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Late-Onset Alzheimer Risk Variants in Memory Decline, Incident Mild Cognitive Impairment and Alzheimer Disease

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Abstract

Background—Genome-wide association studies (GWAS) of late-onset Alzheimer's disease (LOAD) identified risk variants. We assessed the association of nine variants with memory and progression to mild cognitive impairment (MCI) or LOAD (MCI/LOAD).

Methods—Older Caucasians, cognitively normal at baseline and longitudinally evaluated at Mayo Clinic Rochester and Jacksonville, were assessed for associations of genetic variants with memory decline (n=2,262) using linear mixed models and for incident MCI/LOAD (n=2,674) with Cox proportional hazards models. Each variant was tested both individually and collectively using a single weighted risk score.

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Results—*APOE*- ϵ 4 was significantly associated with worse memory at baseline ($\beta=-0.88$, $p=2.78E-03$) and increased rate of 5-year decline ($\beta=-1.43$, $p=3.71E-06$) with highly significant overall effect on memory ($p=3.88E-09$). *CLU*-locus risk allele rs11136000-G was associated with worse memory at baseline ($\beta=-0.51$, $p=0.012$), but not with increased rate of decline. *CLU* allele was also associated with incident MCI/LOAD (hazard ratio=HR=1.14, $p=0.049$) in sensitivity analysis. *MS4A6A*-locus risk allele rs610932-C was associated with increased incident MCI/LOAD in primary analysis (HR=1.17, $p=0.016$) and had suggestive association with lower baseline memory ($\beta=-0.35$, $p=0.08$). *PICALM*-locus risk allele rs3851179-G had nominally significant HR in both primary and sensitivity analysis, but with a protective estimate. LOAD risk alleles *ABCA7*-rs3764650-C and *EPHA1*-rs11767557-A associated with increased rates of memory decline in the subset of subjects with a final diagnosis of MCI/LOAD. Risk scores excluding *APOE* were not significant, whereas *APOE*-inclusive risk scores associated with worse memory and incident MCI/LOAD.

Conclusions—The collective influence of the nine top LOAD GWAS variants on memory decline and progression to MCI/LOAD appears limited. Given the significant associations observed with *APOE*- ϵ 4, discovery of the biologically functional variants at these loci may uncover stronger effects on memory and incident disease.

Keywords

Alzheimer's disease; memory; mild cognitive impairment; genetic risk; association; cognitive decline

1. Introduction

Genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) at 20 genetic loci in addition to *APOE* ϵ 4, that are associated with late-onset Alzheimer's disease (LOAD) risk in large case-control series(1-6). These twenty SNPs are unlikely to be functional variants, but are rather markers that tag the biologically functional genetic variation at these loci(7). In addition, although the LOAD risk GWAS loci are identified by the names of the nearest genes, the identities of the LOAD risk genes remain to be established. Although uncovering the pathophysiologic mechanisms that underlie the LOAD risk conferred by the GWAS loci awaits discovery of the functional variants and the disease genes, the GWAS variants can nonetheless be evaluated for their effects on biological quantitative phenotypes of AD. This endophenotype approach offers an opportunity to investigate these variants for their influence on key functional outcomes associated with this complex disease, thereby providing not only additional support for their role in AD risk, but potentially also information on their mechanistic effects.

Cognitive phenotypes constitute an important category of endophenotypes for AD. Current conceptualization of the dynamic changes in AD biomarkers posits that subtle cognitive decline begins prior to the clinical diagnosis of mild cognitive impairment (MCI) and certainly AD (8, 9). Genetic variants that influence cognitive decline in these preclinical stages of AD may serve as predictive factors for this disease. Indeed, *APOE* ϵ 4, which is the strongest, common genetic risk factor for LOAD, associates with cognitive decline prior to the diagnosis of MCI/AD(10-13). Consistent with the model of clinical progression of AD

from preclinical cognitive decline to MCI, and then AD(9), *APOE* ϵ 4 is also associated with increased incidence of MCI(14), AD(15) or dementia(16).

