion criteria.

Data on *APOE* ϵ_4 allele and outcome will be summarized in a 2 x 2 table. In this table, the number of people who are carriers of at least 1 *APOE* ϵ_4 allele and number of noncarriers who remained stable or progressed to AD will be specified. Information on the specific *APOE* genotype and type of dementia at follow-up will be summarised in another table. The data will be cross-checked for consistency. Authors will be contacted in case of incomplete data. Excel, Endnote and RevMan will be used to manage the data.

Assessment of methodological quality

We will assess the methodological quality of each study using QUADAS-2 tool (Whiting 2011), as recommended by the Cochrane Collaboration. The tool consists of four domains: Patient selection; Index test; Reference standard; and Patient flow. Each domain is assessed in terms of risk of bias, with the first three domains also considered in terms of applicability (<http://www.bris.ac.uk/quadas/quadas-2> Appendix 3). The components of each of these domains and a rubric that details how judgements concerning risk of bias are made are detailed in Appendix 4. Certain key areas important to qualify assessment are participant selection, blinding and missing data. We recognise that QUADAS-2 may still develop and that as yet standard RevMan software does not incorporate QUADAS-2 functionality.

We will pilot QUADAS-2 assessment on 2 papers. If agreement is poor, we will refine the questions. QUADAS-2 data will not be used to form a summary quality score. We will produce a narrative summary describing numbers of studies that found high/low/unclear risk of bias as well as concerns regarding applicability, which we will assess using Appendix 5 .

Statistical analysis and data synthesis

We will apply the DTA framework for the analysis of a single test and extract the data from a study into a 2x2 table, showing the binary test results cross-classified with the binary reference standard and ignoring any censoring that might have occurred. We acknowledge that such a reduction in the data may represent a significant oversimplification. We will therefore adopt an intention to diagnose (ITD) approach as well. If possible, we would present what the result would be if all dropouts would have developed dementia, and if all dropouts would not have developed dementia. We may also need to assume that the proportion of positive and negative test results is the same in the unknown as the known participants in order to do this.

We will use data from the 2x2 tables abstracted from the included studies (true positive=TP, false negative=FN, false positive=FP, true negative=TN) and entered into RevMAN to calculate the sensitivities, specificities and their 95% confidence intervals. We will

also present individual study results graphically by plotting estimates of sensitivities and specificities in both a forest plot and a receiver operating characteristic (ROC) space. If more than one threshold is published in primary studies, we will report accuracy estimates for all thresholds.

If there are sufficient data, we will meta-analyse the pairs of sensitivity and specificity. The preferred approach would be the hierarchical summary ROC curve (HSROC) method proposed by Rutter and Gatsonis (Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy, chapter 10) (Macaskill 2010) because implicit thresholds are expected in primary studies. We will conduct these analyses in Stata, R and RevMan software (Stata Corporation 2011; R Core Team 2012; Review Manager 2012) with support from the UK DTA Support Unit. If there are common thresholds across included studies, we will also consider the bivariate random effects approach (Reitsma 2005). When a primary study reports more than one threshold result, we will only select the threshold nearer to the upper left point of the ROC curve for the meta-analysis. We are aware that this data-driven method for threshold selection could lead to an overestimation of diagnostic accuracy (Leeftang 2008).

We will explore the implications of any summary accuracy estimates not affected by heterogeneity emerging by considering the numbers of false positive and false negatives in populations with different prevalence of MCI and by presenting the results as natural frequencies and using alternative metrics such as likelihood ratios and predictive values.

We will prepare a summary of results table irrespectively.

Investigations of heterogeneity

The potential sources of heterogeneity, which will be used as framework for investigating heterogeneity, include:

- Spectrum of patients (mean age, gender, Mini-Mental State Examination (MMSE) score)
- Background prevalence of *APOE* ϵ_4 allele
- Definition of MCI

- Length of follow-up
- Reference standards used
- Exclusion criteria of the study

For diagnostic odds ratios, heterogeneity will be assessed by the Chi-square test, Q . The amount of heterogeneity for each outcome measure will be calculated using DerSimonian-Laird, with Tau as an estimate for the standard deviation of the underlying true outcomes across studies. The proportion of variation due to heterogeneity will likewise be computed and expressed as I^2 -square.

As heterogeneity may also be a result of correlation between outcomes (e.g. high sensitivity is correlated with low specificity), a bivariate model will be used and results presented in ROC space. Descriptive analysis will be done by visual examination of the forest plot of sensitivity and specificity, and the ROC plot. Subgroup analyses will be performed if there are sufficient number of studies included. Further analysis will be done by including the potential sources of heterogeneity as covariates in the HSROC model.

Sensitivity analyses

We will visually inspect the forest plots for outliers and investigate the influence of study quality on the overall diagnostic accuracy of the index test. Studies at high risk of bias as assessed using QUADAS-2 and the Standards for Reporting Diagnostic Accuracy (STARD) (Bossuyt 2003; Appendix 4; Appendix 6) will be systematically excluded, and the analyses repeated with every exclusion of a study to test if the results would be consistent.

A separate sensitivity analysis will be performed by excluding data-driven threshold selection studies to evaluate their effects on the overall diagnostic accuracy of the index test.

Assessment of reporting bias

Reporting bias will not be investigated because of current uncertainty about how it operates in test accuracy studies and the interpretation of existing analytical tools such as funnel plots.

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APPENDICES

Appendix 1 Reference standards for the diagnosis of AD dementia

Pathological criteria

Braak stage I/II indicates normal condition; stage III/IV, cognitive impairment; and stage V/VI, dementia (Murayama 2004). The CERAD differentiates between neuritic and diffuse plaques, classifies neocortical NP as 0, A, B, and C, and in addition uses clinical information in making the diagnosis. A CERAD stage C means abundant NP and corresponds to dementia in the elderly. The NIA-RIA categorises dementia in terms of likelihood and combines the Braak and CERAD criteria. Braak stage I/II and CERAD NP stage A corresponds to low probability of AD; stage III/IV and B indicates middle probability of AD; and stage V/VI and C is regarded as high probability of AD (Murayama 2004).

Clinical criteria

The NINCDS-ADRDA key features for the diagnosis of probable AD are impairment in at least two cognitive domains, progressive impairment in memory and other cognitive functions not owing to other physical or neurological conditions, normal consciousness, with age of onset between 40 and 90 years of age (McKhann 1984). The supportive features are progressive worsening of specific cognitive functions, such as language, goal-oriented activity, and perception, which is also confirmed by the patients' family; and abnormal results of electroencephalogram (EEG), computed tomography (CT) or magnetic resonance imaging (MRI), or cerebrospinal fluid (CSF) examination (McKhann 1984). Possible AD refers to dementia, based on clinical examination and MMSE or comparable tests score. It course has a clinical presentation. A diagnosis of possible AD is made in the absence of other neurological, psychiatric or general physical impairment that could be causing the dementia; or in the presence of a general physical impairment of brain disorder that could result in dementia but is, in this case, not the cause of the dementia; or in the presence of a confirmed single gradually progressive serious cognitive impairment without other cause (McKhann 1984).

Appendix 2 Medline search strategy

MEDLINE (Ovid SP) search strategy

1. exp Cohort Studies/
2. cohort.ti,ab.
3. longitudinal.ti,ab.
4. follow-up.ti,ab.
5. year risk.ti,ab.
6. (prospective adj2 (study or analysis or evaluation)).ti,ab.
7. epidemiologic studies/
8. "observational study".ti,ab.
9. "preclinical detection".ti,ab. OR predict*.ti.
10. Disease Progression/ OR Risk/
11. or/1-10
12. (dement* OR "cognit* impair*").ti.
13. (alzheimer* or AD).ti.
14. exp Dementia/di [Diagnosis]
15. exp Dementia/ep [Epidemiology]
16. *Cognition Disorders/di [diagnosis]
17. ((endpoint* or outcome*) adj6 (dement* or alzheimer* or AD)).ab.
18. (conversion adj4 (dement* or alzheimer*)).ab.
19. (convert* adj4 (dement* or alzheimer* or AD)).ab.
20. (predict* adj6 (dement* or alzheimer* or AD)).ab.
21. (progress* adj4 ("to dement*" or "to alzheimer*" or "to AD")).ab.
22. or/12-21
23. "Predictive Value of Tests"/
24. (dement* or alzheimer* or AD or "cognit* impair*").ab.
25. 23 and 24
26. Neuropsychological Tests/
27. (dement* or alzheimer* or AD or "cognit* impair*").ab.
28. 26 and 27
29. (neuropsycholog* adj4 (dement* or alzheimer* or AD or "cognit* impair*")).ab.
30. or/22,25,28,29
31. 11 and 30
32. (animals not (humans and animals)).sh.
33. 31 not 32

Search narrative: This search strategy (above) has been kept deliberately very broad – utilizing only two main concepts: time (concept A) and the dementia aspect/outcome (concept B). *APOE-e4* will not be included in the search terms because this allows the search to be more sensitive to studies that do not contain the term *APOE-e4* in their abstracts yet provide information on *APOE* genotyping in the main text.

Potential studies for inclusion were initially identified from published non-Cochrane reviews and background literature. This generated a reference set of 35 potential (and probable) studies for inclusion to use to test the search strategy detailed above. All 35 studies are identified by the above strategy. We then further tested the strategy against a set of potential studies that had been identified through an index-test-based search. The yield from the above strategy will be very high. However, a significant proportion of references retrieved will not need to be screened as they will have already been screened from the monthly search detailed below.

Parallel to running non-index test based searches in the databases listed in the search methods section, the offline version of the Cochrane Dementia and Cognitive Improvement Group's specialized register,

Appendix 2 Medline search strategy *Continued*

ALOIS, will be searched for all longitudinal studies in which the presence of *APOE* ϵ_4 allele was measured. ALOIS is made up of both intervention and diagnostic test accuracy (DTA) studies. The DTA side of ALOIS is still under development and is not 100% comprehensive but the MEDLINE search used to identify diagnostic tests (in both longitudinal and normal cross-sectional studies) in any setting, is run and screened every month.

The MEDLINE (Ovid SP) strategy for ALOIS is:

1. "word recall".ti,ab.
2. "7-minute screen".ti,ab.
3. "6 item cognitive impairment test".ti,ab.
4. "6 CIT".ti,ab.
5. "AB cognitive screen".ti,ab.
6. "abbreviated mental test".ti,ab.
7. "ADAS-cog".ti,ab.
8. AD8.ti,ab.
9. "inform* interview".ti,ab.
10. "animal fluency test".ti,ab.
11. "brief alzheimer* screen".ti,ab.
12. "brief cognitive scale".ti,ab.
13. "clinical dementia rating scale".ti,ab.
14. "clinical dementia test".ti,ab.
15. "community screening interview for dementia".ti,ab.
16. "cognitive abilities screening instrument".ti,ab.
17. "cognitive assessment screening test".ti,ab.
18. "cognitive capacity screening examination".ti,ab.
19. "clock drawing test".ti,ab.
20. "deterioration cognitive observee".ti,ab.
21. "Dem Tect".ti,ab.
22. "fuld object memory evaluation".ti,ab.
23. "IQCODE".ti,ab.
24. "mattis dementia rating scale".ti,ab.
25. "memory impairment screen".ti,ab.
26. "minnesota cognitive acuity screen".ti,ab.
27. "mini-cog".ti,ab.
28. "mini-mental state exam*".ti,ab.
29. "mmse".ti,ab.
30. "modified mini-mental state exam".ti,ab.
31. "3MS".ti,ab.
32. "neurobehavioural cognitive status exam*".ti,ab.
33. "cognistat".ti,ab.
34. "quick cognitive screening test".ti,ab.
35. "QCST".ti,ab.
36. "rapid dementia screening test".ti,ab.
37. "RDST".ti,ab.
38. "repeatable battery for the assessment of neuropsychological status".ti,ab.
39. "RBANS".ti,ab.
40. "rowland universal dementia assessment scale".ti,ab.
41. "rudas".ti,ab.
42. "self-administered gerocognitive exam*".ti,ab.
43. ("self-administered" and "SAGE").ti,ab.
44. "self-administered computerized screening test for dementia".ti,ab.

Appendix 2 Medline search strategy *Continued*

45. "short and sweet screening instrument".ti,ab.
46. "sassi".ti,ab.
47. "short cognitive performance test".ti,ab.
48. "syndrome kurztest".ti,ab.
49. "six item screener".ti,ab.
50. "short memory questionnaire".ti,ab.
51. ("short memory questionnaire" and "SMQ").ti,ab.
52. "short orientation memory concentration test".ti,ab.
53. "s-omc".ti,ab.
54. "short blessed test".ti,ab.
55. "short portable mental status questionnaire".ti,ab.
56. "spmsq".ti,ab.
57. "short test of mental status".ti,ab.
58. "telephone interview of cognitive status modified".ti,ab.
59. "tics-m".ti,ab.
60. "trail making test".ti,ab.
61. "verbal fluency categories".ti,ab.
62. "WORLD test".ti,ab.
63. "general practitioner assessment of cognition".ti,ab.
64. "GPCOG".ti,ab.
65. "Hopkins verbal learning test".ti,ab.
66. "HVL".ti,ab.
67. "time and change test".ti,ab.
68. "modified world test".ti,ab.
69. "symptoms of dementia screener".ti,ab.
70. "dementia questionnaire".ti,ab.
71. "7MS".ti,ab.
72. ("concord informant dementia scale" or CIDS).ti,ab.
73. (SAPH or "dementia screening and perceived harm*").ti,ab.
74. or/1-73
75. exp Dementia/
76. Delirium, Dementia, Amnestic, Cognitive Disorders/
77. dement*.ti,ab.
78. alzheimer*.ti,ab.
79. AD.ti,ab.
80. ("lewy bod*" or DLB or LBD).ti,ab.
81. "cognit* impair*".ti,ab.
82. (cognit* adj4 (disorder* or declin* or fail* or function*)).ti,ab.
83. (memory adj3 (complain* or declin* or function*)).ti,ab.
84. or/75-83
85. exp "sensitivity and specificity"/
86. "reproducibility of results"/
87. (predict* adj3 (dement* or AD or alzheimer*)).ti,ab.
88. (identif* adj3 (dement* or AD or alzheimer*)).ti,ab.
89. (discriminat* adj3 (dement* or AD or alzheimer*)).ti,ab.
90. (distinguish* adj3 (dement* or AD or alzheimer*)).ti,ab.
91. (differenti* adj3 (dement* or AD or alzheimer*)).ti,ab.
92. diagnos*.ti.
93. di.fs.
94. sensitivit*.ab.

Appendix 2 Medline search strategy *Continued*

95. specificit*.ab.
96. (ROC or "receiver operat*").ab.
97. Area under curve/
98. ("Area under curve" or AUC).ab.
99. (detect* adj3 (dement* or AD or alzheimer*)).ti,ab.
100. sROC.ab.
101. accura*.ti,ab.
102. (likelihood adj3 (ratio* or function*)).ab.
103. (conver* adj3 (dement* or AD or alzheimer*)).ti,ab.
104. ((true or false) adj3 (positive* or negative*)).ab.
105. ((positive* or negative* or false or true) adj3 rate*).ti,ab.
106. or/85-105
107. exp dementia/di
108. Cognition Disorders/di [Diagnosis]
109. Memory Disorders/di
110. or/107-109
111. *Neuropsychological Tests/
112. *Questionnaires/
113. Geriatric Assessment/mt
114. *Geriatric Assessment/
115. Neuropsychological Tests/mt, st
116. "neuropsychological test*".ti,ab.
117. (neuropsychological adj (assess* or evaluat* or test*)).ti,ab.
118. (neuropsychological adj (assess* or evaluat* or test* or exam* or battery)).ti,ab.
119. Self report/
120. self-assessment/ or diagnostic self evaluation/
121. Mass Screening/
122. early diagnosis/
123. or/111-122
124. 74 or 123
125. 110 and 124
126. 74 or 123
127. 84 and 106 and 126
128. 74 and 106
129. 125 or 127 or 128
130. (animals not (humans and animals)).sh.
131. 129 not 130

The concepts for this are:

A Specific neuropsychological tests

B General terms (both free text and MeSH) for tests/testing/screening

C Outcome: dementia diagnosis (unfocused MeSH with diagnostic sub-headings)

D Condition of interest: Dementia (general dementia terms both free text and MeSH – exploded and unfocused)

E Methodological filter: not used to limit all search

The concept combinations are:

1. (A OR B) AND C
2. (A OR B) AND D AND E
3. A AND E

Appendix 3 Data extraction forms

Appendix 3.1 General information of the study

Author:	
Source:	
MCI concept:	
Setting:	
Population:	
Inclusion criteria:	
Exclusion criteria:	
Baseline information	Follow-up information
Number of people (N):	Period (number of years): ± (range:)
Age: ±	Number of people who completed the follow-up: (%)
Sex (n): (%) females (%) males	Number of people who became demented: (%)
Education (years): ±	Number of people who progressed to AD-type dementia: (%)
MMSE: ±	

Table legend: MCI=mild cognitive impairment; MMSE=Mini-Mental State Examination; AD=Alzheimer’s disease

Appendix 3.2 Demographic information according to outcome

	AD+ (n=)	AD- (n=)	P-value	Remarks
Age at baseline (range)	± ()	± ()		
Sex (% female)	n= (%)	n= (%)		
Education, years	±	±		
MMSE at baseline	±	±		

Table legend: AD+ = with Alzheimer’s disease; AD- = without Alzheimer’s disease; MMSE=Mini-Mental State Examination

Appendix 3.3 Genotyping data: APOE ε4 allele carriership

	ε4+	ε4-
AD+	n= (%)	n= (%)
AD-	n= (%)	n= (%)

Table legend: ε4+ = carriers of at least 1 APOE ε4 allele; ε4- = noncarriers of the APOE ε4 allele; AD+ = with Alzheimer’s disease; AD- = without Alzheimer’s disease

Appendix 3.4 Genotyping data: APOE genotype

	$\epsilon 2\epsilon 2$	$\epsilon 2\epsilon 3$	$\epsilon 2\epsilon 4$	$\epsilon 3\epsilon 3$	$\epsilon 3\epsilon 4$	$\epsilon 4\epsilon 4$
AD+	n= (%)	n= (%)	n= (%)	n= (%)	n= (%)	n= (%)
AD-	n= (%)	n= (%)	n= (%)	n= (%)	n= (%)	n= (%)
non-AD+ (specify type)	n= (%)	n= (%)	n= (%)	n= (%)	n= (%)	n= (%)
nodem	n= (%)	n= (%)	n= (%)	n= (%)	n= (%)	n= (%)

Table legend: $\epsilon 2\epsilon 2$ =APOE2 $\epsilon 2$ allele; $\epsilon 2\epsilon 3$ =APOE2 $\epsilon 3$ allele; $\epsilon 2\epsilon 4$ =APOE2 $\epsilon 4$ allele; $\epsilon 3\epsilon 3$ =APOE3 $\epsilon 3$ allele; $\epsilon 3\epsilon 4$ =APOE3 $\epsilon 4$ allele; $\epsilon 4\epsilon 4$ =APOE4 $\epsilon 4$ allele; AD+ = with Alzheimer's disease; AD- = without Alzheimer's disease; non-AD+ = with dementia but not of the Alzheimer-type; nodem= not demented

Appendix 4 Assessment of methodological quality table QUADAS-2 tool

Domain	Patient selection	Index test	Reference standard	Flow and timing
Description	Describe methods of patient selection: Describe included patients (prior testing, presentation, intended use of index test and setting):	Describe the index test and how it was conducted and interpreted	Describe the reference standard and how it was conducted and interpreted	Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram): Describe the time interval and any interventions between index test(s) and reference standard
Signalling questions (yes/no/unclear)	Was a consecutive or random sample of patients enrolled?	Were the index test results interpreted without knowledge of the results of the reference standard?	Is the reference standard likely to correctly classify the target condition?	Was there an appropriate interval between index test(s) and reference standard?
	Was a case-control design avoided?	If a threshold was used, was it pre-specified?	Were the reference standard results interpreted without knowledge of the results of the index test?	Did all patients receive a reference standard?
	Did the study avoid inappropriate exclusions?			Did all patients receive the same reference standard?
				Were all patients included in the analysis?

Appendix 4 Assessment of methodological quality table QUADAS-2 tool *Continued*

Domain	Patient selection	Index test	Reference standard	Flow and timing
Risk of bias: High/low/ unclear	Could the selection of patients have introduced bias?	Could the conduct or interpretation of the index test have introduced bias?	Could the reference standard, its conduct, or its interpretation have introduced bias?	Could the patient flow have introduced bias?
Concerns regarding applicability: High/low/ unclear	Are there concerns that the included patients do not match the review question?	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Are there concerns that the target condition as defined by the reference standard does not match the review question?	

Appendix 5 Anchoring statements for quality assessment of *APOE* ϵ_4 allele diagnostic studies

Category	Review Question	Inclusion Criteria
Patients	Participants with mild cognitive impairment, no dementia	Participants fulfilling the criteria for the clinical diagnosis of MCI at baseline
Index Test	<i>APOE</i> genotype	<i>APOE</i> genotype
Target Condition	Alzheimer’s disease dementia (conversion from MCI to Alzheimer’s disease dementia) Any other forms of dementia (conversion from MCI to any other forms of dementia)	Alzheimer’s disease dementia (conversion from MCI to Alzheimer’s disease dementia) Any other forms of dementia (conversion from MCI to any other forms of dementia)
Reference Standard	<u>AD dementia</u> Pathological: Braak, CERAD, NIA-RIA Clinical: NINCDS-ADRDA; DSM; ICD <u>Other dementia</u> NINDS-AIREN; Lund; McKeith; DSM; ICD	<u>AD dementia</u> Pathological: Braak, CERAD, NIA-RIA Clinical: NINCDS-ADRDA; DSM; ICD <u>AD dementia</u> NINDS-AIREN; Lund; McKeith; DSM; ICD
Outcome	N/A	Data to construct 2X2 table
Study Design	N/A	Longitudinal cohort studies and Nested case-control studies if they incorporate a delayed verification design (case-control nested in cohort studies)

Table: Review question and inclusion criteria

1. Anchoring statements to assist with assessment for risk of bias

Patient selection

Was the sampling method appropriate?

Where sampling is used, the designs least likely to cause bias are consecutive sampling or random sampling. Sampling that is based on volunteers or selecting subjects from a clinic or research resource is prone to bias.

Weighting: High risk of bias ('no')

Was a case-control or similar design avoided?

Designs similar to case control that may introduce bias are those designs where the study team deliberately increase or decrease the proportion of subjects with the target condition, which may not be representative. For example a population study may be enriched with extra dementia subjects from a secondary care setting, which are typically more diseased. Some case control methods may already be excluded if they mix subjects from various settings.

Weighting: High risk of bias ('no')

Are exclusion criteria described and appropriate?

The study will be automatically graded as unclear if exclusions are not detailed (pending contact with study authors). Where exclusions are detailed, the study will be graded as "low risk" if exclusions are felt to be appropriate by the review authors. Certain exclusions common to many studies of dementia are: medical instability; terminal disease; alcohol/substance misuse; concomitant psychiatric diagnosis; other neurodegenerative condition. Exclusions are not felt to be appropriate if 'difficult to diagnose' patients are excluded.

Post hoc and inappropriate exclusions will be labeled "high risk" of bias.

Weighting: High risk ('no')

Index test

Was APOE genotyping performed without knowledge of clinical dementia diagnosis?

Terms such as "blinded" or "independently and without knowledge of" are sufficient and full details of the blinding procedure are not required.

Interpretation of the results of the index test may be influenced by knowledge of the results of reference standard. If the index test is always interpreted prior to the reference standard then the person interpreting the index test cannot be aware of the results of the reference standard and so this item could be rated as 'yes'.

For certain index tests the result is objective and knowledge of reference standard should not influence result, for example level of protein in cerebrospinal fluid, in this instance the quality assessment may be "low risk" even if blinding was not achieved.

Weighting: High risk ('no')

Reference standard

Is the assessment used for clinical diagnosis of dementia acceptable?

Commonly used international criteria to assist with clinical diagnosis of dementia include those detailed in DSM-IV and ICD-10. Criteria specific to dementia subtypes include but are not limited to NINCDS-ADRDA criteria for Alzheimer's dementia; McKeith criteria for Lewy Body dementia; Lund criteria for frontotemporal dementias; and the NINDS-AIREN criteria for vascular dementia. Where the criteria used for assessment is not familiar to the review authors or the Cochrane Dementia and Cognitive Improvement group ('unclear') this item should be classified as "high risk of bias".

Weighting: High risk ('no')

Was clinical assessment for dementia performed without knowledge of the APOE genotype results?

Terms such as "blinded" or "independently and without knowledge of" are sufficient and full details of the blinding procedure are not required. Interpretation of the results of the reference standard may be influenced by knowledge of the results of index test.

Weighting: High risk ('no')

Patient flow

Was there an appropriate interval between APOE genotyping and clinical dementia assessment?

As we test the accuracy of the APOE ϵ_4 allele for MCI progression to

dementia, there will always be a delay between the index test and the reference standard assessments. The time between reference standard and index test will influence the accuracy (Okello 2009; Visser 2006; Geslani 2005), and therefore we will note time as a separate variable (both within and between studies) and will test its influence on the diagnostic accuracy. We have set a minimum mean time to follow-up assessment of nine months. If more than 30% of subjects of subjects have assessment for MCI conversion before nine months this item will score 'no'.

Weighting: High risk ('no')

Did all subjects get the same assessment for dementia regardless of APOE genotyping result?

There may be scenarios where subjects who score "test positive" on index test have a more detailed assessment. Where dementia assessment differs between subjects this should be classified as high risk of bias.

Weighting: High risk (no)

Were all patients who underwent APOE genotyping included in the final analysis?

If the number of patients enrolled differs from the number of patients included in the 2x2 table then there is the potential for bias. If patients lost to follow-up differ systematically from those who remain, then estimates of test performance may differ. If drop outs these should be accounted for; a maximum proportion of drop outs to remain low risk of bias has been specified as 20%.

Weighting: High risk ('no')

Were missing or uninterpretable APOE genotyping results reported?

Where missing or uninterpretable results are reported, and if there is substantial attrition (we have set an arbitrary value of 50% missing data), this should be scored as 'no'. If those results are not reported, this should be scored as 'unclear' and authors will be contacted.

Weighting: High risk ('no' and 'unclear')

2. Anchoring statements to assist with assessment for applicability

Patient selection

Were included patients representative of the general population of interest?

The included patients should match the intended population as described in the review question. The review authors should consider population in terms of symptoms; pre-testing; potential disease prevalence; setting

We recognise that identifying all MCI patients in a given population may be particularly hard to achieve; therefore the information about the judgements for this criterion is particularly likely to be sub-optimal. We expect that all included studies will be sub-optimally reported to some degree. If there is a clear ground for suspecting an unrepresentative spectrum the item should be rated poor applicability.

Index test

Are there concerns that the index test differs from the review question?

In this review we are looking at *APOE* genotype ascertained at baseline. The genotype will not change at follow-up. If the accuracy of the index test was based on genotype results, this item should be rated high applicability.

*Were sufficient data on *APOE* genotyping given for the test to be repeated in an independent study?*

Variation in technology, test execution, and test interpretation may affect estimate of accuracy. A common method of genotyping *APOE* is by extracting genomic DNA from EDTA anti-coagulated blood and amplifying this via polymerase chain reaction. DNA may also be extracted from buccal cell sample. Different genotyping platforms may be used. These platforms have generally comparable accuracy. In addition, the background, and training/expertise of the assessor should be reported and taken in consideration. If *APOE* genotyping was not performed consistently this item should be rated poor applicability.

Reference standard

Was clinical diagnosis of dementia made in a manner similar to current clinical practice?

For many reviews, inclusion criteria and assessment for risk of bias will already have assessed the dementia diagnosis. For certain reviews an applicability statement relating to reference standard may not be applicable. There is the possibility that a form of dementia assessment, although valid, may diagnose a far larger proportion of subjects with disease than usual clinical practice. In this instance the item should be rated poor applicability.

Appendix 6 Standards for Reporting of Diagnostic Accuracy (STARD) checklist

Section and Topic		
TITLE/ABSTRACT KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.
Methods		
<i>Participants</i>	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?
<i>Test methods</i>	7	The reference standard and its rationale.
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.

Appendix 6 Standards for Reporting of Diagnostic Accuracy (STARD) checklist *Continued*

Methods Continued		
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).
	13	Methods for calculating test reproducibility, if done.
Results		
Participants	14	When study was performed, including beginning and end dates of recruitment.
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.
	20	Any adverse events from performing the index tests or the reference standard.
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).
	22	How indeterminate results, missing data and outliers of the index tests were handled.
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.
	24	Estimates of test reproducibility, if done.
DISCUSSION	25	Discuss the clinical applicability of the study findings.

Neuropsychiatric symptoms are common in mild cognitive impairment. In our study, agitation, irritability and reduced depression were related to APOE-ε4.

LS Elias-Sonnenschein, IHGB Ramakers, FRJ Verhey, C Graff, GB Frisoni, P Scheltens,
FM Nobili, M Damian, H Soininen, L Spuru, RW Jones, M Olde Rikkert, G Wilcock,
H Hampel, P Aalten, LM Shaw and JQ Trojanowski for the Alzheimer's Disease
Neuroimaging Initiative, PJ Visser
Submitted

CHAPTER 4

Neuropsychiatric symptoms related to the
APOE ϵ_4 allele in subjects with mild cognitive
impairment in the DESCRIPA and ADNI cohorts

ABSTRACT

Background: Neuropsychiatric symptoms are common in mild cognitive impairment (MCI) and Alzheimer's disease (AD). Their relation with the apolipoprotein E (*APOE*) ϵ_4 allele, a major genetic risk factor for AD, in MCI remains unclear.

Methods: Subjects with MCI were selected from the multicenter "Development of Screening Guidelines and Criteria for Predementia Alzheimer's Disease" (DESCRIPA) and the "Alzheimer's Disease Neuroimaging Initiative" (ADNI) studies. Neuropsychiatric symptoms were assessed using the Neuropsychiatric Inventory (NPI). We tested the relation between *APOE*, the presence of neuropsychiatric symptoms (NPI score >0 , pooled cohort) and the presence of clinically significant symptoms (NPI score >3 , DESCRIPA cohort) by generalized linear models corrected for age, gender and study site.

Results: In the pooled cohort ($N=696$), *APOE*- ϵ_4 was correlated with the presence of agitation (OR=1.51, 95% CI 1.22-1.87, $P<0.001$). Sub-analysis in subjects with amnesic MCI showed in addition correlation between *APOE*- ϵ_4 and irritability (OR=1.24, 95% CI 1.09-1.42, $P=0.001$). In DESCRIPA, *APOE*- ϵ_4 was related with clinically significant delusions (OR=2.74, 95% CI 1.48-5.06, $P=0.001$) and with reduced clinically significant depression (OR=0.64, 95% CI 0.48-0.85, $P=0.002$).

Conclusion: At the MCI stage, *APOE*- ϵ_4 is associated with agitation, irritability and delusions and inversely with depression. These symptoms in part may be related to underlying AD pathology.

Keywords: Neuropsychiatric symptoms, *APOE*, mild cognitive impairment, Alzheimer's disease, agitation, delusion, depression, irritability

INTRODUCTION

Neuropsychiatric symptoms are common in subjects with mild cognitive impairment (MCI).^{1,2} Depression, apathy, agitation and aggression are prevalent symptoms that have been reported in both subjects with MCI and Alzheimer's disease (AD).³⁻⁵ Previous studies in subjects with AD indicated that neuropsychiatric symptoms may be associated with the apolipoprotein E ϵ_4 allele (*APOE* ϵ_4 allele), one of the major genetic risk factors for AD.⁶⁻¹² While it is conceivable that the same neuropsychiatric symptoms that are associated with the *APOE* ϵ_4 allele in AD are manifested in MCI, the role of *APOE* in the neuropsychiatric symptomatology of subjects with MCI is unclear and has not been investigated to date. The aim of our study is therefore to investigate the full spectrum of neuropsychiatric and behavioral symptoms in relation to the *APOE* ϵ_4 allele in subjects with MCI.

METHODS

Subjects

Selected were subjects with MCI from the "Development of Screening Guidelines and Criteria for Predementia Alzheimer's Disease" (DESCRIPA) study, and from the "Alzheimer's Disease Neuroimaging Initiative" (ADNI). DESCRIPA is a European multicenter prospective cohort study concerned with developing screening guidelines and clinical criteria for the early detection of AD in subjects with MCI. It included at baseline subjects aged 55 years or older who were referred to a memory clinic because of cognitive impairment not due to dementia according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV)¹³ criteria or to any psychiatric, somatic or neurological disorder that may have caused the cognitive impairment.¹⁴ Subjects with severe depression were excluded from DESCRIPA. For the present study, we defined MCI as an overall Clinical Dementia Rating (CDR)¹⁵ score of 0.5. We selected subjects from the 13 DESCRIPA centers who were assessed for neuropsychiatric symptoms with the Neuropsychiatric Inventory (NPI) (n=402) of whom 386 had MCI. Of these, 304 (59% females) had available *APOE* genotype data. Subjects with *APOE* data had lower Mini-Mental State Examination (MMSE)¹⁶ scores (26.9 ± 2.4 in subjects genotyped for *APOE* vs. $27.6 \pm$

1.8 in those not assessed for the *APOE* genotype, $P=0.004$), while age and education did not differ between the groups ($70.5y \pm 8.1$ vs. $70.6y \pm 7.1$, $p=0.88$ for age and $10y \pm 4.2$ vs. $10.2y \pm 4.7$, $P=0.66$ for education).

ADNI data used in the preparation of this article were obtained from the ADNI database (adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a \$60 million, 5- year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research, approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years and 200 people with early AD to be followed for 2 years." For up-to-date information, see www.adni-info.org.

ADNI included at baseline subjects aged 55 to 90 years clinically diagnosed with probable AD according to the National Institute of Neurological and Communication Disorders-Alzheimer's Disease and Related Disorders Association (NINCDS- ADRDA)¹⁷ criteria, amnesic MCI (aMCI), or normal cognition.¹⁸ Among the exclusion criteria were the presence of neurological disease other than AD, brain lesions or head trauma, the use of psychoactive medication, major depression and psychotic features as detailed in the ADNI

protocol (<http://www.adni-info.org>). Subjects with aMCI who had available NPI scores and *APOE* data (n=392) were included.

At baseline, all subjects underwent routine physical and neurological examinations; and were assessed using the MMSE and other standardized neuropsychological tests, as described elsewhere.^{14,}
¹⁸ The medical ethical commission of the participating centers has approved all procedures, and all participants gave their written informed consent.

Assessment of neuropsychiatric symptoms

We used the 12-item NPI,¹⁹ which is informant-rated and evaluates the following symptoms: delusions, hallucination, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor behavior, nighttime behavior, and appetite/eating abnormalities. The DESCRIPA study made use of the full NPI, which indicated the presence or absence of a symptom (scored as 1=present and 0=absent), its frequency (range: 1-4, wherein 1=occasionally, 2=often, 3=frequently, and 4=very frequently) and severity (range: 1-3, corresponding respectively to mild, moderate, and severe). In cases wherein a symptom was present, a composite score for this symptom was computed by multiplying the frequency by the severity. A composite score of ≥ 4 is considered clinically significant, that is, the symptom is considered severe enough to disrupt a subject's functioning and merits treatment.²⁰ In the ADNI study, the NPI-questionnaire (NPI-Q)²¹ was used, which only indicated whether or not a symptom was present, and the symptom severity. The NPI-Q was scored as symptom present or absent. The NPI-Q has been cross-validated with the NPI and has been shown to have good test-retest reliability,²¹ which allowed us to pool the scores for both instruments.

***APOE* genotyping**

APOE genotype was determined on genomic DNA extracted at baseline from 10 ml EDTA anti-coagulated blood and amplified using the TaQMan polymerase chain reaction technique (Applied Biosystems, Foster City, CA) for the ADNI cohort,¹⁸ and the SNaPshot technique for the DESCRIPA cohort.²²

Table 1 Subject characteristics and prevalence of neuropsychiatric symptoms

Characteristic	Pooled cohorts <i>N</i> =696	DESCRIPA <i>n</i> =304	ADNI <i>n</i> =392
Age, y	72.9 ± 8.0	70.6 ± 8.1	74.7 ± 7.4
Gender, % f	46	59	35
Education, y	13.2 ± 4.7	10.0 ± 4.2	15.7 ± 3.1
MMSE	27.0 ± 2.1	26.9 ± 2.4	27.0 ± 1.8
<i>APOE</i> ε4 ⁺ /ε4 ⁻ (% ε4 ⁺)	349/347 (50)	139/165 (46)	210/182 (54)
Prevalence of neuropsychiatric symptoms, <i>n</i> (%), <i>n</i> ε4 ⁺ /ε4 ⁻			
Delusions	21 (3), 10/11	16 (5), 8/8	5 (1), 2/3
Hallucinations	11 (2), 6/5	10 (3), 5/5	1 (0.3), 1/0
Agitation	122 (18), 72/50	50 (16), 27/23	72 (18), 45/27
Depression	187 (27), 94/93	111 (37), 48/63	76 (19), 46/30
Anxiety	172 (25), 92/80	101 (33), 48/53	71 (18), 44/27
Euphoria	25 (4), 11/14	14 (5), 5/9	11 (3), 6/5
Apathy	148 (21), 78/70	92 (30), 47/45	56 (14), 31/25
Disinhibition	52 (7), 29/23	22 (7), 8/14	30 (8), 21/9
Irritability	204 (29), 108/96	96 (32), 46/50	108 (28), 62/46
Aberrant motor behavior	39 (6), 23/16	19 (6), 13/6	20 (5), 10/10
Night-time behavior	139 (20), 64/75	94 (31), 38/56	45 (12), 26/19
Eating abnormalities	90 (13), 49/41	45 (15), 25/20	45 (12), 24/21

Abbreviations: f=female, y=years, MMSE=Mini-Mental State Examination, ε4⁺=*APOE* ε4 allele carrier, ε4⁻=*APOE* ε4 allele noncarrier

Note: All data are mean ± standard deviation, unless otherwise specified.

