Alzheimer's & Dementia 12 (2016) 872-881

Alzheimer's & Dementia

Featured Article

# Alzheimer's disease risk variants modulate endophenotypes in mild cognitive impairment

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**Abstract**

**Introduction:** We evaluated the effect of Alzheimer's disease (AD) susceptibility loci on endophenotypes closely related with AD pathology in patients with mild cognitive impairment (MCI).
**Methods:** We selected 1730 MCI patients from four independent data sets. Weighted polygenic risk scores (PGS) were constructed of 18 non-apolipoprotein E (*APOE*) AD risk variants. In addition, we determined *APOE* genotype. AD endophenotypes were cognitive decline over time and cerebrospinal fluid (CSF) biomarkers (aβ, tau, ptau).
**Results:** PGS was modestly associated with cognitive decline over time, as measured by mini-mental state examination (MMSE) ($\beta \pm SE: -0.24 \pm 0.10$; $P = .012$), and with CSF levels of tau and ptau (tau: $1.38 \pm 0.36$, $P = 1.21 \times 10^{-4}$; ptau: $1.40 \pm 0.36$, $P = 1.02 \times 10^{-4}$).
**Discussion:** In MCI, we observed a joint effect of AD susceptibility loci on nonamyloid endophenotypes, suggesting a link of these genetic loci with neuronal degeneration in general rather than with Alzheimer-related amyloid deposition.


*Keywords:* Alzheimer's disease; Mild cognitive impairment; Polygenic risk score; Endophenotypes; Genetic risk variants

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http://dx.doi.org/10.1016/j.jalz.2016.01.006

## 1. Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disease caused by genetic and environmental factors. The estimated genetic component (heritability) of sporadic late onset AD is 60%–80% [1]. Over the past 5 years, an increasing number of common genetic AD risk variants (minor allele frequency >5%) has been identified by genome wide association studies (GWAS) [2–6]. Each of the identified variants individually confers a small effect (odds ratio <1.5) on susceptibility to AD, thereby limiting their predictive value in clinical setting. Computation of a polygenic risk score (PGS) appears a suited strategy to improve predictive value of these genetic effects, because it provides a cumulative effect score based on the individual susceptibility variants. The robustness of this strategy has been shown in psychiatric disorders, including schizophrenia and bipolar disorders [7], and previous studies have investigated the usability of PGS in the prediction of conversion from mild cognitive impairment (MCI) to AD [8,9]. A complementary approach assesses the relationship between genetic risk variants and intermediate phenotypes (endophenotypes), such as cerebrospinal fluid (CSF) biomarkers or cognitive decline, which are more proximal to specific events in the pathologic pathways involved in AD pathogenesis [10]. This strategy enhances further identification of the underlying molecular mechanisms associated with the AD susceptibility loci. Research has shown that a PGS without apolipoprotein E (*APOE*) (non-*APOE* PGS) was significantly associated with lower levels of CSF amyloid-beta-42 (Aβ) in AD patients (n = 222) but not with CSF levels of total tau (tau) or tau phosphorylated at threonine-181 (ptau) [11]. A finding contradicted by a study with 338 AD patients, which showed an association between non-*APOE* PGS and increased CSF levels of tau and ptau but not with CSF levels of Aβ [12]. In addition, a large population-based study with non-demented subjects from the Rotterdam Study ($n$ = 5171) has identified a marginal joint effect of non-*APOE* PGS on memory [13].

To date, little research has been performed on the effect of PGS on endophenotypes of AD in patients with MCI. Linking genetic risk factors of AD to pathologic pathways acting at the MCI stage will be crucial for the development of effective treatments and improved definitions of at-risk groups.

The work described here used an alternative approach by exploring the effect of joined AD susceptibility variants on different endophenotypes of AD in patients with MCI. A PGS out of 18 known AD risk GWAS loci (i.e. *CR1, BIN1, INPP5D, MEF2C, CD2AP, NME8, ZCWPW1, EPHA1, PTK2B, CLU, MS4A6A, PICALM, SORL1, FERMT2, SLC24A4/RIN3, ABCA7, CD33, CASS4*) was created, and we investigated associations with two types of endophenotypic markers of AD; CSF biomarkers (Aβ, tau, and ptau) and cognitive decline over time (as measured by the mini-mental state examination [MMSE] [14], and the word list learning test with immediate and delayed recall).

## 2. Methods

### 2.1. Participants

For the present study, 1730 MCI patients were selected from four cohorts: 242 patients from the Amsterdam Dementia Cohort (ADC), 421 from the Dementia Competence Network (DCN), 342 from the study on Aging, Cognition, and Dementia (AgeCoDe), and 725 from Fundació ACE (ACE) (Table 1). The patients were included based on the following inclusion criteria: (1) baseline diagnosis of MCI; (2) availability of longitudinal cognitive assessment including MMSE and word list learning test with immediate and delayed verbal recall; (3) availability of genotyped single-nucleotide polymorphisms (SNPs) for PGS; (4) availability of information concerning conversion (yes/no); and (5) at least 1-year follow-up.