Studies that evaluate the influence of the LOAD risk GWAS loci variants on cognitive endophenotypes are emerging. *CR1* locus variant rs6656401(17), and a coding variant in LD with it(18) were associated with episodic memory decline in a longitudinal cohort of >1,600 elderly subjects. In another study that evaluated *CLU*, *CR1* and *PICALM* loci SNPs, *CLU* and *CR1* variants associated with more rapid global cognitive decline and *PICALM* with earlier age at midpoint of cognitive decline(19) in 1,831 subjects. In a relatively small cohort of 95 cognitively normal subjects who developed MCI or AD, those with the risky *CLU* allele had a more rapid cognitive decline(20).

These studies are informative; however to date there are no reports that investigate the rate of memory decline for association with the larger number of published LOAD GWAS risk loci either individually or as a single weighted risk score. At the time of our study, nine loci were reported from LOAD GWAS(2-5). In our study, we evaluate a longitudinally followed cohort of >2,000 elderly, Caucasian subjects, who were cognitively normal at baseline, for association of memory decline and with incident MCI/LOAD with these nine LOAD GWAS variants. We also investigate their ability to discriminate between subjects that develop MCI/LOAD from those that do not. Our findings provide a paradigm for the assessment of LOAD risk variants for their effects on memory decline and progression to MCI/LOAD both individually and collectively as weighted risk scores.

2. Methods and Subjects

2.1 Subjects

We assessed an elderly, Caucasian cohort of subjects all of whom were clinically normal at baseline and followed by Behavioral Neurologists either at Mayo Clinic Rochester, Minnesota (MCR) or Mayo Clinic Jacksonville, Florida (MCJ). Incident MCI was diagnosed according to Petersen criteria(21) and clinically possible or probable AD was determined according to NINCDS-ADRDA criteria(22). All subjects underwent 2 or more clinical evaluations. The 30-minute delayed recall scores (LMDR) from the Wechsler Memory Scale-Revised(23) Logical Memory subtest were used as the cognitive endophenotypes. For the analyses assessing progression to MCI/LOAD, a total of 2,674 subjects were evaluated. For the memory analysis (n=2,262), patients were excluded if they had fewer than 2 LMDR scores or if their LMDR score at their initial assessment was 0. The demographics of all subjects are shown in Table 1. All studies were approved by Mayo Clinic's Institutional Review Board.

2.2 Genotyping

The most significant LOAD risk GWAS SNPs from 9 loci(1-5) near *CLU*, *PICALM*, *CR1*, *ABCA7*, *BINI*, *MS4A6A*, *EPHA1*, *CD2AP*, and *CD33*, in addition to two SNPs defining *APOE* alleles (rs429358 and rs7412) were genotyped using TaqMan® assays. The genotype frequencies of the SNPs are depicted in Supplementary Table 1.

2.3 Statistical Analysis

All analyses were conducted with each genetic variant tested individually, as well as with weighted risk scores. Two weighted risk scores, both of which included all 9 LOAD risk GWAS SNPs, but one with and one without *APOE* ε4, were calculated based on previously reported odds ratio (OR) estimates from large AD risk GWAS(4) or their follow-up studies(24-26), according to the following formula: $\text{Score}_i = \sum (n_{ij} * \log(\text{OR}_j))$ for the *i*th patient, where: n_{ij} = number of risk alleles for the *i*th patient and *j*th SNP; OR_j =odds ratio for the *j*th SNP. The contribution of each variant to the risk score is shown in Supplementary Table 2. Those subjects missing two or more SNPs were excluded from the risk score analyses. If a subject was missing only one SNP, the mean number of risk alleles for that SNP across all other subjects was used in the calculation of the risk score for that subjects.

Linear mixed-effects models with subject-specific random slopes and intercepts were used to evaluate associations of each variant and the risk scores with LMDR. The models included time from the initial LMDR assessment as the time scale in 5-year increments with site (MCJ=1, MCR=0), age at baseline, gender (male=1, female=0), and years of education as covariates. Unless *APOE* ε4 was being evaluated for association, number of *APOE* ε4 alleles was also included as a covariate in all models. The impact of each covariate in the model on trends in LMDR over time was evaluated through the inclusion of a time interaction term for each variable. Coefficients (β) for the intercept are interpreted as the effect of each additional risk allele on the baseline LMDR score, where the risk allele was identified from LOAD risk GWAS(1-6). Coefficients for the slope are interpreted as changes in the 5-year rate of LMDR for each additional risk allele or 1 standard deviation increase in the risk score. For each genetic variant, we performed a likelihood ratio test to compare the fit of the full model with a reduced model omitting the genetic variant and its time interaction to evaluate whether there was an overall effect of the genetic variant on LMDR.