Statistical analyses

The IBM SPSS version 19 for Mac Os X (SPSS Inc., USA, 2010) was used. Group comparisons on mean age, education and MMSE scores were performed using independent samples t-test. Generalized linear models (GLM) corrected for age, gender and study site were used to analyze the relation between *APOE*-ε4 carriership and the presence of neuropsychiatric symptoms (item score of 0 vs. item score > 0). These analyses were conducted in the pooled DESCRIPA and ADNI cohorts, and in each cohort separately. When the correlation between *APOE* and neuropsychi-

atric symptom was significant, we tested for possible interaction effects with age and gender.

In the DESCRIPA study, the relation between *APOE*- ϵ_4 carriership and the presence of clinically significant neuropsychiatric symptoms (composite score ≥ 4) was also analyzed. We also examined the effects of psychopharmacological medications (anti-depressives and anxiolytics/sedatives) by subgroup analyses to investigate whether results significantly differed from those who did and did not use these medications.

Because the ADNI study only included subjects with aMCI and the DESCRIPA study included both subjects with aMCI and non-amnesic MCI (naMCI), we repeated the GLM analyses after exclusion of subjects with naMCI. The significance level for the main effects between *APOE* and neuropsychiatric symptoms was set at $P=0.004$, which was obtained by dividing the preset $P=0.05$ by the 12 neuropsychiatric items, following Bonferroni correction for multiple testing.

RESULTS

The pooled DESCRIPA and ADNI cohorts consisted of 696 subjects. Their baseline characteristics and symptom prevalence are summarized in Table 1. Of the 349 subjects who carried at least 1 *APOE* ϵ_4 allele (50%), 73 (10% of the whole sample) were homozygotes. The most common neuropsychiatric symptoms were irritability (29%), depression (27%), and anxiety (25%). In ADNI, agitation was also a common symptom (18%). The least common symptoms in both the pooled cohorts and independent cohorts were euphoria, delusions and hallucinations (all $<5\%$). In general, the prevalence of neuropsychiatric symptoms in DESCRIPA was higher compared to that in ADNI. In the pooled DESCRIPA and ADNI cohorts, *APOE* ϵ_4 allele carriers more often had symptoms of agitation compared to noncarriers (21% vs. 14%, OR=1.51, 95% CI 1.22-1.87, $P<0.001$) (Table 2). Exploratory analysis suggested an interaction between agitation and age (uncorrected $P=0.04$), as well as between agitation and gender (uncorrected $P=0.02$). The *APOE* ϵ_4 allele was related to agitation among subjects who were older than 70 years (OR=1.80, 95% CI 1.29-2.50, $P=0.001$) but not in younger subjects. Agitation was more common among males than females (68% vs. 32%) but was significantly related to the *APOE* ϵ_4 allele only among females

Table 2 Association between *APOE* ϵ_4 allele and neuropsychiatric symptoms in MCI

NPI Items	Presence of symptoms ^a			Clinically significant symptoms ^b
	Pooled cohorts OR (95% CI)	ADNI OR (95% CI)	DESCRIPA OR (95% CI)	DESCRIPA OR (95% CI)
Delusions	0.92 (0.49-1.72)	0.55 (0.09-3.37)	1.33 (0.75-2.38)	2.74** (1.48-5.06)
Hallucinations	1.38 (0.65-2.93)	---	1.50 (0.68-3.34)	1.63 (0.43-6.13)
Agitation	1.51** (1.22-1.87)	1.59 (0.94-2.71)	1.37 (0.84-2.24)	0.75 (0.40-1.38)
Depression	0.94 (0.68-1.30)	1.37 (0.82-2.29)	0.79 (0.58-1.07)	0.64** (0.48-0.85)
Anxiety	1.19 (0.88-1.61)	1.47 (0.86-2.50)	1.18 (0.72-1.93)	1.36 (0.78-2.36)
Euphoria	0.77 (0.34-1.75)	1.06 (0.32-3.54)	0.67 (0.15-3.01)	---
Apathy	1.13 (0.86-1.48)	1.16 (0.65-2.07)	1.37 (0.83-2.25)	0.47 (0.47-1.48)
Disinhibition	1.25 (0.66-2.39)	2.11 (0.94-4.75)	0.64 (0.40-1.03)	--- ^c
Irritability	1.13 (0.91-1.39)	1.25 (0.79-1.97)	1.06 (0.71-1.58)	1.25 (0.74-2.11)
Aberrant motor behavior	1.47 (0.70-3.09)	0.89 (0.36-2.20)	2.85* (1.10-7.39)	2.21 (0.68-7.14)
Night-time behavior	0.79 (0.52-1.21)	1.16 (0.61-2.18)	0.77 (0.37-1.63)	0.61 (0.24-1.59)
Eating abnormalities	1.30 (0.93-1.82)	1.08 (0.57-2.04)	1.75* (1.02-3.03)	1.74 (0.61-4.94)

Abbreviations: NPI=neuropsychiatric inventory, OR=odds ratio, CI=confidence interval

- ^a item score ≥ 1 .
- ^b composite score ≥ 4 .
- ^c not computed because of insufficient number of subjects.
- * $P < 0.05$, no longer significant after Bonferroni correction
- ** $P < 0.004$, remained significant after Bonferroni correction

(OR=2.57, 95% CI 1.44-4.57, $P=0.001$). In order to correct for possible effects of cognitive impairment severity, we repeated analysis with correction for MMSE score post hoc. The results were essentially the same. The *APOE* ϵ_4 allele was associated with agitation (OR=1.54, 95% CI 1.25-1.90, $P < 0.001$). There was no significant interaction between the *APOE* ϵ_4 allele and MMSE score (OR=1.14, 95% CI 0.93-1.40, $P=0.22$).

In the DESCRIPA cohort, *APOE* ϵ_4 allele carriers more often had aberrant motor behavior (OR=2.85, 95% CI 1.10-7.39, $P=0.03$) and eating

abnormalities (OR=1.75, 95% CI 1.02-3.03, $P=0.04$). Similar results were obtained when we adjusted for the use of anxiolytic/sedatives. None of the results remained significant after Bonferroni correction, indicating that *APOE* is not a robust predictor of aberrant motor behavior and eating abnormalities in this cohort. These symptoms were also not related with the use of antidepressives/antipsychotics. Analyses with clinically significant symptoms as outcome showed that *APOE* ϵ_4 allele carriers less often had clinically significant symptoms of depression (OR=0.64, 95% CI 0.48-0.85, $P=0.002$). These associations remained statistically significant after correcting for multiple testing. No significant interaction was found between depression, and age ($P=0.76$) and gender ($P=0.25$). Adjusting for the use of antidepressants yielded similar results: symptoms of depression were higher among *APOE* ϵ_4 allele noncarriers. We observed that *APOE* ϵ_4 allele carriers had more frequent symptoms of delusions compared to noncarriers, but the small number of subjects with delusions did not allow for meaningful analyses.

In ADNI, the *APOE* ϵ_4 allele was not significantly associated with any neuropsychiatric symptom.

We repeated the analyses in the pooled DESCRIPA and ADNI cohorts ($n=545$) after exclusion of subjects with naMCI in DESCRIPA ($n=151$). The association between agitation and *APOE*- ϵ_4 status remained essentially the same. In addition, *APOE*- ϵ_4 was now associated with irritability (OR=1.24, 95% CI 1.09-1.42, $P=0.001$). There was no significant interaction with age and gender ($P=0.63$ and $P=0.75$, respectively).

In subjects with aMCI ($n=153$, 62% females, mean age=71.5 $\pm$ 8.0, mean education=9.9 $\pm$ 4.0y, mean MMSE=26.4 $\pm$ 2.4) from the DESCRIPA cohort, *APOE* ϵ_4 allele carriers frequently had symptoms of delusions (OR=3.81, 95% CI 1.88-7.69, $P<0.001$). Other symptoms were not associated with *APOE*.

DISCUSSION

Our findings show that the previously reported associations between the *APOE* genotype and the neuropsychiatric symptoms of agitation, irritability, depression and delusions in AD^{8, 23-26} are already evident at the MCI stage. The prevalence of these symptoms in our study is in accordance

with the literature.^{1, 2} The symptoms were less frequent but had similar patterns in dementia and AD.^{2, 27}

Agitation and irritability are common neuropsychiatric symptoms in MCI^{1, 3, 4} and have been reported to be more prevalent in AD than in MCI.^{28, 29} In our study, the *APOE* ϵ_4 allele was also related to agitation and irritability. This is in line with previous findings in AD showing the *APOE* ϵ_4 allele to be a risk factor for agitation and aggression.^{8, 23, 24}

Age and gender interacted with the *APOE* ϵ_4 allele and agitation. While age has been shown to modify the effect of *APOE* on conversion from MCI to AD and on AD neuropathology,³⁰ it has been reported to be unrelated to neuropsychiatric symptoms in MCI.³¹ A follow-up study may help clarify whether agitation among elderly *APOE*- ϵ_4 carriers signals early pathology or is independent of the neuropsychiatric symptom. The *APOE* ϵ_4 allele was significantly associated with agitation among females, although agitation was more prevalent among males. This suggests that agitation among males is independent of the *APOE*- ϵ_4 status.

Depression has been reported to be highly prevalent in MCI compared to cognitively normal subjects.⁴ It has been associated with increased risk of developing MCI and conversion to AD although others did not find this association.^{32, 33} In the present study, we found that clinically significant symptoms of depression were less common among *APOE* ϵ_4 allele carriers. Our findings do not support the risk for depression in the presence of the *APOE* ϵ_4 allele among subjects with MCI. This is consistent with studies in AD that showed a protective effect of the *APOE* ϵ_4 allele on depression.^{9, 34}

The relation between clinically significant delusions and *APOE* has also been reported in subjects with AD.^{25, 26} In contrast, a recent genome-wide association study of AD with psychosis³⁵ and a review of currently known genetic basis of psychosis in AD¹¹ reported that neither *APOE*- ϵ_4 status nor *APOE*- ϵ_4 dose were associated with psychosis. Delusions and hallucinations are commonly seen in AD^{36, 37} but are less common in MCI,^{1, 4, 38} as confirmed by the low prevalence of these symptoms in our study. *Post hoc* analyses showed that delusions mainly occurred in subjects with aMCI. Because subjects with aMCI are more likely to progress to AD than subjects with naMCI,¹ our findings suggest that delusions signal early underlying pathology, although the low prevalence of

delusions does not allow firm conclusions.

There are several possible explanations for the relation between *APOE* and neuropsychiatric symptoms. The *APOE* ϵ_4 allele may cause neuropsychiatric symptoms through its effects on brain atrophy. The *APOE* ϵ_4 allele has been shown to correlate with increased rate of hippocampal atrophy among subjects with MCI.^{39, 40} Neuroimaging studies showed that in aMCI, delusions and disinhibition were correlated with gray matter (GM) atrophy in the medial temporal lobe and anterior cingulate gyrus.²⁷ These confirm findings in mild AD, wherein agitation, delusions and also apathy have been reported to correlate with cortical atrophy.⁴¹ Depression was not associated with GM atrophy in aMCI.²⁷ Among older people with no dementia but with late-life depression, depression was related to hippocampal neuronal loss but not to Alzheimer pathology.⁴²

Alternatively, the *APOE* ϵ_4 allele may cause neuropsychiatric symptoms through its effect on beta amyloid processing. It has been shown to increase beta-amyloid deposition in the frontal cortex of subjects who were not demented.⁴³ We previously showed that irritation and agitation were related to abeta concentrations in the cerebrospinal fluid of subjects with MCI.⁴⁴

Neuropsychiatric symptoms may also be due to the effect of *APOE* on synaptic acetylcholine concentrations and cholinergic function.⁴⁵⁻⁴⁷ *APOE*- ϵ_4 has been shown to interact with the cholinergic system by altering the activity of acetylcholinesterase (AChE) and choline acetyltransferase (ChAT), which are needed for the formation of acetylcholine.^{48, 49} In AD, AChE and ChAT activities have been reported to be lower among *APOE*- ϵ_4 carriers, indicating cholinergic neurodegeneration,^{47, 48} although increased cholinergic function among *APOE* carriers have also been reported in subjects with MCI and mild AD.^{49, 50} Reduced cholinergic function has been correlated with increased agitation and reduced depression, the latter being a result of disrupted neural mechanisms for expressing depression.⁴⁵ This is consistent with studies that showed that a hypercholinergic state reduced agitation and induced depression due to reduced noradrenergic activity.^{50, 51} This observation is consistent with our study in which the *APOE* ϵ_4 allele was related to increased agitation and reduced depression.

The effect of the *APOE* genotype on neuropsychiatric symptoms may also result from the subjects' psychological reaction to incipient dementia.

In a *post hoc* analysis, disease severity as assessed by the MMSE did not change our results. Given the small range in the MMSE score of subjects included in the analyses, the MMSE may not be a sensitive tool for measuring disease severity that could affect the manifestation of neuropsychiatric symptoms.

We performed our study in two independent cohorts. The relation between agitation and *APOE* genotype was similar in both cohorts although it did not reach statistical significance in each cohort separately. In examining the effects of medication and exclusion of subjects with naMCI in DESCRIPA, we obtained essentially similar findings.

As far as we know, ours is the first study to investigate the association between *APOE* and the full spectrum of neuropsychiatric and behavioral symptoms in MCI. Among the strengths of this study are the large sample size, two independent well-defined study populations, and the use of the same well-validated assessment tool in both cohorts. These enabled us to perform various statistical analyses while correcting for covariates and the effects of the multicenter nature of the pooled cohorts. We were able to examine the relationship between *APOE* and neuropsychiatric symptoms according to presence and clinical significance of symptoms. Because the subjects in our study were recruited from memory centers or research settings, our findings may have limited generalizability outside of these settings. Neuropsychiatric symptoms may be transient and may also reflect the subjects' reaction to cognitive changes. Longitudinal study will help clarify the role of *APOE* in neuropsychiatric symptoms among subjects with MCI and determine whether they have an increased risk of converting to AD or other types of dementia.

In conclusion, our findings show that at the MCI stage, the symptoms of agitation and delusions reported in AD and associated with the *APOE* ϵ_4 allele were already evident. The association between *APOE* and agitation is modest. Clinically significant symptoms of delusions among aMCI *APOE*- ϵ_4 carriers warrant further investigation to find out if these subjects are at an increased risk of developing AD or other dementia.

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Abnormal concentrations of amyloid beta ($A\beta$) and tau may indicate underlying AD pathology.

We reviewed the effects of the most compelling genetic risk factors for AD on $A\beta$ and tau.

CHAPTER 5

Relationship between genetic risk factors and markers for Alzheimer's disease pathology

ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by neuritic plaques (main constituent: amyloid beta, A β) and neurofibrillary tangles (hyperphosphorylated tau protein) in the brain. Abnormalities in A β and tau can be measured upon neuropathological examination, in cerebrospinal fluid (CSF) or by positron emission tomography (PET). Etiologically, a growing body of evidence suggests that susceptibility to AD is genetically controlled. However, the precise nature of the underlying risk genes and their relation to AD biomarkers remains largely elusive. To this end, we performed a qualitative review of 17 studies (covering 47 polymorphisms in 26 genes) and investigated the potential relation between the most compelling AD risk genes and markers for A β and tau in CSF, PET imaging and neuropathological examination. Of all covered genes, only *APOE* and *PICALM* showed consistent effects on A β but not on tau, while no obvious effects were observed for *CLU*, *CR1*, *ACE*, *SORL* and *MAPT*.

Keywords: Alzheimer, AlzGene, genetics, amyloid, biomarker, CSF, genetics, GWAS, pathology, SNP, tau

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by senile or neuritic plaques and neurofibrillary tangles (NFTs).^{1,2} The main component of neuritic plaques is amyloid beta ($A\beta$), whereas NFTs consist of hyperphosphorylated tau (p-tau) protein.^{2,3} Abnormalities in $A\beta$ and tau can be measured on neuropathological examination, or in-vivo in cerebrospinal fluid (CSF) or by positron emission tomography (PET) imaging.⁴⁻⁷ Genetically, AD represents a so-called complex disease showing a great deal of genetic (and phenotypic) heterogeneity. To date, more than 250 autosomal dominant mutations have been identified in genes that affect $A\beta$ production or metabolism (*APP*, *PSEN1*, *PSEN2*; see ⁸ for a recent review). However, these mutations account for only a small fraction of the disease. Recently, a number of genetic risk factors for AD has been identified but their relation to AD pathology remains unclear.⁹ Here, we performed a qualitative review of studies – including genome-wide association studies (GWAS)— investigating the potential role of genetic risk factors in the pathophysiology of AD by correlating them with in-vivo amyloid and tau biomarkers and neuropathological markers for AD.

AD pathophysiology

According to the amyloid cascade hypothesis, AD is the result of an imbalance between the production and clearance of $A\beta$ in the brain.^{2,10} Aggregated $A\beta$ is supposed to be neurotoxic and to initiate a cascade of events, including abnormal tau phosphorylation and aggregation, oxidative stress, inflammation and dysregulation of lipid metabolism.⁶ The soluble oligomeric forms of $A\beta$, such as dimers of high molecular weight oligomers, are considered the main toxic species.¹¹⁻¹³ They have been reported to interfere with synaptic functioning¹¹ and to have a higher correlation with synaptic loss and cognitive impairment severity compared to non-soluble aggregated $A\beta$.¹³

Different isoforms of the $A\beta$ peptide are generated from the amyloid precursor protein (APP) by α , β and γ secretases. In the non-amyloidogenic pathway, APP cleavage by α -secretase occurs within the $A\beta$ region, thus precluding the formation of $A\beta$.¹⁴ In the amyloidogenic metabolic pathway, APP is first cleaved in its extracellular domain by

the β -site APP cleaving enzyme 1 (BACE1; a.k.a. β -secretase) to generate soluble APP (sAPP) containing the entire A β sequence,¹⁵ which is subsequently cleaved in the transmembrane region by γ -secretase to produce A β_{1-40} and A β_{1-42} .¹

In AD, especially A β_{1-42} seems to be important as it is more prone to aggregate compared to other isoforms.¹ The increase in aggregated A β may be caused by either increased production, disturbance of the post-translational modification or reduced clearance of A β .^{1, 2} Abnormal CSF concentrations of A β_{1-42} can indicate if there is abnormal trafficking of this peptide and are correlated to amyloid plaques, whereas total tau (t-tau) and phosphorylated tau (p-tau) concentrations are related to neuronal degeneration.¹⁶ CSF A β_{1-42} is decreased while t-tau and p-tau are increased among subjects with AD.² This AD profile has been shown to be predictive of progression to AD among subjects in the pre-dementia stage.¹⁷ Compared to A β_{1-42} , the A β_{1-42} /A β_{40} ratio has been reported as a better predictor of incipient AD,¹⁸ whereas p-tau/A β_{1-42} and t-tau/A β_{1-42} ratios are better predictors of AD than each marker individually have been reported to distinguishing those with AD from healthy controls.¹⁹⁻²¹

Genetics of AD

AD presents clinically as familial AD with Mendelian disease transmission or sporadic AD with no obvious familial aggregation (non-Mendelian).⁹ Familial AD with Mendelian inheritance usually have an onset age of below 65 years (early onset familial AD, EOFAD) and account for less than 5% of all AD cases.^{3, 9, 22} The majority of AD cases are of the non-Mendelian forms ('sporadic AD') and typically have an onset age after age 65 years (late-onset AD, LOAD).⁸ It is thought that familial AD is caused by abnormalities in the production of A β , while sporadic AD is mainly the result of inefficient A β clearance from the brain.²

Mendelian forms of AD

Highly penetrant mutations in *APP*, presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) genes have been identified to cause autosomal dominant EOFAD.^{2, 23} The *APP* gene encodes APP, whose physiological role remains largely elusive but which has been reported to promote neuritic outgrowth and synaptogenesis.²⁴ AD-causing APP mutations are typi-

cally located near the A β -sequence and affect proteolytic processing by BACE1 and/or γ -secretase. This typically leads to an increase in the A β_{1-42} /A β_{1-40} ratio, with or without a concomitant increase in total A β -levels.²⁵ Presenilins, i.e. *PSEN1* and *PSEN2*, encode proteins that constitute the catalytic center of the γ -secretase complex.²⁶ Mutations in *PSEN1* and *PSEN2* alter the proteolytic site for APP cleavage thereby also affecting the A β_{1-42} /A β_{1-40} ratio.²⁵ Eventually, the changes induced by the currently known missense mutations in all three genes result in A β accumulation and deposition in neuritic plaques.^{1, 3, 27-30} Non-AD type dementia has also been linked to *PSEN1* as not all mutations in *PSEN1* lead to increased A β production.³¹ *APP*, *PSEN1* and *PSEN2* mutations account for less than 5% of all early onset familial AD, and for 30-50% of all AD cases.³²

A recent study has identified mutations in the sortilin-related receptor (*SORL1*) gene among patients with autosomal dominant early onset AD.³³ *SORL1* encodes the protein SorLA, which is involved in modulating A β peptide production,³³ and is part of a group of protein-trafficking molecules in the endocytic and retromer pathways, which are sites for A β peptide production.³⁴ These novel data suggest that *SORL1* may represent a genetic risk factor for both early and late-onset forms of AD, although these findings still await independent replication.

Non-Mendelian forms of AD

Association studies using disease state as outcome

Hypothesis-driven candidate gene association studies, e.g. focused on proteins suspected or known to be involved in the production or clearance of A β , have led to the discovery of the association between the apolipoprotein E (*APOE*) gene and AD in 1993.³⁵ *APOE* has three isoforms (ϵ_2 , ϵ_3 and ϵ_4) and six genotypes ($\epsilon_2\epsilon_2$, $\epsilon_2\epsilon_3$, $\epsilon_2\epsilon_4$, $\epsilon_3\epsilon_3$, $\epsilon_3\epsilon_4$ and $\epsilon_4\epsilon_4$) resulting from a combination of two single nucleotide polymorphisms (SNPs). *APOE*, particularly its ϵ_4 -allele, has been observed to have a high-avidity binding to A β .^{36, 37} *APOE* binds to A β in cerebrospinal fluid (CSF), transports cholesterol and other lipids in the brain, and modulates neuronal repair. The *APOE* ϵ_4 allele is less efficient in carrying out these functions.^{1, 2} In addition, this allele has been reported to stimulate aggregation of A β *in vitro* and deposition of amyloid plaques *in*

vivo.^{38, 39} Until recently, only the ϵ_4 -allele of *APOE* had been established as a bona fide genetic risk factor for sporadic AD.^{36, 40-42} At the pre-dementia stage, the *APOE* ϵ_4 allele has been correlated with a moderately increased risk of developing AD-type dementia.⁴³

GWAS in the past years have identified a number of SNPs associated with clinical AD or with AD biomarkers. Compared to *APOE*, these SNPs exert only small risk effects, which when taken in combination, may have a considerable role in the development of AD. Early case-control GWAS with sample sizes of typically less than 1,000 have identified other susceptible loci⁴⁴⁻⁵⁰ (for a review, see Bertram 2010⁹ and the AlzGene database⁴¹). Except for *APOE*, these findings could not be replicated in more recently conducted GWAS including much larger sample sizes. Instead, these latter studies have identified other genetic risk factors which can now be considered as established AD risk genes: clusterin (*CLU*),^{23, 51} phosphatidylinositol binding clathrin assembly protein (*PICALM*),^{51, 52} siglec3 (*CD33*),^{53, 54} and ATP-binding cassette subfamily A member 7 (*ABCA7*), membrane-spanning 4 domains, subfamily A, member 6A (*MS4A6A*), membrane-spanning 4-domains, subfamily A, member 4E (*MS4A4E*), EPH receptor A1 (*EPHA1*), and CD2-associated protein (*CD2AP*).^{53, 55}

Association studies using biomarker levels as outcome

Biomarker association studies aim to identify susceptibility loci correlated with biomarker levels, either as binary ('normal' vs. 'abnormal') or quantitative (e.g. raw or normalized concentrations) traits in a given study population. As for the disease state studies outlined above, there exist a number of hypothesis-driven studies⁵⁶⁻⁵⁸ and, more recently, genome wide analyses in the form of GWAS. How to interpret the results from either approach remains a challenge, given the numerous susceptibility loci that have been identified but not replicated, the inconsistency in findings and the heterogeneity of the trait in the population(s) under study.

METHODS

Search strategy and inclusion criteria

Three approaches were used to identify genes for inclusion in this review. First, we selected SNPs from genes listed in the 'Top Results' list of AlzGene,⁴¹ a regularly updated online database of published genetic association studies in AD. These included *APOE*, *CLU*, *PICALM*, *CD33*, *ABCA7*, *MS4A6A*, *MS4A4E*, *CD2AP*, bridging integrator 1 (*BIN1*) and complement receptor (3b/4b) 1 (*CR1*). On AlzGene, cross-study meta-analyses for disease risk (but not quantitative) association studies are provided for all polymorphisms with a minor allele frequency of more than 1% in healthy controls and for which genotype data in four or more independent case-control samples are available for the same polymorphism.⁴¹

Second, we chose a number of additional candidate genes for AD, which were selected since multiple independent studies indicated that these could represent promising genetic risk factors for AD. These genes are angiotensin-converting enzyme (*ACE*), microtubule-associated protein tau (*MAPT*), translocase of outer mitochondrial membrane 40 homolog (*TOMM40*) and *SORL1*.^{34, 56, 59-68} *ACE* is an endopeptidase associated with hypertension and cardiovascular diseases.⁶⁹ At the same time, it mediates the degradation of $A\beta_{1-42}$ to $A\beta_{1-40}$. Inhibition of *ACE* results in increased $A\beta$ deposition.⁷⁰ *MAPT* codes for the tau proteins, which stabilizes microtubules and maintains the integrity of neurons, axonal transport and polarity.⁷¹ Tau hyperphosphorylation and aggregation results in NFTs, the overabundance of which is one of the causes of neurodegeneration. *TOMM40* controls the outer mitochondrial membrane pore subunit (Tom40) through which proteins enter the mitochondria, and as such is crucial for the survival of the mitochondria.⁷² Mitochondrial dysfunction that leads to APP accumulation and $A\beta$ cellular influx has been hypothesized to be the key pathological event in AD.⁷²⁻⁷⁴ *SORL1* is involved in $A\beta$ production, as previously explained.

Finally, we identified SNPs highlighted in published biomarker GWAS.⁷⁵⁻⁷⁷ In all instances, we arbitrarily limited the number of SNPs to be included for each gene to five.

The PubMed database was then searched for publications in English using the broad terms 'Alzheimer', 'SNPs', 'biomarker' and

'genes'. The search yielded 240 hits, covering the period 2000-2012. Their titles and abstracts were screened. Sixty studies pertaining to diseases other than Alzheimer and/or to animal studies, and 2 studies not written in English were excluded. Of the remaining 178, we further excluded 40 studies that did not have data on biomarkers; 42 studies of SNPs that were not selected for our review; 23 studies that were reviews, commentaries or part of a seminar series; 16 studies that measured mRNA, gene or protein expression; 10 neuroimaging studies; 11 studies that did not have genetic data; 7 studies on cognition; 2 studies on plasma markers; 10 other irrelevant studies; and 3 duplicates. Studies that had data on AD-related SNPs and biomarker correlations according to abovementioned criteria were included. Additional references were identified from the reference list of the reviewed articles. For *CR1* and *SORL1*, more than five different SNPs were identified that had been tested in relation to AD markers. For *CR1* only SNPs that were listed in AlzGene were included in the review.

For *SORL1*, only the SNPs that were statistically significantly correlated with clinical AD in a recent meta-analysis were selected.³⁴ From the AlzGene 'Top Results' loci, studies with biomarker outcome have been conducted on *APOE*, *BIN1*, *CLU*, *CR1*, and *PICALM*. ORs originating from case-control studies of the SNPs from the top AlzGene loci or from the candidate genes from the AlzGene meta-analysis are shown in Table 1. The final list of 26 genes and 47 SNPs included in our review is shown in Tables 2-4.

Data extraction

A data extraction file was completed for each study. Studies were classified into three categories: (1) AlzGene 'Top Result' genes with biomarkers as outcome, (2) candidate genes with biomarkers as outcome, and (3) GWAS with biomarkers as outcome. The outcomes for biomarkers were A β and tau, specified as to whether they were measured in CSF, PET imaging or on neuropathological examination. If studies provided multiple outcome measures for a single marker, we only chose the outcome measure that was most indicative of AD pathology. The odds ratio or p-value, uncorrected for multiple testing if available, for the biomarker correlation was extracted. Information on minor and risk alleles

Table 1 Odds ratios from AlzGene meta-analyses for SNPs included in this review

Gene	SNP	Minor	Risk	OR (95% CI)
AlzGene 'Top Results'^a				
<i>APOE</i>		$\epsilon 2$	$\epsilon 4$	3.68 (3.30-4.11)
<i>BIN1</i>	rs744373	G	G	1.17 (1.13-1.20)
<i>CLU</i>	rs11136000	A	G	0.89 (0.86-0.91)
<i>CR1</i>	rs3818361	T	T	1.15 (1.11-1.20)
	rs1408077	A	A	1.13 (1.06-1.20)
	rs6656401	A	A	1.19 (1.09-1.30)
<i>PICALM</i>	rs3851179	A	G	0.88 (0.86-0.91)
	rs541458	C	T	0.88 (0.85-0.91)
	rs7110631 ^b	na	na	na
Other SNPs in AD candidate genes				
<i>ACE</i>	rs1800764	C	T	0.88 (0.80-0.97)
	rs4291	T	A	0.88 (0.81-0.96)
	rs4343	G	A	0.96 (0.82-1.12)
<i>SORL1</i>	rs2070045 (SNP19)	G	G	1.12 (1.04-1.20)
	rs641120 (SNP10)	T	C	0.93 (0.87-1.00)
	rs661057 (SNP4)	C	T	0.92 (0.85-0.99)
	rs668387 (SNP8)	T	C	0.93 (0.88-0.99)
	rs689021 (SNP9)	A	G	0.94 (0.89-1.00)
<i>MAPT</i>	rs2471738	T	T	1.14 (0.98-1.33)
	rs242557	A	A	1.10 (0.98-1.23)
<i>MAPT H1/H2</i>	rs1052553	H2 (G)	H2 (G)	1.01 (0.94-1.07)
<i>TOMM40</i>	rs157580	G	A	0.60 (0.56-0.65)
	rs157581	C	C	2.57 (2.15-3.06)
	rs2075650	G	G	2.79 (2.38-3.27)

Abbreviations: SNP=single nucleotide polymorphism; OR=Odds ratio; CI=confidence interval; na=not available

^a For more details, consult the AlzGene database.⁴¹

^b One study 87 used rs7110631 as proxy for this SNP (not included in meta-analysis).

were obtained from the AlzGene for SNPs with available meta-analysis data. In general, an odds ratio of <1 indicates that the allele is protective, whereas an odds ratio of >1 is suggestive of a risk allele.

Results are presented per study population (AD, mild cognitive impairment,¹² controls and combined), as applicable. Other demographic data extracted were study setting (clinical, population or mixed), cohort and mean age of the study sample.

RESULTS

Seventeen studies were included in the review, of which 14 assessed biomarkers in CSF, 1 with amyloid PET, and 5 by neuropathological examination. Data on pathology originated from three prospective cohort studies while two came from convenience sampling. Four studies were performed in the general population, four in a clinical setting, and nine in a mixed setting. Three studies only included healthy controls, two studies with only AD, and five studies included subjects with MCI. The remaining studies selected subjects from multiple diagnostic categories and presented data for the pooled sample ($n=7$) or for separate diagnostic groups ($n=4$).

One study classified subjects as CSF positive (CSF⁺) or CSF negative (CSF⁻), regardless of the AD, MCI or control diagnosis. CSF⁺ were subjects with a low CSF A β_{1-42} (<192 pg/ml) and high p-tau (>23 pg/ml), which indicated AD pathology, whereas CSF⁻ were those with high CSF A β_{1-42} (>192 pg/ml) and low p-tau (<23 pg/ml), which was an unlikely AD profile.⁷⁸ Subjects who were neither CSF⁺ nor CSF⁻ were excluded from the study.

Another study used ¹¹C-Pittsburgh Compound-B-Positron Emission Tomography (PiB-PET).⁷⁹ PiB binds to fibrillar A β and thus measures A β deposition in the brain.

One study measured senile plaques and NFTs from various areas of the brain.⁸⁰ We included here only the results for the middle frontal lobe as this is a more vulnerable area in AD compared to other brain areas measured.

A GWAS classified subjects with a Braak score of 2 or lower as low Braak, and those with a score of 4 or higher as high Braak.⁷⁷ Subjects with a score of 3 were excluded.

Biomarkers and top results AlzGene SNPs

APOE

In CSF, the *APOE* ϵ_4 allele was consistently linked with lower $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratio. It did not correlate with total $A\beta$ concentration.⁵⁶ One population study using (PiB-PET) reported higher PiB retention among cognitively normal *APOE* ϵ_4 allele carriers.⁷⁹ In neuropathological studies, $A\beta$ deposition in senile plaques was related to carriership of the *APOE* ϵ_4 allele as well.⁸⁰⁻⁸² Except in one study,⁸³ the effect of *APOE* on $A\beta$ was present in both AD, MCI, control or mixed populations.

The *APOE* ϵ_4 allele also correlated with the AD profile of low $A\beta$ and high p-tau levels in CSF⁷⁸ or with a combined neuropathological score of plaques and tangles in a mixed population of subjects with AD, MCI and controls.⁸⁰

Results for tau in CSF were inconsistent. Two studies did not find a link with tau or p-tau in AD, MCI and controls,^{83, 84} while two studies did in a combined AD and control population.^{84, 85} Moreover, in one study, the correlation was in the opposite direction, i.e. noncarriers of the *APOE* ϵ_4 allele had higher tau concentrations.⁸⁴ Another study reported that the effect of the *APOE* ϵ_4 allele on p-tau among subjects with AD was moderated by age.⁸⁶ The relation between tau and neuropathology was similarly inconsistent. A study with a mixed population found tau in NFT to correlate with the *APOE* ϵ_4 allele,⁸⁰ but these findings were not confirmed by another study with a mixed population⁸² and two population studies found no correlation.^{77, 81}

BIN1

BIN1 belongs to the Bin1/amphiphysin/RVS167 (BAR) family of proteins that are involved in clathrin-mediated endocytosis and apoptosis.²⁵ Only one study tested the relation between *BIN1* and biomarkers.⁷⁸ The study showed that rs744373 was not linked with the AD profile of abnormal $A\beta$ and p-tau.

CLU

CLU is an extracellular chaperone molecule involved in lipid transport and in the aggregation as well as clearance of $A\beta$ through endocytosis.²⁵ Like *APOE*, *CLU* binds soluble $A\beta$ and is present in amyloid plaques.

Data were available for four independent studies testing eight biomarker associations in rs11136000 in *CLU*.^{78, 82, 84, 87} This SNP did not correlate with Aβ and tau in CSF,^{78, 84} nor with plaques and tangles on neuropathology.^{82, 87}

CR1

CR1 regulates the complement cascade and has a role in Aβ clearance by binding Aβ oligomers to its C3b complement factor.²⁵ Aβ in plasma has also been reported to bind to C3b, suggesting a role in clearing pathogens from the bloodstream.⁸⁸ Data were available for 5 independent studies testing 12 biomarker associations in 3 SNPs.^{78, 82, 84, 87, 89} *CR1* rs1408077 was marginally correlated with abnormal Aβ and p-tau in CSF in a combined population of subjects with AD and MCI, and controls ($P=0.04$).⁷⁸ This SNP did not correlate with any other marker in CSF nor on neuropathology in the other studies. There was no correlation between rs3818361 and abnormal Aβ and p-tau in CSF, as well as between rs6656401 and markers in neuropathology.