The ADC cohort included 242 MCI patients who visited the memory clinic of the Alzheimer center of the VU University Medical Center (VUmc) between 2000 and 2013 [19]. In short, all patients underwent an extensive standardized dementia assessment, including medical history, informant-based history, physical and neurologic examination, laboratory tests, neuropsychological assessment including the MMSE, and the Dutch version of the Rey auditory verbal learning task (including immediate and delayed recall) [15], CSF investigation and magnetic resonance investigation (MRI) of the brain. Diagnosis was made in a consensus meeting without prior knowledge of the CSF results. For the diagnosis of MCI, Petersen's criteria were used until the beginning of 2012 [20], when the National Institute on Aging–Alzheimer's Association (NIA-AA) criteria for MCI [21] were implemented. In general, follow-up is organized in such a way that patients are monitored on an annual basis in a standardized fashion. Progression to probable AD was diagnosed based on the NINCDS-ADRDA criteria [22,23].

The DCN cohort included 421 MCI patients who were recruited at 14 university hospital memory clinics across Germany between 2003 and 2005 [24]. Baseline assessment comprised extensive neuropsychological tests, including those of the consortium to establish a registry for Alzheimer's disease (CERAD) [16], MMSE and immediate and delayed verbal recall and structural MRI scans of the brain. CSF was collected from all consenting participants. MCI was diagnosed according to the consensus criteria by the international working group (IWG) on MCI [25]. Minor changes in complex activities of daily living were tolerated. Clinical diagnoses of MCI subtypes were made by team conferences at the local study centers. Follow-up assessments were performed at 12 and 24 months. Conversion to probable AD was diagnosed based on the NINCDS-ADRDA criteria.

The AgeCoDe cohort included 342 MCI patients who were recruited from general practice registries across six study centers in Germany between 2002 and 2003 [26,27]. All AgeCoDe participants were assessed using the

Table 1
Characteristics of 1729 MCI patients from four different data sets

| Characteristic | ADC | DCN | AgeCoDe | ACE |
|---|---|---|---|---|
| N | 242 | 421 | 342 | 725 |
| Female, n (%) | 90 (37) | 170 (40) | 239 (70) | 495 (68) |
| Conversion, n (%) | 96 (40) | 75 (18) | 145 (42) | 407 (56) |
| Age (y)* | 66.5 ± 7.6 | 65.5 ± 8.6 | 81.2 ± 4.1 | 76.56 ± 6.9 |
| MMSE† | 26.6 ± 2.3 | 27.4 ± 2.1 | 26.0 ± 2.1 | 25.6 ± 2.9 |
| Word list learning immediate recall† | 30.8 ± 7.8 | 17.3 ± 4.4 | 16.2 ± 4.6 | 18.7 ± 5.9 |
| Word list learning delayed recall† | 3.8 ± 2.6 | 5.3 ± 2.3 | 4.1 ± 2.4 | 2.1 ± 2.4 |
| Time follow-up (y) | 2.7 ± 1.5 | 2.5 ± 3.9 | 5.2 ± 2.3 | 4.2 ± 2.4 |
| ≥1 *APOE* ε4, n (%) | 130 (54) | 151 (36) | 91 (27) | 259 (36) |
| CSF (n)‡ | 218 | 173 | — | — |
| CSF Aβ (ng/L) | 664.3 ± 294.7§ | 770.1 ± 331.3¶ | — | — |
| CSF ptau (ng/L) | 72.9 ± 37.0# | 62.0 ± 32.6** | — | — |
| CSF tau (ng/L) | 492.1 ± 342.2†† | 401.2 ± 253.5‡‡ | — | — |

Abbreviations: ADC, Amsterdam Dementia Cohort; DCN, Dementia Compentence Network; AgeCoDe, study on Aging, Cognition, and Dementia; ACE, Fundació ACE; n, number; SD, standard deviation; MMSE, mini-mental state examination [14]; CSF, cerebrospinal fluid; Aβ, amyloid-beta; ptau, tau phosphorylated at threonine 181; tau, total tau; ng/L, nanogram/liter.

NOTE. Displayed are mean ± standard deviation. ADC: Dutch version of the Rey auditory verbal learning task: immediate recall = range 0–75; delayed recall = range 0–15 [15]. DCN: CERAD: immediate recall = range 0–30; delayed recall = range 0–10 [16]. AgeCoDe: CERAD: immediate recall = range 0-30; delayed recall = range 0–10 [16]. ACE: Wechsler memory scale, third edition: immediate recall = range 0–48; delayed recall = range 0–12 [17,18].

*groups differed significantly by Kruskal Wallis non-parametric test, $P < .01$.

†displayed are the raw test scores, but statistical analyses were performed with z-scores.

‡displayed are the raw values, but statistical analyses were performed with z-scores of the log transformed CSF biomarker values.

§n = 218.

¶n = 173.

#n = 217.

**n = 169.

††n = 217.

‡‡n = 170.

structured interview for diagnosis of dementia of Alzheimer type, multi-infarct dementia, and dementia of other etiology according to DSM-IV and ICD-10 (SIDAM) [28,29]. The SIDAM contains a 55-item neuropsychological test battery, including all 30 items of the MMSE. Diagnoses were assigned at a consensus conference with the interviewer and an experienced geriatrician or geriatric psychiatrist. All subjects were diagnosed with MCI according to the IWG MCI criteria. Each participant is assessed at baseline and at 18-month follow-up visits thereafter. The diagnosis of AD was established according to the NINCDS-ADRDA criteria for probable AD.

The ACE cohort included 725 MCI patients who were recruited and assessed at the Diagnostic Unit of Fundació ACE (Barcelona, Spain) between January 2006 and July 2013. All participants received standardized neurobehavioral examinations, including neurologic examination, MMSE, and neuropsychological assessment by the neuropsychological battery of Fundació ACE (NBACE) [17,18] including word list learning test from the Wechsler Memory Scale–Third Edition (WMS-III) without the interference list and neuroimaging by MRI or computed tomography(CT) scans. Medical records were reviewed to classify them as MCI according to Petersen's criteria [20,30] and to the CHS cognition study criteria [31,32]. All diagnoses were assigned at a consensus conference. Annual follow-up was performed, with the diagnoses of AD established according to the NINCDS-ADRDA criteria for probable AD.

For all four data sets, the study protocols were approved by the local ethic committees of the participating medical centers, and written informed consent was obtained from all study participants or their legal guardians before inclusion.

### 2.2. Neuropsychological assessment

In the four cohorts, cognitive functions were assessed by different neuropsychological test batteries. We used MMSE as a measure for global cognitive decline, available in all data sets. For memory, we used the verbal word list learning test with immediate and delayed recall. As different versions were used to test verbal word list learning, z-scores relative to baseline z-scores were computed to enable comparison.

### 2.3. Cerebrospinal fluid

For ADC ($n = 218$), CSF analyses were performed at the Neurochemistry Laboratory Department of Clinical Chemistry, VUmc [33]. Within 2 hours, CSF samples were centrifuged at 2100 g for 10 minutes at 4°C. The performance of the assays is monitored with two internal quality control pools of surplus CSF (high and low biomarker values). For DCN (n = 173), CSF was centrifuged for 10 minutes

(2000 g at 4°C). All CSF samples were sent to the Department of Psychiatry in Erlangen for quantification [34]. For both data sets, levels of CSF Aβ, tau, and ptau were determined using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) (Innogenetics, Gent, Belgium). Mean values of the CSF biomarkers are listed in Table 1. CSF was not available for Fundació ACE and AgeCoDe cohort, and these two data sets were therefore not included in the CSF biomarker analysis.

### 2.4. DNA extraction and SNP genotyping

DNA was isolated from peripheral whole blood using standard methods. Single-nucleotide polymorphisms (SNPs) selection was based on a review of the literature. Here, only those SNPs in loci identified by GWAS or meta-GWAS efforts were selected. To avoid missing loci, for all the loci selected for PGS construction, whenever possible, alternative SNPs in linkage disequilibrium (LD) were also selected (i.e. LD proxies). This additional SNP thus served as a backup in the event that the primary selected SNP failed in the Sequenom assay. Further details on the references used to select SNPs, the genotyping procedures, and genotyping quality control are provided in the Supplementary Material. The Sequenom technology genotyping methods are described elsewhere [35].

SNPs rs7412 and rs429358, which determine the different *APOE* isoforms were not encoded as individual SNPs, using instead the *APOE* ε2/ε3/ε4 diplotype conventional nomenclature. All individuals included in this study had *APOE* genotype.

### 2.5. Polygenic risk scores

After quality control (see Supplementary Material), allele information and odds ratios of the 18 SNPs were extracted from the IGAP article (see Supplementary Table 1.2) [5]. Deliberately, we have calculated the PGS without *APOE*, because associations between *APOE* ε4 genotype and both cognitive decline and CSF biomarkers (especially Aβ) are well known, risking that any given effect of the PGS would in fact reflect an already known effect of *APOE* ε4 only. A polygenic score ($z_{pgs}$) for an individual was calculated by the sum of manifested risk alleles of all considered SNPs, each one weighted with the logarithm of its odds ratio.

$$z_{pgs} = \frac{\sum_{i\varepsilon\{snps\}} z_i \ln OR_i}{\sum_{i\varepsilon\{snps\}} \ln OR_i},$$

Here, the risk allele was characterized as the one that increases the odds of AD susceptibility in case-control analysis. To keep the results interpretable, the score was divided by the sum of the odds ratios' logarithms, such that the resulting score was a number between zero and two. Missing genotypes were imputed with their expected values, which is given by two times the risk allele frequency [7]. The latter was taken from dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) for the CEU population.