Table 2 Qualitative summary of biomarker studies assessing AlzGene ‘top results’ loci

Gene	SNP	Study	Setting (cohort)	Sample (n)	Mean age (SD)	Out-come	
<i>APOE</i>	ε4+ vs. ε4-	Alexopoulos 2011 ⁸⁶	Mixed (ADNI)	AD (87) MCI (166) controls (100)	75 (8) 75 (7) 76 (5)	Aβ	
						tau	
						tau	
		Bennett 2003 ⁸¹	Population (ROS)	AD (51) no dementia (77)	88 (5) ^a 83 (7) ^a	Aβ	
						tau	
		Guo 2012 ⁸³	Clinical	AD (44) MCI (61)	66 (10) 65 (9)	Aβ	
						tau	
		Kantarci 2012 ⁷⁹	Population (MCSA)	Cognitively normal (408)	79	Aβ	
		Kauwe 2009 ⁵⁶	Clinical (WU)	AD (88) controls (225)	68 (12)	Aβ	

PICALM

PICALM is involved in clathrin-mediated endocytosis. It has been suggested to modify APP or its proteases by altering the trafficking of vesicle-associated membrane protein (VAMP), which in turn results in the increased production of A β .⁹⁰ Moreover, *PICALM* has been reported to influence A β neurotoxicity by regulating endocytosis.⁹¹

Data were available for four independent studies testing eight biomarker associations in three SNPs.^{78, 82, 84, 87} *PICALM* SNPs correlated with CSF A β_{1-42} ⁸⁴ or aggregated A β on neuropathological examination^{82, 87} in all studies. It did not correlate with CSF tau or tangles on neuropathological examination.^{82, 87} The combination of abnormal A β_{1-42} and p-tau in CSF was linked with rs541458 but not with rs3851179.⁷⁸ A neuropathological study found a correlation between AD pathology load of plaques and tangles with rs7110631, which was used in that study as a surrogate marker for rs3851179.⁸⁷

	Marker (modality)	Result				Comment
		AD	MCI	Controls	Combined	
	A β_{1-42} (CSF)	$P<0.001$	$P<0.001$	$P<0.001$	---	
	p-tau (CSF)	ns	$P<0.01$	$P<0.01$	---	
	t-tau (CSF)	ns	$P=0.02$	$P=0.02$	---	
	NP (PA)	---	---	---	odds=2.69 (1.01-7.13)	Adjusted for age, gender and education
	NFT (PA)	---	---	---	odds=2.41(0.92-6.33)	
	A β_{1-42} (CSF)	ns	$P<0.001$	---	---	
	tau (CSF)	ns	ns	---	---	
	A β_{1-42} (average binding to A β deposition)	---	---	$P<0.001$	---	
	A β_{1-42} /A β_{1-40} (CSF)	---	---	---	$P<0.0001$	

Table 2 Qualitative summary of biomarker studies assessing AlzGene ‘top results’ loci
Continued

Gene	SNP	Study	Setting (cohort)	Sample (n)	Mean age (SD)	Out-come	
						Aβ	
		Kauwe 2010 ⁸⁵	Clinical (WU) Mixed (ADNI)	AD (97) controls (248) AD (154) controls (103)	74 (8) 66 (11) 75 (8) 77 (5)	Aβ	
						tau	
		Kok 2011 ⁸²	Mixed (TASTY)	AD, other dementia, brain donors (484-530)	63 ^b	Aβ	
						tau	
		Schott 2011 ⁷⁸	Mixed (ADNI)	AD (88) MCI (163) controls (75)	75 (1)	AD profile	
		Wider 2012 ⁸⁰	Mixed (MCJ brain bank)	AD (517) other dementia (114) mixed (131) ^d	81 ^e	Aβ	
						tau	
	ε4 vs. ε3	Schjeide 2011 ⁸⁴	Mixed	AD (200) controls (169) combined (395)	---	Aβ	
				AD (199) controls (170)		tau	
		Kramer 2011 ⁷⁷	Population	controls (299)	85 (7)	tau	
		Schott 2011 ⁷⁸	Mixed (ADNI)	AD (88) MCI (163) controls (75)	75 (1)	AD profile	
<i>BIN1</i>	rs744373	Schott 2011 ⁷⁸	Mixed (ADNI)	AD (88) MCI (163) controls (75)	75 (1)	AD profile	
<i>CLU</i>	rs11136000	Chibnik 2011 ⁸⁷	Population (ROS, MAP)	No dementia (553)	88 (6) ^a	Aβ	

	Marker (modality)	Result				Comment
		AD	MCI	Controls	Combined	
	Total A β (CSF)	---	---	---	$P=0.16$	
	A β_{1-42} (CSF)	---	---	---	$P<0.0001$ (WU) $p<0.0001$ (ADNI)	
	p-tau (CSF)	---	---	---	$P=0.0006$ (WU) $P<0.0001$ (ADNI)	
	Total SP (PA)	---	---	---	OR=3.2 (2.05-4.90), $P<0.0001$	Adjusted for age
	NFT (PA)	---	---	---	ns	
	Abnormal A β and p-tau (CSF) ^c	---	---	---	OR=9.84 (5.48-17.67), $p<0.001$	
	SP (PA)	---	---	---	$P<0.001$	Adjusted for age and gender
	NFT (PA)	---	---	---	$P=0.001$; $P<0.001$	Adjusted for age and gender
	A β_{1-42} (CSF)	$P=0.06$	---	$P=2.4\times 10^{-11}$	$P=7.15\times 10^{-16}$	
	t-tau (CSF)	$P=0.82$	---	$P=0.49$	$P=0.003$	
	NFT (PA)	---	---	$P=0.46$	---	
	Abnormal A β and p-tau (CSF) ^c	---	---	---	OR=8.32 (4.61-15.01), $P<0.001$	
	Abnormal A β and p-tau (CSF) ^c	---	---	---	OR=0.87 (0.60-1.26), $P=0.36$	
	NP (PA)	---	---	$P=0.64$	---	Adjusted for age, gender and education

Table 2 Qualitative summary of biomarker studies assessing AlzGene ‘top results’ loci
Continued

Gene	SNP	Study	Setting (cohort)	Sample (n)	Mean age (SD)	Out-come	
						AD profile	
						tau	
		Kok 2010 ⁸²	Mixed (TASTY)	AD, other dementia, brain donors (484-530)	63 ^b	Aβ	
						tau	
		Schjeide 2011 ⁸⁴	Mixed	AD (187-196) controls (187-188) combined (413)	---	Aβ	
					---	tau	
		Schott 2011 ⁷⁸	Mixed (ADNI)	AD (88) MCI (163) controls (75)	75 (1)	AD profile	
CR1	rs1408077	Brouwers 2012 ⁸⁹	Clinical	AD (296)	75 (9)	Aβ	
						tau	
						tau	
						tau	
		Kok 2011 ⁸²	Mixed (TASTY)	AD, other dementia, brain donors (484-547)	63 ^b	Aβ	
						tau	
		Schott 2011 ⁷⁸	Mixed (ADNI)	AD (88) MCI (163) controls (75)	75 (1)	AD profile	
	rs3818361	Schott 2011 ⁷⁸	Mixed (ADNI)	AD (88) MCI (163) controls (75)	75 (1)	AD profile	
	rs6656401	Chibnik 2011 ⁸⁷	Population (ROS, MAP)	No dementia (553)	88 (6) ^a	Aβ	

	Marker (modality)	Result				Comment
		AD	MCI	Controls	Combined	
	Global plaque and tangle score (PA)	---	---	$P=0.98$	---	
	NFT (PA)	---	---	$P=0.28$	---	
	SP total (PA)	---	---	---	OR=1.1 (0.73-1.76), $P=0.57$	Interaction with APOE. Dominant model.
	NFT (PA)	---	---	---	ns	
	A β_{1-42} (CSF)	$P=0.78$	---	$P=0.26$	$P=0.50$	
	t-tau (CSF)	$P=0.52$	---	$P=0.39$	$P=0.25$	
	Abnormal A β and p-tau (CSF) ^c	---	---	---	OR=0.87 (0.60-1.26), $P=0.36$	
	A β_{1-42} (CSF)	$P=0.44$	---	---	---	Adjusted P -value, corrected for age, disease duration, gender, APOE status
	t-tau (CSF)	ns	---	---	---	
	p-tau (CSF)	ns	---	---	---	
	Total SP (PA)	---	---	---	OR=0.9 (0.62-1.54), $P=0.92$	Dominant model
	NFT (PA)	---	---	---	ns	
	Abnormal A β and p-tau (CSF) ^e	---	---	---	OR=1.59 (1.01-2.49), $P=0.04$	
	Abnormal A β and p-tau (CSF) ^c	---	---	---	OR=1.41 (0.91-2.17), $P=0.12$	
	NP (PA)	---	---	$P=0.28$	---	Adjusted for age, gender and education

Table 2 Qualitative summary of biomarker studies assessing AlzGene ‘top results’ loci
Continued

Gene	SNP	Study	Setting (cohort)	Sample (n)	Mean age (SD)	Out-come	
						AD profile	
						tau	
		Schjeide 2011 ⁸⁴	Mixed	AD (200-202) controls (187-188) combined (416)	---	Aβ	
						tau	
PICALM	rs3851179	Kok 2010 ⁸²	Mixed (TASTY)	AD, other dementia, brain donors (484-546)	63 ^b	Aβ	
						tau	
		Schott 2011 ⁷⁸	Mixed (ADNI)	AD (88) MCI (163) controls (75)	75 (1)	AD profile	
	rs541458	Schjeide 2011 ⁸⁴	Mixed	AD (195-196) controls (191-190) combined (412-413)	---	Aβ	
						tau	
		Schott 2011 ⁷⁸	Mixed (ADNI)	AD (88) MCI (163) controls (75)	75 (1)	AD profile	
	rs7110631	Chibnik 2011 ⁸⁷	Population (ROS, MAP)	No dementia (553)	88 (6) ^a	Aβ	
						Aβ	
						tau	

Abbreviations: SNP=single nucleotide polymorphism; SD=standard deviation; AD=Alzheimer’s disease; MCI=mild cognitive impairment; CSF=cerebrospinal fluid; Aβ=amyloid beta; t-tau=total tau; p-tau=phosphorylated tau; SP=senile plaques; NP=neuritic plaques; DP=diffuse plaques; NFT=neurofibrillary tangles; PA=pathology; p=p-value; OR ()=odds ratio (95% confidence interval); ns=not significant; PiB-PET=11C-Pittsburgh Compound-B-Positron Emission Tomography; TASTY=Tampere Autopsy Study; ADNI=Alzheimer’s Disease Neuroimaging Initiative; ROS=Religious Order Study; MCSA=Mayo Clinic Study of Aging; MAP=Rush Memory Aging Project; WU=Washington University cohort; MCJ=Mayo Clinic Jacksonville brain bank for neurodegenerative disorders

	Marker (modality)	Result				Comment
		AD	MCI	Controls	Combined	
	Global plaque and tangle score (PA)	---	---	$P=0.34$	---	
	NFT (PA)	---	---	$P=0.37$	---	
	$A\beta_{1-42}$ (CSF)	$P=0.88$	---	$P=0.08$	$P=0.81$	
	t-tau (CSF)	$P=0.41$	---	$P=0.74$	$P=0.39$	
	Total SP (PA)	---	---	---	OR=0.60, (0.41-0.95) $P=0.03$	Dominant model
	NFT (PA)	---	---	---	ns	
	Abnormal $A\beta$ and p-tau (CSF) ^c	---	---	---	OR=0.82 (0.58-1.18), $P=0.29$	
	$A\beta_{1-42}$ (CSF)	$P=0.01$	---	$P=0.74$	$P=0.002$	
	t-tau (CSF)	$P=0.85$	---	$P=0.76$	$P=0.20$	
	Abnormal $A\beta$ and p-tau (CSF) ^c	---	---	---	OR=0.68 (0.47-0.98), $P=0.04$	
	NP (PA)	---	---	$P=0.009$	---	Adjusted for age, gender and education
	Global plaque and tangle score (PA)	---	---	$P=0.03$	---	
	NFT (PA)	---	---	$P=0.37$	---	

^a Mean age at death.

^b Based on N=603.

^c Subjects classified as CSF+ (that is, low $A\beta_{1-42}$ and high p-tau) or CSF- (that is, high $A\beta_{1-42}$ and low p-tau).

^d Mixed population for this study consisted of subjects with Parkinson's disease (n=50); non-Parkinson movement disorders (n=20); and cognitively normal, mild cognitive impairment and psychiatric disorders (n=61).

^e Median age at death.

Biomarkers and SNPs in other candidate genes

ACE

Only one case-control study investigated the relation between *ACE* and CSF $A\beta_{1-42/1-40}$ ratio.⁵⁶ In a combined population, only rs1800764 showed a marginal effect on $A\beta_{1-42}/A\beta_{1-40}$ ($P=0.01$). Rs4291 and rs4242 were not linked with CSF $A\beta$.

SORL1

SORL1 was the best-studied candidate gene in relation to biomarkers with data from four independent studies, all performed with CSF samples. Of the top seven *SORL1* SNPs previously reported to be linked with AD,³⁴ data on the correlation with CSF markers were available for rs661057 (SNP4), rs668387 (SNP8), rs689021 (SNP9), rs641120 (SNP10) and rs2070045 (SNP19) but not for rs11218304 (SNP5) and rs12285364 (SNP19).

One study reported a link between SNP4 and tau concentrations in subjects with AD ($P=0.03$)⁸³ (Table 3). In another study, SNP8 correlated with $A\beta_{1-42}$ in subjects with AD ($P=0.04$).⁸⁶ This study found SNP8, SNP9 and SNP10 to interact with *APOE*- ϵ_4 , which affected $A\beta_{1-42}$ concentrations. Given the small sample sizes and large number of comparisons performed in these studies, these results may represent false positive findings.

SNP4 was not linked with tau among subjects with MCI. SNP4, SNP8, SNP9, SNP10 and SNP19 did not correlate with any other CSF markers in controls and among subjects with AD. One study reported increased *SORL1* levels for subjects with MCI who were homozygous minor allele carriers of SNP8 (TT), SNP9 (AA) and SNP10 (CC) (all $P=0.04$).⁸³ In summary, except in one study,⁸³ it appears that *SORL1* markers do not correlate with CSF $A\beta$ and tau.

MAPT

Data were available for three independent studies testing seven biomarker associations in two SNPs.^{56, 57, 80} *MAPT* H1/H2 rs1052553 correlated with NFT ($P=0.03$) on neuropathological examination and was marginally linked with $A\beta$ in senile plaques in a mixed population study.⁸⁰ The other *MAPT* variants (rs2471738, rs7210728, and rs242557) were not related to biomarkers in CSF nor to neuropathology in the other studies.

TOMM4o

Data were available for four CSF studies from two independent cohorts (including three studies based on ADNI data) testing eight biomarker associations in three SNPs.^{56, 75, 76, 78} *TOMM4o* rs157580 and rs2075650 correlated with CSF $A\beta_{1-42}$ or the ratio of $A\beta$ and tau or p-tau in four biomarker GWAS analyses based on ADNI data.^{75, 76, 78} These GWAS studies did not find an association with CSF tau. Rs207560 further had significant effects on the combination of abnormal CSF $A\beta_{1-42}$ and p-tau in ADNI data, which the study reported as likely to be driven by $A\beta_{1-42}$ given that rs207560 correlated with $A\beta$ but no with tau. Rs157581 was not associated with the CSF $A\beta_{1-42}/1-40$ ratio in a cohort from Washington University.⁵⁶ These results generally reflect those reported for *APOE*, which is expected given the physical proximity (~2,000 base pairs) and degree of linkage disequilibrium between the *APOE* and *TOMM4o* loci.

GWAS biomarkers

Three GWAS have been performed that used CSF markers or neuropathological markers as outcome.

One study in the ADNI cohort with CSF markers as outcome found that CSF $A\beta_{1-42}$ in controls was related with *ARLB5B* rs11015839, *CYP19A1* rs2899472 and *NCAM2* rs1022442.⁷⁵ CSF t-tau was associated with *CDH4* rs4925189 in subjects with AD, and with polymorphisms at *FLJ21511* (rs2768975 and rs6850199) in subjects with MCI. CSF p-tau correlated with different genetic variants in subjects with AD (*UNC5C* rs12643654), subjects with MCI (*CHN2* rs121724 and *MTUS1* rs7842088), and controls (*UPP2* rs2074955).

Another GWAS using CSF data from the ADNI cohort combined controls and subjects with AD and MCI and found that genetic variants in *EPC2* correlated with CSF t-tau (rs10171238, rs1374441, rs2121433 and rs4499362) and the t-tau/ $A\beta_{1-42}$ ratio (rs10171238 and rs4499362).⁷⁶

Both GWAS with ADNI data used *APOE* status as covariate. It is noteworthy that although their sample size was comparable (N=390 vs. N=377) and the same genetic data were studied, the outcomes were highly divergent.

Table 3 Qualitative summary of biomarker studies assessing SNPs in other AD candidate genes

Gene	SNP	Study	Setting (cohort)	Subjects (n)	Mean age (SD)	Out-come	
ACE	rs1800764	Kauwe 2009 ⁵⁶	Clinical (WU)	AD (88) controls (225)	68 (12)	Aβ	
	rs4291	Kauwe 2009 ⁵⁶	Clinical (WU)	AD (88) controls (225)	68 (12)	Aβ	
	rs4343	Kauwe 2009 ⁵⁶	Clinical (WU)	AD (88) controls (225)	68 (12)	Aβ	
SORL1	rs661057 (SNP4)	Alexopoulos 2011 ⁸⁶	Mixed (ADNI)	AD (87) MCI (166) controls (100)	76 (5) 75 (7) 75 (8)	Aβ	
						tau	
						tau	
		Guo 2012 ⁸³	Clinical	AD (44) MCI (61)	66 (10) 65 (9)	Aβ	
						tau	
		Kauwe 2010 ⁸⁵	Clinical, mixed (WU, ADNI)	AD (251) controls (351)	75 (8) 69 (9)	Aβ	
	rs668387 (SNP8)	Alexopoulos 2011 ⁸⁶	Mixed (ADNI)	AD (87) MCI (166) controls (100)	76 (5) 75 (7) 75 (8)	Aβ	
						tau	
						tau	
		Guo 2012 ⁸³	Clinical	AD (44) MCI (61)	66 (10) 65 (9)	Aβ	
						tau	
		Kauwe 2010 ⁸⁵	Clinical, mixed (WU, ADNI)	AD (251) controls (351)	75 (8) 69 (9)	Aβ	

	Marker (modality)	P-value				Comment
		AD	MCI	Control	Combined	
	$A\beta_{1-42}/A\beta_{1-40}$ (CSF)	---	---	---	$P=0.014^a$	Adjusted for $APOE-\epsilon_4$ status and CDR. Dominant model
	$A\beta_{1-42}/A\beta_{1-40}$ (CSF)	---	---	---	$P=0.22^a$	Adjusted for $APOE-\epsilon_4$ status and CDR
	$A\beta_{1-42}/A\beta_{1-40}$ (CSF)	---	---	---	$P=0.22^a$	Adjusted for $APOE-\epsilon_4$ status and CDR
	$A\beta_{1-42}$ (CSF)	ns ^b	ns ^b	ns ^b	---	
	t-tau (CSF)	ns	ns	ns	---	
	p-tau (CSF)	ns	ns	ns	---	
	$A\beta_{1-42}$ (CSF)	ns	ns	---	---	
	tau (CSF)	$P=0.03$	$P=0.19$	---	---	Homozygous minor allele carriers vs. other allele carriers. Indication of gene dose effect.
	$A\beta_{1-42}$ (CSF)	---	---	---	$P=0.21^a$	Adjusted for age, $APOE-\epsilon_4$ status and CDR
	$A\beta_{1-42}$ (CSF)	$P=0.04^c$	ns ^c	ns ^c	---	SNP interacted with $APOE-\epsilon_4$ ($P=0.03$)
	t-tau (CSF)	ns	ns	ns	---	
	p-tau (CSF)	ns	ns	ns	---	
	$A\beta_{1-42}$ (CSF)	ns	ns	---	---	SNP associated with increased SORL1 levels among homozygous minor allele carriers with MCI ($P=0.04$) but not with AD ($P=0.42$).
	tau (CSF)	ns	ns	---	---	
	$A\beta_{1-42}$ (CSF)	---	---	---	$P=0.32^a$	Adjusted for age, $APOE-\epsilon_4$ status and CDR

Table 3 Qualitative summary of biomarker studies assessing SNPs in other AD candidate genes
Continued

Gene	SNP	Study	Setting (cohort)	Subjects (n)	Mean age (SD)	Out-come	
	rs689021 (SNP9)	Alexopoulos 2011 ⁸⁶	Mixed (ADNI)	AD (87) MCI (166) controls (100)	76 (5) 75 (7) 75 (8)	Aβ	
						tau	
						tau	
		Guo 2012 ⁸³	Clinical	AD (44) MCI (61)	66 (10) 65 (9)	Aβ	
						tau	
		Kauwe 2010 ⁸⁵	Clinical, mixed (WU, ADNI)	AD (251) controls (351)	75 (8) 69 (9)	Aβ	
	rs641120 (SNP10)	Alexopoulos 2011 ⁸⁶	Mixed (ADNI)	AD (87) MCI (166) controls (100)	76 (5) 75 (7) 75 (8)	Aβ	
						tau	
						tau	
		Guo 2012 ⁸³	Clinical	AD (44), MCI (61)	66 (10) 65 (9)	Aβ	
						tau	
	rs2070045 (SNP19)	Alexopoulos 2011 ⁸⁶	Mixed (ADNI)	AD (87) MCI (166) controls (100)	76 (5) 75 (7) 75 (8)	Aβ	
						tau	
						tau	
		Guo 2012 ⁸³	Clinical	AD (44) MCI (61)	66 (10) 65 (9)	Aβ	
						tau	
		Kauwe 2010 ⁸⁵	Clinical, mixed (WU, ADNI)	AD (251) controls (351)	75 (8) 69 (9)	Aβ	
		Kolsch 2008 ⁹⁹	Clinical (DCN)	AD (153)	71 (8)	Aβ	
<i>MAPT</i>	rs2471738	Kauwe 2009 ⁵⁶	Clinical (WU)	AD (88) controls (225)	68 (12)	Aβ	

	Marker (modality)	P-value				Comment
		AD	MCI	Control	Combined	
	A β_{1-42} (CSF)	ns ^c	ns ^c	ns ^c		SNP interacted with APOE- ϵ_4 ($P=0.04$)
	t-tau (CSF)	ns	ns	ns	---	
	p-tau (CSF)	ns	ns	ns	---	
	A β_{1-42} (CSF)	ns	ns	--	---	
	tau (CSF)	ns	ns	--	---	
	A β_{1-42} (CSF)	---	---	---	$P=0.34^a$	Adjusted for age, APOE- ϵ_4 status and CDR
	A β_{1-42} (CSF)	ns ^b	ns ^b	ns ^b	---	SNP interacted with APOE- ϵ_4 ($P<0.001$).
	t-tau (CSF)	ns	ns	ns	---	
	p-tau (CSF)	ns	ns	ns	---	
	A β_{1-42} (CSF)	ns	ns	--	---	
	tau (CSF)	ns	ns	--	---	
	A β_{1-42} (CSF)	ns ^b	ns ^b	ns ^b	---	
	t-tau (CSF)	ns	ns	ns	---	
	p-tau (CSF)	ns	ns	ns	---	
	A β_{1-42} (CSF)	ns	ns	--	---	
	tau (CSF)	ns	ns	--	---	
	A β_{1-42} (CSF)	---	---	---	$P=0.29^a$	Adjusted for age, APOE- ϵ_4 status and CDR
	A β_{1-42} (CSF)	$P=0.11$	---	---	---	Adjusted for APOE- ϵ_4 status and MMSE
	Total A β (CSF)	---	---	---	$P=0.65^a$	Adjusted for APOE- ϵ_4 status and CDR

Table 3 Qualitative summary of biomarker studies assessing SNPs in other AD candidate genes
Continued

Gene	SNP	Study	Setting (cohort)	Subjects (n)	Mean age (SD)	Out-come	
	rs7210728	Cruchaga 2010 ⁵⁷	Clinical, mixed (WU, ADNI)	AD and non-demented (493)	72 (7)	tau	
	rs242557	Wider 2012 ⁸⁰	Mixed (MCJ brain bank)	AD (517) other dementia (114) mixed (131) ^c	81	A β	
						tau	
<i>MAPT H1/H2</i>	rs1052553	Wider 2012 ⁸⁰	Mixed (MCJ brain bank)	AD (517) other dementia (114) mixed (131) ^c	81	A β	
						tau	
<i>TOMM40</i>	rs157580	Kim 2010 ⁷⁶	Mixed (ADNI)	AD (96) MCI (176) controls (102)	75 (8) 75 (8) 76 (5)	A β	
	rs157581	Kauwe 2009 ⁵⁶	Clinical (WU)	AD (88) control (225)	68 (12)	A β	
	rs2075650	Schott 2011 ⁷⁸	Mixed (ADNI)	AD (88) MCI (163) controls (75)	75 (1)	AD profile	
		Han 2010 ⁷⁵	Mixed (ADNI)	AD (172) MCI (109) Controls (109)	71 70 72	A β	
						tau	
						tau	
		Kim 2010 ⁷⁶	Mixed (ADNI)	AD (96) MCI (176) controls (102)	75 (8) 75 (8) 76 (5)	A β	
						tau	

	Marker (modality)	P-value				Comment
		AD	MCI	Control	Combined	
	$A\beta_{1-42}/A\beta_{1-40}$ (CSF)	---	---	---	$P=0.46^a$	Adjusted for <i>APOE</i> - ϵ_4 status and CDR
	p-tau (CSF)	---	---	---	$P=0.11$	Dominant model
	SP (PA)	---	---	---	$P=0.94$	Adjusted for age, gen- der and <i>APOE</i> status
	NFT (PA)	---	---	---	$P=0.33$	Adjusted for age, gen- der and <i>APOE</i> status
	SP (PA)	---	---	---	$P=0.048^c$	Adjusted for age, gen- der and <i>APOE</i> status
	NFT (PA)	---	---	---	$P=0.03^c$	Adjusted for age, gen- der and <i>APOE</i> status
	$A\beta_{1-42}$ (CSF)	---	---	---	$P=1 \times 10^{-06}$	GWAS study with bio- marker as outcome
	$A\beta_{1-42}/A\beta_{1-40}$ (CSF)	---	---	---	$P=0.25^a$	Adjusted for <i>APOE</i> - ϵ_4 status and CDR
	Abnormal $A\beta$ and p-tau (CSF) ^d	---	---	---	OR=4.30, (2.61-7.06), $P<0.001$	
	$A\beta_{1-42}$ (CSF)	ns	ns	$P=3.03 \times 10^{-07}$	---	GWAS study with bio- marker as outcome
	t-tau (CSF)	ns	ns			
	p-tau (CSF)	ns	ns			
	$A\beta_{1-42}$ (CSF)	---	---	---	$P=1 \times 10^{-06}$	GWAS study with bio- marker as outcome
	t-tau (CSF)	---	---	---	ns	

Table 3 Qualitative summary of biomarker studies assessing SNPs in other AD candidate genes
Continued

Gene	SNP	Study	Setting (cohort)	Subjects (n)	Mean age (SD)	Out-come	
						AD profile	

Abbreviations: SNP=single nucleotide polymorphism; SD=standard deviation; OR=odds ratio (95% confidence interval); AD=Alzheimer’s disease; MCI=mild cognitive impairment; Aβ=amyloid beta; t-tau=total tau; p-tau=phosphorylated tau; CSF=cerebrospinal fluid; PA=neuropathology; SP=senile plaques; NFT=neurofibrillary tangles; CDR=clinical dementia rating; ns=not significant; WU=Washington University; ADNI=Alzheimer’s Disease neuroimaging Initiative; DCN=German Dementia Competence Network; GWAS=genome-wide association study

- ^a Not adjusted for multiple testing.
- ^b Not significant after applying Bonferroni or other correction for multiple testing.
- ^c Mixed population for this study consisted of subjects with Parkinson’s disease (n=50); non-Parkinson movement disorders (n=20); and cognitively normal, mild cognitive impairment and psychiatric disorders (n=61).
- ^d Subjects were classified as CSF+ (that is, low Aβ₁₋₄₂ and high p-tau) or CSF- (that is, high Aβ₁₋₄₂ and low p-tau).

Table 4 Qualitative summary of genome-wide association studies using biomarkers as outcome

Study	Setting	Subjects (n)	Mean age (SD)	Modality	Out-come	
Han 2010 ^{75b}	Mixed (ADNI)	AD (172)	71	CSF	t-tau	
					p-tau	
					p-tau	
		MCI (109)	70	CSF	t-tau	
					t-tau	
					p-tau	
		Controls (109)	72	CSF	Aβ ₁₋₄₂	
					Aβ ₁₋₄₂	
					Aβ ₁₋₄₂	
					p-tau	

	Marker (modality)	P-value				Comment
		AD	MCI	Control	Combined	
	p-tau/A β_{1-42} (CSF)	---			$P=1\times 10^{-06}$	Effect likely driven by A β_{1-42}

	Gene	SNP	Minor allele ^a	P-value	Comment
	<i>CDH4</i>	rs4925189	G	6.45×10^{-07}	All P-values adjusted for age and APOE status
	<i>UNC5C</i>	rs12643654	G	4.95×10^{-07}	
	<i>CHN2</i>	rs121724	G	1.45×10^{-07}	
	<i>FLJ21511</i>	rs2768975	G	1.96×10^{-07}	
		rs6850199	A	3.18×10^{-07}	
	<i>MTUS1</i>	rs7842088	G	2.12×10^{-07}	
	<i>ARL5B</i>	rs11015839	A	6.45×10^{-07}	
	<i>CYP19A1</i>	rs2899472	A	1.90×10^{-07}	
	<i>NCAM2</i>	rs1022442	A	2.75×10^{-07}	
	<i>UPP2</i>	rs2074955	C	1.09×10^{-07}	

Table 4 Qualitative summary of genome-wide association studies using biomarkers as outcome
Continued

Study	Setting	Subjects (n)	Mean age (SD)	Modality	Out- come	
Kim 2010 ^{76c}	Mixed (ADNI)	AD (96) MCI (176) controls (102)	75 (8) 75 (8) 76 (5)	CSF	t-tau/Aβ ₁₋₄₂	
					t-tau	
					t-tau/Aβ ₁₋₄₂	
					t-tau	
					t-tau	
					t-tau	
Kramer 2011 ^{77d}	Population	controls (299)	85 (7)	PA	tau in NFT	

Abbreviations: SD=standard deviation; SNP=single nucleotide polymorphism; AD=Alzheimer’s disease; MCI=mild cognitive impairment; ADNI=Alzheimer’s Disease Neuroimaging Initiative; CSF=cerebrospinal fluid; Aβ=amyloid beta; t-tau=total tau; p-tau=phosphorylated tau; PA=pathology; NFT=neurofibrillary tangles

^a Obtained from the National Center for Biotechnology information (NCBI), when not specified in the study.
^b The study also reported an association between SNPs in *TOMM40* and CSF biomarkers (see Table 3) and an association between *APOE*-ε4 allele dose and CSF Aβ₁₋₄₂. The *APOE*-ε4 allele dose was not associated with CSF t-tau and p-tau.
^c The study also reported an association between SNPs in *TOMM40* and CSF biomarkers (see Table 3) and an association between *APOE* rs429358 and CSF Aβ₁₋₄₂, p-tau and ratio of Aβ/tau and Aβ₁₋₄₂/t-tau.
^d The study also reported no association between *APOE* alleles and tangles (see Table 2).

	Gene	SNP	Minor allele ^a	P-value	Comment
	<i>EPC2</i>	rs10171238	C	1×10^{-06}	All P-values unadjusted. <i>APOE</i> status used as covariate.
				1×10^{-06}	
		rs4499362	T	1×10^{-06}	
				1×10^{-06}	
		rs1374441	C	1×10^{-06}	
		rs2121433	A	1×10^{-06}	
	<i>ADRA1</i>	rs1481650	G	2.52×10^{-05}	Subjects classified as low Braak (score of 2 or lower) or high Braak (score of 4 or higher); subjects with Braak score of 3 excluded. All P-values adjusted for gender and age.
	<i>C1QTNF1</i>	rs877874	C	1.74×10^{-05}	
	<i>MSRA</i>	rs11782819	T	3.23×10^{-06}	
	<i>RBBP6</i>	rs11649538	A	2.00×10^{-05}	
	<i>RELN</i>	rs4298437	T	1.53×10^{-06}	
		rs6943822	C	1.21×10^{-05}	
		rs6951875	A	5.15×10^{-06}	
	<i>TMEM65</i>	rs10956170	A	1.57×10^{-05}	
	<i>TMPRSS2</i>	rs7278439	T	1.33×10^{-05}	

Kramer and colleagues performed a GWAS with Braak stages of NFTs as outcome in subjects from the general population.⁷⁷ They reported correlations with polymorphisms in 7 different genes (Table 4). They also investigated the effects of *APOE* on neuropathology by testing whether the *APOE* ϵ_4 allele frequency of subjects with a high Braak score differed significantly from those with a low Braak score. There was no significant difference between the two groups ($p=0.56$), which they explained as likely due to the advance age of their subjects.

DISCUSSION

We investigated the effect of 47 SNPs from 26 genes on AD markers in CSF, amyloid PET or neuropathology. Our main conclusion is that – similar to the well-established and very highly significant effect on disease risk – effects were strongest for *APOE* and less evident/consistent for the other genes.

APOE was the most frequently tested gene in relation to AD markers. Despite the limited number of studies we included in this review, we found a consistent correlation between the *APOE* ϵ_4 allele and abnormal amyloid, but its effect on tau remains questionable. This latter result is in agreement with observations from *in vitro* and animal studies typically not showing any correlations between tau and the *APOE*- ϵ_4 allele and the findings that *APOE* is associated with aggregated A β .

TOMM40 was strongly suggested to be associated with CSF A β ₁₋₄₂ but not with tau. This pattern is similar to that observed for the *APOE* ϵ_4 allele. *TOMM40*, located in chromosome 19, is in linkage disequilibrium with *APOE*. That is, DNA sequence variants (such as SNPs) in one locus are strongly correlated with SNPs at the other locus. Some authors have discussed the possibility that genetic variants in *TOMM40* exert their effects independent of *APOE*,^{63, 92-94} while other studies have not confirmed this.^{95, 96}

CR1 marginally correlated with CSF A β . There was no further indication of a link between the SNPs we selected for inclusion in the study and other AD biomarkers. Brouwers and colleagues⁸⁹ have analyzed a range of other SNPs that were not included in the present study. Their findings suggested that SNPs in 'linkage disequilibrium

block 4' were associated with CSF $A\beta_{1-42}$. However, rs1408077, which is a SNP from this block included in our review, was not associated with CSF $A\beta_{1-42}$ in their study or in any of the selected studies. Two other SNPs (rs12034383 and rs11803956) showed marginal correlation with $A\beta$, while no link with biomarkers was found with any of the other tested SNPs rs11118131, rs11118166, rs11118167, rs12034598, rs1746659, rs3738468, rs4844610, rs646817 and rs6691117.

CLU was not associated with $A\beta$. Its lack of correlation with tau is consistent with the report of another study.⁹⁷ For *PICALM*, there was a moderately strong suggestion of correlation with abnormal $A\beta$ in CSF or with neuropathology from independent studies. It was not associated with tau. *BIN1* did not correlate with the AD profile of abnormal $A\beta$ and p-tau, but there was only one study available, which was insufficient to base a meaningful conclusion.

With respect to the candidate genes assessed here, previous studies have reported that *ACE* rs1800764, rs4343 and rs4291 maybe associated with risk for AD.^{59, 60, 98} However, the overall effect of these *ACE* polymorphisms on AD biomarkers remains inconclusive and is certainly much less compelling in terms of statistical significance than the associations observed with the GWAS-derived loci discussed above.

The effect of *SORL1* variants on AD biomarkers equally remains inconclusive. Moreover, $A\beta$ concentrations have been reported to be affected by the interaction between *SORL1* and *APOE*.⁸⁶ *SORL1* is an apolipoprotein E receptor.⁸⁸ However, another study which corrected for *APOE* status and which had substantial statistical power still did not detect any correlation between *SORL1* polymorphisms and CSF $A\beta_{1-42}$.⁸⁵ Other SNPs that have been studied but did not yield significant results in relation to biomarkers were rs1010159, rs11600875, rs1699102, rs2282649, rs3824968, *SORL1*-18ex26.^{56, 83, 85, 86, 99} Thus, in addition to being at best a modest genetic risk factor for AD, the presence of substantial allelic heterogeneity makes it much more difficult to conclusively assess the potential link between *SORL1* and AD compared to other genes.³⁴

Studies of *SORL* haplotypes showing correlations with AD biomarkers^{83, 99, 100} may yield more insight as to how these polymorphisms affect pathophysiology.

Similarly, there is no strong evidence yet for the effect of *MAPT* polymorphisms on AD biomarkers, although the limited number of studies on this gene makes it difficult to reach more definitive conclusions.

The few currently available biomarker GWAS identified a range of genes but except for *APOE* and *TOMM40*, these have not been identified by previous GWAS that used a case-control design with clinical AD as outcome. This may be due to a number of factors including small effect size of the variant under study, small sample size, imprecision in the methods of reliably determining biomarker 'levels'. Thus, a number of the proposed findings may be false positive. Alternatively, the currently inconclusive findings may indicate that different sets of genes and/or polymorphisms modulate biomarker vs. susceptibility outcomes. Further, it is noteworthy that in the two GWAS performed with ADNI data no noteworthy overlap in association signals was reported. Given that both datasets were identical or at least largely overlapping in terms of included subjects as well as measured phenotypes and genotypes this discrepancy appears striking. Replication studies in larger samples are direly needed to reassess these findings.

Given the complexity of the considered traits and body of literature in the field, our study is, naturally, not without limitations. First, the small number of studies included limits our ability to distinguish true from possibly erroneous signals. However, our final collection of studies was determined following a systematic review of the literature, so this factor is beyond our control. Second, owing to the small number of available studies, we only conducted a *qualitative* analysis of the published data on genetic risk factors and markers for A β and tau. Once more data become available, future analyses should also include a *quantitative* synthesis of all available data, similar to what is done the field of genetic association studies in AD.⁴³ Third, we only focused on what we consider the "best-established" risk genes for AD to date. It needs to be emphasized that a wide range of additional genes has been investigated in relation to AD biomarkers but a careful review of these studies was outside the scope of this review. Finally, we only concentrated on markers for amyloid and tau, but it may also be worthwhile to focus on cognition and brain atrophy as useful endpoints in future assessments of this kind.^{72, 101}

Conclusions and future perspectives

Of the genes included in our study, *APOE* was by far most strongly and most consistently correlated with biomarkers and neuropathological markers of AD, in particular those that correlate with $A\beta$ pathology. It needs to be resolved whether the similar effects observed with polymorphisms in *TOMM40* – which are highly correlated with those in *APOE* – represent genuinely independent effects. If that were the case, it would strengthen the notion that mitochondrial dysfunction may represent one of the key pathological events in AD.⁷² Of all other potential gene-biomarker effects assessed here, it appears that only the potential relation between *PICALM* and $A\beta$ deserves further exploration. *PICALM* has been posited to promote $A\beta$ synthesis through endocytosis regulation, which leads to changes in $A\beta$ concentrations.^{23, 88}

The finding that *BIN1*, *CLU*, *CR1*, *ACE*, *SORL* and *MAPT* did not correlate with $A\beta$ or tau biomarkers may suggest that these genes exert their effects on AD through pathways other than those directly related to tau and $A\beta$. The lack of correlation, however, may also result from the small number of studies available for this review and the small sample sizes in most studies.

In general, the current lack of consistent patterns for genes other than *APOE* and *TOMM40* to correlate with biomarkers in GWAS strongly argues for performing future studies on a much larger scale. Just like GWAS assessing the genetic architecture underlying disease susceptibility, this will require forming national and international collaborations that will allow sharing, pooling and harmonizing raw data. Future collaborative efforts should also attempt to harmonize the techniques employed to determine biomarker phenotypes in order to reduce the chance of bias due to technical imprecision. However, it needs to be emphasized that ‘bigger’ does not always equal ‘better’, as the correlation between most of today’s biomarkers with the underlying pathophysiology is less than perfect. Thus, future studies also need to focus on identifying novel markers that more closely and specifically reflect the underlying disease process. Ideally, these biomarkers should be easy to measure with high precision, while at the same time being only minimally invasive and most cost effective than current variables. This idealized future may seem long ahead and will necessitate a broad range of

new developments in the fields of high-throughput proteomics, specimen ascertainment, and bioinformatics. However, recent advances in DNA sequencing methodology – which now allows researchers to decipher whole genomes at base-pair resolution at constantly increasing precision and cost-efficiency – have shown that revolutionary progress is sometimes closer than it may appear. Applying these technologies on large collections of carefully ascertained study cohorts will lay the foundation to develop better and more reliable disease biomarkers. In the meantime, efforts are ongoing to consolidate published materials on AD-related biomarkers and making these publicly available in an 'AlzBiomarkers' database, which will follow the same general idea as the AlzGene database. All these developments will hopefully lead to a vast improvement of our means for early detection and monitoring of the biochemical consequences elicited by AD and other devastating neurodegenerative diseases.

Executive summary

Alzheimer's disease (AD) is a neurodegenerative disease

- Neuritic plaques, mainly composed of aggregated A β , and neurofibrillary tangles, consisting of hyperphosphorylated tau, are characteristic lesions in AD.
- AD markers can be measured in-vivo or upon neuropathological examination.

AD pathophysiology

- AD results from the imbalance in the production and clearance of A β in the brain.
- A β_{1-40} and A β_{1-42} are produced by β and γ -secretase cleavage of the β -amyloid precursor protein. A β_{1-42} is prone to aggregate.

Genetics of AD

- Less than 5% of all AD cases are caused by autosomal dominant mutations following Mendelian disease transmission.
- Non-Mendelian forms of AD caused by the interplay of genetic (and non-genetic) susceptibility factors of small effect sizes represent the vast majority of cases.

Biomarkers and top results AlzGene SNPs

- *APOE* and *PICALM* correlated with abnormal A β but not with tau in CSF and in neuritic plaques.
- *CR1* was marginally correlated with A β and tau.
- *BIN1* and *CLU* did not correlate with A β or with tau.

Biomarkers and other SNPs in AD candidate genes

- *ACE*, *SORL1* and *MAPT* had no conclusive effect on A β and tau.
- *TOMM40* correlated with CSF A β , but *TOMM40* is in strong linkage disequilibrium with *APOE*.

Genome-wide association studies (GWAS) with biomarker outcomes

- Except for *APOE/TOMM40*, findings from GWAS assessing disease risk currently show no or little overlap with findings from biomarker GWAS.
- Other GWAS signals currently show weak, inconsistent or no correlation with AD markers.

Conclusion

- *APOE* is currently the only gene that correlates strongly with biomarkers and neuropathological markers of AD, while the correlation with *PICALM* is less definitive.
- Larger scale studies using current and future biomarkers are needed to elucidate the role of susceptibility genes on the pathophysiology of AD.

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*Changes in A β and tau levels, hippocampal volume
and memory occur at different stages of AD.*

*We tested how these may be related to genetic risk
factors for AD.*

LS Elias-Sonnenschein, C Graff, RW Jones, PG Kehoe, AK Wallin, FM Nobili,
M Olde Rikkert, P Scheltens, L Spuru, G Wilcock, K Blennow, R Wolz,
FRJ Verhey, PJ Visser
Under review

CHAPTER 6

Biomarker and cognitive correlates of AD risk
genes in nondemented individuals with cognitive
impairment

ABSTRACT

Background

Genome-wide association studies (GWAS) in recent years have identified single nucleotide polymorphisms (SNPs) associated with Alzheimer's disease (AD). To understand how these loci relate to key markers of AD pathology, we tested their correlation with key markers of AD pathology in cerebrospinal fluid (CSF) $A\beta_{1-42}$ and t-tau, hippocampal volume and delayed memory in nondemented individuals with cognitive impairment in a memory clinic setting. Furthermore, we tested the risk associated with these loci for progression to AD.

Methods

We tested the top 10 AlzGene risk variants in *APOE*, *ABCA7*, *BIN1*, *CD2AP*, *CD33*, *CLU*, *CR1*, *MS4A4E*, *MS4A6A* and *PICALM*. DNA was genotyped on Sequenom i-Plex platform. CSF (n=66) $A\beta_{1-42}$ and t-tau concentrations were measured using commercially available sandwich ELISAs; hippocampal volume (n=58) using automated learning embeddings for atlas propagation (LEAP); and delayed memory (n=265) using a word list-learning task. We used mixed and logistic regression models, and applied false discovery rate (FDR) correction for multiple testing.

Results

APOE ϵ_4 allele carriers had a 2.5-fold increased risk of progressing to AD, and showed decreased $A\beta_{1-42}$ levels ($P=0.001$) and $A\beta_{1-42}$ /t-tau ratio ($P=0.007$). The other SNPs showed weak effects. None of the SNPs correlated with t-tau. *PICALM* homozygous risk allele carriers had decreased $A\beta_{1-42}$ concentrations ($P=0.02$) and low $A\beta_{1-42}$ /t-tau ratio ($P=0.05$). Hippocampal volume was reduced among *CLU* risk allele carriers ($P=0.02$), but was larger among *CR1* risk allele carriers ($P=0.01$). *CD2AP* risk allele carriers had poorer delayed memory ($P=0.02$) while risk allele carriers of *ABCA7* performed better on memory tasks ($P=0.02$). Except for *APOE*, none of these results remained statistically significant after FDR correction.

Conclusion

Only *APOE* predicted progression to AD, showed a consistent relation to AD markers, and correlated significantly with CSF $A\beta_{1-42}$. The weak association of the other top GWAS SNPs with clinical outcome suggests that these may be of limited prognostic value for nondemented individuals with cognitive impairment.

Keywords: genetics, SNPs, cognitive impairment, Alzheimer, CSF, amyloid beta, tau, hippocampal atrophy, memory

INTRODUCTION

Alzheimer's disease (AD) of the non-Mendelian form has been shown to also have a strong genetic component.^{1,2} Apart from the apolipoprotein E (*APOE*) ϵ_4 allele, which is to date the strongest known genetic risk factor for AD, single nucleotide polymorphisms (SNPs) associated with AD have been identified in the past years through genome-wide association studies (GWAS) and other high-throughout approaches to genetics. *ABCA7*, *BIN1*, *CD2AP*, *CD33*, *CLU*, *CR1*, *MS4A4E*, *MS4A4/MS4A6A* and *PICALM* have now also been considered as established risk genes for AD.³⁻⁸ Their effects on markers of AD pathology are not clearly understood and warrant further research.

The aim of the current study was to investigate the relation between AD risk genes and key measures of AD pathology in cerebrospinal fluid (CSF), magnetic resonance imaging (MRI) and cognition. We tested CSF $A\beta_{1-42}$, CSF total tau (t-tau) and hippocampal volume as biomarkers for AD, and delayed memory as a clinical marker.

CSF $A\beta_{1-42}$ and tau reflect key pathological processes in the brain. In AD, CSF $A\beta_{1-42}$ concentrations are decreased and correlate with the presence of neuritic plaques, which are one of the pathological hallmarks of AD. Hyperphosphorylated tau (p-tau) levels are increased in CSF and correlate with neurofibrillary tangles (NFTs).^{9,10} Abnormal p-tau and total tau (t-tau) concentrations are also related to neuronal degeneration.¹¹⁻¹³ Abnormally decreased concentrations of $A\beta_{1-42}$ and increased levels of tau are regarded as supporting features for the diagnosis of AD in the revised National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.^{14,15}

CSF $A\beta_{1-42}$ and tau levels have been reported to differ significantly between *APOE* ϵ_4 allele carriers and noncarriers who progressed to AD.¹³ In AD, *PICALM* has been shown to affect $A\beta_{1-42}$ but not tau,¹⁶ while *CR1* has been reported to have no effect on both $A\beta_{1-42}$ and tau.¹⁷ In another study of a mixed sample of AD, MCI and controls, *CR1* and *PICALM*, but not *BIN1*, have been related to the CSF AD profile of abnormal $A\beta$ and tau levels.¹⁸

Another biomarker of AD pathology is hippocampal volume. The hippocampus is one of the earliest brain regions affected by AD.^{19,}
²⁰ Hippocampal atrophy is already present in patients with cognitive impairment but with no dementia, and predicts progression to AD among subjects with mild cognitive impairment (MCI)²¹ and in a memory clinic setting.^{22, 23} The *APOE* ϵ_4 allele and *PICALM* have been found to significantly correlate with reduced hippocampal volume in a combined sample of cognitively normal controls, MCI nonconverters, MCI converters and patients with AD from a general population.²⁴ In the same study, *BIN1* has been reported to marginally correlate with decreased hippocampal volume, while no significant effect was observed for *CR1*.²⁴

Clinically, progressive memory loss is a key feature of AD and is already evident among patients in the predementia stage. A relation between decline in delayed memory and the *APOE* ϵ_4 allele has been reported among cognitively impaired elderly in a population study.²⁵ The *APOE* ϵ_4 allele has been shown to significantly correlate with poorer immediate and delayed memory in normal aging,²⁶ but affected only delayed memory and not other memory scores in familial AD.²⁷ *APOE*, *CR1*, *PICALM*, *BIN* and *CLU* have been reported to exert no significant effects on episodic memory among cognitively normal controls.²⁸ However, a combination of the *APOE* ϵ_4 allele with the homozygote major alleles of *CR1*, *PICALM*, *BIN* and *CLU* have been observed to correlate with significantly low episodic memory.²⁸

The biomarkers and cognitive measure we selected for the present study become abnormal in different stages of AD. According to the amyloid cascade hypothesis, CSF $A\beta_{1-42}$ become abnormal first, while abnormal tau, hippocampal atrophy and memory decline occur later.^{29, 30}

We investigated the relation of established AD risk genes in the AlzGene top results loci with CSF $A\beta_{1-42}$ and t-tau, hippocampal volume and delayed memory and risk for progression to AD in nondemented patients with cognitive impairment from a memory clinic setting. These patients have a high risk for progressing to AD although not all will have AD pathology.³¹

METHODS

Study population

Cohort

Participants were recruited from the 'Development of Screening Guidelines and Criteria for Predementia Alzheimer's Disease' (DESCRIPA) study, which is a multicenter prospective cohort study by the European AD Consortium tasked to develop screening guidelines and clinical criteria for the diagnosis of predementia AD.³² DESCRIPA included patients aged 55 years or older who were referred to memory clinics because of cognitive complaints but did not fulfill the clinical criteria for dementia according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) and the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA).^{33, 34} Participants who had memory complaints that could not be objectified through cognitive tests were diagnosed with subjective cognitive impairment (SCI).³⁵ Those who obtained a total clinical dementia rating (CDR) of 0.5 were diagnosed with mild cognitive impairment (MCI). Exclusion criteria were any psychiatric, somatic or neurological disorder, or a history of alcohol abuse that may have caused the cognitive impairment.³² Participants underwent clinical, neurological, neuropsychiatric, neuropsychological and functional assessments. A subset of participants underwent neuroimaging, provided CSF or venous blood for analyses. Contraindications for CSF collection were coagulation disorder, use of anticoagulants, spinal deformations or presence of neurological disorders that could lead to increased intracranial pressure. The local medical ethics committee of the participating centers approved the procedures. All participants gave their written informed consent.

Follow-up assessment was performed annually up to five years. Progression to AD-type dementia at follow-up was diagnosed according to the DSM-IV³³ and NINCDS-ADRDA criteria.³⁴

Selection of participants

For the present study, we included all nondemented participants with cognitive impairment for whom venous blood for DNA extraction and CSF, MRI scan or scores on a memory domain test were available.

Of the 881 DESCRIPA participants from 20 centers, genotype data were available for 287 from 9 centers. Participants who were genotyped were slightly younger than those who were not genotyped (mean 69.3 vs. 70.8 years, $P=0.01$). The two groups did not differ in terms of education and Mini-Mental State Examination (MMSE)³⁶ score ($P>0.05$).

Of the 287 participants, we included for the current study 265 (MCI=244, SCI=21) from 9 centers who had available scores on a delayed recall memory test, 66 (MCI=57, SCI=9) from 6 centers who provided CSF samples and 58 (MCI=53, SCI=5) from 5 centers who had MRI scans. Participants with CSF data were younger than those without CSF data (68.4 vs. 70.8 years, $P<0.001$). The two groups were similar in terms of education and MMSE score ($P>0.05$). Participants with MRI scans had less years of education and lower MMSE scores compared to those without MRI scans (9.2 vs. 10.8 years, $P=0.03$ for education and 27.1 vs. 27.6, $P=0.003$ for MMSE score). Their age was comparable ($P>0.05$). Participants who were and were not assessed for memory performance differed in age (69.9 vs. 73.5 years, $P<0.001$), but had comparable education and mean MMSE score ($P>0.05$).

Gene selection

We selected for analyses *APOE*, rs744373 in bridging integrator 1 (*BIN1*), rs11136000 in clusterin (*CLU*), rs3764650 in ATP-binding cassette, subfamily A, member 7 (*ABCA7*), rs3818361 in complement component receptor 1 (*CR1*), rs3851179 in phosphatidylinositol binding clathrin assembly protein (*PICALM*), rs610932 and rs670139 in the membrane-spanning 4-domains, subfamily A gene cluster (*MS4A6A* and *MS4A4E*, respectively), rs3865444 in CD33 molecule (*CD33*), and rs9349407 in CD2-associated protein (*CD2AP*) (for a description of the functions of the genes, see ³⁷⁻³⁹). These loci were listed as top 10 in AlzGene. The up-to-date online AlzGene database provides meta-analyses of published genetic association studies for AD for all polymorphisms with a minor allele frequency of $>1\%$ in healthy controls and for which genotype data for the same polymorphism is available in at least four case-control studies.⁴⁰ The 'top results' lists polymorphisms with a nominally significant summary odds ratio (OR), which are subsequently ranked according to the interim criteria of the Human Genome Epidemiology Network (HuGENet).⁴⁰ The criteria

developed by HuGENet, also known as Venice criteria, takes into consideration the amount of evidence (operationalized as sample size, statistical power or false discovery rate); extent and consistency of replication (as assessed among others by measures of heterogeneity); and protection from potential sources of bias (such as population stratification and genotyping error) in assessing the epidemiologic credibility of the genetic association.⁴¹ In the present study, we used the term 'risk allele' as the risk allele reported in AlzGene meta-analysis of genetic association studies.

Genetic analysis

Genomic DNA was extracted from EDTA anticoagulated blood and amplified by polymerase chain reaction (PCR). A total of 2.5 ng of genomic DNA with the SNP site of interest was amplified in a 5 µl total volume of reaction mixture containing forward and reverse primers. Residual dNTPs were dephosphorylated with shrimp alkaline phosphatase (SAP). Extension primers were added following SAP treatment. Multiplex assays for amplification and extension primers were designed using the Sequenom MassARRAY Assay Design 3.1 software. DNA samples, with a number of duplicates as controls, were randomly placed on 384-well plates. SNPs were genotyped on Sequenom i-Plex platform (Sequenom Inc., Hamburg, Germany). *APOE* genotype was determined through the combination of two SNPs, rs7412 and rs429358.⁴² The bioanalysts who performed the genotyping were blinded to clinical information.

Chi-square test of genotypic frequencies showed that the SNPs selected for the present study were in Hardy-Weinberg equilibrium ($P > 0.05$). All samples included had a call rate of $\geq 90\%$.

Genotypic frequencies of the minor allele variant was $< 20\%$ for 90% of the SNPs tested. This indicated that assessing the effects of the SNPs on AD markers through an overall analysis or using a recessive model would yield unreliable results due to the small number of homozygous carriers of the minor allele which reduced statistical power for these models. In this case, it is not unusual to assume a dominant model.⁴³ We therefore assessed carrier risk against the major genotype in a dominant model, wherein carriers of homozygous major alleles were compared with carriers of the pooled homozygous minor and heterozygous alleles. For *APOE*, participants were dichotomized as ϵ_4 allele carriers vs. noncarriers.

Cognitive assessment

We used delayed memory as a clinical marker. Memory performance was assessed according to the neuropsychological protocol at each center. Consequently, the tests used varied. Of the 265 participants with genotype and cognitive data, 200 were tested using the Rey Auditory Verbal Learning Test,⁴⁴ 42 took the Buschke Selective Reminding Test⁴⁵ and 23 underwent the Hopkins Verbal Learning Tests.⁴⁶ To pool the data, raw scores were transformed into z-scores adjusted for age, gender and education using normative data that were locally collected or were published.³² A z-score of -1.5 corresponds to a score of 1.5 standard deviation (SD) below the expected score after correction for age, gender and education. Z-scores were used in the analyses.

CSF collection and analysis

CSF was obtained through lumbar puncture of the L3/L4 or L4/L5 intervertebral space. The samples were centrifuged and stored in sterile polypropylene tubes at -80 degree Celsius until analyses. A β_{1-42} and t-tau concentrations were measured using commercially available INNOTEST enzyme-linked immunosorbent assays (ELISAs) (Innotest β -amyloid₍₁₋₄₂₎, Innotest hTau Ag, Innogenetics, Ghent, Belgium). All CSF measurements were performed at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital in Mölndal, Sweden by bioanalysts who were unaware of the clinical data.

Concentrations below 500 pg/ml for CSF A β_{1-42} and above 375 pg/ml for CSF t-tau were considered abnormal.⁴⁷ For SNPs showing significant correlation with CSF markers, we further examined whether a CSF profile for AD was present. This was done by computing the ratio of CSF A β_{1-42} to t-tau and defining scores lower than 1.14 as indicative of an CSF AD profile.^{48, 49}

Neuroimaging acquisition and analysis

All MRI scans were acquired at 1.0 or 1.5T and included a T1-weighted three-dimensional gradient echo sequence and a fast fluid attenuated inversion recovery (FLAIR) sequence.⁵⁰ Hippocampal volume was measured using automated learning embeddings for atlas propagation (LEAP). Details of this method have been described elsewhere.⁵¹ For the

present study, we used the sum of the left and right hippocampal volume corrected for intracranial pressure using the Montreal Neurological Institute space.⁵² Hippocampal volume of less than 5300 cm³⁴⁹ was considered abnormal. All measurements using LEAP were performed at the Department of Computing, Imperial College in London by experienced technicians who were blinded to clinical information.

Statistical analysis

The IBM SPSS Statistics version 19 (IBM Corp., NY, USA) for Mac OS X and Stata 11.2 (Stata Corp., TX, USA) was used for the statistical analyses. Group differences in terms of mean age, education and MMSE score were assessed using independent samples t-test. The Hardy-Weinberg equilibrium was computed using Chi-square test. Progression to AD was assessed through logistic regression, with correction for age and gender. In examining the effects of SNPs on the markers of AD pathology, we used mixed models to adjust for confounders.

Prior to statistical analyses, we assessed normality of data distribution using Shapiro-Wilk. Data for $A\beta_{1-42}$ and t-tau levels were skewed ($P < 0.001$) and were thus transformed into their natural logarithms to normalize distribution. Log transformed CSF data were used in the statistical analyses. Age and gender were included as covariates for all analyses, and adjustment for education was done for the cognitive measure. Correction for center was made for analyses involving centers with more than one participant.

The unadjusted statistical significance level was $P < 0.05$. We corrected for multiple testing using the procedure of Benjamini and Liu for controlling the false discovery rate (FDR).^{53, 54} FDR is the expected proportion of false positives among all significant results,⁵⁵ which we defined in the present study as no more than 5%. In this method, the observed P -values were ranked from smallest to largest and successively compared with FDR adjusted P -values (P -adj). The P -adj were computed using the formula: $\min(0.05, 0.05 \times \text{total number of SNPs tested} / (\text{total number of SNPs tested} + 1 - \text{rank of the observed } P\text{-value}))$.⁵³ Observed P -values that were equal or smaller than P -adj were considered significant.

RESULTS

Characteristics of the study population

Table 1 shows the demographic characteristics of the study population per subgroup. Of the 66 participants with available CSF, 41 (62%) had abnormally reduced $A\beta_{1-42}$ concentrations, 30 (45%) had abnormally elevated t-tau levels and 22 (33%) had abnormally low CSF $A\beta_{1-42}$ /t-tau ratio. Ten of the 58 participants (17%) with MRI scans had abnormally reduced hippocampal volume. Of the 265 participants who underwent cognitive assessment, 110 (41%) performed below the expected score for the delayed memory test (z-score of -1.5 or lower).

The genotype and minor allele frequencies of participants with available memory test score, which constituted the biggest subgroup, are presented in Table 2. Participants with data on CSF biomarkers and hippocampal volume had similar genotypic distributions (Supplementary Table 1).

Of the 151 participants seen for follow-up assessment (average follow-up 2.6 years, $SD=1.1$), 19% ($n=29$) progressed to AD within 5 years, with 12% of the conversion occurring within the first 2 years (average conversion 2.5 years, $SD=1.1$).

Table 1 Baseline characteristics of the study population

Characteristic	CSF subgroup	n	Hippocampal volume subgroup	n	Memory subgroup	n
Age (years)	68.0 (8.2)	66	69.3 (8.6)	58	69.2 (8.0)	265
Sex (female)		33		35		159
Education (years)	11.3 (4.3)	66	9.5 (4.0)	58	10.7 (4.5)	265
MMSE score	27.7 (2.4)	66	27.9 (2.1)	58	27.5 (2.3)	265
Memory z-score	-0.63 (1.27)	60	-1.25 (1.08)	56	-1.14 (1.40)	265
$A\beta_{1-42}$ level, pg/ml	464 (213)	66	482 (219)	15	462 (222)	60
Total tau level, pg/ml	383 (276)	66	423 (342)	15	380 (256)	60
Hippocampal volume, cm^3	5996 (787)	15	6132 (714)	58	6183 (673)	56

All data are mean (standard deviation) unless otherwise specified. CSF, cerebrospinal fluid; n, number of cases; MMSE, Mini-Mental State Examination; $A\beta$, amyloid beta.

Table 2 Genotype and minor allele frequencies of subgroup with memory test scores

Gene	SNP	Cases, <i>n</i>	Genotype	Genotypic frequencies, %	MAF, %
<i>CR1</i>	rs6656401	265	GG/GA/AA	55.1/39.2/5.7	25
<i>BIN1</i>	rs744373	259	TT/TC/CC	49.8/41.7/8.5	29
<i>CD2AP</i>	rs9349407	262	GG/GC/CC	53.0/40.5/6.5	27
<i>CLU</i>	rs11136000	264	CC/CT/TT	36.7/43.9/19.3	41
<i>MS4A4E</i>	rs670139	261	CC/CA/AA	36.4/54.0/9.6	37
<i>MS4A6A</i>	rs610932	260	CC/CA/AA	29.2/48.0/22.7	47
<i>PICALM</i>	r3851179	262	GG/GA/AA	45.0/45.8/9.2	32
<i>ABCA7</i>	rs3764650	251	TT/TG/GG	80.1/18.3/1.6	11
<i>CD33</i>	rs3865444	259	GG/GT/TT	50.6/39.4/10.0	30
<i>APOE</i>		258	0ε4/1ε4/2ε4 ^a	63.6/31.0/5.4	... ^b

SNP, single nucleotide polymorphism; *n*, number of cases; MAF, minor allele frequency; *CR1*, complement component receptor 1; *BIN1*, bridging integrator 1; *CD2AP*, CD2-associated protein; *CLU*, clusterin; *MS4A4E*, membrane-spanning 4-domains subfamily A member 4E; *MS4A6A*, membrane-spanning 4-domains subfamily A member 6A; *PICALM*, phosphatidylinositol binding clathrin assembly protein; *ABCA7*, ATP-binding cassette, subfamily A, member 7; *CD33*, CD33 molecule; *APOE*, apolipoprotein E.

^a Presented as ε4 allele carriership, wherein 0ε4=noncarriers, 1ε4=carrier of one ε4 allele, 2ε4=carriers of two ε4 alleles.

^b Minor allele frequency not computed because *APOE* is tri-allelic.

Relation of SNPs to CSF biomarkers

CSF Aβ₁₋₄₂

Only the *APOE* ε4 allele was significantly correlated to reduced concentration of Aβ₁₋₄₂ after adjusting for multiple testing (*P*=0.001; Table 3). *PICALM* was correlated with decreased Aβ₁₋₄₂ concentrations among homozygous risk allele carriers (*P*=0.02), but this effect did not pass FDR correction.

Risk allele carriers of *CD2AP* showed a trend of reduced Aβ₁₋₄₂ (*P*=0.06). A clear opposite effect was observed for *BIN1*, wherein carriers of the risk allele had increased Aβ₁₋₄₂ concentrations (*P*=0.69; Figure 1a).

CSF tau

None of the SNPs studied were significantly related with CSF t-tau (Figure 1b, Table 3). The strongest effects were seen for *CLU* and *APOE*. Increased t-tau levels were observed among *CLU* homozygous risk allele carriers ($P=0.15$; Table 3) and *APOE* ϵ_4 allele carriers ($P=0.17$).

CSF AD profile

SNPs in *APOE* and *PICALM* showed correlation with individual CSF measures. We therefore tested whether there was also a correlation between these SNPs and CSF $A\beta_{1-42}$ /t-tau ratio. We used continuous CSF values in the computations. The *APOE* ϵ_4 allele significantly correlated with the ratio ($P=0.007$), while a marginal correlation was observed for the risk allele of *PICALM* ($P=0.05$). We did not adjust for multiple testing because this was a sub-analysis involving two SNPs.

Relation of SNPs to hippocampal volume

The SNPs tested did not show a consistent pattern of correlation to hippocampal volume (Figure 1c, Table 3). Only *CLU* correlated with decreased hippocampal volume among homozygote risk allele carriers ($P=0.02$), although this effect did not remain significant after FDR correction. The ϵ_4 allele of *APOE* showed marginal correlation with reduced hippocampal volume ($P=0.08$). *CR1* risk allele carriers had larger hippocampal volume ($P=0.01$) instead of the expected reduced volume. This effect was no longer significant after correction for multiple testing.

Relation of SNPs to delayed memory

The majority of the SNPs we tested were not related to delayed memory performance (Figure 1d, Table 3). A lower memory performance was seen for *CD2AP* risk allele carriers ($P=0.02$), while risk allele carriers of *ABCA7* performed better on memory tasks ($P=0.02$). These effects did not withstand multiple test correction. *APOE* ϵ_4 allele carriers showed poorer memory compared to noncarriers, although this result was not significant ($P=0.08$).

Table 3 Effects of SNPs on markers of AD pathology

Chr, Gene	SNP	Genotype	A β ₁₋₄₂ , pg/ml Mean (SD)	P-value DM	T-tau, pg/ml Mean (SD)	
1, <i>CR1</i>	rs6656401	GG	482 (215)	0.21	384 (292)	
		AG	455 (217)		394 (270)	
		<u>AA</u>	293 (124)		385 (209)	
2, <i>BIN1</i>	rs744373	TT	428 (213)	0.69	375 (244)	
		CT	473 (197)		452 (317)	
		<u>CC</u>	544 (243)		271 (264)	
6, <i>CD2AP</i>	rs9349407	GG	526 (254)	0.06	376 (312)	
		GC	405 (165)		406 (252)	
		<u>CC</u>	475 (102)		340 (95)	
8, <i>CLU</i>	rs11136000	<u>CC</u>	460 (225)	0.53	447 (312)	
		CT	433 (202)		385 (271)	
		TT	555 (208)		252 (184)	
11, <i>MS4A4E</i>	rs670139	CC	486 (226)	0.63	300 (242)	
		CA	457 (213)		419 (300)	
		<u>AA</u>	444 (205)		402 (158)	
11, <i>MS4A6A</i>	rs610932	<u>CC</u>	433 (201)	0.40	361 (287)	
		CA	448 (205)		442 (292)	
		AA	542 (250)		276 (200)	
11, <i>PICALM</i>	rs3851179	<u>GG</u>	384 (170)	0.02	311 (218)	
		GA	527 (222)		551 (303)	
		AA	582 (318)		384 (289)	
19, <i>ABCA7</i>	rs3764650	TT	464 (227)	0.86	383 (257)	
		TG	467 (123)		398 (409)	
		<u>GG</u>	464 ^a		296 ^a	
19, <i>CD33</i>	rs3865444	<u>GG</u>	447 (203)	0.57	397 (318)	
		GT	504 (254)		371 (239)	
		TT	433 (118)		479 (207)	
19, <i>APOE</i>		$\epsilon\epsilon$ ^b	547 (234)	0.001^{c*}	317 (258)	
		1ϵ ^b	372 (134)		434 (272)	
		<u>2ϵ</u> ^b	321 (130)		604 (298)	

AD, Alzheimer's disease; Chr=chromosome; SNPs, single nucleotide polymorphisms; A β , amyloid beta; SD, standard deviation; T-tau, total tau; DM, dominant model.

AlzGene risk allele underlined and in bold, presented for reference purposes. Genotypes for the current study presented in the following order: major/major, major/minor, minor/minor. Analyses were corrected for age, gender, education and study center, as appropriate. Adjustment for education was done only for memory. No correction for study site was done for hippocampal volume because in one of the study sites there was only one participant.

	P-value DM	Hippocampal volume, cm ³ Mean (SD)	P-value DM	Memory score Mean (SD)	P-value DM
	0.78	5909 (788)	0.01	-1.13 (1.38)	0.74
		6452 (480)		-1.21 (1.46)	
		6113 (670)		-0.83 (1.28)	
	0.69	6212 (669)	0.45	-1.17 (1.33)	0.77
		5969 (792)		-1.15 (1.45)	
		6434 (528)		-1.02 (1.66)	
	0.55	6240 (715)	0.43	-0.92 (1.32)	0.02
		6068 (733)		-1.27 (1.42)	
		6001 (710)		-2.27 (1.47)	
	0.15	5866 (812)	0.02	-1.30 (1.57)	0.27
		6375 (531)		-1.24 (1.26)	
		6058 (768)		-0.64 (1.26)	
	0.76	6042 (696)	0.43	-1.09 (1.53)	0.95
		6256 (670)		-1.15 (1.36)	
		5863 (922)		-1.30 (1.11)	
	0.25	6306 (646)	0.32	-1.25 (1.32)	0.42
		6093 (74)		-1.17 (1.38)	
		6068 (679)		-0.98 (1.57)	
	0.29	6133 (542)	0.99	-1.08 (1.31)	0.23
		6024 (824)		-1.20 (1.48)	
		6646 (878)		-1.24 (1.55)	
	0.64	6175 (687)	0.47	-1.24 (1.37)	0.02
		6010 (853)		-0.87 (1.55)	
		6177 ^a		0.43 (1.54)	
	0.99	6194 (721)	0.32	-1.09 (1.38)	0.85
		6162 (538)		-1.18 (1.45)	
		5407 (1043)		-1.27 (1.48)	
	0.17 ^c	6200 (697)	0.08 ^c	-1.10 (1.33)	0.08 ^c
		5923 (631)		-1.24 (1.45)	
		5312 (910)		-1.88 (1.69)	

*Significant after False Discovery Rate correction.

^a Only 1 ABCA7 rs3764650 GG carrier had available CSF and hippocampal volume measurement;

^b Presented as APOE ϵ_4 allele carriership, wherein 0 ϵ_4 = noncarriers, 1 ϵ_4 =carrier of one ϵ_4 allele, 2 ϵ_4 =carriers of two ϵ_4 alleles;

^c Result for APOE ϵ_4 allele carriers vs. noncarriers.

Figure 1a

Relation between AD risk genes and markers in CSF Aβ1-42. All data are means.

Figure 1b

Relation between AD risk genes and markers in t-tau. All data are means.

Figure 1c

Relation between AD risk genes and markers in hippocampal volume. All data are means.

Figure 1d

Relation between AD risk genes and markers in delayed memory. All data are means.

Effects of SNPs on progression to AD

The risk effects related to the SNPs studied for progression to AD are presented in Table 4. As a reference, we also showed the odds ratio for AD from the AlzGene.

Only the ϵ_4 allele of *APOE* was a significant predictor of AD (OR 2.5, 95% CI 1.06-6.02, uncorrected $P=0.04$). *CD2AP*, *MS4A4E*, *CLU*, *MS4A6A* and *PICALM* showed an association with AD line with AlzGene findings, although this was not statistically significant. The outcomes for *CR1*, *BIN1*, *ABCA7* and *CD33* were in the opposite direction of what would be expected based on AlzGene meta-analyses.

Table 4 Predictive value of SNPs for progression to AD within 5 years (n=29)

Chr, Gene	SNP/allele	OR (95% CI)	P-value	AlzGene OR (95% CI)
1, <i>CR1</i>	rs6656401	0.62 (0.25-1.50)	0.29	1.19 (1.09-1.30)
2, <i>BIN1</i>	rs744373	0.55 (0.23-1.34)	0.19	1.17 (1.13-1.20)
6, <i>CD2AP</i>	rs9349407	1.70 (0.70-4.02)	0.24	1.12 (1.08-1.16)
8, <i>CLU</i>	rs11136000	0.55 (0.23-1.33)	0.19	0.89 (0.86-0.91)
11, <i>MS4A4E</i>	rs670139	1.34 (0.52-3.45)	0.54	1.08 (1.05-1.11)
11, <i>MS4A6A</i>	rs610932	0.52 (0.20-1.34)	0.17	0.90 (0.88-0.93)
11, <i>PICALM</i>	rs3851179	0.89 (0.37-2.12)	0.79	0.88 (0.86-0.91)
19, <i>ABCA7</i>	rs3764650	0.28 (0.06-1.34)	0.11	1.23 (1.18-1.28)
19, <i>CD33</i>	rs3865444	1.41 (0.58-3.42)	0.45	0.89 (0.84-0.95)
19, <i>APOE</i>	ϵ_4	2.53 (1.06-6.02)	0.04	3.68 (3.30-4.11)

Dominant model used in the analyses. Odds ratio from AlzGene presented for reference purposes. SNPs, single nucleotide polymorphisms; AD, Alzheimer’s disease; Chr, chromosome; OR, odds ratio; CI, confidence interval.

Post hoc analyses

Our study sample was composed of patients with MCI and SCI. When we excluded subjects with SCI, the results remained essentially the same.

DISCUSSION

We tested the effects of the top 10 AlzGene loci on progression to AD in nondemented patients with cognitive impairment, and performed a

correlational study of these loci with $A\beta_{1-42}$ and tau in CSF, hippocampal volume and delayed memory.

Relation to CSF biomarkers

CSF $A\beta_{1-42}$ and tau

In the analyses with CSF biomarkers, the SNPs we tested generally showed some weak effects on CSF $A\beta_{1-42}$ but not on t-tau. We found that $A\beta_{1-42}$ concentrations were significantly reduced among *APOE* ϵ_4 allele carriers in our cohort. This is in accordance with previous findings in MCI in a clinical setting,⁵⁶⁻⁵⁸ and in a mixed clinical and population setting.⁵⁹ In the largest study so far, the CSF level of $A\beta_{1-42}$ decreased with approximately 100 pg/ml for each *APOE* ϵ_4 allele,⁶⁰ which is similar to that in the present study.