### 2.6. Statistics

Preparation and cleaning of the data sets were performed in IBM, Statistics 20.0, for Mac (SPSS Inc., USA). Statistical association analysis was performed by using the statistical program R (www.r-project.org). Data sets were compared by the Kruskal–Wallis non-parametric test and CSF mean values by Student *t* test.

Associations between PGS and baseline cognition and cognitive performance over time were assessed by linear mixed models (LMM). LMM has increased statistical power as it accounts for within-person correlation over time, allows inter-individual differences in number of assessments and differences in time between assessments [36]. The model included terms for PGS, time, and the interaction between PGS and time. Random effects were subject ID and time. The beta-estimates ± standard error for PGS represent the association between the PGS and baseline cognitive performance, whereas the interaction between PGS and time represents the association between PGS and cognitive performance over time. All analyses were performed with *z* scores and adjusted for age at baseline, gender, and education (low, middle, high). Finally, for all four studies, the beta estimates and standard errors were combined in a meta-analysis using the fixed effects model if the test of heterogeneity proved to be non-significant (i.e. $P \geq .05$), and the random effects model if the test proved to be significant [37,38].

To assess the relationship between PGS and CSF biomarkers, we performed linear regression analyses. Because CSF markers were not normally distributed, they were first normalized by log transformation. The analyses were performed with z-scores and were adjusted for the covariates age at baseline and gender. Then, we performed meta-analyses.

Subsequently, we repeated all analyses after stratification for dichotomized *APOE* ε4 genotype and for progression to AD dementia. Our a priori hypothesis for this stratification was that the associations between PGS and the different AD endophenotypes would behave differently in these subgroups. MCI patients who converted to other types of dementia were not included in the group of progressors to AD dementia but were included in the group of the nonprogressors.

For both LMM and linear regression analyses, the effect of PGS on the five endophenotypic markers of AD was considered to be significant after Bonferroni correction for the number of association tests performed ($0.05/5 = P < .01$). For the stratification analyses, the effects were considered to be significant if $P < .0025$ ($= 0.05/(4 \times 5)$).

Additionally, we performed exploratory per-SNP univariate regression analysis between the CSF biomarkers and the 18 dichotomized SNPs. Rationale behind this analysis was to find out whether individual SNPs with extremely strong effect sizes could be held responsible for the detected

association with the combined PGS. The effects of individual variants were considered significant after Bonferroni correction for the number of association tests performed ($0.05/(3 \times 18) = P < 9.26 \times 10^{-4}$).

## 3. Results

In the meta-analyses, PGS showed a modest association with cognitive decline over time, as assessed by change of MMSE over time (beta-estimate ± standard error: −0.24 ± 0.10, *P* value = .012), but not with memory assessed by the verbal word list learning test with immediate or delayed recall (Table 2). For the CSF biomarkers, the PGS analysis did not reveal an association with CSF levels of Aβ (−0.65 ± 0.39, *P* = .090), but we observed an association between PGS and higher CSF levels of both tau and ptau (tau: 1.38 ± 0.36, $P = 1.21 \times 10^{-4}$, and ptau: 1.40 ± 0.36; $P = 1.02 \times 10^{-4}$; Table 3). As expected, we confirmed the reported association between *APOE* ε4 carrier status and all three CSF biomarkers (all $P < .001$).

We repeated the association analyses between PGS and the five endophenotypes with stratification for *APOE* ε4 status and progression to AD dementia. The stratification was based on theoretical ground, as we detected no significant interaction between PGS and *APOE* ε4 status or between PGS and progression to AD dementia. The stratification of the association analysis between PGS and cognitive measurements did not show any significant results (Supplementary Tables 2.1 and 2.2). Stratification of the effect of PGS on CSF levels revealed that the effect was stronger in *APOE* ε4 noncarriers (tau: 1.47 ± 0.48; *P* = .002, and ptau: 1.65 ± 0.52; *P* = .001) and in subjects who did not progress to AD dementia (tau: 1.42 ± 0.38, $P = 1.59 \times 10^{-4}$ and ptau: 1.49 ± 0.39, $P = 1.55 \times 10^{-4}$; Supplementary Table 2.3).

Finally, the exploratory per-SNP univariate regression analysis between PGS and the CSF biomarkers revealed an association between SNP rs11218343 (Sortilin-related receptor gene (*SORL1*)) and higher CSF levels of tau and ptau in the meta-analysis including the ADC and DCN data sets. This association did not survive the Bonferroni correction (see Table 4 for the meta-analysis and Supplementary Tables 2.4 and 2.5 for the separate analyses).

## 4. Discussion

Identification of the role of AD susceptibility loci in molecular pathophysiological pathways will enhance our knowledge concerning AD pathogenesis. In our study, we investigated the effect of PGS on five endophenotypic markers of AD.