APOE did not correlate with t-tau in our patient population. Changes in tau have been reported to occur in the later phase of AD pathogenesis.¹³ Previous studies have shown that *APOE* is involved in amyloid rather than tau pathology.^{56, 61} In the present study, the *APOE* ϵ_4 allele was also related with the CSF $A\beta_{1-42}$ /t-tau ratio which could be attributed to significantly reduced $A\beta_{1-42}$ levels and nonsignificant increase in t-tau concentrations.

PICALM was related to reduced CSF $A\beta_{1-42}$ levels in our cohort (unadjusted $P=0.02$), although this effect did not remain significant after multiple test correction. *PICALM* has been reported to increase $A\beta$ production and affect $A\beta$ neurotoxicity through endocytic regulation.^{62, 63} Neuropathological examination of a sample population composed mainly of individuals with no dementia has found a correlation between *PICALM* and amyloid but not tau pathology. A population study of individuals with no dementia that used a surrogate marker (rs7110631 for rs3851179) likewise obtained significant correlation with $A\beta$ but not tau upon brain autopsy. Another loci in *PICALM* (rs541458) has been linked with CSF $A\beta_{1-42}$ in AD but not in cognitively healthy controls, whereas no correlation was found with CSF t-tau.¹⁶ These results suggest that *PICALM* affects amyloid and this effect could be detected in the absence of dementia.

In *PICALM*, we found that the low CSF $A\beta_{1-42}$ /t-tau ratio was driven by decreased $A\beta_{1-42}$ concentration among homozygous risk allele carriers, despite the fact that these subjects also had decreased t-tau

levels. The correlation was marginal ($P=0.05$). In a previous study, it was not rs3851179 but another loci in *PICALM* (rs541458) that was related to a CSF AD profile.⁶⁴

CD2AP marginally correlated with reduced CSF A β_{1-42} (unadjusted $P=0.06$). *CD2AP* has a role in synapse formation in the immune system.³⁸ It has been reported to interact with an A β toxicity modifier gene (*SYNJ1*)⁶⁵ and has been suggested to contribute to AD pathogenesis through synaptic dysfunction and cell membrane processes.⁶⁶

None of the SNPs we tested correlated with t-tau. Abnormalities in tau concentrations have been linked to a later event in the pathogenesis of AD^{13, 22, 67} rather than in predementia stages, which could explain why we found no effect of the SNPs on tau.

Relation to hippocampal volume

None of the SNPs tested significantly correlated with reduced hippocampal volume after multiple test correction. We observed a nonsignificant decrease in hippocampal volume in relation to *CLU*, *APOE* and *CR1*.

CLU is an apolipoprotein involved in lipid transport³⁸. It binds soluble A β and is present in amyloid plaques.⁶⁸ It is interesting to note that *CLU* affected hippocampal volume more than CSF A β_{1-42} in our cohort. As mentioned, decrease in hippocampal volume is a later event in the AD pathogenesis.

The *APOE* ϵ_4 allele marginally correlated with reduced hippocampal volume in our cohort. This is in line with previous studies reporting hippocampal volume loss related to *APOE* in MCI and among cognitively healthy control subjects but not in AD.^{69, 70}

CR1 has a suggested role in neuroinflammation and in A β clearance since it binds A β oligomers to its C3b complement factor.^{38, 71} In the present study, *CR1* homozygous nonrisk allele carriers had reduced hippocampal volume. This finding is not supported by literature and may have been the result of our small sample size. *CR1* has not been shown in previous studies to affect the hippocampus.^{24, 72} In a study of young healthy individuals, homozygous risk allele carriers of *CR1* rs6656401 have been shown to have reduced gray matter in the entorhinal cortex.⁷² A similar finding for another loci in *CR1* (rs1408077) has been reported in another study in a mixed clinical and population setting.²⁴ In the same study, rs1408077 was not related to hippocampal atrophy.

Relation to delayed memory

CD2AP showed correlation to delayed memory at an unadjusted *P*-value. *CD2AP* has not been shown in a previous study to significantly affect memory, although this study pertained to nondemented individuals in a general population.⁷³ It is conceivable that subtle effects of *CD2AP* on memory are more detectable in a population of nondemented individuals with cognitive impairment in a clinical setting, such as our cohort. Further research is needed to verify the influence of *CD2AP* on memory.

We observed a weak correlation between *APOE* and delayed memory ($P=0.08$). *APOE* does not appear to directly affect cognition. Rather, it influenced CSF $A\beta_{1-42}$ concentrations in our cohort. In MCI, *APOE* has been correlated with poor episodic memory in the presence of low CSF $A\beta_{1-42}$.⁷⁴ A high cerebral $A\beta$ load rather than *APOE* has been reported to strongly affect episodic memory among cognitively healthy elderly.⁷⁵ However, NFTs rather than amyloid deposition have been hypothesized to be related to cognitive decline.⁷⁶ This has been shown in clinicopathological studies wherein *APOE* has been correlated with $A\beta$ deposition and NFTs.^{77, 78} In turn, NFTs have been associated with cognitive decline and the clinical diagnosis of AD.⁷⁹ Our cohort consisted of nondemented patients, and because tangle formation is a later pathogenic event in AD, this partly explains why we found no significant correlation with delayed memory.

The observation that risk allele carriers of *ABCA7* rs3764650 appeared to have better delayed recall compared to noncarriers was unexpected but may be due to the small number of risk allele carriers in our cohort. A recent study has found no correlation between *ABCA7*, memory and other cognitive domains among nondemented individuals in a population setting.⁷³

Effects on clinical AD as outcome

Only *APOE* was a significant predictor of AD in our cohort. Carriers of the ϵ_4 allele had a 2.5-fold risk of progressing to AD compared to noncarriers. This approximates the findings of a previous study of the predictive accuracy of the ϵ_4 allele for progression from MCI to AD-type dementia.⁸⁰ A few loci we tested conferred weak effects on the risk of developing AD, in line with AlzGene cross-sectional findings. Other genes,

however, showed an opposite effect. These genes, including *ABCA7*, also showed an opposite effect with delayed recall. We tested four markers that become abnormal in different stages of AD. Only *APOE* showed a consistent pattern for all markers. *CD2AP* showed both an effect on CSF $A\beta_{1-42}$ and memory, but for the other SNPs, patterns were less consistent. The observation that SNPs correlated with some markers but not with others may indicate that the SNPs are associated with different pathophysiological mechanisms, but larger studies are needed to confirm this.

Our study was performed in a memory clinic population of nondemented patients. Some of these may have early stage AD while others may have cognitive impairments due to other causes. It is likely that the association between SNPs and AD markers will be dependent on the sample and stage of AD, as markers will vary with disease stage. This may also explain some of the discrepant findings with studies performed in other settings. The observation that SNPs had a very weak association with clinical outcome suggests that they are of limited use for giving patients with SCI or MCI a prognosis.

Strengths and limitations

One of the strengths, and a novel aspect, of the present study is the combination of MCI and SCI patients in a memory clinic setting. This patient population is at risk of progressing to AD, yet has not been studied before in relation to AD risk genes and key markers of AD pathology. Another strength is that we examined four markers that reflect different stages of AD. Our small sample size and the subsequent use of a dominant genetic model in the analyses is a limitation. Our findings must be interpreted in the exploratory context of the present study.

Conclusion

Only *APOE* predicted progression to AD, showed a consistent effect on AD markers, and correlated significantly with CSF $A\beta_{1-42}$. *APOE* showed stronger effects on all outcomes compared to recently discovered AD genetic risk factors. This is in line with stronger association with clinical AD according to AlzGene. Given the weak effects of the newly discovered genetic risk factors on AD, larger studies are needed to better understand the relation between these genes and AD pathophysiology.

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*Genetic variations--so-called SNPs-- in APOE,
MS4A4A and CLU were related to abnormally low
A β 1-42 levels in Finnish patients with AD.*

LS Elias-Sonnenschein, S Helisalmi, T Natunen, A Hall, T Paajanen, S-K Herukka,
M Laitinen, AM Remes, AM Koivisto, KM Mattila, T Lehtimäki, FRJ Verhey,
PJ Visser, H Soininen, M Hiltunen
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CHAPTER 7

Genetic loci associated with Alzheimer's disease
and cerebrospinal fluid biomarkers in a Finnish
case-control study

ABSTRACT

Background: To understand the relation between genetic risk factors for Alzheimer's disease (AD) and their influence on biomarkers for AD, we examined the association between AD in the Finnish cohort and single nucleotide polymorphisms (SNPs) from top AlzGene loci, genome-wide association studies (GWAS), and candidate gene studies; and tested the correlation between these SNPs and AD markers $A\beta_{1-42}$, total tau (t-tau), and phosphorylated tau (p-tau) in cerebrospinal fluid (CSF).

Methods: We tested 24 SNPs for genetic association with clinical AD in our cohort comprised of 890 AD patients and 701-age matched healthy controls using logistic regression. For the correlational study with biomarkers, we tested 36 SNPs in a subset of 222 AD patients with available CSF using mixed models. Statistical analyses were adjusted for age, gender and *APOE* status. False discovery rate for multiple testing was applied. All participants were from academic hospital and research institutions in Finland.

Results: *APOE-ε4*, *CLU* rs11136000 and *MS4A4A* rs2304933 correlated with significantly decreased $A\beta_{1-42}$ (corrected $P < 0.05$). At an uncorrected $P < 0.05$, *PPP3R1* rs1868402 and *MAPT* rs2435211 were related with increased t-tau; while *SORL1* rs73595277 and *MAPT* rs16940758, with increased p-tau. Only *TOMM40* rs2075650 showed association with clinical AD after adjusting for *APOE-ε4* ($P = 0.007$), but not after multiple test correction ($P > 0.05$).

Conclusions: We provide evidence that *APOE-ε4*, *CLU* and *MS4A4A*, which have been identified in GWAS to be associated with AD, also significantly reduced CSF $A\beta_{1-42}$ in AD. None of the other AlzGene and GWAS loci showed significant effects on CSF tau. The effects of other SNPs on CSF biomarkers and clinical AD diagnosis did not reach statistical significance. Our findings suggest that *CLU* and *MS4A4A* influence both AD risk and CSF $A\beta_{1-42}$.

Keywords: Alzheimer, genetics, single nucleotide polymorphisms, amyloid, tau, biomarkers, cerebrospinal fluid, case-control

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease with a complex etiology. Neuritic plaques mainly composed of aggregated amyloid beta ($A\beta$) and neurofibrillary tangles (NFTs) resulting from hyperphosphorylated tau protein (p-tau) are pathological hallmarks of AD.¹ $A\beta_{1-42}$ tends to aggregate more compared to other $A\beta$ isoforms.² Total tau (t-tau) concentrations in cerebrospinal fluid (CSF) have been suggested to indicate the extent of neuronal damage, while p-tau levels reflected the phosphorylated state of tau.³ P-tau and t-tau levels signal axonal degeneration.^{4,5} In AD, the concentration of CSF $A\beta_{1-42}$ is decreased, which is supposed to reflect sequestration of $A\beta_{1-42}$ in amyloid plaques in the brain,⁶ while t-tau and p-tau levels are increased.¹

Genetically, AD is differentiated into Mendelian and non-Mendelian forms. AD with a Mendelian disease transmission typically has an early onset age (below 65 years) and caused by rare, highly penetrant mutations in the amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), or presenilin 2 (*PSEN2*) genes. The prevalence of AD due to *APP*, *PSEN1*, and *PSEN2* mutations is less than 5%.⁷ The majority of AD cases are of the non-Mendelian forms, which have been reported to have a strong genetic component.⁸ The apolipoprotein E (*APOE*) ϵ_4 allele is the strongest known genetic risk factor for AD.

Other high-risk genetic variants have been identified in genome-wide association studies (GWAS) (for review, see⁹). In addition, previous studies showed that a number of candidate genes correlated with $A\beta$ or tau. However, the relation between these AD risk genes and AD biomarkers remains ambiguous. With the exception of *APOE* and translocase of outer mitochondrial membrane 40 homolog (*TOMM40*), single nucleotide polymorphisms (SNPs) identified in case-control GWAS with clinical AD as outcome have not been replicated in GWAS with biomarkers as outcome.¹⁰

In this study, we first investigated whether previously reported genetic risk factors for AD were associated with AD risk in a Finnish case-control cohort. Second, we tested in the AD group the effects of these variants on the AD markers $A\beta_{1-42}$ and tau in CSF. We selected SNPs from AlzGene and GWAS, and from candidate genes that previously showed a relation with CSF $A\beta_{1-42}$ and tau.

METHODS

Study population

The Finnish-AD is a multicenter cohort comprised of 890 AD patients and 701 age-matched healthy controls from Kuopio, Oulu and Tampere in Finland. All patients were diagnosed with probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.¹¹ AD patients with an early onset did not show conclusive evidence of autosomal dominant transmission or mutations in *APP*, *PSEN1* or *PSEN2*. Control subjects had no symptoms of cognitive impairment based on clinical interview and neuropsychological examination. Of the 890 AD patients, 222 from Kuopio provided CSF for our study.

The Ethics Committee of the North-Savo Hospital District, Kuopio, Finland, approved the study. The physician and/or the study nurse gave written information of the study and explained the study protocol to the patient and caretaker if available. All participants provided written informed consent. A next of kin, caretakers or guardians consented on behalf of participants whose capacity to consent was compromised. In these cases, also the patient's own opinion was asked and considered, and the patient was recruited in the study only when he or she also agreed with this. The ethics committee approved this informed consent procedure.

Gene selection

We selected genes based on their reported association with AD or effect on CSF A β and tau. We included the 10 'top results' loci from AlzGene, which is an online database providing meta-analyses of published genetic association studies (for AlzGene top results criteria, see¹²), and selected the most promising SNPs from GWAS or other candidate gene studies. *APOE*, *CR1*, *BIN1*, *CD2AP*, *CLU*, *MS4A4E*, *MS4A6A*, *PICALM*, *ABCA7* and *CD33* were from AlzGene (for gene names, see Supplementary table). Analyses with *APOE* alone were performed for reference purposes only because *APOE* is an established susceptibility gene for sporadic AD.¹²⁻¹⁴ Functions of the AlzGene variants in relation to AD have been previously described.^{10, 15, 16} *MS4A4A*, *EXOC3L2* and *MTHFD1L* have

been shown in GWAS to be associated with AD¹⁷⁻¹⁹ but have not been included in the AlzGene top list to date.

CYP19A and *TOMM40* have been shown in GWAS with bio-markers as outcome to be related to A β , whereas *EPC2* and *RELN* were associated with tau.²⁰⁻²² *CYP19A* has also been reported to increase AD risk.²³

The candidate genes we selected that have been studied in relation to CSF biomarkers were *ACE*, *IDE*, *MAPT*, *SORL1*, *CYP46A1*, *BDNF*, *TF*, *PPP3R1* and another *TOMM40* polymorphism. The effects of *ACE*, *MAPT*, *SORL1* and *TOMM40* on A β and tau have been reported in a review (see¹⁰). *PPP3R1* has been correlated with increased p-tau.⁴ *BDNF* was linked with decreased total A β while *TF* was related to decreased A β_{1-42} /A β_{1-40} ratio.²⁴ *CYP46A1* has been associated with AD²⁵ and correlated with A β_{1-42} .²⁶ *IDE* has been reported to decrease A β_{1-42} in AD.²⁷ Moreover, it has been associated with AD risk²⁸ and neuropathological A β deposition.²⁹

Thirty-six SNPs in 25 genes were included in the analyses with CSF biomarkers (Supplementary table). For the genetic association analyses, we tested 24 genetic variants, excluding those in *ABCA7*, *BIN1*, *BDNF*, *CD2AP*, *CD33*, *CYP46*, *CLU*, *CR1*, *EPHA1*, *EXOC3L2*, *MS4A4E* and *MS4A6A* because these genes were previously studied in the Finnish population and found to be associated with AD either in the Finnish cohort alone or in multicenter GWAS.^{17, 25, 30-32}

Genotyping

DNA was extracted from peripheral blood with EDTA and amplified using polymerase chain reaction technique. DNA samples were randomly placed on 384-well plates and genotyped using Sequenom iPLEX platform (Sequenom, Hamburg, Germany) at University of Eastern Finland (UEF) in Kuopio. Patients and controls were dichotomized as *APOE*- ϵ_4 carriers or noncarriers.

Quality control procedures included using duplicates and negative controls, filtering on individual sample and SNP call rate, and testing whether the SNPs were in Hardy-Weinberg equilibrium (HWE). Samples with an average call rate of 90% were included. SNPs for the association analysis were in Hardy-Weinberg equilibrium ($P > 0.001$).

CSF analysis

CSF was obtained through lumbar puncture performed at UEF and the Kuopio University Hospital. $A\beta_{1-42}$, t-tau, and p-tau levels were measured using commercially available INNOTEST enzyme-linked immunosorbent assays (ELISAs) (Innotest β -amyloid₍₁₋₄₂₎, Innotest hTau Ag, and Innotest Phospho-tau_(181P), Innogenetics, Ghent, Belgium). All measurements were performed in one laboratory at UEF.

The relation between SNPs and biomarkers was assessed only for the AD group because the number of controls with available CSF ($n=30$) was too small to allow for meaningful analyses.

Statistical analysis

SPSS version 19 (Chicago, IL, USA) was used for the statistical analyses. We performed power calculations for the genetic association analyses.³³ Simulation analysis yielded more than 80% power to achieve ~1.3-1.5 risk effect at $P=0.05$, indicating that our case-control sample size was sufficient to find moderate genetic association with AD. The same risk effect for the false discovery rate (FDR) corrected $P=0.005$ yielded more than 50% power. For quantitative trait association,³⁴ we computed ~80% power for an effect size of 60-70 pg/ml for the dominant model at $P=0.05$ and 50% power at FDR corrected $P=0.005$. Similarly for t-tau, the effect size was 120-150 pg/ml and for p-tau 15-20pg/ml for a power of 80% at $P=0.05$ and 50% when FDR corrected ($P=0.005$).

Genetic association with AD was examined using Pearson chi-square test for multiple group comparisons and binary logistic regression for pairwise comparisons in univariate and multivariate analysis adjusted for age, sex, and *APOE-ε4* status. The relation between SNPs and biomarkers was assessed through mixed models for multiple group comparisons and pairwise comparisons, with correction for confounders. Normality was tested and assumed for biomarkers.

For the binary association, we first performed an overall test that provided information on genotype differences between cases and controls, and multiple comparison tests. For the quantitative association, we compared differences in mean biomarker values between genotypes in the overall test. Based on genotype frequencies, we opted to use a dominant model. Minor genotype frequencies for both genetic associa-

tion and CSF biomarker analyses were 0-15% for 40-50% of the SNPs studied. This reduces statistical power of a recessive model in most SNPs. All analyses were first corrected for age and sex, and then repeated with corrections for age, sex, and *APOE*- ϵ 4 status.

We used FDR correction for multiple testing, following the method of Benjamini and Hochberg.³⁵ Corrections were based on the number of SNPs tested and were performed separately for binary and quantitative associations. Observed *P*-values were ranked from smallest to largest. Adjusted *P*-values were successively computed in a step-up manner, starting from the second largest *P*-value, as follows: observed *P*-value (total number of SNPs tested/rank). Statistical significance was set at FDR adjusted $P < 0.05$.

RESULTS

Characteristics of the cohort

Table 1 shows the demographic characteristics of the participants. AD patients with available CSF did not differ from patients without CSF in terms of onset age ($P = 0.07$) and Mini-Mental State Examination³⁶ score ($P = 0.77$).

Genetic association with AD

APOE conferred a significant AD risk of 6.30 times higher among ϵ 4 allele carriers compared to noncarriers ($P < 0.0001$, Table 2). Age and gender did not affect the results (OR=6.25, 95% CI 5.22-8.15, $P < 0.0001$).

TOMM40 rs157580, rs2075650 and rs8106922 indicated association with AD risk in the univariate analyses (FDR $P < 0.05$). A protective effect was observed for G allele carriers of rs157580 and rs8106922, and a risk effect for G allele carriers of rs2075650. Only rs2075650 remained significant in the multivariate analysis (unadjusted $P < 0.007$) but did not pass FDR correction ($P > 0.05$).

Results for *MAPT* rs16940758 suggested no association with AD (FDR adjusted $P > 0.05$), although AD and control groups differed in multiple comparison test. *PPP3R1* rs1868402 and *ACE* rs4293 showed association with AD in multivariate analysis (unadjusted $P = 0.03$ for rs1868402 and $P = 0.01$ for rs4293) but did not remain significant after FDR adjustment. Overall and univariate analyses with rs1868402 and rs4293 were not significant.

Table 1 Characteristics of study participants

Characteristics	AD (N=890)	Control (N=701)	AD with CSF subgroup (n=222)
Age, mean (SD), y	69.8 (8.2) ^a	69.1 (6.2) ^b	69 (8) ^a
Female sex (%)	596 (67)	420 (60)	149 (67)
MMSE score, mean (SD)	19 (5)	-	19 (6)
APOE ε2/ε3/ε4 allelic distribution, %	2/53/45	4/80/16	-
Aβ ₁₋₄₂ level (SD), pg/ml		735 (195) ^c	443 (158) ^d
Phosphorylated tau level (SD), pg/ml		63 (26) ^e	84 (36) ^f
Total tau level (SD), pg/ml		311 (143) ^g	546 (269) ^h

Abbreviations: AD=Alzheimer’s disease; CSF=cerebrospinal fluid; N, n=sample size; SD=standard deviation; y=years; MMSE=Mini-Mental State Examination.

- ^a Onset age.
- ^b Age at examination.
- ^c Available for 32 control subjects.
- ^d Available for 222 AD patients.
- ^e Available for 30 control subjects.
- ^f Available for 151 AD patients.
- ^g Available for 30 control subjects.
- ^h Available for 159 AD patients.

Effects of SNPs on CSF Aβ₁₋₄₂

APOE ε₄ allele carriers had significantly reduced CSF Aβ₁₋₄₂ (FDR adjusted $P<0.05$, Table 3). Apart from APOE, only CLU significantly affected Aβ₁₋₄₂ among the AlzGene top loci. Carriers of rs11136000 major allele (C, risk allele in AlzGene meta-analysis) showed significantly decreased Aβ₁₋₄₂ (FDR adjusted $P<0.05$).

Of the SNPs identified in GWAS but not in the top AlzGene loci, only MS4A4A correlated with Aβ₁₋₄₂. Minor allele carriers (C, risk allele in GWAS) of rs2304933 showed significantly decreased Aβ₁₋₄₂ levels (FDR adjusted $P<0.05$) compared to major allele (A) carriers. The correlation was strengthened when corrected for APOE-ε₄ status.

Decreased $A\beta_{1-42}$ levels among *EXOC3L2* rs597668 minor allele carriers (C, risk allele in GWAS) were observed but the correlation did not pass FDR filter ($P > 0.05$).

Risk allele carriers of *TOMM40* SNPs identified through GWAS with biomarkers as outcome and through other candidate gene studies had decreased $A\beta_{1-42}$ concentrations, but this was not independent of *APOE-ε4* status (FDR adjusted $P > 0.05$).

The rest of the SNPs did not exhibit conclusive effects on $A\beta_{1-42}$ concentrations.

Effects of SNPs on CSF tau

None of the SNPs from the top AlzGene loci significantly correlated with CSF t-tau and p-tau. The strongest effect was for *PICALM* rs642949 and t-tau ($P = 0.06$, adjusted for age, sex, and *APOE-ε4* status), with increased t-tau levels among minor allele (C) carriers (Table 4). *APOE ε4* allele carriers also showed a nonsignificant increase in t-tau concentrations ($P = 0.08$).

None of the polymorphisms in other GWAS with clinical AD as outcome were linked with t-tau and p-tau.

From GWAS SNPs with biomarker as outcome, none of the results attained statistical significance. *EPC2* rs1374441 and t-tau showed the strongest effect. T-tau levels increased among minor allele (C, risk allele in GWAS) carriers ($P = 0.06$, corrected for age, sex, and *APOE-ε4* status).

Of the SNPs selected from other candidate genes, marginal correlations at FDR unadjusted $P < 0.05$ were obtained for polymorphisms in *PPP3R1*, *SORL1* and *MAPT*. T-tau levels increased among carriers of *PPP3R1* rs1868402 major allele (T, FDR adjusted $P > 0.05$). The effect was strengthened when corrected for *APOE-ε4* status.

SORL1 rs73595277 minor allele carriers (G) had increased p-tau (FDR adjusted $P > 0.05$). Effects of *SORL1* slightly decreased with *APOE-ε4* correction.

In *MAPT*, minor allele carriers of rs2435211 (T) had increased t-tau levels and minor allele carriers of rs16940758 had increased p-tau levels (FDR adjusted $P > 0.05$ for both SNPs). Correcting for *APOE-ε4* status enhanced the effects. These results, however, did not remain significant after FDR correction (adjusted $P > 0.05$ for both SNPs).

Table 2 Single nucleotide polymorphisms associated with Alzheimer’s disease risk

Chr, Gene	SNP	Genotypes	Cases (n) genotypic frequencies, %	MAF, %	Controls (n) genotypic frequencies, %	
2, <i>EPC2</i>	rs1374441	TT/TC/CC	(868) 58/36/6	24	(681) 59/35/6	
2, <i>EPC2</i>	rs4499362	CC/CT/TT	(875) 57/37/6	25	(689) 58/35/7	
2, <i>PPP3R1</i>	rs1868402	TT/TC/CC	(853) 53/39/8	27	(668) 56/38/6	
3, <i>TF</i>	rs1049296	CC/CT/TT	(866) 81/18/1	10	(680) 81/18/1	
6, <i>MTHFD1L</i>	rs11754661	GG/GA/AA	(878) 92/8/0	4	(690) 92/8/0	
7, <i>RELN</i>	rs4298437	CC/CT/TT	(879) 52/38/10	29	(690) 53/37/10	
10, <i>IDE</i>	rs1887922	TT/TC/CC	(871) 72/26/2	15	(683) 75/23/2	
11, <i>MS4A4A</i>	rs2304933	CC/CA/AA	(882) 35/48/17	41	(690) 34/49/17	
11, <i>MS4A4A</i>	rs4938933	TT/TC/CC	(872) 59/36/5	23	(683) 57/36/7	
11, <i>PICALM</i>	rs3851179	GG/GA/AA	(878) 45/43/12	34	(691) 43/42/15	
11, <i>PICALM</i>	rs642949	TT/TC/CC	(868) 58/36/6	24	(681) 58/37/5	
11, <i>SORL1</i>	rs2070045	TT/TG/GG	(883) 57/37/6	25	(695) 54/39/7	
11, <i>SORL1</i>	rs3824968	TT/TA/AA	(673) 44/39/17	36	(568) 42/43/15	
11, <i>SORL1</i>	rs73595277	CC/CG/GG	(873) 79/20/1	11	(688) 78/20/2	
15, <i>CYP19A</i>	rs2899472	CC/CA/AA	(883) 60/35/5	22	(690) 60/35/5	
17, <i>ACE</i>	rs4293	AA/AG/GG	(866) 30/50/20	45	(682) 26/53/21	
17, <i>MAPT</i>	rs1467967	AA/AG/GG	(869) 43/45/12	34	(685) 42/45/13	
17, <i>MAPT</i>	rs16940758	CC/CT/TT	(886) 65/31/4	20	(700) 63/30/7	
17, <i>MAPT</i>	rs2435211	CC/CT/TT	(880) 37/48/15	39	(691) 37/47/16	
17, <i>MAPT</i>	rs7521	AA/AG/GG	(869) 25/50/25	50	(683) 26/50/24	
19, <i>TOMM40</i>	rs157580	AA/AG/GG	(872) 69/29/2	17	(685) 54/39/7	
19, <i>TOMM40</i>	rs2075650	AA/AG/GG	(881) 44/44/12	34	(689) 74/23/3	
19, <i>TOMM40</i>	rs8106922	AA/AG/GG	(886) 43/44/13	35	(695) 26/43/31	
19, <i>APOE</i>		ε2/ε3/ε4	(890) 29/51/20	--- ^c	(701) 72/24/4	

Abbreviations: Chr=chromosome; SNP=single nucleotide polymorphism; n=number of cases; MAF=minor allele frequency; OR=odds ratio; CI=confidence interval; 0ε4=*APOE* ε4 allele non-carriers; 1ε4=carriers of 1 *APOE* ε4 allele; 2ε4=carriers of 2 *APOE* ε4 alleles.

Multiple comparison analysis (overall) was calculated by Pearson chi-square test. Univariate and multivariate analyses were calculated using binary logistic regression assuming a dominant model. Multivariate analyses were adjusted for age, sex and *APOE*-ε4 status. Analyses testing *APOE* adjusted for age and gender. Results for *APOE* are shown for comparative purposes only.

	MAF, %	AD risk Overall <i>P</i> -value	Genetic model	Univariate analysis OR (95% CI)	<i>P</i> -value	Multivariate analysis OR (95% CI)	<i>P</i> -value
	24	0.87	TT vs. TC+CC	1.04 (0.85-1.28)	0.69	1.03 (0.82-1.29)	0.83
	24	0.52	CC vs. CT+TT	1.05 (0.86-1.29)	0.61	1.05 (0.84-1.30)	0.69
	25	0.30	TT vs. TC+CC	1.13 (0.92-1.38)	0.25	1.29 (1.01-1.62)	0.03 ^b
	10	0.48	CC vs. CT+TT	0.99 (0.77-1.28)	0.93	0.96 (0.72-1.27)	0.76
	4	0.44	GG vs. GA+AA	1.10 (0.76-1.59)	0.61	1.06 (0.71-1.59)	0.77
	29	0.98	CC vs. CT+TT	1.02 (0.83-1.24)	0.86	0.90 (0.72-1.13)	0.35
	14	0.27	TT vs. TC+CC	1.19 (0.95-1.50)	0.13	1.27 (0.99-1.64)	0.07
	41	0.89	AA vs. AC+CC	0.95 (0.77-1.18)	0.66	1.06 (0.84-1.34)	0.61
	25	0.12	TT vs. TC+CC	0.93 (0.76-1.14)	0.48	1.02 (0.81-1.27)	0.89
	36	0.32	GG vs. GA+AA	0.93 (0.76-1.14)	0.50	0.97 (0.77-1.21)	0.77
	23	0.59	TT vs. TC+CC	1.02 (0.83-1.25)	0.84	1.00 (0.80-1.26)	0.97
	27	0.41	TT vs. TG+GG	0.88 (0.72-1.07)	0.20	0.87 (0.69-1.08)	0.21
	36	0.42	TT vs. TA+AA	0.93 (0.74-1.12)	0.51	0.95 (0.74-1.22)	0.70
	12	0.48	CC vs. CG+GG	0.96 (0.75-1.22)	0.74	0.84 (0.64-1.11)	0.22
	22	0.98	CC vs. CA+AA	1.01 (0.82-1.23)	0.96	1.02 (0.82-1.28)	0.85
	47	0.20	AA vs. AG+AA	0.82 (0.66-1.03)	0.08	0.72 (0.56-0.93)	0.01 ^a
	35	0.86	AA vs. AG+GG	0.98 (0.78-1.17)	0.67	0.96 (0.77-1.20)	0.72
	22	0.03 ^b	CC vs. CT+TT	0.90 (0.73-1.11)	0.33	0.84 (0.67-1.06)	0.13
	38	0.77	CC vs. CT+TT	1.03 (0.84-1.26)	0.80	0.98 (0.78-1.23)	0.84
	49	0.83	AA vs. AG+GG	1.05 (0.84-1.32)	0.68	1.02 (0.79-1.31)	0.89
	27	<0.0001 ^a	AA vs. AG+GG	0.53 (0.43-0.65)	<0.0001 ^a	0.76 (0.60-0.96)	0.02 ^b
	15	<0.0001 ^a	AA vs. AG+GG	3.63 (2.92-4.50)	<0.0001 ^a	1.46 (1.11-1.92)	0.007 ^b
	53	<0.0001 ^a	AA vs. AG+GG	0.46 (0.37-0.57)	<0.0001 ^a	1.05 (0.81-1.37)	0.60
	--- ^c	<0.0001 ^a	0E4 VS. 1E4+2E4	6.30 (5.06-7.85)	<0.0001 ^a	6.25 (5.22-8.15)	<0.0001 ^a

^a Significant at false discovery rate adjusted $P < 0.05$.

^b Not significant after false discovery rate correction.

^c Minor allele frequency not computed because *APOE* is tri-allelic.

Table 3 Effects of SNPs on cerebrospinal fluid Aβ1-42 in Alzheimer’s disease

Chr, Gene	SNP	Genotypes	Genotype, n	
Top AlzGene loci				
1, <i>CR1</i>	rs6656401	GG/GA/ <u>AA</u>	127/82/8	
2, <i>BIN1</i>	rs744373	TT/TC/ <u>CC</u>	117/96/8	
2, <i>BIN1</i>	rs7561528	GG/GA/AA	99/97/25	
6, <i>CD2AP</i>	rs9349407	GG/GC/ <u>CC</u>	132/73/15	
8, <i>CLU</i>	rs11136000	<u>CC</u> /CT/TT	75/112/32	
11, <i>MS4A4E</i>	rs670139	AA/AC/ <u>CC</u>	83/111/27	
11, <i>MS4A6A</i>	rs610932	<u>CC</u> /CA/AA	116/87/17	
11, <i>PICALM</i>	rs642949	TT/TC/CC	130/79/11	
11, <i>PICALM</i>	rs3851179	<u>GG</u> /GA/AA	96/104/21	
19, <i>ABCA7</i>	rs3752246 [§]	CC/CG/GG	165/40/4	
19, <i>CD33</i>	rs3865444	<u>GG</u> /GT/TT	89/111/21	
19, <i>APOE</i>		ε2/ε3/ <u>ε4</u>	50/111/61	
Selection GWAS SNPs not in top AlzGene loci				
6, <i>MTHFD1L</i>	rs11754661	GG/GA/AA	206/15/0	
11, <i>MS4A4A</i>	rs2304933 [‡]	CC/CA/ <u>AA</u>	72/107/43	
11, <i>MS4A4A</i>	rs4938933	TT/TC/CC	125/81/14	
19, <i>EXOC3L2</i>	rs597668	TT/TC/ <u>CC</u>	97/98/24	
GWAS with biomarkers as outcome				
2, <i>EPC2</i>	rs1374441	TT/TC/CC	119/85/15	
2, <i>EPC2</i>	rs4499362	CC/CT/TT	119/89/13	
15, <i>CYP19A</i>	rs2899472	CC/CA/AA	132/79/11	
7, <i>RELN</i>	rs4298437	CC/CT/TT	111/83/27	
19, <i>TOMM40</i>	rs157580	<u>AA</u> /AG/GG	150/68/3	
19, <i>TOMM40</i>	rs2075650	AA/AG/ <u>GG</u>	98/100/24	
Other candidate genes				
2, <i>PPP3R1</i>	rs1868402	TT/TC/CC	121/75/18	
3, <i>TF</i>	rs1049296	CC/CT/ <u>TT</u>	176/40/4	
10, <i>IDE</i>	rs1887922	TT/TC/ <u>CC</u>	167/52/2	
11, <i>BDNF</i>	rs6265	GG/GA/ <u>AA</u>	128/45/3	
11, <i>SORL1</i>	rs2070045	TT/TG/ <u>GG</u>	122/86/13	
11, <i>SORL1</i>	rs3824968	TT/TA/ <u>AA</u>	58/66/35	
11, <i>SORL1</i>	rs73595277	CC/CG/GG	183/36/2	
14, <i>CYP46A1</i>	rs754203	TT/TC/CC	103/98/20	

	Mean (SD) Aβ ₁₋₄₂ level, pg/ml, per genotype	P-value	
		Overall	DM
	446(168) /443(142) /370(150)	0.48 (0.21)	0.82 (0.96)
	446(148) /440(161) /451(266)	0.97 (0.92)	0.94 (1.00)
	447(152) /441(163) /443(173)	0.92 (0.71)	0.85 (0.77)
	440(158) /450(152) /444(202)	0.97 (0.88)	0.84 (0.84)
	400(116) /459(166) /497(194)	0.005 (0.01)	0.003^a (0.005)
	465(164) /436(155) /408(152)	0.18 (0.18)	0.13 (0.11)
	447(153) /443(163) /410(176)	0.67 (0.74)	0.73 (0.51)
	459(165) /432(148) /346(116)	0.07 (0.06)	0.12 (0.11)
	441(172) /453(149) /412(140)	0.52 (0.34)	0.95 (0.61)
	445(160) /446(159) /433(281)	0.99 (0.83)	0.90 (0.91)
	437(169) /448(155) /441(135)	0.87 (0.78)	0.65 (0.48)
	532 (214)/441(136)/ 374 (95)	<0.001	<0.001^a
	440(156) /490(189) /--- ^c	0.20 (0.07)	0.20 (0.07)
	494(192) /428(133) /395(130)	0.002 (0.001)	0.001^a (<0.0001)
	453(160) /435(152) /411(188)	0.60 (0.54)	0.41 (0.29)
	472(192) /422(123) /423(129)	0.08 (0.56)	0.02 (0.28)
	440(172) /456(147) /411(109)	0.52 (0.38)	0.67 (0.58)
	443(172) /445(140) /441(155)	0.98 (1.00)	0.96 (0.98)
	454(163) /433(152) /378(122)	0.36 (0.22)	0.27 (0.19)
	439(137) /432(156) /498(230)	0.17 (0.21)	0.86 (0.79)
	426(146) /476(175) /593(202)	0.04 (0.27)	0.02^b (0.12)
	485(195) /418(112) /377(104)	0.002 (0.24)	0.001^a (0.19)
	447(173) /448(145) /418(131)	0.70 (0.56)	0.81 (0.53)
	449(165) /412(120) /539(146)	0.22 (0.37)	0.42 (0.42)
	442(138)/ 437(196)/ 747(457)	0.03 (0.04)	0.82 (0.94)
	430(156) /453(173) /432(142)	0.79 (0.62)	0.49 (0.35)
	440(149) /453(166) /403(195)	0.58 (0.53)	0.89 (0.89)
	438(130) /442(158) /457(206)	0.92 (0.87)	0.85 (0.68)
	449(168) /421(102) /397(76)	0.65 (0.87)	0.35 (0.60)
	447(155) /467(160) /456(176)	0.85 (0.66)	0.72 (0.54)

Table 3 Effects of SNPs on cerebrospinal fluid Aβ1-42 in Alzheimer’s disease *Continued*

Chr, Gene	SNP	Genotypes	Genotype, n	
17, <i>ACE</i>	rs4293	AA /AG/GG	60/107/54	
17, <i>MAPT</i>	rs16940758	CC/CT/TT	146/68/8	
17, <i>MAPT</i>	rs2435211	CC/CT/TT	71/118/32	
17, <i>MAPT</i>	rs1467967	AA /AG/GG	97/98/25	
17, <i>MAPT</i>	rs7521	AA/AG/ GG	62/112/46	
19, <i>TOMM40</i>	rs8106922	AA /AG/GG	113/91/18	

Abbreviations: Chr=chromosome; SNP=single nucleotide polymorphism; n=number of cases; SD=standard deviation; DM=dominant model; GWAS=genome-wide association study Risk allele according to AlzGene meta-analyses or study source in **bold** and underscored. Information on risk allele was not available for all studies. *P*-values based on mixed model analyses adjusted for age and gender; values in parenthesis () adjusted for age, gender and

Table 4 Effects of SNPs on cerebrospinal fluid tau in Alzheimer’s disease

Chr, Gene	SNP	Genotypes	Geno- type, n	Mean (SD) t-tau level, pg/ml, per genotype	
Top AlzGene loci					
1, <i>CR1</i>	rs6656401	GG/GA/ AA	93/57/7	512(246) /579(275) /682(456)	
2, <i>BIN1</i>	rs744373	TT/TC/ CC	80/74/5	576(287) /514(251) /547(224)	
2, <i>BIN1</i>	rs7561528	GG/GA/AA	68/72/19	583(281) /511(247) /544(305)	
6, <i>CD2AP</i>	rs9349407	GG/GC/ CC	94/54/10	551(281) /537(245) /566(305)	
8, <i>CLU</i>	rs11136000	CC /CT/TT	59/79/19	523(230) /543(274) /564(299)	
11, <i>MS4A4E</i>	rs670139	AA/AC/ CC	60/81/18	556(286) /519(233) /634(351)	
11, <i>MS4A6A</i>	rs610932	CC /CA/AA	77/71/9	572(281) /525(250) /537(337)	
11, <i>PICALM</i>	rs3851179	GG /GA/AA	75/69/15	583(301) /518(243) /488(194)	
11, <i>PICALM</i>	rs642949	TT/TC/CC	94/56/8	499(221) /612(313) /525(223)	
19, <i>ABCA7</i>	rs3752246	CC/CG/GG	119/25/4	532(267) /570(275) /568(185)	
19, <i>CD33</i>	rs3865444	GG /GT/TT	63/81/14	587(305) /503(202) /622(394)	
19, <i>APOE</i>		ε2 /ε3/ ε4	37/79/43	468 (289)/537(249)/630 (269)	
Selection GWAS SNPs not in AlzGene top					
6, <i>MTHFD1L</i>	rs11754661	GG/GA/AA	149/10/0	550(268) /492(301) /--- ^a	

	Mean (SD) Aβ ₁₋₄₂ level, pg/ml, per genotype	P-value	
		Overall	DM
	451(184) / 451(154) / 421(136)	0.26 (0.67)	0.56 (0.91)
	439(169) / 449(141) / 467(99)	0.90 (0.84)	0.68 (0.57)
	420(136) / 456(167) / 450(171)	0.29 (0.27)	0.12 (0.17)
	446(168) / 451(161) / 409(100)	0.58 (0.57)	0.81 (0.99)
	419(105) / 456(179) / 449(165)	0.37 (0.33)	0.17 (0.14)
	417(126) / 461(169) / 520(236)	0.02 (0.75)	0.02^b (0.55)

APOE-ε4 status. Analyses testing APOE adjusted for age and gender. Results for APOE are shown for comparative purposes only.