The main finding of our study is that PGS obtained from AD susceptibility loci modulate endophenotypes closely related with AD pathology in patients with MCI, i.e., cross-sectional CSF levels of tau and ptau, and to a lesser degree cognitive decline over time. Both effects appeared to be stronger in the *APOE* ε4 noncarriers and in the patients nonprogressing to AD dementia. Because the interactions between PGS and *APOE* ε4 status and between PGS and progression to AD dementia were both not significant, we cannot draw any strong conclusions from this stratification analyses.

First, we found a modest association with MMSE, which is known to test global cognition, in contrast to the word list learning test, which is thought to be more specific of AD. A previous study revealed that a PGS created out of 9 AD risk loci and *APOE* was associated with lower baseline memory and increased rate of memory decline as measured by word list learning test with delayed recall. However, the association did not sustained significance after excluding

Table 2
Estimated effect of PGS on baseline cognition and cognitive change over time

| | MMSE | | | Word list learning immediate recall | | | Word list learning delayed recall | | |
|---|---|---|---|---|---|---|---|---|---|
| | β ± S.E | *P* | $I^2$ | β ± S.E | *P* | $I^2$ | β ± S.E | *P* | $I^2$ |
| Estimated baseline performance | | | | | | | | | |
| ADC | 0.10 ± 0.44 | .827 | | 0.06 ± 0.48 | .894 | | −0.52 ± 0.48 | .280 | |
| DCN | 0.58 ± 0.38 | .125 | | 0.44 ± 0.33 | .180 | | 0.44 ± 0.34 | .197 | |
| AgeCoDe | −0.07 ± 0.34 | .845 | | −0.19 ± 0.35 | .596 | | −0.51 ± 0.35 | .147 | |
| ACE | −0.44 ± 0.27 | .108 | | −0.39 ± 0.25 | .126 | | −0.49 ± 0.27 | .065 | |
| Meta-analysis | −0.06 ± 0.17 | .747 | 39% | −0.09 ± 0.16 | .600 | 28% | −0.27 ± 0.17 | .108 | 48% |
| Estimated change over time | | | | | | | | | |
| ADC | −0.73 ± 0.25 | .005 | | −0.34 ± 0.20 | .088 | | −0.32 ± 0.20 | .108 | |
| DCN | −0.02 ± 0.29 | .938 | | 0.07 ± 0.16 | .654 | | 0.22 ± 0.16 | .165 | |
| AgeCoDe | −0.25 ± 0.23 | .291 | | −0.03 ± 0.10 | .757 | | −0.05 ± 0.08 | .556 | |
| ACE | −0.16 ± 0.12 | .196 | | −0.08 ± 0.07 | .252 | | −0.05 ± 0.07 | .434 | |
| Meta-analysis | −0.24 ± 0.10 | *.012* | 35% | −0.07 ± 0.05 | .190 | 0% | −0.04 ± 0.05 | .383 | 37% |

Abbreviations: PGS, polygenic risk score; ADC, Amsterdam Dementia Cohort; DCN, Dementia Competence Network; AgeCoDe, Study on Aging, Cognition, and Dementia; ACE, Fundació ACE; MMSE, mini-mental state examination.

NOTE. Data are presented as β ± standard error (S.E.), with calculations performed with z scores. *P* values are given for the models corrected for age, gender, and education (low, middle, high). Multiple testing correction by Bonferroni (0.05/5 tests); $P < .01$ was considered significant. Meta-analysis: the fixed effect model was applied.

Table 3
Estimated effect of PGS on CSF biomarkers

| | CSF Aβ | | | | | | CSF tau | | | | | | CSF ptau | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *APOE* ε4 | | | PGS | | | *APOE* ε4 | | | PGS | | | *APOE* ε4 | | | PGS | | |
| | β ± S.E | *P* | $I^2$ | β ± S.E | *P* | $I^2$ | β ± S.E | *P* | $I^2$ | β ± S.E | *P* | $I^2$ | β ± S.E | *P* | $I^2$ | β ± S.E | *P* | $I^2$ |
| ADC | −0.97 ± 0.12 | 2.93E−13 | | −0.82 ± 0.49 | .094 | | 0.49 ± 0.13 | 2.18E−04 | | 1.74 ± 0.45 | 1.43E−04 | | 0.44 ± 0.13 | 7.55E−04 | | 1.69 ± 0.45 | 2.59E−04 | |
| DCN | −0.55 ± 0.15 | 2.93E−04 | | −0.38 ± 0.62 | .546 | | 0.41 ± 0.15 | 6.26E−03 | | 0.74 ± 0.60 | .217 | | 0.38 ± 0.15 | .012 | | 0.91 ± 0.59 | .125 | |
| Meta-analysis | −0.80 ± 0.10* | **3.00E−04*** | 79% | −0.65 ± 0.39 | .090 | 0% | 0.46 ± 0.10 | **3.12E−06** | 0% | 1.38 ± 0.36 | **1.21E−04** | 44% | 0.42 ± 0.10 | **2.20E−05** | 0% | 1.40 ± 0.36 | **1.02E−04** | 10% |

Abbreviations: PGS, polygenic risk score; CSF, cerebrospinal fluid; ADC, Amsterdam Dementia Cohort; DCN, Dementia Competence Network. Aβ, amyloid-beta; ptau, phosphorylated tau at threonine 181; tau, total tau.

NOTE. Data are presented as β ± standard error (S.E.), with calculations performed with z-scores of log transformed CSF values. *P*-values are given for the models corrected for age, gender. Multiple testing correction by Bonferroni (0.05/5 tests); $P < .01$ was considered significant (bold). Meta-analysis: In general, the fixed effect model was applied.

*The random effect model was applied because test of heterogeneity $P \geq .05$.

the *APOE* effect [39]. Albeit small effect sizes, our results may suggest that AD susceptibility loci may affect more global cognition, also encompassing nonmemory domains, rather than memory alone. Supporting this observation, a previous study with longitudinal data from the Rotterdam Study ($n = 360$) detected a stronger effect of non-*APOE* PGS on risk of the nonamnestic subtype of MCI compared to the amnestic subtype, suggesting a joint effect of the AD risk variants on aspecific neurodegenerative processes rather than on AD [13]. Alternatively, testing several cognitive domains at the same time with the MMSE may have more power to detect association with our PGS than focusing on one single-memory domain.

Second, looking at endophenotypes more closely related to AD pathology, we observed in the meta-analysis a nonsignificant trend toward association between the joined AD susceptibility loci and lower CSF levels of Aβ. Our study does not replicate a recent study, describing a marginal effect of a non-*APOE* PGS on CSF levels of Aβ levels in a sample of 222 patients with dementia due to AD ($P = .04$) [11]; however, we detected the same direction of effect in our sample. One potential explanation may be that we included a more heterogeneous group such as MCI patients, whereas the former study explored the effect of PGS in patients with dementia due to AD. It is reasonable to assume that the genetic variants behave differently in MCI compared to AD dementia as it has been suggested for the *CLU* gene contained in our PGS [40]. However, as CSF Aβ levels reach a plateau level early on in the disease, we would have hypothesized that a relationship between PGS and CSF Aβ levels would be more likely associated in a sample of MCI patients than in a sample of patients with AD dementia.

The main association of PGS was found with higher CSF levels of tau and ptau, which might be interpreted as markers of more downstream AD pathophysiology and/or as markers of neurodegeneration in general. Given their function, it seems plausible that the AD risk variants recently discovered by the IGAP consortium are probably involved in several nonamyloidogenic pathologic pathways, such as immune response and inflammation, lipid transport, endocytosis, cholesterol, and tau processing pathways [5]. Our finding of a relationship between joined AD susceptibility loci and higher CSF levels of tau and ptau, reinforces the involvement of AD susceptibility variants in nonamyloidogenic pathways. Hypothetically, the combined AD risk loci modulate the tau pathologic pathway in absence of overt disease, possibly by acting in parallel with the amyloidogenic pathway and causing tau aggregation or neurofibrillary tangle formation. These pathologic changes result in neuronal damage as reflected by increased (p)tau levels in CSF and by the first signs of MCI [41]. Previous studies have described the presence of biomarkers of neurodegeneration in general, independent from amyloid-beta pathology, in normal elderly [42–44] and the presence of tau biomarkers in specific in *Drosophila* models [41,45]. In addition, there is a lively debate on the clinical value and

Table 4
Per-SNP regression analysis with CSF biomarkers in the meta-analysis of ADC and DCN