- ^a Significant at false discovery rate corrected $P < 0.05$.
- ^b No longer significant after false discovery rate correction.
- ^c No cerebrospinal fluid measured because none of the participants carried the rs11754661 AA genotype.

	P-value		Geno- type, n	Mean (SD) p-tau level, pg/ml, per genotype	P-value	
	Overall	DM			Overall	DM
	0.48 (0.44)	0.28 (0.30)	90/53/7	81(33) / 88(35) / 103(66)	0.53 (0.48)	0.40 (0.41)
	0.29 (0.26)	0.11 (0.10)	76/69/6	86(36) / 81(35) / 100(44)	0.41 (0.41)	0.47 (0.45)
	0.26 (0.26)	0.10 (0.10)	65/66/20	86(34) / 82(37) / 86(38)	0.87 (0.87)	0.63 (0.62)
	0.95 (0.89)	0.95 (0.84)	90/50/10	86(39) / 82(31) / 87(41)	0.87 (0.83)	0.74 (0.69)
	0.77 (0.65)	0.47 (0.36)	59/72/18	83(36) / 83(35) / 88(40)	0.88 (0.85)	0.80 (0.72)
	0.20 (0.25)	0.68 (0.78)	55/77/19	83(37) / 83(35) / 93(37)	0.49 (0.50)	0.70 (0.66)
	0.57 (0.67)	0.30 (0.42)	74/66/9	84(35) / 83(33) / 96(62)	0.73 (0.76)	0.83 (0.75)
	0.49 (0.40)	0.25 (0.20)	72/65/14	86(39) / 84(36) / 76(20)	0.80 (0.76)	0.76 (0.72)
	0.11 (0.10)	0.07 (0.06)	89/54/7	79(32) / 93(39) / 75(37)	0.13 (0.14)	0.15 (0.15)
	0.98 (0.98)	0.83 (0.89)	111/26/4	81(36) / 93(37) / 82(30)	0.43 (0.45)	0.25 (0.56)
	0.11 (0.12)	0.12 (0.10)	61/75/14	87(45) / 82(26) / 87(43)	0.62 (0.60)	0.37 (0.34)
	0.11	0.08	36/75/40	78(42) / 84(35) / 91(31)	0.54	0.36
	0.48 (0.39)	0.48 (0.39)	141/10/0	85(36) / 72(38) / --- ^a	0.26 (0.22)	0.26 (0.22)

Table 4 Effects of SNPs on cerebrospinal fluid tau in Alzheimer's disease *Continued*

Chr, Gene	SNP	Genotypes	Geno- type, n	Mean (SD) t-tau level, pg/ml, per genotype	
11, <i>MS4A4A</i>	rs2304933	CC/CA/ <u>AA</u>	54/76/29	526(293) /534(233) /611(309)	
11, <i>MS4A4A</i>	rs4938933	TT/TC/CC	86/64/8	561(270) /509(238) /567(347)	
19, <i>EXOC3L2</i>	rs597668	TT/TC/ <u>CC</u>	73/70/14	538(270) /562(271) /446(136)	
GWAS with biomarkers as outcome					
2, <i>EPC2</i>	rs1374441	TT/TC/ <u>CC</u>	87/60/10	511(239) /567(269) /631(387)	
2, <i>EPC2</i>	rs4499362	CC/CT/TT	88/62/9	539(293) /559(242) /528(221)	
15, <i>CYP19A</i>	rs2899472	CC/CA/AA	98/51/10	534(274) /567(235) /557(387)	
7, <i>RELN</i>	rs4298437	CC/CT/TT	73/63/23	519(255) /588(264) /516(318)	
19, <i>TOMM40</i>	rs157580	<u>AA</u> /AG/GG	113/43/2	567(281) /492(226) /329(250)	
19, <i>TOMM40</i>	rs2075650	AA/AG/ <u>GG</u>	74/71/14	505(274) /573(251) /623(315)	
Other candidate genes					
2, <i>PPP3R1</i>	rs1868402	TT/TC/CC	95/50/9	515(173) /624(316) /497(264)	
3, <i>TF</i>	rs1049296	CC/CT/ <u>TT</u>	128/27/3	547(285) /543(189) /563(310)	
10, <i>IDE</i>	rs1887922	TT/TC/ <u>CC</u>	117/40/2	547(275) /551(259) /368(145)	
11, <i>BDNF</i>	rs6265	GG/GA/ <u>AA</u>	98/30/2	543(266) /492(182) /777(350)	
11, <i>SORL1</i>	rs2070045	TT/TG/ <u>GG</u>	86/63/9	583(288) /503(236) /532(270)	
11, <i>SORL1</i>	rs3824968	TT/TA/ <u>AA</u>	38/41/28	530(284) /522(245) /557(223)	
11, <i>SORL1</i>	rs73595277	CC/CG/GG	132/25/2	529(241) /624(388) /698(16)	
14, <i>CYP46A1</i>	rs754203	TT/TC/CC	73/74/11	564(281) /526(257) /479(116)	
17, <i>ACE</i>	rs4293	<u>AA</u> /AG/GG	43/82/34	601(262) /506(263) /574(284)	
17, <i>MAPT</i>	rs16940758	CC/CT/TT	108/46/5	526(264) /579(282) /657(258)	
17, <i>MAPT</i>	rs2435211	CC/CT/TT	50/86/23	483(184) /562(34) /621(370)	
17, <i>MAPT</i>	rs1467967	AA/AG/ <u>GG</u>	64/74/20	577(303) /515(251) /552(215)	
17, <i>MAPT</i>	rs7521	AA/AG/ <u>GG</u>	42/84/32	543(240) /534(266) /554(281)	
19, <i>TOMM40</i>	rs8106922	<u>AA</u> /AG/GG	78/66/15	570(257) /526(257) /512(375)	

Abbreviations: Chr=chromosome; SNP=single nucleotide polymorphism; n=number of cases; SD=standard deviation; t-tau=total tau; p-tau=phosphorylated tau; DM=dominant model; GWAS=genome-wide association studies.

Risk allele according to AlzGene meta-analyses or study source in bold and underscored. Information on risk allele was not available for all studies. All CSF values are means (standard deviation). *P*-values based on mixed model analyses adjusted for age and gender; values in parenthe-

	P-value		Geno- type, n	Mean (SD) p-tau level, pg/ml, per genotype	P-value	
	Overall	DM			Overall	DM
	0.46 (0.39)	0.60 (0.56)	50/71/30	81(40) /85(35) /88(33)	0.18 (0.79)	0.56 (0.55)
	0.41 (0.51)	0.18 (0.24)	82/60/8	86(37) /82(33) /80(40)	0.59 (0.61)	0.36 (0.39)
	0.30 (0.18)	0.73 (0.32)	70/64/15	85(41) /84(33) /78(20)	0.72 (0.58)	0.54 (0.37)
	0.14 (0.13)	0.06 (0.06)	85/55/9	80(30) /86(38) /102(63)	0.17 (0.16)	0.13 (0.13)
	0.68 (0.61)	0.41 (0.35)	86/58/7	84(37) /86(36) /73(26)	0.53 (0.48)	0.61 (0.57)
	0.76 (0.77)	0.77 (0.79)	90/51/10	84(38) /86(31) /80(44)	0.69 (0.68)	0.84 (0.84)
	0.17 (0.23)	0.13 (0.15)	68/62/21	83(35) /90(37) /73(35)	0.13 (0.16)	0.53 (0.60)
	0.27 (0.42)	0.15 (0.21)	107/41/2	85(36) /83(38) /15(53)	0.56 (0.66)	0.86 (0.96)
	0.52 (0.96)	0.26 (0.81)	72/66/13	81(36) /87(36) /87(32)	0.80 (0.92)	0.59 (0.91)
	0.07 (0.04)	0.05 (0.03)^b	91/46/9	82(33) /93(41) /78(40)	0.24 (0.20)	0.20 (0.17)
	0.83 (0.84)	0.55 (0.56)	120/27/3	82(35) /94(41) /80(43)	0.53 (0.51)	0.35 (0.32)
	0.63 (0.68)	0.97 (0.92)	112/37/2	84(35) / 87(39) / 65(34)	0.69 (0.71)	0.80 (0.76)
	0.50 (0.50)	0.45 (0.41)	92/29/2	84(37) /79(31) /121(57)	0.45 (0.45)	0.75 (0.74)
	0.41 (0.43)	0.18 (0.20)	83/58/9	86(36) /81(36) /89(36)	0.84 (0.85)	0.79 (0.80)
	0.55 (0.57)	0.50 (0.55)	36/41/24	87(43) /83(36) /89(29)	0.72 (0.72)	0.96 (0.99)
	0.18 (0.23)	0.07 (0.09)	127/22/2	82(32) /98(53) /91(40)	0.11 (0.12)	0.04^b (0.05)
	0.64 (0.73)	0.37 (0.46)	69/71/10	81(34) /88(39) /73(17)	0.36 (0.32)	0.31 (0.26)
	0.08 (0.09)	0.31 (0.20)	41/78/32	86(29) /83(41) /84(33)	0.84 (0.89)	0.96 (0.94)
	0.11 (0.10)	0.06 (0.05)	105/41/5	80(36) /93(37) /90(24)	0.08 (0.08)	0.03^b (0.02)^b
	0.08 (0.05)	0.03^b (0.02)^b	47/81/23	80(32) /84(34) /95(49)	0.27 (0.22)	0.22 (0.20)
	0.59 (0.50)	0.37 (0.28)	61/70/19	89(40) /80(35) /84(26)	0.62 (0.59)	0.36 (0.32)
	0.91 (0.92)	0.78 (0.71)	41/76/33	85(30) /78(32) /95(46)	0.07 (0.07)	0.98 (0.92)
	0.75 (0.82)	0.49 (0.93)	36/75/40	87(35) /82(33) /82(53)	0.88 (0.94)	0.63 (0.90)

sis () adjusted for age, gender and *APOE*- ϵ_4 status. Analyses testing *APOE* adjusted for age and gender. Results for *APOE* are shown for comparative purposes only.

^a No cerebrospinal fluid measured because none of the participants carried the rs11754661 AA genotype.

^b No longer significant after false discovery rate correction.

DISCUSSION

We performed a case-control genetic association analysis of 25 AD risk variants and tested the effects of 36 risk variants on CSF $A\beta_{1-42}$, t-tau, and p-tau in the AD group.

Only *APOE* and *TOMM40* showed genetic association with AD. However, *TOMM40* is in linkage disequilibrium with *APOE* and did not exhibit an effect independent of *APOE*, in accordance with recent evidence.³⁷

Of the AlzGene top loci, *CLU* correlated significantly with decreased $A\beta_{1-42}$. Previous studies have not found the same effect, which may be attributed to their smaller sample size.^{38, 39} Consistent with GWAS findings, the minor allele of *CLU* exerted a protective effect on AD risk,³⁰ and on CSF $A\beta_{1-42}$ in the Finnish cohort. *CLU* did not significantly affect tau. *CLU* binds soluble $A\beta$ and plays a role in $A\beta$ clearance and aggregation,¹⁵ which could partly explain why it primarily affected $A\beta_{1-42}$.

PICALM affects $A\beta$ concentration in the brain through endocytic processes. Marker rs3851179 in *PICALM* has been reported to correlate with CSF $A\beta_{1-42}$ in another study, the major allele (G) being the risk allele.³⁹ In our cohort, we found no indication of a correlation between rs3851179 and CSF markers. Instead, rs642949 minor allele carriers (C) had decreased $A\beta_{1-42}$ and increased t-tau levels, although these were not statistically significant. The C allele of rs642949 has been reported to exert a risk effect in a case-control study.⁴⁰

APOE and the other top AlzGene loci did not correlate with t-tau and p-tau in our cohort.

Of the GWAS SNPs not in AlzGene top loci, *MS4A4A* showed significant correlation with $A\beta_{1-42}$ but not with tau. *MS4A4A* belongs to the *MS4A* cluster.¹⁹ Not much is known yet about *MS4A4A* rs2304933 but its effect on $A\beta_{1-42}$ is consistent with our genetic association analysis results suggesting a risk effect of the minor (C) allele. The mechanisms by which *MS4A4A* affect CSF $A\beta_{1-42}$ levels need further investigation. As far as we know, studies of the relation between *MS4A4A* rs2304933 and CSF markers have not been published before.

The decrease in $A\beta_{1-42}$ levels among C allele carriers of *EXO-C3L2* rs597668 is interesting. Although not statistically significant (FDR adjusted $P > 0.05$), it conferred with a previous study identifying the C al-

lele as a risk allele in the Finnish population.³¹ The C allele has also been reported in another study to promote AD progression.⁴¹

Among GWAS SNPs with biomarker as outcome, *TOMM40* SNPs correlated with CSF $A\beta_{1-42}$ but not with tau, which was consistent with previous findings.^{20, 21} None of the *TOMM40* SNPs remained significant after *APOE* correction. This makes it likely that the *TOMM40* effects could be attributed to *APOE*, given the close proximity of *TOMM40* to *APOE*.

EPC2, which correlated with t-tau in biomarker GWAS,²¹ showed no effect on CSF t-tau and also $A\beta_{1-42}$ in our cohort. The aforementioned GWAS had a much smaller AD group than in our cohort. This and the variability in population could account for the different findings.

The SNPs from candidate genes were not related to CSF $A\beta_{1-42}$. For tau, marginal correlations were obtained for *PPP3R1*, *SORL1* and *MAPT* at an FDR unadjusted $P < 0.05$. Although none passed the FDR filter, the small effects observed were suggestive of a trend for variants in these three genes. *PPP3R1* is a protein phosphatase and is the calcium binding regulatory subunit of calcineurin.⁴² Calcineurin is involved in modulating tau phosphorylation.⁴³ A previous study found *PPP3R1* to correlate with p-tau.⁴ In our cohort, we found an effect on t-tau but the effect on p-tau was weaker. This difference in results could partly be attributed to variability in population.

SORL1 binds to ApoE and plays a role in $A\beta_{1-42}$ production.⁴⁴ Numerous SNPs in *SORL1* have been studied in relation to AD⁴⁵ and CSF biomarkers⁴⁴ but results so far have been inconclusive.¹⁰ One study found that rs3824968 in *SORL1* significantly reduced $A\beta_{1-42}$ in AD⁴⁶ whereas another study reported no correlation.⁴⁷ *SORL1* appears to exert small effects likely to be detected only in mega-analysis of pooled samples⁴⁸ or in haplotypes.⁴⁹ In general, single polymorphism in *SORL1* did not correlate with CSF $A\beta_{1-42}$ and tau.¹⁰ Our result relating *SORL* with p-tau may be due to chance.

MAPT codes for tau proteins. Aggregated hyperphosphorylated tau proteins are a component of NFTs. Consistent with the findings of another study, we found *MAPT* to correlate with CSF t-tau.⁵⁰

We noted a number of changes in the strength of correlation between SNPs and biomarkers when we corrected for *APOE*. As mentioned above, *TOMM40* was no longer associated with AD and CSF $A\beta_{1-42}$

after *APOE* correction. This confirms previous finding that *TOMM40* is in strong linkage disequilibrium with *APOE*. The effect of *EXOC3L2* on CSF $A\beta_{1-42}$ became nonsignificant after correcting for *APOE*. This suggests that the effect was not independent of *APOE*. Due to our small sample size, our result could also be a false positive. For the other SNPs, we found no or minor changes on either AD risk or correlation with CSF markers after *APOE* correction. This means that the effects of these SNPs were independent of *APOE*, or that our sample size was too small to detect interaction effects.

We tested the relation between SNPs and CSF biomarkers in AD patients who were already demented. We found few SNP-related differences in CSF $A\beta$ because $A\beta$ may already be strongly decreased in demented patients. The effect of genetic risk factors on amyloid metabolism may be more evident in predementia stages. Although AD has been related to changes in tau, we only found weak correlations between the studied SNPs and tau. Of interest is the weak correlation of *MAPT* and *PPP3R1* with tau, which confirms the role of these SNPs in tau metabolism. We note here that *MAPT* and *PPP3R1* also showed weak associations with clinical AD in our case-control genetic association analyses, which suggest that the studied loci in these genes contribute to dementia risk. In the analyses with biomarkers, none of the AlzGene or other GWAS SNPs were related to tau, suggesting that these SNPs have no clear effect on tau metabolism.

Our study had the unique design that we tested SNPs both for genetic association in a clinical case-control design and for correlation with CSF biomarkers. This is one of the strengths of our exploratory study. Another strength was the large selection of high-risk SNPs identified by GWAS or reported in other candidate gene studies covering different possible pathophysiological pathways. The small sample size remained a limitation. We had sufficient power for finding moderate associations, but a larger sample is needed for detecting the very small effect sizes of the other SNPs studied.

In conclusion, we provide evidence that *APOE*, *CLU* and *MS4A4A*, which have been identified in GWAS to be associated with AD, also significantly affected CSF $A\beta_{1-42}$. To our knowledge, ours is the first study to report on the correlation between *MS4A4A* and CSF $A\beta_{1-42}$.

None of the AD risk genes studied showed significant effects on CSF tau. The nonsignificant trends in *PPP3R1* and *MAPT* in relation to tau may be due to our small sample size rather than genuine lack of risk effects. Collaboration on a larger scale is necessary to ascertain the effects of the aforementioned SNPs and identify reliable genetic risk variants for AD markers in CSF.

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Supplementary table Genetic variants included in the study. All were tested for biomarker analyses. SNPs tested in previous studies for genetic association with AD were not included in the present study, as indicated.

Gene	SNP	Genetic association
Top AlzGene loci		
1, <i>CR1</i>	rs6656401	Previously tested. Significant in GWAS ^{a[1]}
2, <i>BIN1</i>	rs744373	Previously tested. Significant in replication study ^{a[2]}
2, <i>BIN1</i>	rs7561528	Gene previously tested in GWAS ^{a[3]}
6, <i>CD2AP</i>	rs9349407	Previously tested. Significant in GWAS ^{a[3]}
8, <i>CLU</i>	rs11136000	Previously tested. Significant in GWAS ^{a[1]}
11, <i>MS4A4E</i>	rs670139	Previously tested. Significant in GWAS ^{a[3]}
11, <i>MS4A6A</i>	rs610932	Previously tested. Significant in GWAS ^{a[3]}
11, <i>PICALM</i>	rs3851179	Included in current study
11, <i>PICALM</i>	rs642949	Included in current study
19, <i>ABCA7</i>	rs3752246	Previously tested. Significant in GWAS ^{a[3]}
19, <i>CD33</i>	rs3865444	Previously tested. Significant in GWAS ^{a[3]}
19, <i>APOE^b</i>	ε2/ε3/ε4	Included in current study
GWAS		
6, <i>MTHFD1L</i>	rs11754661	Included in current study
11, <i>MS4A4A</i>	rs2304933	Included in current study
11, <i>MS4A4A</i>	rs4938933	Included in current study
19, <i>EXOC3L2</i>	rs597668	Previously tested. Significant in replication study ^{a[2]}
2, <i>EPC2</i>	rs1374441	Included in current study
2, <i>EPC2</i>	rs4499362	Included in current study
15, <i>CYP19A</i>	rs2899472	Included in current study ^{c[4]}

Supplementary table Continued. Genetic variants included in the study. All were tested for biomarker analyses. SNPs tested in previous studies for genetic association with AD were not included in the present study, as indicated.

Gene	SNP	Genetic association
7, <i>RELN</i>	rs429837	Included in current study
19, <i>TOMM40</i>	rs157580	Included in current study
19, <i>TOMM40</i>	rs2075650	Included in current study
Other candidate genes		
2, <i>PPP3R1</i>	rs1868402	Included in current study
3, <i>TF</i>	rs1049296	Included in current study
10, <i>IDE</i>	rs1887922	Included in current study ^{c[5]}
11, <i>BDNF</i>	rs6265	Previously tested. Not significant in genetic association analysis ^{a[6]}
11, <i>SORL1</i>	rs2070045	Included in current study
11, <i>SORL1</i>	rs3824968	Included in current study
11, <i>SORL1</i>	rs73595277	Included in current study
14, <i>CYP46a</i>	rs754203	Previously tested. Significant in candidate gene analysis ^{c[7]}
17, <i>ACE</i>	rs4293	Included in current study ^{c[8]}
17, <i>MAPT</i>	rs16940758	Included in current study
17, <i>MAPT</i>	rs2435211	Included in current study
17, <i>MAPT</i> haplotype	rs1467967	Included in current study
17, <i>MAPT</i> haplotype	rs7521	Included in current study
19, <i>TOMM40</i>	rs8106922	Included in current study

Abbreviations: Chr, chromosome; SNP, single nucleotide polymorphism; CSF, cerebrospinal fluid; GWAS, genome-wide association study

Gene names: *CR1*, complement component receptor 1; *BIN1*, bridging integrator 1; *CD2AP*, CD2-associated protein; *CLU*, clusterin; *MS4A4E*, membrane-spanning 4-domains, subfamily A, member 4E; *MS4A6A*, membrane-spanning 4-domains, subfamily A, member 6A; *PICALM*, phosphatidylinositol binding clathrin assembly protein; *ABCA7*, ATP-binding cassette, subfamily A, member 7; *CD33*, CD33 molecule; *MTHFD1L*, methylenetetrahydrofolate dehydrogenase 1-like; *RELN*, reelin; *MS4A4A*, membrane-spanning 4-domains, subfamily A, member 4A; *EXOC3L2*, exocyst complex component 3-like 2; *EPC2*, enhancer of polycomb homolog 2; *CYP19A*, cytochrome P450, family 19, subfamily a, polypeptide 1; *TOMM40*, translocase of outer mitochondrial membrane 40 homolog; *PPP3R1*, protein phosphatase 3, regulatory subunit B, alpha; *TF*, transferin; *IDE*, insulin-degrading enzyme; *BDNF*, brain-derived neurotrophic factor; *SORL1*, sortilin-related receptor;

CYP46a, cytochrome P₄₅₀, family 46, subfamily A, polypeptide; *ACE*, angiotensin I converting enzyme 1; *MAPT*, microtubule-associated protein tau; *TOMM40*, translocase of outer mitochondrial membrane 40 homolog; *APOE*, apolipoprotein E

^a Gene previously genotyped in GWAS or SNP genotyped in genetic association studies that included the Finnish cohort and excluded from the genetic association analysis in the present study but included in the biomarker analysis.

^b *APOE* tested for reference purposes.

^c Gene previously genotyped in the Finnish cohort but with different SNP(s). Present SNP included in the genetic association and biomarker analysis.

Number in table text [] denotes references.

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We previously found that MS₄A₄A correlated with abnormally low A β ₁₋₄₂ levels in patients with AD. We aimed to replicate the findings in an independent cohort composed of various diagnostic groups.

CHAPTER 8

Effects of *MS4A* variants on cerebrospinal fluid
 $A\beta_{1-42}$ in the EDAR cohort

ABSTRACT

Background

Single nucleotide polymorphisms (SNPs) in the membrane-spanning 4-domains, subfamily A (*MS4A*) gene cluster have been identified in genome-wide association studies (GWAS) to be associated with Alzheimer's disease (AD). We recently reported that *MS4A4A* rs2304933, but not *MS4A4E* rs670139 and *MS4A6A* rs2304933, significantly reduced cerebrospinal fluid (CSF) amyloid beta 1-42 isoform ($A\beta_{1-42}$) in a Finnish AD population. We aim to replicate these findings in an independent cohort composed of various diagnostic groups.

Methods

We included 243 participants (78 AD, 93 mild cognitive impairment, 48 other type of dementia, 24 cognitively healthy control subjects) from the European multicenter EDAR study. Genomic DNA was extracted from EDTA anticoagulated blood using polymerase chain reaction technique. SNPs were genotyped on Sequenom I-plex platform. CSF was obtained through lumbar puncture and analysed using multiplex xMAP Luminex platform. Statistical analyses were performed using mixed models, corrected for age, sex, *APOE*- $\epsilon 4$ status and study site. Adjustment for multiple testing was set at a false discovery rate adjusted $P < 0.05$.

Results

The mean CSF $A\beta_{1-42}$ levels of homozygote risk allele carriers are lower compared to other genotypes for *MS4A4E* rs670139, *MS4A6A* rs610932 and *MS4A4A* rs2304933. AD cases generally had lower CSF $A\beta_{1-42}$ concentrations compared to the other diagnostic groups and control subjects. Variant rs610932 significantly correlated with CSF $A\beta_{1-42}$ (unadjusted $P = 0.001$, FDR adjusted $P < 0.05$) in a dominant model. A trend between CSF $A\beta_{1-42}$ and rs670139 (unadjusted $P = 0.12$) and rs2304933 (unadjusted $P = 0.08$) was observed in a recessive model. There was no interaction with age, sex, *APOE*- $\epsilon 4$ status and diagnosis for all analyses (all $P > 0.05$).

Conclusion

Our findings suggest that polymorphisms in the *MS4A* gene may influence CSF A β ₁₋₄₂. This supports results from GWAS studies that this gene is a genetic risk factor for AD. Replication on a larger scale will further determine the influence of SNPs in the *MS4A* gene cluster on AD.

Keywords: *MS4A*, genetics, biomarker, amyloid, cerebrospinal fluid, Alzheimer, replication study

INTRODUCTION

Genetic loci in *MS4A4A*, *MS4A4E* and *MS4A6A* from the membrane-spanning 4-domains, subfamily A (*MS4A*) gene cluster have been identified in genome-wide association studies (GWAS) to be associated with clinical Alzheimer's disease (AD).¹⁻³ The role of *MS4A* on AD pathogenesis merits further investigation. The *MS4A* cluster, which is located in chromosome 11q12, includes at least 16 known genes of which some have been shown to be involved in immunity while the rest have no known specific functions. *MS4A* family members share common genomic features.² They have been suggested to form part of oligomeric cell surface proteins and are important in cellular signal transduction.⁴ *MS4A4A*, *MS4A4E* and *MS4A6A* have been reported to play a role in immunity, being structurally related to the immunoglobulin E receptor and to CD20,⁵ which is a B-lymphocyte cell surface molecule.⁶ Inflammatory responses in AD have been related to amyloid beta (A β) oligomers and deposition.⁷⁻¹⁰ In the present study, we focused on testing the correlation between A β in cerebrospinal fluid (CSF) and variants in *MS4A* that have been associated with AD.

We recently reported that a single nucleotide polymorphism (SNP) in *MS4A4A* (rs2304933), but not in *MS4A4E* (rs670139) and *MS4A6A* (rs2304933), was significantly related to reduced cerebrospinal fluid (CSF) amyloid beta (A β) in a Finnish AD population.¹¹ Our purpose is to replicate this study in an independent cohort composed of various diagnostic groups.

METHODS

Study population

Cohort

Participants for the current study were recruited from the 'beta amyloid oligomers in the early diagnosis of AD and as marker for treatment response' (EDAR) study, a European multicenter prospective cohort study (for general information, see www.edarstudy.eu) with 15 partners across Europe. The goal of EDAR is to develop new diagnostic markers for AD that can be used for the early diagnosis and for the monitoring of treatment response in drug trials. The project focuses on A β oligomers in CSF, serum and plasma, and the effect of genes involved in A β

processing on these oligomers. EDAR recruited participants with MCI, AD-type dementia, non-AD dementia and cognitively healthy controls from memory centers. MCI was broadly defined to include referral to a memory clinic because of cognitive impairments not due to dementia according to Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV)¹¹ criteria at baseline, neurological or endocrine disorders, or severe depression. MCI patients aged above 60 years were included. For dementia, the inclusion criteria were age 40 years and above, Mini-Mental State Examination (MMSE)¹² score above 18, a diagnosis of probable or possible AD according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)¹³ criteria, diagnosis of fronto-temporal criteria according to Neary criteria, diagnosis of vascular dementia according to the National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN)¹⁴ criteria, or diagnosis of Lewy body disease according to McKeith¹⁵ criteria. Cognitively healthy control subjects were those aged above 40 years who obtained an overall Clinical Dementia Rating (CDR)¹⁶ of 0, an MMSE score above the 10th percentile for age and education corrected norms, did not fulfill a clinical diagnosis of dementia according to DSM-IV criteria, and had no obvious causes of cognitive impairment such as stroke, depression or endocrine disorders. Participants underwent clinical, neurological, neuropsychiatric, neuropsychological and functional assessments at baseline, 9 months and 18 months. Venous blood, CSF samples and MRI scans were available for a subset of participants. The local ethics committee approved the study procedures and all participants gave their informed consent.

Selection of participants

For the present study, we included 243 participants with available venous blood for DNA extraction and for whom baseline CSF A β ₁₋₄₂ levels were measured and diagnosis was made. The selection process is shown in Figure 1.

Participants who were genotyped did not differ from those who were not genotyped in terms of age (mean 69.1 $\pm$ 8.6 vs. 69.3 $\pm$ 9.2

Figure 1 Selection of participants

Abbreviations: CSF=cerebrospinal fluid; AD=Alzheimer’s disease; MCI=mild cognitive impairment

years, $P=0.93$) and years of education (mean 10.9 ± 4.1 vs. 10.0 ± 4.5 years, $P=0.33$). Those who were genotyped scored lower on the MMSE than those who were not genotyped (25.3 ± 4.0 vs. 27.1 ± 2.9 , $P=0.02$). Participants with CSF data did not differ significantly from those who had no available CSF measurements in terms of age, years of education and MMSE score (all $P>0.05$).

Genetic analysis

We genotyped three SNPs in the *MS4A* cluster: rs670139 in *MS4A4E*, rs610932 in *MS4A6A* and rs2304933 in *MS4A4A*. These SNPs are in linkage disequilibrium.² *APOE* genotype was determined by a combination of two SNPs, rs7412 and rs429358.

Genomic DNA was extracted from EDTA anticoagulated blood using the polymerase chain reaction technique. DNA samples were randomly placed on 384-well plates. SNPs were genotyped on Sequenom i-Plex platform (Sequenom Inc., Hamburg, Germany). Genotyping was performed blinded to clinical data. Genetic data were subjected to quality control. The SNPs included for the present study were in Hardy-Weinberg equilibrium (Chi-square test, $P > 0.05$) and had a call rate of $\geq 90\%$.

CSF collection and analysis

CSF samples, obtained through lumbar puncture of the L3/L4 or L4/L5 intervertebral space, were centrifuged and stored in sterile polypropylene tubes at -80 degree Celsius until analyses. All A β ₁₋₄₂ concentrations were measured using multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics immunoassays (INNO-BIA AlzBio3 Ghent, Belgium) in a laboratory at the Vrije Universiteit Medical Center (VUmc) in Amsterdam, Netherlands. The bioanalysts who performed the analyses were blinded to clinical information.

For the present study, we considered CSF A β ₁₋₄₂ concentrations below 389 pg/ml to be abnormal. This cut-off has been determined at the VUmc to best differentiate between patients with subjective cognitive impairment and AD, with a sensitivity of 95% and a specificity of 58%.

Statistical analysis

We used IBM SPSS Statistics version 19 (IBM Corp., NY, USA) for Mac OS X for the statistical analyses. Group differences in terms of mean age, education and MMSE score were assessed using independent samples t-test.

The distribution of CSF A β ₁₋₄₂ distribution was skewed (Shapiro Wilk test, $P < 0.001$). We therefore used log-transformed values in the statistical analyses. We assessed the relation between SNPs and CSF

$A\beta_{1-42}$ in the pooled sample by applying dominant and recessive models. This allowed us to test the effects of heterozygosity and of having two risk alleles. Although an additive model is commonly used in genetic analyses, we did not include this model because it has been shown to have less power in detecting recessive effects, especially when the minor genotype frequency is low,³⁷ which was the case in our study. Moreover, a statistically nonsignificant result for an additive model does not preclude association, because in general, genes do not act in a simple additive manner but through complex networks and interactions.¹⁸

We used mixed models in the statistical analyses. The covariates were age (continuous variable), sex (dichotomized as male vs. female), diagnosis (categorized as control, MCI, AD and other dementia) and *APOE-ε4* status (dichotomized as carriers of at least one ϵ_4 allele vs. noncarriers). Study site was included as a random effect. Given the exploratory nature of the study, we likewise tested whether there was interaction between SNPs and age, sex, *APOE-ε4* status and diagnosis. We corrected for multiple testing using the false discovery rate (FDR) according to the procedure of Benjamini and Hochberg.³⁹ An FDR adjusted *P*-value of $P < 0.05$ was considered significant.

RESULTS

The characteristics of the study participants per diagnostic group are summarized in Table 1. The genotype frequencies for the studied SNPs are likewise presented in the table. The group with other dementia consisted of 24 (50%) participants with frontotemporal dementia, 13 (27%) with dementia of the Lewy body type, 9 (19%) with vascular dementia, 1 (2%) with posterior cortical atrophy, and 1 (2%) with corticobasal degeneration. Compared to controls, participants with MCI and other dementia had lower MMSE score and years of education (all $P < 0.05$) but the three groups did not differ in age ($P > 0.05$). AD cases were older than MCI and control subjects and had lower MMSE score (all $P < 0.05$).