| | | | | | CSF Aβ | | CSF tau | | CSF ptau | |
|---|---|---|---|---|---|---|---|---|---|---|
| SNP | chr | Minor | Major | Risk | β ± S.E | *P* | β ± S.E | *P* | β ± S.E | *P* |
| rs6656401 | 1 | A | G | A | 0.03 ± 0.10 | .789 | 0.05 ± 0.10 | .614 | 0.08 ± 0.10 | .422 |
| rs744373 | 2 | C | T | C | 0.11 ± 0.09 | .248 | −0.03 ± 0.10 | .736 | 0.07 ± 0.10 | .450 |
| rs35349669 | 2 | T | C | T | 0.06 ± 0.11 | .629 | 0.02 ± 0.12 | .839 | −0.06 ± 0.12 | .613 |
| rs190982 | 5 | G | A | A | −0.13 ± 0.10 | .193 | 0.04 ± 0.10 | .691 | 0.02 ± 0.10 | .815 |
| rs10948363 | 6 | G | A | G | −0.11 ± 0.10 | .235 | 0.17 ± 0.10 | .078 | 0.12 ± 0.10 | .216 |
| rs2718058 | 7 | G | A | A | 0.04 ± 0.10 | .696 | 0.01 ± 0.10 | .943 | 0.05 ± 0.10 | .651 |
| rs1476679 | 7 | C | T | T | −0.01 ± 0.10 | .934 | 0.08 ± 0.10 | .409 | 0.07 ± 0.10 | .469 |
| rs10808026 | 7 | A | C | C | −0.05 ± 0.10 | .581 | 0.01 ± 0.10 | .901 | 0.03 ± 0.10 | .795 |
| rs28834970 | 8 | C | T | C | 0.11 ± 0.10 | .255 | −0.01 ± 0.10 | .950 | −0.01 ± 0.10 | .960 |
| rs11136000 | 8 | T | C | C | −0.04 ± 0.09 | .655 | 0.09 ± 0.10 | .371 | 0.17 ± 0.10 | .076 |
| rs4938933 | 11 | C | T | T | −0.02 ± 0.10 | .820 | 0.24 ± 0.10 | .015 | 0.27 ± 0.10 | .007 |
| rs3851179 | 11 | A | G | G | −0.09 ± 0.09 | .339 | 0.08 ± 0.10 | .419 | 0.03 ± 0.10 | .743 |
| rs11218343 | 11 | C | T | T | 0.06 ± 0.17 | .727 | 0.56 ± 0.18 | *.001* | 0.62 ± 0.18 | *.001* |
| rs17125944 | 14 | C | T | C | −0.22 ± 0.12 | .071 | 0.08 ± 0.13 | .541 | 0.05 ± 0.13 | .708 |
| rs10498633 | 14 | T | G | G | −0.04 ± 0.10 | .720 | 0.17 ± 0.10 | .101 | 0.22 ± 0.10 | .035 |
| rs3752246 | 19 | G | C | G | −0.03 ± 0.10 | .801 | −0.13 ± 0.10 | .212 | −0.15 ± 0.10 | .155 |
| rs3865444 | 19 | T | G | G | 0.02 ± 0.09 | .832 | 0.02 ± 0.10 | .873 | −0.03 ± 0.10 | .728 |
| rs7274581 | 20 | C | T | T | 0.02 ± 0.13 | .885 | 0.11 ± 0.13 | .405 | 0.00 ± 0.13 | .982 |

Abbreviations: CSF, cerebrospinal fluid; Aβ, amyloid-beta; tau, total tau; ptau, tau phosphorylated at threonine 181; chr, chromosome.

NOTE. Data are presented as β ± standard error (S.E.), with calculations performed with z-scores of log transformed CSF values. *P*-values are given for the models corrected for age, gender. Multiple testing correction by Bonferroni (0.05/(3 × 18 tests)); $P < 9.26 \times 10^{-4}$ was considered significant. Meta-analysis: the fixed effect model was applied. Beta-estimates ± standard error for the risk alleles are displayed. Effects are always given with respect to risk allele, where the risk allele is defined as odds ratio >1 in the IGAP 2013 analysis [5]. Results with borderline significance are depicted in italics.

meaning of SNAP (suspected non-Alzheimer pathology) in patients with MCI, and the joint AD susceptibility loci might contribute to the brain changes observed in SNAP [46–48].

When we evaluated the SNPs in the univariate models, the intronic SNP rs11218343 in *SORL1* was modestly associated with higher CSF levels of tau and ptau, although the result did not sustain the correction for multiple testing. In MCI, *SORL1* variants have earlier been found to be associated with (p)tau levels in CSF [49]. However, for our exploratory per-SNP analysis, our sample seems to be underpowered to detect associations between the individual genetic risk variants and the AD endophenotypes. On the other hand, this finding underscores our assumption that the susceptibility loci individually have little effect on AD biomarkers, but combined in one PGS, they impose stronger effects on endophenotypes of AD sustaining over different data sets.

Strengths of our study are the large sample size and different endophenotypes of AD used, including longitudinal cognitive data. By using LMM, we took into account all available cognitive data points at follow-up, thereby making use of each patient's individual cognitive trajectory and maximizing our statistical power. By using different data sets, we were able to increase the number of MCI subjects included. However, this also encompasses a drawback of heterogeneity due to different study set up and settings across the data sets. For example AgeCoDe is a large population based sample, whereas ADC, DCN, and ACE represent memory clinic-based samples. Furthermore, owing to the different initial inclusion criteria of the studies, we detected significant differences in mean age and conversion rate. On the other hand, including different data sets also enhances external validity and hence clinical relevance. Another limitation is our relatively small follow-up window, by which we may have missed the slow progressors to AD dementia. To investigate the effect of the genetic risk variants in different stage of disease, i.e., the preclinical stage, in MCI and in AD dementia, more longitudinal research with large data sets is warranted.