Of the 243 participants, abnormal CSF $A\beta_{1-42}$ levels were observed in 48 (52%) with MCI, 57 (73%) with AD-type dementia, 25 (52%) with other dementia and 6 (25%) control subjects. Participants with AD had lower CSF $A\beta_{1-42}$ concentration compared to all the other diagnostic groups ($P < 0.005$). MCI participants had lower CSF $A\beta_{1-42}$ levels compared

Table 1 Characteristics of study participants

Characteristic	All cases	AD-type dementia	MCI	Other dementia	Control subjects
n	243	78	93	48	24
Age (SD), years	69 (9.2)	72.1 (8.7)	69 (8.7)	67.6 (10.6)	65.5 (8.1)
Female, n (%)	104 (43)	35 (45)	44 (47.3)	13 (27)	12 (50)
Education (SD), years	9.8 (4.5)	9.3 (4.1)	9.7 (4.3)	9.4 (4.7)	11.8 (3.7)
MMSE score (SD)	25.3 (3.8)	22.8 (3.6)	27.1 (2.2)	23.6 (4.3)	29.1 (1.0)
A β ₁₋₄₂ level (SD), pg/ml	370 (152)	308 (139)	387 (140)	392 (167)	470 (132)
<i>APOE</i> - ϵ 4 status, n (%):					
noncarrier	125 (51)	41 (53)	46 (50)	28 (58)	10 (42)
at least 1 ϵ 4	103 (42)	33 (42)	42 (45)	18 (38)	10 (42)
Genotype frequencies (n), %:					
<i>MS4A4E</i> rs670139, CC/CA/AA	(230) 32/53/13	(77) 30/54/16	(88) 28/53/18	(43) 39/49/12	(22) 36/55/9
<i>MS4A6A</i> rs610932, CC/CA/AA	(230) 31/50/19	(74) 30/53/17	(91) 34/45/21	(43) 28/49/23	(22) 23/50/27
<i>MS4A4A</i> rs2304933, CC/CA/AA	(231) 39/51/10	(77) 31/61/8	(90) 40/50/10	(44) 50/36/14	(20) 40/45/15

Abbreviations: AD=Alzheimer's disease; MCI=mild cognitive impairment; n=sample size;

SD=standard deviation; MMSE= Mini-Mental State Examination

Note: All data are means (SD), unless otherwise specified.

to controls ($P=0.01$), but did not differ with the A β ₁₋₄₂ levels of those with other type of dementia ($P=0.86$).

Figure 2 shows that in the pooled sample, the mean CSF A β ₁₋₄₂ levels of homozygote risk allele carriers are lower compared to other genotypes for *MS4A4E* rs670139, *MS4A6A* rs610932 and *MS4A4A* rs2304933. Using a dominant model, rs610932 showed significant correlation with CSF A β ₁₋₄₂ ($P=0.001$, FDR adjusted $P<0.05$, Table 2). Its effect on A β ₁₋₄₂ for each diagnostic group is presented in Figure 3. A pattern of lower A β ₁₋₄₂ is seen among homozygous risk allele carriers of rs610932 in all diagnostic groups, except in AD. Using a recessive model, we observed a trend between CSF A β ₁₋₄₂ and rs670139 ($P=0.12$) and rs2304933 ($P=0.08$). We found no interaction with age, sex, *APOE*- ϵ 4 status and diagnosis for all analyses (all $P>0.05$).

Table 2 Influence of MS4A single nucleotide polymorphisms on CSF Aβ1-42

Gene	SNP	Geno- type	Mean (SD) Aβ1-42 level in pg/ml, per genotype	Genetic model	P-value
MS4A4E	rs670139	CC/ CA/ AA	369 (151)/ 384 (149)/ 337 (150)	Recessive	0.12
MS4A6A	rs610932	CC/ CA/ AA	333 (151)/ 387 (150)/ 373 (150)	Dominant	0.001*
MS4A4A	rs2304933	CC / CA/ AA	376 (150)/ 370 (153)/ 350 (159)	Recessive	0.08

Abbreviations: CSF=cerebrospinal fluid; SNP=single nucleotide polymorphism; Aβ=amyloid beta; SD=standard deviation

Note: Risk allele according to AlzGene and other literature underlined and in bold.

* Significant at a false discovery rate adjusted $P<0.05$.

Figure 2 Mean CSF Aβ1-42 concentration of the pooled sample shown per genotype for polymorphisms in three MS4A genes

Figure 3 Mean CSF A β ₁₋₄₂ concentration per genotype shown per diagnostic group for *MS4A6A* rs610932

DISCUSSION

We found that for all SNPs studied, homozygote risk allele carriers had reduced CSF A β ₁₋₄₂ concentration (rs610932) or showed a trend of lower A β ₁₋₄₂ levels (rs670139 and rs2304933) compared to heterozygotes and nonrisk allele carriers. Variant rs610932 in *MS4A6A* significantly correlated to decreased CSF A β ₁₋₄₂ level in a dominant model. We did not detect this effect in our earlier study with a Finnish AD population. For *MS4A4A* rs2304933, a trend of decreased CSF A β ₁₋₄₂ levels was observed in a recessive model but not in a dominant model. In our previous study, we found a significant influence of this SNP in a dominant model. For *MS4A4E*, the nonsignificant correlation between CSF A β ₁₋₄₂ and homozygous risk allele carriers of rs670139 is consistent with our results in the Finnish AD study. The difference in findings has several possible explanations. One is that the genotypic distribution varied between EDAR and the Finnish cohort. For instance, 31% of participants in EDAR were homozygous risk allele carriers for rs610932 compared to 53% in the

Finnish AD cohort. Another possibility is that the effect of this SNP on CSF A β_{1-42} varies with diagnosis. In EDAR, we included participants with a range of diagnoses, of which 78 had AD while in the Finnish cohort, all subjects ($N=222$) had AD. Our discrepant findings may also be caused by the fact that *MS4A4A*, *MS4A4E* and *MS4A6A* are in linkage disequilibrium. Given our small sample size and the possible weak effects of *MS4A* loci on AD, one SNP may become predictive by chance.

In conclusion, our findings suggest that polymorphisms in the *MS4A* gene may influence CSF A β_{1-42} . This supports results from GWAS studies that this gene is a genetic risk factor for AD. Replication on a larger scale can further help to determine the influence of this gene on AD. There are possibly other genetic risk factors within this linkage disequilibrium block influencing AD pathogenesis, also through pathways other than amyloid metabolism. Further research is needed to discover this.

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We assessed the influence of genetic risk factors on key markers of AD pathology in various diagnostic groups. These markers become abnormal at different stages of AD. Our findings may serve as a basis for further research.

CHAPTER 9

General discussion

RATIONALE FOR THE THESIS

The main objective of the thesis was to investigate the influence of genetic risk factors on the pathophysiology and clinical manifestation of Alzheimer’s disease (AD). While several AD risk genes have been identified, their effects on AD pathophysiology remain unclear. We examined this by correlating the effects of AD risk genes with key measures of AD pathology, including cerebrospinal fluid (CSF) markers, hippocampal atrophy, memory performance and neuropsychiatric symptoms. As the relation between genetic risk factors and AD may differ depending on the stage of the disease, we used cohorts with different severities of cognitive impairment.

Table 1 Summary of effects of top 10 AlzGene loci on AD risk and key markers of AD pathology

Chr, Gene SNP	CSF Aβ ₁₋₄₂			CSF t-tau			
	MCI	AD	Other	MCI	AD	Other	
1, <i>CR1</i> rs6656401	NS	NS	Trend in controls ^{a,b}	NS	NS	NS in controls ^a	
2, <i>BIN1</i> rs744373	NS	NS		NS	NS		
6, <i>CD2AP</i> rs9349407	Trend ^b	NS		NS	NS		
8, <i>CLU</i> rs11136000	NS	Significant ^f	NS in AD and controls ^a	NS	NS	NS in AD and controls ^a	
11, <i>MS4A4E</i> rs670139	NS	NS	NS in pooled sample ^g	NS	NS		
11, <i>MS4A6A</i> rs610932	NS	NS	Significant in pooled sample ^g	NS	NS		
11, <i>PICALM</i> rs3851179	Significant ^e	NS		NS	Trend		
19, <i>ABCA7</i> rs3764650	NS	NS		NS	NS		
19, <i>CD33</i> rs3865444	NS	NS		NS	NS		

This chapter presents a summary of our main findings in the context of current knowledge, relates these findings to genes in AD pathophysiology, and discusses the implications and methodological considerations.

SUMMARY OF MAIN FINDINGS IN THE CONTEXT OF CURRENT KNOWLEDGE

Table 1 summarizes our findings of the effects of the top 10 AlzGene loci on key markers in AD pathophysiology and on prediction from MCI to AD. The influence of candidate loci on CSF biomarkers is shown in Table 2.

	Hippocampal volume	Delayed memory	Neuropsychiatric symptoms in MCI	Association with clinical AD in Finnish case-control study	Predictive value for progression from MCI to AD
	MCI	MCI			
	Significant ^a	NS	--- ^c	Significant in multicenter GWAS ^d	NS
	NS	NS	--- ^c	Significant in replication study ^d	NS
	NS	Significant ^e	--- ^c	Significant in multicenter GWAS ^d	NS
	Significant ^e	NS	--- ^c	Significant in multicenter GWAS ^d	NS
	NS	NS	--- ^c	Significant in multicenter GWAS ^d	NS
	NS	NS	--- ^c	Significant in multicenter GWAS ^d	NS
	NS	NS	--- ^c	NS	NS
	NS	Significant ^e	--- ^c	Significant in multicenter GWAS ^d	NS
	NS	NS	--- ^c	Significant in multicenter GWAS ^d	NS

Table 1 Summary of effects of top 10 AlzGene loci on AD risk and key markers of AD pathology
Continued

Chr, Gene SNP	CSF Aβ ₁₋₄₂			CSF t-tau			
	MCI	AD	Other	MCI	AD	Other	
19, APOE-ε4	Significant ^f	Significant ^f	Significant in controls ^a	NS	Trend ^b	Incon- sistent effects ^a	

Abbreviations: AD=Alzheimer’s disease; chr=chromosome; SNP=single nucleotide polymorphism; CSF=cerebrospinal fluid; MCI=mild cognitive impairment; NS=not significant

Note: MCI data from *Chapters* 2 (meta-analysis), 4 (DESCRIPA and ADNI), and 6 (DESCRIPA); AD data from *Chapter* 7 (FINN-AD); Other data from *Chapters* 5 (review) and 8 (EDAR). Analyses for MCI group adjusted for age and gender; analyses for AD group adjusted for age, gender and APOE-ε4 status (except where APOE itself was tested)

- ^a Based on systematic review in *Chapter* 5.
- ^b Trend defined as $P \geq 0.05$ and $P < 0.10$.
- ^c Only APOE was tested for correlation with neuropsychiatric symptoms.
- ^d Gene previously genotyped in GWAS or SNP genotyped in genetic association studies that included the Finnish cohort and excluded from the genetic association analysis.
- ^e Significant at an unadjusted $P < 0.05$.
- ^f Significant at a false discovery rate adjusted $P < 0.05$.
- ^g EDAR cohort which consisted of participants with MCI, AD-type dementia, and other dementia in a clinical setting, and cognitively healthy controls.
- ^h Based on meta-analysis in *Chapter* 2.

	Hippocampal volume	Delayed memory	Neuropsychiatric symptoms in MCI	Association with clinical AD in Finnish case-control study	Predictive value for progression from MCI to AD
	MCI	MCI			
	Trend ^b	Trend ^b	Increased agitation, irritability and delusion; decreased depression	Significant. ^f ~6x increased risk	Significant. ^{f,h} ~2.5 increased risk

Table 2 Summary of effects of SNPs in other GWAS and candidate genes on CSF biomarkers in AD

Chr, Gene	A β ₁₋₄₂	T-tau	P-tau
GWAS not in top 10 AlzGene			
6, <i>MTHFD1L</i>	NS	NS	NS
11, <i>MS4A4A</i>	Significant for rs2304933 ^a NS for A β ₁₋₄₂ in pooled sample ^b	NS	NS
19, <i>EXOC3L2</i>	NS	NS	NS
GWAS with biomarkers as outcome			
2, <i>EPC2</i>	NS	Trend for rs1374441 ^c	NS
7, <i>RELN</i>	NS	NS	NS
15, <i>CYP19A</i>	NS	NS	NS
19, <i>TOMM40</i> (rs157580, rs2075650)	NS	NS	NS
Other candidate genes			
2, <i>PPP3R1</i>	NS	Trend for rs1868402 ^c	NS
3, <i>TF</i>	NS	NS	NS
10, <i>IDE</i>	NS	NS	NS
11, <i>BDNF</i>	NS	NS	NS
11, <i>SORL1</i>	NS	Trend for rs73595277 ^c	Trend for rs73595277 ^c
14, <i>CYP46a</i>	NS	NS	NS
17, <i>ACE</i>	NS	NS	NS

Table 2 Summary of effects of SNPs in other GWAS and candidate genes on CSF biomarkers in AD *Continued*

Chr, Gene	A β_{1-42}	T-tau	P-tau
17, MAPT	NS	Significant for rs2435211 ^d , trend for rs16940758 ^c	Significant for rs16940758 ^d
19, TOMM40 (rs8106922)	NS	NS	NS

Abbreviations: SNPs=single nucleotide polymorphisms; GWAS=genome-wide association study; CSF=cerebrospinal fluid; AD=Alzheimer’s disease

Note: Data from *Chapters 7* (FINN-AD) and *8* (EDAR)

- ^a Significant at a false discovery rate adjusted $P<0.05$
- ^b EDAR cohort consisting of participants with MCI, AD-type dementia, other dementia in a clinical setting, and cognitively healthy controls.
- ^c Trend defined as $P\geq 0.05$ and $P<0.10$.
- ^d Significant at an unadjusted $P<0.05$.

Predictive value of top AlzGene loci for developing AD-type dementia in individuals with mild cognitive impairment (MCI)

We performed analyses in two studies. In the first study (*Chapter 2*), we assessed the predictive value of *APOE* in a large meta-analysis of prospective studies of subjects with MCI. We found an odds ratio (OR) of 2.3, indicating moderate association. The sensitivity and positive predictive value were low, indicating that *APOE* genotyping has limited clinical value but is useful for trials aiming to select subjects who are at higher risk for progressing to AD-type dementia. The predictive value of *APOE* in our meta-analysis was affected by age and follow-up length. With advancing age, the percentage of *APOE*- ϵ_4 carriers among subjects who progressed to AD decreased. This is consistent with previous findings¹ and could mean that carriers of the ϵ_4 allele may have either progressed to AD or have died at a younger age.² A previous study has shown that there is a North-South gradient in *APOE* prevalence in Europe.³ We modeled this effect using meta-regression and found that the back-

ground prevalence of *APOE* influenced the predictive accuracy. Sensitivity increased while specificity decreased with a higher background prevalence. It is therefore important to take into account the prevalence of *APOE* in the population tested when interpreting the results.

In the second study (*Chapter 6*), we tested the predictive accuracy of the top 10 AlzGene loci in nondemented individuals with cognitive impairment from a memory clinic setting from the DESCRIPA cohort. Only *APOE* predicted AD-type dementia with an OR very similar to that obtained in our meta-analysis (OR $\approx$ 2.5). The other top AlzGene loci showed much weaker risk effects that were not statistically significant. Our results are consistent with those of another study assessing disease progression in MCI.⁴ Nonetheless, based on AlzGene meta-analyses, it is unlikely that other SNPs would independently exert an effect greater than *APOE*. A population-based study of nondemented individuals has found that the combined effect sizes of the individual top AlzGene loci only marginally improved prediction of AD, but we have not tested this in the present study.⁵

We noted a number of results that were the opposite of AlzGene findings, namely, a seemingly protective effect of *CR1* and *BIN1* and risk effect of *CD33* in our cohort. This could be explained by the very small number of risk allele carriers in the SNPs studied in these genes in our cohort.

Genetic association with clinical AD

We also tested the association of 24 genetic variants with clinical AD in the Finnish population composed of AD patients and age-matched healthy control subjects (*Chapter 7*). Only *APOE* and *TOMM40* were associated with clinical AD. Carrying at least one ϵ_4 allele increased the risk of AD to more than six times. This result was not affected by age and gender. The high odds ratio we obtained, which was comparable to those reported in other Caucasian populations,⁶⁻⁸ could be attributed to the high *APOE* prevalence in the Finnish population. *TOMM40* showed genetic association with AD, but this effect disappeared after correction for *APOE* genotype. *TOMM40* is in linkage disequilibrium with *APOE* and our results were in accordance with recent evidence showing that the possibility of finding an effect that was not due to *APOE* in the *APOE*

region was unlikely.⁹ Other candidate genes were not associated with AD in the Finnish population, which is in line with AlzGene findings (for genes not included in top list) and the fact that they did not show significant effects in large GWAS.

In multicenter genetic association studies or GWAS involving the Finnish population, associations have been reported for the top AlzGene loci in *ABCA7*, *BIN1*, *CD2AP*, *CD33*, *CLU*, *CR1*, *MS4A4E* and *MS4A6A*.¹⁰⁻¹²

Correlation with CSF biomarkers

We tested the correlation between CSF markers and top AlzGene loci both in MCI and AD; a range of candidate loci only in AD; and variants in the *MS4A* cluster in a pooled sample consisting of participants with MCI, AD-type dementia, other dementia and cognitively healthy controls. The findings for the top 10 AlzGene loci in MCI and AD are summarized in Figure 1a-d. The plots also highlight the differences in CSF marker concentrations between MCI and AD. While CSF A β_{1-42} concentrations are in the same range in MCI and in AD, the distribution in MCI is more spread out. Tau levels are clearly lower in MCI than in AD. The variation is also smaller.

CSF A β_{1-42}

Of the top AlzGene, only *APOE-ε4* is significantly related to reduced CSF A β_{1-42} in both MCI and AD (*Chapters 6 and 7*). In MCI (*Chapter 6*), A β_{1-42} concentrations were significantly decreased among *APOE ε4* allele carriers whereas marginal reductions at an unadjusted *P*-value were observed among *PICALM* risk allele carriers. Both genes have been reported to play a role in A β aggregation: *APOE-ε4* through its high avidity binding to A β yet less efficient function in A β clearance,¹³⁻¹⁵ and *PICALM* through endocytotic processes.^{16, 17} Homozygote risk allele carriers of the other top AlzGene loci tended to have lower A β_{1-42} levels, but these effects were not significant.

In AD (*Chapter 7*), *APOE* and *CLU*, which is another apolipoprotein that binds soluble A β and is involved in A β aggregation,¹⁸ were significantly related to reduced CSF A β_{1-42} concentrations.

Figure 1a MCI in DESCRIPA cohort

Figure 1b AD in FINN-AD cohort

Figure 1a-b The plots show the mean CSF Aβ₁₋₄₂ levels in mild cognitive impairment (MCI, 1a) and in Alzheimer’s disease (AD, 1b) for the top 10 AlzGene loci. Genetic variants for which the minor allele is the risk allele are represented by straight lines (—) while variants for which the major allele is the risk allele are shown in broken lines (---).

Figure 1c

Figure 1d

Figure 1c-d The plots show the mean t-tau levels in mild cognitive impairment (MCI, 1c) and in Alzheimer's disease (AD, 1d) for the top 10 AlzGene loci. Genetic variants for which the minor allele is the risk allele are represented by straight lines (—) while variants for which the major allele is the risk allele are shown in broken lines (---).

There were few other SNP-related differences because CSF A β may already be strongly reduced in demented patients, as indicated by the low variability in CSF A β ₁₋₄₂ values.

Of the other GWAS SNPs, *TOMM40* and *EXOC3L2* likewise correlated with decreased A β ₁₋₄₂ levels in AD, but these effects were not independent of *APOE*.

A notable finding is the relation between SNPs in the *MS4A* gene family and CSF A β ₁₋₄₂. Significant reductions in CSF A β ₁₋₄₂ were found in relation to *MS4A4A* in AD (*Chapter 7*), and *MS4A6A* in a pooled sample of MCI, AD, other dementia and cognitively healthy control subjects (*Chapter 8*). The SNPs we studied in *MS4A4A* and *MS4A6A* have been identified in GWAS with clinical AD as outcome,¹⁹ but as far as we know, there has been no studies yet reporting their correlation with AD biomarkers. *MS4A4A* and *MS4A6A* are in linkage disequilibrium, and are 2 of the 16 known gene members of the *MS4A* cluster. The *MS4A* gene family is poorly characterized.²⁰ Other variants of this cluster have been found to be associated with AD in non-Caucasian population.²¹ A role in immunity has been suggested for other variants,^{20 22-25} however, it is beyond the scope of the current thesis to investigate this. In the thesis, we showed that *MS4A4A* and *MS4A6A*, which have been associated with clinical AD, also have an effect on CSF A β ₁₋₄₂.

CSF tau

The top AlzGene loci were not significantly correlated to CSF tau in both MCI and AD (*Chapters 6 and 7*). Although tau levels among homozygous risk allele carriers in MCI were generally higher than normal, and were abnormal in AD, they were not or were only weakly correlated with the top AlzGene. This suggests that the top AlzGene loci contribute to the development of AD through pathways other than directly affecting tau.

Of interest are the trends we found for polymorphisms in candidate genes, *PPP3R1* and *MAPT*, in relation to CSF tau in AD (*Chapter 7*). *PPP3R1* plays a role in modulating tau phosphorylation via calcineurin.^{26, 27} *MAPT*, in turn, codes for tau proteins,²⁸ as will be discussed in more detail below. The nonsignificant trends may indicate that the effects of these variants are subtle and requires a much bigger sample in order to attain significance. Our results nevertheless point to the need to further inves-

tigate the role of candidate genes in tau metabolism, as SNPs in these genes have been previously shown to affect disease progression.^{29, 30} Meanwhile, we also observed elevated tau levels in relation to *SORL1*. This finding may be due to chance as *SORL1*, which binds to ApoE, is not normally associated with tau.

Correlation with hippocampal volume

None of the top 10 AlzGene loci significantly correlated with hippocampal volume in MCI (*Chapter 6*). The lack of significant correlations with the top AlzGene loci does not necessarily mean that these variants do not affect the hippocampus but more likely that our sample size was too small to detect robust effects on the one hand. On the other hand, hippocampal atrophy is a later event in the pathogenesis of AD and may not yet be evident in our cohort.

We observed nonsignificant associations between hippocampal volume and risk allele carriers of *CLU* and *APOE*, and nonrisk allele carriers of *CR1*. *CLU* has been associated with brain atrophy in both MCI and AD.³¹ *CLU* has also been suggested to affect episodic memory through hippocampal dysfunction.³² In our cohort, *CLU* was related to hippocampal volume and not to memory.

The nonsignificant correlation between hippocampal volume and the *APOE* ϵ_4 allele in our cohort is consistent with previous findings. Hippocampal volume loss among *APOE* ϵ_4 allele carriers with MCI³³ and among cognitively healthy individuals³⁴ has been previously reported. *APOE* does not directly lead to hippocampal volume loss. Rather, a neuropathologic study has shown that hippocampal atrophy among carriers of the *APOE* ϵ_4 allele occurs through a complex mediation process wherein *APOE* affected CSF A β that, in turn, resulted in changes in tau,³⁵ eventually leading to tangle formation and hippocampal atrophy.

The reduced hippocampal volume among homozygous non-risk allele carriers of *CR1* was the opposite of what we expected and not in line with previous studies. It may be due to chance. *CR1* has been suggested to play a protective role in AD by binding A β oligomers to its C3b complement factor.^{18, 37} A protective effect of the *CR1* SNP (rs6656401) that we studied is not likely based on its effects on CSF A β_{1-42} and tau in the present cohort and also in the Finnish AD population.

Correlation with clinical markers in MCI

Delayed memory

We tested the top AlzGene loci for influence on delayed memory (*Chapter 6*). None of the SNPs were significantly related to memory after multiple test correction. *CD2AP* and *ABCA7* showed effects on memory at an unadjusted *P*-value, while a trend was observed for *APOE*.

CD2AP correlated with worse delayed memory performance. *CD2AP* has not been shown in a previous study to significantly affect memory, although this study pertained to nondemented individuals in a general population.⁵ It is conceivable that subtle effects of *CD2AP* on memory are more detectable in a population of nondemented individuals with cognitive impairment in a clinical setting, such as our cohort. Further research is needed to verify the influence of *CD2AP* on memory.

The higher delayed memory performance we observed among *ABCA7* risk allele carriers compared to noncarriers is apparently the result of the very small number of risk allele carriers in our cohort (1.6% genotype frequency for homozygous risk allele carriers and 18.3% for heterozygous carriers). The risk allele of *ABCA7* has further not been found to significantly affect memory in the population-based study described above.⁵

We observed a weak correlation between the *APOE* ϵ_4 allele and delayed memory. Current knowledge suggests that *APOE* does not directly affect cognition. Rather, *APOE* has been reported to affect $A\beta$ which, through a cascade of events, leads to $A\beta$ deposition, tangle formation and the clinical manifestation of cognitive decline.^{35, 36, 38} In our study, *APOE* was significantly related to reduced CSF $A\beta_{1-42}$. *APOE*- ϵ_4 carriers had higher tau levels, smaller hippocampal volume and lower delayed memory performance, although the correlation was not significant. These results could be conceived in the context of events shown in Figure 2.

Our results do not rule out the effects of other AD risk genes on cognition. Rather, they suggest that the effects may be subtle, indirect or occur through different pathophysiological mechanisms, as in the case of *APOE*.

Figure 2 Schematic diagram of the influence of *APOE* on amyloid beta and the series of events that lead to the clinical manifestation of cognitive decline

Neuropsychiatric symptoms

The *APOE* ϵ_4 allele was significantly related with agitation and irritability, and with clinically significant symptoms of heightened delusions and reduced depression in MCI (*Chapter 4*). Clinically significant symptoms are severe enough to disrupt daily functioning and merit treatment.³⁹ Agitation and irritability have been reported to be related with reduced CSF $A\beta_{1-42}$ in MCI.⁴⁰ The correlation we found in our study may very well reflect the effect of *APOE* on CSF $A\beta_{1-42}$, and thus AD, rather than directly on agitation. *APOE*- ϵ_4 was related to reduced risk of depression in MCI.

This is consistent with reported findings in AD.^{41, 42} The relation between *APOE* and delusion must be interpreted with caution because of the very small number of individuals in our study who had delusions.

GENES IN AD PATHOPHYSIOLOGY

As discussed in the previous section, we found a number of associations between genetic risk factors and AD biomarkers. In this section, we will discuss how these findings relate to the role of genetic risk factors in AD pathophysiology. As model, we use the amyloid cascade (Figure 2). According to this model, abnormal accumulation of A β in plaques triggers a series of responses, including tau phosphorylation and aggregation⁴³ that, in turn, lead to the formation of neurofibrillary tangles (NFTs). Cognitive decline, which is a later event in AD pathogenesis, has been shown to correlate with NFTs.³⁶

Top 10 AlzGene loci

Only *APOE*- ϵ_4 consistently showed a relation to the markers we tested. We interpret this in the context of recent *in vivo*^{44, 45} and neuropathological^{35, 36, 38} findings that *APOE* mainly affects amyloid and does not directly affect tau, hippocampal atrophy, memory, and presumably neuropsychiatric symptoms as discussed earlier. We found that indeed, *APOE*- ϵ_4 was strongly correlated with CSF A β_{1-42} and showed weaker correlation with the other markers we studied, which are considered as downstream markers. *APOE*- ϵ_4 has been shown to have a high avidity binding to A β ,^{14, 46} to be less efficient in transporting A β across the blood-brain barrier,¹³ and to stimulate A β aggregation *in vitro* and amyloid plaque deposition *in vivo*.^{13, 15, 47} *APOE*- ϵ_4 has also been shown to increase the formation of soluble A β oligomers,^{48, 49} which have been reported to be highly neurotoxic.^{13, 50, 51} Abnormal accumulation of A β in plaques triggers a series of responses, including tau phosphorylation and aggregation⁴³ that, in turn, lead to the formation of neurofibrillary tangles (NFTs). Cognitive decline, which is a later event in AD pathogenesis, has been shown to correlate with NFTs,³⁶ as previously discussed and summarized in Figure 2.

CLU correlated with reduced CSF A β_{1-42} level in AD and reduced hippocampal volume in MCI in the present thesis. Previous studies

have not found a relation between *CLU*, and A β and tau in CSF⁵²⁻⁵⁴ or in plaques and tangles.^{55, 56} No common coding variant in *CLU* has been found to date that could explain the genetic association with AD.^{57, 58} Instead, genetic variations that affect expression levels of clusterin have been suggested to influence the risk of developing AD.^{18, 57, 59} We did not measure clusterin levels in the current thesis, but we explain our findings in view of this knowledge. *CLU* has generally been reported to exert a protective role in AD.^{58, 60, 61} *CLU* codes for multifunctional proteins that among others, act as chaperone of A β in the brain.^{58, 62} Clusterin is involved in lipid transport, guards against A β aggregation through mechanisms that include binding to soluble A β in CSF, stabilizing A β peptides, inhibiting formation of A β oligomers and amyloid fibrils, mediating A β clearance from the brain through endocytosis and transporting A β across the blood- brain barrier.^{18, 58, 61-63} However, in the presence of excessive A β peptides or oligomers, clusterin has been shown to increase A β aggregation.⁵⁸ While this is on the one hand detrimental for the brain, on the other hand, it has also been reported to prevent excessive neuroinflammation,⁵⁸ thus conferring to a certain extent a protective effect. Elevated clusterin levels have been found to correlate with reduced CSF A β_{1-42} in AD but not in controls, suggesting that clusterin levels are related to neuritic plaques.⁶⁴ In a clinicopathological study, increased clusterin levels in the hippocampus of AD patients have been reported, but this did not correlate with neuritic plaque density, and thereby not with A β .⁶⁵ Meanwhile, other studies have provided *in vivo* and *in vitro* evidence that A β enhanced clusterin expression.^{59, 66} Increased clusterin levels in MCI has been suggested as neuroprotective in that it corresponded to slower brain atrophy rates; whereas in AD, the correlation between elevated clusterin levels and brain atrophy has been attributed to failure of neuroprotection.⁶⁷ Based on the aforementioned studies, we conclude that clusterin appears to be an indicator, rather than an initiator, of AD pathology.

PICALM in the present thesis decreased CSF A β_{1-42} concentration in MCI and showed a trend of increased tau level in AD. The influence of *PICALM* on A β in CSF and on neuritic plaques upon neuropathological examination has been confirmed by other studies.⁵⁴⁻⁵⁶ *PICALM* is involved in clathrin-mediated endocytosis. It has been shown to

influence A β neurotoxicity by regulating endocytosis.¹⁶ It has also been suggested to indirectly increase A β production by modifying A β precursor protein or its proteases through altered trafficking of vesicle-associated membrane protein 2 (VAMP2).¹⁷ Previous studies have reported that VAMP2 trafficking is critical for synaptic integrity.^{68, 69} *PICALM* has been posited to influence AD risk through synaptic dysfunction.⁷⁰ Synaptic dysfunction has been related to tau aggregation and hyperphosphorylation.⁷¹ While it is conceivable that the trend we observed for CSF tau in the current thesis could be explained by the indirect role of *PICALM* on synaptic integrity, other studies showed no relation between *PICALM* and tau in CSF^{53, 54} and NFTs upon neuropathological examination.^{55, 56}

CD2AP was related to decreased delayed memory in MCI in our study. In addition, we observed a trend of reduced CSF A β_{1-42} level in MCI. To our knowledge, no literature to date has linked *CD2AP* with cognition and A β . A previous study found no correlation between cognition and the combined risk of top GWAS SNPs including *CD2AP*.⁵ Like *PICALM*, *CD2AP* is involved in clathrin-mediated endocytosis,⁷² and has a suggested role in synaptic dysfunction and cell membrane processes.⁷⁰ *CD2AP* functions as a scaffold adaptor protein¹⁰ and as such has a role in regulating signal transduction. The involvement of *CD2AP* in A β processing is not straightforward but has been posited to occur within the complex complement cascade network.²⁵ *CD2AP* has been described as forming part of the complement system and an immune network through its association with CD2, which is a T-lymphocyte marker protein, that facilitates communication between cells.²⁵ In addition, *CD2AP* has been linked with atherosclerosis, which is an AD risk factor, via its binding with other proteins that are exploited by bacterial pathogens.²⁵

CR1 was related to increased hippocampal volume in MCI in the present thesis. We attributed this result to chance given that the rest of our findings did not support a protective effect of the SNP we studied. A different variant in *CR1* has been suggested to be neuroprotective.⁷³ Meanwhile, a trend of decreased A β_{1-42} concentration among control subjects has been reported in previous research.⁵⁴ The precise nature of how *CR1* contributes to AD risk has been the subject of numerous studies. *CR1*, with as complement factors C3b and C4b, is a member of the complement system receptors.^{18, 25} The complement system is part

of the innate immune system,⁷⁴ which is the body's first line of defense against pathogens. Complement activation has been reported as an early event in the development of AD pathology.⁷⁵ *CR1* aids in eliminating immune complexes containing C3b and C4b, and in clearing A β by binding A β oligomers to its C3b complement factor.¹⁸ A β_{1-42} in plasma has also been reported to adhere to C3b.⁷⁶ Apart from being actively involved in clearing pathogens, *CR1* also plays a role in complement inhibition, thereby subduing the inflammatory response and limiting the damage to surrounding tissues.⁷⁴ Excessive and chronic activation of the complement system is detrimental as it damages surrounding tissues.⁷⁴ Moreover, it has been reported to lead to neuropathological changes that increase the phosphorylation of tau and ultimately leads to neuronal death.^{74, 77} In this sense, *CR1* appears to have a protective role in AD, although this needs to be verified by further research. Previous work has shown reduced complement inhibitors in AD.⁷⁷ Furthermore, it has been suggested that in AD, disruptive complement signaling and the presence of A β leads to a series of events wherein more complement factors are secreted which, in turn, results in chronic inflammatory reaction.⁷⁷

The *ABCA7* risk allele correlated with better memory in the current thesis. We explained this finding as probably due to the very small number of risk allele carriers in our study. *ABCA7* has not been shown in previous work to be associated with cognition.⁵ *ABCA7* has also not been reported to be involved in A β metabolism,⁷⁰ although it has been shown to inhibit the secretion of A β *in vitro*.⁷⁸ Several studies have suggested that *ABCA7* possibly contributes to AD pathophysiology by its involvement in the complement system and lipoprotein-related mechanisms.^{19, 25, 70, 79} *ABCA7* codes an ATP-binding cassette (ABC) transporter mainly found in immune network cells.²⁵ One of its substrates is ApoE. The main function of *ABCA7* appears to be phagocytosis of apoptotic cells, thereby serving as defense mechanism against pathogens.²⁵ It has been reported to be involved in the efflux of lipids from cells to lipoprotein particles,⁷⁹ but not in the efflux of cholesterol.⁸⁰ However, it has been shown to mediate efflux of cholesterol to lipid-laden ApoE,²⁵ which opens further research possibilities for understanding the contribution of *ABCA7* and its interaction with other risk factors to AD pathophysiology.

We studied polymorphisms in three *MS4A* genes, of which two are included in the top 10 AlzGene (*MS4A4E* and *MS4A6A*) and another (*MS4A4A*) has been identified in GWAS to be associated with clinical AD. We will discuss these three genes, which are in linkage disequilibrium, together in this section. *MS4A4A* and *MS4A6* reduced CSF A β ¹⁻⁴² concentrations respectively in AD and in a pooled sample of cognitively healthy controls, MCI, AD and other dementia cases in the present thesis. To our knowledge, *MS4A* has not been previously reported to affect CSF A β . Previous studies of *MS4A* genes have been in relation to immunity.²²⁻²⁵ The *MS4A* family consists of at least 16 known genes¹⁰ clustered along chromosome 11q12. All *MS4A* family members code for proteins with multiple membrane-spanning domains,⁸¹ form part of oligomeric cell surface proteins and play a role in cellular signal transduction.²² *MS4A4A*, *MS4A4E* and *MS4A6A* have been reported to play a role in immunity, being structurally related to the immunoglobulin E receptor and to CD20,²⁵ which is a B-lymphocyte cell surface molecule.¹⁸ These studies suggest a role of immunity mediated by *MS4A* in AD pathophysiology. The precise mechanisms by which this occurs, and how this may have affected CSF A β in our study, deserve further investigation.

BIN1 has been established in various GWAS as a genetic risk factor for AD,^{10, 19, 82-84} but showed no effect on the AD markers we studied. Previous studies likewise found no correlation between *BIN1* and CSF A β and tau,⁵³ or with cognition.^{5, 85} The involvement of *BIN1* in clathrin-mediated endocytosis and apoptosis provides a possible explanation how *BIN1* contributes to AD pathophysiology. *BIN1* encodes proteins belonging to the Bin/Amphiphysin/Rvs (BAR) family adapter that form complexes acting in clathrin-mediated endocytosis.¹⁸ In this process, proteins from the plasma membrane are transported via clathrin-coated vesicles from the cell surface to inside the cell, where they are sorted and targeted to different destinations.⁸⁶ The manner in which the amyloid precursor protein (APP) is processed has been reported to be dependent on endocytosis and intracellular sorting which, in turn, influences A β production.⁸⁶ Other explanations are the involvement of *BIN1* in synaptic formation, neuronal membrane organization and stress signaling^{18, 70, 84} which could be disrupted in AD.

CD33 also did not influence any of the AD markers we tested. *CD33* has previously been studied in relation to cognition, but this likewise did not yield significant result.⁵ *CD33* has been posited to contribute to AD risk via the immune network.²⁵ *CD33* belongs to the sialic acid binding Ig-like lectin (Siglec) family that binds sialic acid and has been reported to regulate both innate⁷⁹ as well as adaptive²⁵ immune responses. Further, *CD33* has been suggested to contribute to the development of AD through synaptic dysfunction and cell membrane processes.⁷⁰

GWAS SNPs not in top 10 AlzGene

EXOC3L2 in our study was related to reduced CSF $A\beta_{1-42}$ levels in AD, but this result became nonsignificant when we corrected for *APOE-ε4* status. *EXOC3L2* is in linkage disequilibrium with *APOE*.⁸⁷ Previous studies have shown that the association between *EXOC3L2* and clinical AD was not independent of *APOE*.^{12, 87} *EXOC3L2* has been shown to facilitate correct vesicle targeting of proteins and lipids during exocytosis, thereby playing a role in neuronal outgrowth and integrity.^{88, 89} *EXOC3L2* has been associated with rapid disease progression in AD.⁹⁰

MTHFD1L showed no correlation to CSF $A\beta_{1-42}$ and tau in our study. *MTHFD1L* has been suggested to affect AD risk through folate-related pathways.⁹¹ *MTHFD1L* encodes a protein that synthesizes tetrahydrofolate in the mitochondria. This folate-pathway has been reported to be involved in the synthesis of homocysteine.⁹² Elevated homocysteine levels have been associated with increased AD risk,⁹³⁻⁹⁵ but also with cardiovascular diseases^{96, 97} that are also risk factors for AD. The influence of *MTHFD1L* on AD risk is therefore possibly mediated by cardiovascular factors. We did not test this in our study, and its ascertainment deserves further research.

GWAS SNPs with biomarkers as outcome

We observed a trend between *EPC2* and increased CSF tau concentration in AD but *EPC2* did not affect $A\beta_{1-42}$. This confirms previous GWAS findings with CSF tau and $A\beta_{1-42}$.⁹⁸ Other *EPC2* variants in GWAS also mainly affected CSF tau and not $A\beta$.⁹⁸ The known functions of *EPC2* do not directly link to tau metabolism or AD. One of the possibilities as to

how *EPC2* contributes to AD pathology is through epigenetics. *EPC2*, which is a member of the polycomb family, is involved in heterochromatin formation, modification of chromatin, and dysregulation of epigenetic mechanisms and has also been implicated in mental retardation.⁹⁸ Further research would help better characterize the relation between *EPC2* and AD.

RELN was not significantly related to CSF biomarkers in the current thesis. A previous clinicopathological study showed that *RELN* was associated with abnormal tau concentration in NFT, particularly in the hippocampus, in AD and interestingly also in cognitively healthy controls.⁹⁹ This suggests that *RELN* is a potential indicator of AD pathology before the onset of dementia. *RELN* codes for reelin, which is part of the ApoE pathway, and which is involved in directing the migration of neurons and their positioning, and in forming and maintaining synapses.⁹⁹⁻¹⁰¹ Previous studies have indicated that *RELN* plays a protective role in AD by (1) increasing long-term potentiation (LTP), which has been suppressed by A β oligomers; and (2) inhibiting tau phosphorylation.^{99, 101} *RELN* has been shown to compensate for A β -induced LTP suppression by increasing the receptor activity of NMDA, thereby reversing the effect of A β and preventing synaptic dysfunction.¹⁰¹ This compensatory mechanism fails at high concentrations of A β .

We studied three polymorphisms in *TOMM40*, of which two (rs157580 and rs2075650) have been identified in GWAS with biomarkers as outcome, and one (rs8106922) is a candidate SNP. In this section, we will discuss all three variants. All *TOMM40* SNPs were related to reduced CSF A β_{1-42} but not tau levels in the present thesis, in accordance with GWAS findings. The GWA studies reported associations that were uncorrected for *APOE*.^{98, 102} As *TOMM40* is in linkage disequilibrium with *APOE*, we repeated our analyses with adjustment for *APOE*- ϵ_4 status and found that indeed, the effects of *TOMM40* SNPs on CSF biomarkers were not independent of *APOE*. *TOMM40* has been suggested to influence AD pathophysiology through mitochondrial dysfunction.¹⁰³ *TOMM40* functions to control the outer mitochondrial membrane pore subunit, through which proteins enter the mitochondria.¹⁰³ Mitochondrial dysfunction has been reported to lead to the

accumulation of APP and the cellular influx of A β .¹⁰³⁻¹⁰⁵ The contribution of *TOMM40* to AD pathophysiology is likely to be influenced by *APOE* or reflects the effects of *APOE*.

CYP19A did not affect CSF biomarkers in AD in our study. This is consistent with GWAS results, which interestingly found an association between *CYP19A* and CSF A β ₁₋₄₂ in control subjects but not in AD and MCI.¹⁰² *CYP19A* encodes cytochrome P450 aromatase, which catalyzes the formation of estrogens from androgens.¹⁰⁶ Aromatase has a neuroprotective role and functions to regulate neural differentiation, plasticity and, through the synthesis of estrogen, also sexual behavior.¹⁰⁷⁻¹⁰⁹ Impaired aromatase expression and estrogen deficiency have been suggested to contribute to AD risk, which has been shown in animal studies to increase the formation of A β plaques.¹¹⁰ As estrogen is involved in modulating kinase and phosphatase activities, it also has a suggested role in inhibiting tau hyperphosphorylation.¹¹¹ The role of estrogen, and consequently gender, as risk factors for AD remain controversial. Longitudinal studies of estrogen therapy among postmenopausal women showed an increased risk of AD.^{112, 113} A gender-specific effect of *CYP19* has been suggested,¹⁰⁶ but another study found no effect of gender on aromatase-related AD risk.¹¹⁴

SNPs from candidate genes

PPP3R1 showed a weak association with AD risk and a trend of elevated tau concentration in AD in our study. A correlation between *PPP3R1* and tau in individuals with A β deposition has been previously reported and found to be associated with disease progression.²⁹ *PPP3R1* appears to contribute to AD pathophysiology via calcineurin, which modulates the phosphorylation of tau.²⁷ *PPP3R1*, which is a protein phosphatase, is the calcium binding regulatory subunit of calcineurin.²⁶

MAPT was related to increased tau in AD and was also weakly associated with AD risk in the current thesis. Previous studies have shown conflicting evidence linking *MAPT* with tau.^{29, 115-117} In one study, a *MAPT* haplotype (H1) has been correlated with reduced AD pathology independent of *APOE*,¹¹⁶ whereas another study found the same variant to be associated with risk of developing MCI.¹¹⁷ What is known is that tauopathy is a feature of AD and that tau dysfunction leads to

neurodegeneration. *MAPT* plays a role in this in that it codes for the microtubule-associated protein tau, which functions to promote and stabilize microtubule assembly, as well as maintain neuronal integrity, axonal transport and polarity.²⁸ In AD, this protein has been reported to be abnormally expressed.¹¹⁸ Tau, which is normally bound to microtubules, has been described to disengage from microtubules and to disassemble during abnormal hyperphosphorylation.¹¹⁸ Moreover, the disengaged tau has been reported to capture normal tau, thus preventing microtubule assembly.¹¹⁸ Aside from these, hyperphosphorylated tau protein is a main component of NFTs, as discussed earlier.

SORL1 was related to increased tau in AD in the present thesis. We attributed this findings to chance because previous correlational studies of individual SNPs in *SORL1* and CSF biomarkers have yielded either conflicting or inconclusive results.¹¹⁹ The influence of *SORL1* on AD risk appears to be very small that detection is likely only by performing haplotype analyses¹²⁰ or by pooling samples in a mega-analysis.¹²¹ Different mechanisms have been suggested by which *SORL1* contributes to AD pathophysiology. One is though APP processing. *SORL1* encodes the vacuolar protein-sorting 10 (VPS10) domain, which is part of a group of protein-trafficking molecules that guide APP into recycling pathways.¹²² Underexpression of *SORL1* has been reported to result in APP being sorted into sites for A β production, wherein APP cleavage by presenilin 1 occurs and A β peptides are produced.¹²²⁻¹²⁵ Another mechanism by which *SORL1* may influence AD pathogenesis is through cholesterol metabolism and its associated cerebrovascular diseases.¹²⁵ *SORL1* likewise encodes a protein belonging to the low-density lipoprotein (LDL) receptor family, which binds ApoE and transports LDL into cells via endocytosis.¹²⁵

TF did not correlate to CSF biomarkers in our study. Previous work has shown that *TF* reduced CSF A β_{1-42} /A β_{1-40} ratio.¹¹⁵ *TF* has been suggested to influence AD risk by modulating A β and through iron overload.¹²⁶ *TF* encodes transferrin, a plasma glycoprotein that binds and transports iron across the blood brain barrier to the brain.¹²⁷ A β , and also tau, have metal binding sites. Metal ions have been reported to foster the aggregation of A β .¹²⁸ Via another mechanism, iron overload due to disrupted iron metabolism has been shown to contribute

to AD pathogenesis by oxidative damage which, in turn, results in reduced protein synthesis in the brain¹²⁹ and ultimately cell death.

BDNF in the current thesis showed no correlation to CSF biomarkers. A correlation between *BDNF* and reduced CSF A β concentration has been previously reported.¹¹⁵ *BDNF* encodes the brain-derived neurotrophic factor (BDNF) protein, which is a member of the neurotrophin nerve growth factor family, that is involved in multiple functions, including promoting neuronal survival and differentiation, axonal and dendritic growth, as well as synaptic transmission and plasticity.¹³⁰ *BDNF* has a neuroprotective function. It is abundantly expressed in the hippocampus, neocortex and hypothalamus,^{131, 132} which are specific areas affected in AD. Decreased *BDNF* protein levels have been reported to influence AD pathophysiology by means of insufficient trophic support that may lead to regional neuronal degeneration, as has been observed in AD.¹³³ Alternately, reduced *BDNF* levels has also been suggested to reflect overall synaptic loss and amyloid deposition in AD.¹³²

CYP46A1 was not related to CSF biomarkers in the present thesis. In previous studies, *CYP46A1* has been shown to affect A β_{1-42} , and interestingly also tau,¹³⁴ both in CSF and upon neuropathological examination.^{134, 135} *CYP46A1* has been posited to contribute to the pathogenesis of AD through cholesterol metabolism. *CYP46A1* encodes the enzyme, cholesterol 24S-hydroxylase (*CYP46*), which is brain-specific. *CYP46* functions to make cholesterol more soluble and easier to eliminate through the blood-brain barrier by hydroxylation of cholesterol into 24-hydroxycholesterol.^{136, 137} Deficits in *Cyp46* has been suggested to result in increased brain cholesterol.¹³⁶ A cholesterol-rich environment has been reported to be favorable for the activities of b-secretase and g-secretase,¹³⁶ which cleave APP, resulting in the production of A β isoforms.

IDE did not correlate to CSF biomarkers in our study. Past research has correlated *IDE* amyloid deposition and NFT in AD.¹³⁸ *IDE* codes for zinc metallopeptidase and is involved in the degradation of intracellular insulin and A β .¹³⁹ It has also been shown to play a role in A β clearance.¹⁴⁰ One of the ways in which deficiencies in the insulin-degrading enzyme, *IDE*, could contribute to AD pathology is by failing to clear A β .¹⁴¹ Another is by failing to degrade A β . This has been observed in cases of

hyperinsulinaemia, wherein insulin competes with A β for IDE.^{141, 142} In both instances, A β can accumulate and aggregate.

ACE in our study marginally correlated with AD risk but showed no effect on CSF biomarkers. While the association between *ACE* and AD risk has been confirmed by several studies,¹⁴³⁻¹⁴⁵ results of other studies on the effects of *ACE* risk variants on biomarkers of AD have been inconclusive.^{115, 146} *ACE* encodes angiotensin I converting enzyme (ACE) 1, and is associated with hypertension and cerebrovascular diseases.¹⁴⁴ *ACE* mediates the degradation of the more toxic A β ₁₋₄₂ isoform to A β ₁₋₄₀.¹⁴⁷ The use of ACE inhibitors has been shown to lead to A β accumulation and deposition,^{147, 148} thereby possibly contributing to AD pathophysiology.

METHODOLOGICAL CONSIDERATIONS

Study design

The main objective of the thesis was to examine the influence of genetic risk factors on clinical and biomarker correlates of AD. As such, the cross-sectional design we used was sufficient. We were able to test the prognostic value of *APOE* for progression to AD through a meta-analysis. Based on the limited follow-up information on clinical outcome for one of the cohorts we studied, we were also able to test the risk associated with developing AD for the top AlzGene loci. For one of the studies, we employed a case-control design, which allowed us to perform genetic association analyses.

In *Chapter 6*, we used a cohort comprised of SCI and MCI. Excluding SCI cases from the analyses yielded similar results. This suggests that at baseline, differences between the two diagnostic groups in terms of the markers studied were not evident. However, as SCI and MCI may represent heterogeneous etiologies, a longitudinal design would help determine whether there are differential effects of the AD susceptibility genes. We were not able to examine the effects of the AD risk genes on biomarkers and clinical markers longitudinally because data on these were not available at the time of the study.

Sample population and setting

The population in our studies included patients with MCI, AD-type dementia, non-AD type dementia and cognitively healthy controls. This has the advantage of testing the effects of AD susceptibility genes on the selected markers in different diagnostic groups. Given the multi-center composition of the cohorts used in the thesis, we included study site as a random effect in the statistical analyses.

Sample size

The sample sizes limited the interpretation of results for some of the studies in the thesis. Our sample size was sufficiently powered for the correlational study with neuropsychiatric symptoms in MCI in *Chapter 4*, the correlational study with memory in MCI in *Chapter 6*, and the genetic association and biomarker correlation analyses in AD in *Chapter 7*. The sample size for the correlational analyses with CSF biomarker and imaging marker in MCI was relatively small, which reduced statistical power and can explain why findings were not significant after multiple test correction. Nevertheless, results were often consistent with those reported in studies using larger samples. This suggests that exploratory studies such as ours could still provide useful information, although replication in larger samples is needed.

Multiple testing

Multiple testing in genetic studies is inevitable. While this guards to a certain extent against false positive findings, it may have resulted in conservative interpretations of results, given that the genes we tested, with the exception of candidate genes, are established AD risk genes which justify independent hypothesis testing. For this reason, some studies did not adjust for multiple testing.^{149, 150, 155} In the thesis, we chose to correct for multiple testing but also to discuss results that were significant at an unadjusted *P*-value or showed notable trends.

Gene selection

A SNP does not necessarily indicate that the gene in which the SNP is found is actually involved in the pathophysiology of a disease. It could also be nearby genes in linkage disequilibrium that could be involved.

In the present thesis, we selected SNPs from genes that have been associated with AD as established in GWAS and AlzGene meta-analyses, or based on their effects on A β and tau as reported in GWAS or previous candidate gene studies. There is nonetheless a wide range of other genes that have been studied in relation to AD and are of interest for clinical and biomarker studies, but this was outside the scope of the current thesis.

Genetic model

We used a dominant genetic model in the analyses for two studies because of the very low minor genotype frequencies (<20% for 90% of SNPs in *Chapter 6*, and $\leq 15\%$ for ~50% of SNPs in *Chapter 7*) in combination with the relatively small sample size. While it is not unusual to assume a dominant model in this case,¹⁵¹ it may have nonetheless masked the effects of some SNPs. As guide in interpreting the results, we have provided continuous data per genotype on CSF biomarkers and hippocampal volume measure, and memory. This enabled qualitative interpretation of the relation between markers and each genotype.

Comparability of measurement instruments

As we made use of different cohorts, measurement instruments differed. This may have influenced the comparison of results between these cohorts, although this may not be a major issue as discussed below.

Genetic analyses

For DESCRIPA, FINN-AD and EDAR, genotyping of genomic DNA from venous blood was done on Sequenom i-Plex MassARRAY platform and subjected to similar quality measures, i.e., filters for call rate, minor allele frequency and Hardy-Weinberg equilibrium. We did not use imputed SNP values. The SNPs in our analyses were genotyped, which optimizes accuracy of data used.

ADNI made use of Illumina 610 Quad array. Information on *APOE* genotype was procured from their database. In *Chapter 4*, we pooled *APOE* data from ADNI and DESCRIPA. As far as we know, there are no conflicting issues between Sequenom and Illumina platforms.

CSF biomarker analyses

CSF was obtained through lumbar puncture in all cohorts. A β and tau levels in DESCRIPA and FINN-AD were measured using commercially available INNOTEST enzyme-linked immunosorbent assays (ELISAs). For DESCRIPA, measurements were performed in one laboratory. But because CSF was obtained from various sites, we adjusted for the possible effect of study site in our statistical analyses. For FINN-AD, CSF collection and measurements were performed in one laboratory following routine protocol, thus minimizing variations in results.

For EDAR, another platform, Luminex, was used. Measurements were centralized in one laboratory. Measurements obtained via Luminex require statistical transformations to make them comparable to ELISA-based systems.

In the thesis, we pooled CSF data only for study sites in the same cohort and did not combine data from different cohorts.

MRI imaging analyses

MRI data was available only for one cohort (DESCRIPA). MRI scans were made following the routine protocol of the study centers. This may have introduced variability. In our multilevel statistical model, we were not able to correct for the possible effect of study site because of the small number of participants per site. Hippocampal volume was measured using automated learning embeddings atlas propagation (LEAP) with corrections for intracranial volume. This, in turn, minimized variability in measurement.

Cognitive measures

We used delayed memory as a clinical marker. Memory performance was assessed according to the neuropsychological protocol at each center. Consequently, the tests used varied. To pool the data, raw scores were transformed into z-scores adjusted for age, gender and education using normative data that were locally collected or were published.¹⁵² We used z-scores in the analyses.

Assessment of neuropsychiatric symptoms

We used the Neuropsychiatric Inventory (NPI), which is a well-validated instrument, to assess neuropsychiatric symptoms in two independent cohorts. DESCRIPA used the full NPI scale that assessed both presence and severity of symptoms, whereas ADNI used the shorter NPI questionnaire (NPI-Q) that only indicated presence of symptoms. As the NPI-Q has been cross-validated with the NPI and has been shown to have good test-retest reliability,¹⁵³ we were able to assess neuropsychiatric symptoms in the pooled cohort using NPI items that only assessed presence of symptoms.

The NPI is an informant-rated instrument. Interpreting the results obtained in this manner must therefore take into consideration that they may reflect the informant's subjective experience.

IMPLICATIONS

The findings of the current thesis have allowed us to gain better understanding of the influence of genetic risk factors on key markers of AD pathology, and to draw several conclusions.

First, compared to *APOE*, the other variants we studied conferred much smaller or no effects on AD risk. They currently have limited diagnostic and predictive value. Only *APOE* ϵ_4 allele was a significant predictor for progression from MCI to AD-type dementia. *APOE*- ϵ_4 , however, has low sensitivity and positive predictive value, which makes genotyping of limited value for predicting AD-type dementia in clinical practice. *APOE* genotyping may be of value in selecting subjects for trials that aim to prevent progression to dementia, as it helps select subjects with a higher risk for progression to AD-type dementia. In turn, in a population where the prevalence of *APOE*- ϵ_4 is high, such as in *Chapter 7*, *APOE* genotyping is clinically useful in identifying those who have AD-type dementia. Testing *APOE* in combination with other AD risk genes may increase predictive accuracy.

Second, *APOE*- ϵ_4 mainly affects $A\beta$ and is thus useful in assessing AD risk but not progression, which is associated with tau. The relation between *APOE*- ϵ_4 and the clinical markers cognition and neuropsychiatric symptoms in MCI may very well reflect underlying $A\beta$ abnormalities.

Third, we presented evidence that *CLU* and *MS4A* correlated with CSF A β_{1-42} . Both have been established as AD risk genes in GWAS and in the 10 top AlzGene. Replication on a larger scale is needed to assess the clinical utility of our findings.

Fourth, CSF A β_{1-42} appears to be a stronger correlate of AD genetic risk factors, compared to the other biomarker and clinical markers we assessed. However, different SNPs exert their influence at different stages of AD pathogenesis. It is thus of clinical relevance to take into consideration the temporal order of pathogenic events and determine appropriate genetic variants to test, if the functions of these variants are known.

Fifth, we included three multicenter studies that had as participants nondemented individuals with cognitive impairment or MCI in a clinical setting. We were particularly interested in this population because they have an increased risk of progressing to AD, although not all of them will develop AD pathology. The effects of AD susceptibility genes on AD risk may differ for other diagnostic groups in a different setting. Moreover, the loci identified in GWAS were associated with clinical AD. These genes may exert effects in AD that are not evident in MCI.

Finally, the clinical utility of genetic risk factors with smaller effects in our study lies in understanding the different mechanisms by which they contribute to AD risk and pathophysiology. In the present thesis, we used the amyloid cascade hypothesis as a model. However, the majority of the variants we tested may have contributed to AD risk via other pathways, notably, immune network, complement system, clathrin-mediated endocytosis, cholesterol and lipid metabolism, as well as tau metabolism. While this does not relegate the role of A β in AD pathogenesis, it emphasizes the need to explore other factors and mechanisms that lead to AD.

FUTURE RESEARCH DIRECTIONS

We assessed the influence of genetic risk factors on key markers of AD pathology in various diagnostic groups. These markers become abnormal at different stages of AD. Our findings may serve as basis for further

research. Replication on a larger scale is needed in order to test the robustness of our results.

The trends or small effects we found for a number of SNPs strongly indicates the need to pool data and perform studies on a much larger scale. As we mentioned in *Chapter 5*, this requires collaborating at national and international levels to allow sharing, pooling, harmonizing raw data, and when feasible, harmonizing techniques for genetic and phenotypic analyses. Databases that are accessible for scientific research are another excellent means of performing studies with a larger sample size.

Our limited follow-up data did not allow us to assess changes in the AD markers in relation to genetic risk factors. A longitudinal design would be more informative, and may possibly yield causal relations. Studies of genetic risk factors known to affect disease progression, such as SNPs involved in tau metabolism, require a longitudinal design. Ideally, such as design includes neuropathologic confirmation. Furthermore, ascertaining genotype may not be sufficient when studying disease progression. Measuring gene expression or protein levels, such as in the case of clusterin, is one way of determining the extent of pathogenesis.

Exploring other markers, such as those pertaining to inflammation or lipid metabolism, in relation to AD susceptibility genes in predementia would further the understanding of AD pathophysiology. Moreover, as genes interact with other genes and with the environment, it is of relevance to perform research as to how this may contribute to AD risk and what intervention strategies could be formulated.

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SUMMARY

SAMENVATTING

Summary

Alzheimer's disease (AD) is the most common cause of dementia. It is characterized by progressive neuronal death, memory loss, behavioral and affective changes, and impairments in activities of daily living. It culminates in dementia.

There is currently no cure for AD-type dementia. Available treatments are aimed at slowing the progression of the disease and address its associated symptoms. Often, treatment is sought when the disease is already at an advanced phase because only then do clinical symptoms become evident. Early diagnosis of AD is important, as drugs with disease-delaying potentials are most effective at the predementia stage.

Early detection of AD entails understanding the complex relationship between risk factors and AD pathophysiology. Recent technological advances have shown the increasing role of genetics in AD susceptibility. In this dissertation, we investigated the influence of genetic risk factors on the pathophysiology and clinical manifestation of Alzheimer's disease (AD). While several AD risk genes have been identified, their effects on AD pathophysiology remain unclear. We examined this by testing the correlations between genetic risk factors for AD, in particular, single nucleotide polymorphisms (SNPs), and key measures of AD pathology, including cerebrospinal fluid (CSF) markers A β and tau, hippocampal atrophy, memory performance and neuropsychiatric symptoms. As the relation between genetic risk factors and AD may differ depending on the stage of the disease, we used cohorts with different severities of cognitive impairment. The specific objectives, methods used and general information on the cohorts are described in *Chapter 1*.

In *Chapter 2*, using meta-analysis, we assessed the usefulness of *APOE* genotyping in predicting progression to AD-type dementia among patients with mild cognitive impairment (MCI) and in selecting subjects for clinical trials aimed at preventing progression to dementia. We found that the *APOE* ϵ_4 allele carriers have more than twice the

risk of developing AD compared to noncarriers. The low sensitivity and positive predictive value indicate that *APOE* genotyping has limited clinical value but is useful for trials aiming to select subjects who are at higher risk for progressing to AD-type dementia.

Chapter 3 is closely related to Chapter 2. It contains the protocol for the diagnostic test accuracy of *APOE* for dementia in the primary care setting. The protocol is written for the Cochrane Collaboration, is meant to serve as guide for similar reviews, and is a prerequisite for the conduct of full reviews.

In *Chapter 4*, we studied the relation between *APOE* ϵ_4 allele and neuropsychiatric symptoms in people with MCI. We found that the *APOE* ϵ_4 allele was significantly related with agitation and irritability, and with reduced depression.

Chapter 5 is a qualitative literature review in various ways: (1) of the possible role of AD genetic risk variants on $A\beta$ and tau were measured *in vivo* in CSF or by positron emission tomography (PET), or (2) upon neuropathological examination. Of the almost 50 genetic variants in 26 genes we included in the review, only *APOE* and *PICALM* showed consistent effects on $A\beta$ but not on tau.

In *Chapter 6*, we investigated the association between the top 10 AlzGene loci, which are considered as established AD risk genes; and key markers of AD pathology in CSF, magnetic resonance imaging and cognition in nondemented individuals with cognitive impairment. Only *APOE* predicted progression to AD, showed a consistent effect on AD markers, and correlated significantly with CSF $A\beta_{1-42}$.

Chapter 7 presents the findings of a case-control genetic association study of 25 SNPs, and correlational study of 36 SNPs with CSF biomarkers in a Finnish cohort. We provide evidence that *APOE*- ϵ_4 , *CLU* and *MS4A4A*, which have been identified in GWAS to be associated with AD, also significantly reduced CSF $A\beta_{1-42}$ in AD. None of the other AlzGene and GWAS loci showed significant effects on CSF tau. The effects of other SNPs on CSF biomarkers and clinical AD diagnosis did not reach statistical significance. Our findings suggest that *APOE*- ϵ_4 , *CLU* and *MS4A4A* influence both AD risk and CSF $A\beta_{1-42}$.

Chapter 8 aimed to replicate the findings in *Chapter 7*, and further investigated the effects of variants in the *MS4A* gene cluster on CSF A β . The study was performed in a cohort consisting of patients with AD, MCI and cognitively healthy individuals. Our findings suggest that polymorphisms in the *MS4A* gene may influence CSF A β ₁₋₄₂. This supports results from GWAS studies that *MS4A* is a genetic risk factor for AD.

Chapter 9 presents a general summary and discussion of our main findings and their role in the context of current knowledge, relates the findings to genes in AD pathophysiology, their implications and the methodological considerations. We conclude with suggestions for future research directions.

Samenvatting

De ziekte van Alzheimer (AD) is de meest voorkomende oorzaak van dementie en wordt gekenmerkt door het progressief afsterven van neuronen, geheugenverlies, veranderingen in gedrag, stemmingswisselingen en toenemende problemen in het dagelijks functioneren. De ziekte leidt uiteindelijk tot dementie.

Op dit moment is er nog geen behandeling om dementie als gevolg van AD te genezen. Beschikbare behandelmethoden zijn erop gericht om de progressie van de ziekte te vertragen en de symptomen te bestrijden. Vaak start de behandeling pas als de ziekte al in een gevorderd stadium is, omdat de klinische symptomen dan pas herkend worden. Vroege diagnostiek van dementie is belangrijk omdat medicijnen die de potentie hebben om de ziekte te kunnen vertragen het meest effectief zijn als het stadium van dementie nog niet is bereikt.

Om AD in een vroeg stadium vast te kunnen stellen, is het noodzakelijk om inzicht te hebben in de complexe relatie tussen risicofactoren en de pathofysiologie van AD. Recente technologische ontwikkelingen hebben de rol van genen op de vatbaarheid voor AD aan het licht gebracht. In deze proefschrift hebben we de invloed onderzocht van genetische risicofactoren, met name 'single nucleotide polymorphisms' (SNPs), op de pathofysiologie en klinische manifestaties van AD. Hoewel er verschillende risicogenen van AD zijn geïdentificeerd, is hun effect op de pathofysiologie van AD nog steeds onduidelijk. We hebben dit onderzocht door de effecten van AD risicogenen te correleren met sleutelindicatoren van AD pathologie, waaronder hersenvocht (cerebrospinal fluid oftewel CSF) markers bèta amyloïde (A β) en tau, hippocampus atrofie, geheugenprestaties en neuropsychiatrische symptomen. Aangezien de relatie tussen genetische risicofactoren en AD kan verschillen tussen ziektestadia, hebben we cohorten gebruikt die verschillen in de mate van cognitieve achteruitgang. De specifieke doelen van het onderzoek, de gebruikte methoden en algemene informatie over de cohorten, staan beschreven in *Hoofdstuk 1*.

In *Hoofdstuk 2* hebben we een meta-analyse uitgevoerd om te onderzoeken hoe bruikbaar *APOE* genotypering is bij het voorspellen van AD-dementie in patiënten met milde cognitief stoornis (mild cognitive Impairment), oftewel MCI. Ook hebben we onderzocht hoe bruikbaar *APOE* genotypering is voor het selecteren van deelnemers aan klinische trials die gericht zijn op het voorkomen van progressie naar AD-dementie. Wij vonden dat het risico op AD meer dan verdubbeld is in dragers van het *APOE* ϵ_4 allel in vergelijking met mensen zonder dit allel. De lage sensitiviteit en positieve voorspellende waarde vormen een indicatie dat *APOE* genotypering van beperkte waarde is voor klinische doeleinden, maar wel bruikbaar is om mensen te selecteren die een hoger risico lopen om AD te ontwikkelen ten behoeve van trials.

Hoofdstuk 3 is nauw verbonden aan Hoofdstuk 2. Het bevat het protocol hoe wij kunnen testen hoe nauwkeurig *APOE* is voor een diagnose dementie in de eerstelijnszorg. Het protocol is geschreven voor de Cochrane Collaboration en is bedoeld als gids voor vergelijkbare reviews. Het is een eerste vereiste voor het uitvoeren van volledige reviews.

In *Hoofdstuk 4*, hebben we de relatie onderzocht tussen het *APOE* ϵ_4 allel en neuropsychiatrische symptomen in mensen met MCI. We vonden dat het *APOE* ϵ_4 allel significant gerelateerd is aan agitatie en prikkelbaarheid, evenals aan verminderde depressie.

Hoofdstuk 5 beschrijft een kwalitatief literatuuronderzoek naar de mogelijke rol van genetische risicofactoren voor AD op A β en tau. A β en tau werden op verschillende manieren gemeten: (1) *in vivo* in CSF of met behulp van positron emissie tomografie (PET); of (2) door neuropathologisch onderzoek. Van de bijna 50 genetische variaties in 26 genen die wij in deze review hebben geïncludeerd, vertoonden alleen *APOE* en *PICALM* consistente effecten op A β , maar niet op tau.

In *Hoofdstuk 6* hebben wij onderzoek gedaan naar de relatie tussen de top 10 AlzGene loci – die worden beschouwd als gevestigde risicogenen voor AD en *key markers* van AD pathologie in CSF, MRI en cognitie in niet demente individuen met cognitieve stoornissen. Alleen *APOE* bleek een voorspeller te zijn voor de ontwikkeling naar AD. Het

vertoonde een consistent effect op AD-markers en vertoonde een significante correlatie met CSF A β_{1-42} .

In *Hoofdstuk 7* presenteren wij de bevindingen van een case-control genetische associatie studie van 25 SNPs, en een correlatie studie van 36 SNPs met CSF biomarkers in een Fins cohort. Wij tonen aan dat *APOE- ϵ_4* , *CLU* en *MS4A4A*, die in GWAS zijn geïdentificeerd als AD-gerelateerd, ook samenhangen met een significante reductie van CSF A β_{1-42} in AD. Geen van de andere AlzGene en GWAS loci vertoonden significante effecten op CSF tau. De effecten van andere SNPs op CSF biomarkers en klinische AD diagnoses waren statistisch niet significant. Onze bevindingen wijzen erop dat *APOE- ϵ_4* , *CLU* en *MS4A4A* invloed hebben op zowel het risico op AD als op CSF A β_{1-42} .

Het doel van *Hoofdstuk 8* was om de bevindingen van Hoofdstuk 7 te repliceren en om de effecten van varianten in het *MS4A* gen-cluster op CSF A β te onderzoeken. Het onderzoek werd uitgevoerd in een cohort dat bestond uit patiënten met AD, MCI en cognitief gezonde individuen. Onze bevindingen suggereren dat polymorphismen in het *MS4A* gen van invloed kunnen zijn op CSF A β_{1-42} . Dit ondersteunt de resultaten van GWAS studies die aangeven dat dit gen een genetische risicofactor is voor AD.

Hoofdstuk 9 geeft eerst een samenvatting en beschouwing van onze belangrijkste bevindingen in de context van de huidige stand van zaken in de wetenschap. Wij relateren de bevindingen aan genen en hun rol in AD pathofysiologie, en gaan in op de implicaties van onze bevindingen. In dit hoofdstuk gaan wij ook in op methodologische overwegingen. Wij sluiten af met suggesties voor toekomstig onderzoek.

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In June 2008, I started my PhD research in Maastricht. In December 2012, I submitted my dissertation. I travelled more than 200,000 kilometers by train between Delft and Maastricht during these years. In a way, doing a PhD and taking the Dutch trains are the same: In cases of delays, try to find alternatives. Each alternative brought its own challenges. Is it then a wonder that I need 6 pages for my acknowledgments?

I would like to thank the many people who contributed to my fascinating journey to understanding Alzheimer and life.

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I was fortunate to work with a close team in Kuopio. Prof. Soininen, Hilkka, your encouraging words, also when you comment on DESCRIPA papers, work like magic. Dr. Hiltunen, Mikko, your energy and enthusiasm are contagious. Together with the whole team there, we produced a nice paper. It has been a pleasure working on it. Dr. Helisalmi, Seppo, you have always found it important that things go well with the research *and* with the researcher. Having lunch is equally important as genotyping SNPs. I went to your lab as a neuropsychologist. I came out totally fascinated with this barely visible thing called DNA.

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Léon, mijn lieve man, dank je dat je altijd bij mij bent. Kom, houd mijn hand vast. Wij gaan verder!

CURRICULUM VITAE AND PUBLICATIONS

Lyzel Samaniego Elias-Sonnenschein was born on August 25, 1970 in Manila, Philippines. She completed her high school at the Dominican St. James Academy. She earned her bachelor and masteral degrees in psychology, with specializations in clinical and experimental psychology, at the University of the Philippines (UP) in Diliman. As a student, she co-founded an organization for street children, and was engaged in charitable activities and medical missions.

While studying for her master's, Lyzel worked in the academe and at several health institutions. Among others, she was an editor at the Ateneo de Manila University, a lecturer in psychology at UP Manila and a psychologist in a private clinic. In 1998, she started working as a researcher for ECPAT International, which is a global network against the commercial sexual exploitation of children. As part of a training team, her work brought her to Africa, Central America and Europe. In 2000, she decided to work as a psychologist in a clinical setting, and joined the memory center of St. Luke's Medical Center. Trained to perform neuropsychological assessments, she became part of a multidisciplinary team that mainly saw patients who had memory complaints. This was the start of her professional interest in Alzheimer.

In 2002, she married Léon Sonnenschein, whom she met in Delft during her trip to Europe for ECPAT. A year later, she came to live in the Netherlands. After learning Dutch, she proceeded to obtaining a master's degree in clinical neuropsychology at the Vrije Universiteit in Amsterdam. For her thesis, she set up a joint research with the Department of Neurology, Academic Medical Center, University of Amsterdam to study cognition in Dutch patients with LGMD2A, a

progressive muscular disease caused by mutations in the *CAPN3* gene. She earned her degree cum laude in 2007. During her studies, she was a board member of Alzheimer Nederland-Delft, Westland and Oostland region. In her portfolio were the Alzheimer Café's in this region.

In 2008, Lyzel joined the Department of Psychiatry and Neuropsychology of Maastricht University to do PhD research on early markers and predictors of Alzheimer's disease. Her research focused on genetic risk factors. As part of her PhD, she went to Kuopio, Finland in 2011 and worked for three months as a visiting researcher at the Institute of Clinical Medicine-Neurology, University of Eastern Finland. This resulted in a collaborative project that is still ongoing. In 2012, she returned twice to Kuopio for work visits.

As part of her interest in education, Lyzel also started a project to develop together with foreign students internationalized tutor material tasks for selected courses in medicine. She is currently working as a researcher at the Department of Psychiatry and Neuropsychology at Maastricht University.

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Elias-Sonnenschein LS, Helisalmi S, Natunen T, Hall A, Paaanen T, Herukka S-K, Laitinen M, Remes AM, Koivisto AM, Mattila KM, Lehtimäki T, Verhey FRJ, Visser PJ, Soininen H, Hiltunen M. Genetic variants associated with Alzheimer's disease and cerebrospinal fluid biomarkers in a Finnish case-control cohort. *PLoS ONE*, 2013 (In Press).

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