In conclusion, we showed that already in the predementia phase of AD, there is a consistent effect of joint AD genetic risk variants on nonamyloid endophenotypes. The results suggest a link between the AD susceptibility loci and the tau pathologic pathway in patients with MCI.

## Acknowledgments

We thank all participants for their contribution to this project. Research of the VUmc Alzheimer Center is part of the neurodegeneration research program of the Neuroscience Campus Amsterdam, the Netherlands. The VUmc Alzheimer Center is supported by Alzheimer Nederland and Stichting VUmc fonds. The clinical database structure was developed with funding from Stichting Dioraphte. E. Louwersheimer receives research funding from Stichting Dioraphte and a travel fellowship from Alzheimer Nederland (WE 15-2014-04). The Fundació ACE research programs are supported by Trinitat Port-Carbó and her family. Fundació ACE collaborates with the Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED, Spain) and is one of the participating centers of the Dementia Genetics Spanish Consortium (DEGESCO). CIBERNED is an Instituto de Salud Carlos

III ISCIII Project. A. Ruiz is supported by grant PI13/02434 (Acción Estratégica en Salud. Instituto de Salud Carlos III (ISCIII). Ministerio de Economía y Competitividad, Spain), and Obra Social "La Caixa" (Barcelona, Spain). T. Becker and M. M. Nöthen are members of the DFG-funded Excellence Cluster ImmunoSensation. The work described in the present publication was performed within the context of the German Research Network on Dementia (KND) and the German Research Network on Degenerative Dementia (KNDD), which are funded by the German Federal Ministry of Education and Research (grants KND: 01GI0102, 01GI0420, 01GI0422, 01GI0423, 01GI0429, 01GI0431, 01GI0433, 01GI0434; grants KNDD: 01GI1007A, 01GI0710, 01GI0711, 01GI0712, 01GI0713, 01GI0714, 01GI0715, 01GI0716, 01ET1006B). Disclosures: None of the sponsors had any role in the design of the study; in the collection, analysis, and interpretation of the data; in the writing of the article; or in the decision to submit the article for publication.

E.L., S.W., A.E., A.L., S.H.-H., M.A., I.H., M.R.-R., J.K., L.F., M.H., E.R., J.W., M.S., S.R.-H., F.J., M.M.N., W.M., T.K., M.W., H.H., T.B., and A.R. report no conflicts of interest. L.T. reports personal fees from Roche. M.B. reports personal fees from Nutricia, Roche, Lilly, Servier, Elan, Janssen/Pfizer, GRIFOLS, and from ARACLON. J. Kornhuber has a patent PCT/EP2004/003963: Diagnosis of Alzheimer's disease issued, a patent EP 1811304 A1: Large Aß-peptide binding particles (LAPS) in diagnosis and therapy of Alzheimer's dementia issued, a patent WO2007/082750 A1: Immunoglobulin-bound Ab-peptides and immunoglobulins-binding Ab-peptides in diagnosis and therapy of Alzheimer's dementia issued, a patent EP 2437067A2: Methods of differentially diagnosing dementias issued, and a patent New formulations for diagnosis of Alzheimer's disease pending. O. Peters reports grants and personal fees from Lilly, Roche, Genentech, Affiris, grants from Lundbeck, and Trx-Pharmaceuticals, personal fees from Piramal and Novartis, outside the submitted work. M.M. Nöthen has a patent on means and methods for establishing a clinical prognosis of diseases associated with the formation of aggregates of Aß1-42 pending. P. Scheltens has received grant support (for the institution) from GE Healthcare, Danone Research, Piramal and MERCK. In the past 2 years, he has received consultancy/speaker fees (paid to the institution) from Lilly, GE Healthcare, Novartis, Forum, Sanofi, Nutricia, Probiodrug, and EIP Pharma. A. Ruiz reports personal fees from Landsteiner Genmed. W. M. van der Flier reports grants from Boehringer Ingelheim, Piramal Imaging, Janssen Stellar, Stichting Dioraphte, Alzheimer Nederland, and ZonMW.

## Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jalz.2016.01.006.

## RESEARCH IN CONTEXT

1. Systematic review: We searched PubMed up to August 2015 combining the terms "Mild Cognitive Impairment (MCI) and polygenic risk scores (PGS)" and "Mild Cognitive Impairment and endophenotypes". Although most published studies have explored the effect of PGS on Alzheimer's disease (AD) endophenotypes in AD patients, very little research using PGS has been devoted to similar endophenotypes in MCI patients.
2. Interpretation: Our study illustrates the utility of exploring the effect of joined AD susceptibility genes on different endophenotypes of AD in well-characterized MCI samples. In this sample, PGS modulated cognitive decline over time and cerebrospinal fluid levels of tau and ptau.
3. Future directions: To explain the observed PGS effect, future studies on these endophenotypes should focus on delineation of the molecular network linking the AD susceptibility genes to tau pathology. This research will be crucial for the development of treatments targeting alternative nonamyloidogenic pathways and improved identification of at-risk groups.

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