Primary analysis for memory associations were conducted on all subjects without discrimination for last diagnosis of MCI/LOAD vs. clinically normal. We also performed secondary analyses where changes in the 5-year rate of LMDR by genotype were estimated separately for subjects with a last diagnosis of MCI/LOAD and those with a last diagnosis of normal. These secondary analyses differed from the primary analyses only in their inclusion of separate time interaction terms by genotype for the two “last diagnoses” categories and another time interaction variable for last diagnosis of MCI/LOAD.

Associations with risk of progression to LOAD or MCI (MCI/LOAD) were evaluated using Cox proportional hazard regression models that included time from baseline as the time scale and adjusted for site, gender, age, years of education, with or without adjustment for the number of *APOE* ε4 alleles, as described above. The primary endpoint for these analyses was the time to first diagnosis of MCI/LOAD; and those subjects who did not develop MCI/LOAD were censored at the time of their last assessment. In sensitivity analyses, those patients who were diagnosed with MCI/LOAD during their longitudinal assessment but whose last diagnosis was neither MCI nor LOAD were censored at their last assessment. There were 77 such patients, 3 of whom had a diagnosis of LOAD and 74 with a diagnosis

of MCI at some point during their longitudinal assessment. Seventy of these subjects had a final diagnosis of “normal” and 7 had a final diagnosis of “other”, and were consequently censored at their last diagnosis in the sensitivity analysis. Hazard ratios (HR) and 95% CI were given for the time-to-MCI/LOAD and for the sensitivity analyses.

Concordance index (c-index) for the prediction of conversion to MCI/LOAD was calculated for the subset of patients who had genotypes for each of the 9 genetic variants (n=2,240). Interpretation of the c-index is similar to the interpretation of the area under the receiver operating characteristic (ROC) curve: a value of 1.0 indicates that the variables in the Cox model perfectly discriminate patients with different outcomes, while a value of 0.5 indicates that the variables contain no predictive information. All analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina). Since 10 variants were tested (*APOE* +9 LOAD GWAS SNPs), P-values <0.005 were considered to be study-wide significant after Bonferroni correction. P-values <0.05 and 0.005 were nominally significant, and those 0.1 and 0.05 were considered to be suggestive.

3. Results

We evaluated elderly, Caucasian subjects who were cognitively normal at baseline and followed longitudinally for association of their memory scores or incident MCI/LOAD with 9 LOAD risk GWAS loci SNPs. Given the restrictions for the cognitive decline analysis based on available LMDR scores, there were less subjects for this analysis compared to those for the time to MCI/LOAD analyses, but the subjects from these analytic groups were overlapping and had similar characteristics (Table 1). Subjects from MCR were older, had fewer years of education, greater male frequency and lower median LMDR scores compared to MCJ subjects. Increased baseline age and male gender were associated with lower baseline LMDR scores, whereas increased years of education were associated with higher scores (Table 2). The MCJ site, where subjects have higher education and are younger, also associated with higher baseline LMDR scores. The MCR site and higher baseline age were associated with faster rate of decline in memory. Male gender had an estimated effect consistent with greater memory decline, but did not reach significance. *APOE* ϵ 4 was strongly associated with lower baseline memory scores and faster decline, with a highly significant overall effect on memory ($p=3.88 \times 10^{-9}$).

When the nine LOAD GWAS risk loci variants were tested for association with longitudinal memory scores while adjusting for *APOE* and the other variables shown in Table 2, only *CLU* locus SNP rs11136000 had nominally significant results (Table 3). The risk allele of this SNP associated with lower baseline LMDR scores. Although this allele was associated with a more negative slope of memory decline, it did not reach significance. *CLU*-rs11136000 had an overall nominally significant effect on memory, though this would not hold up to adjustment for multiple testing. MS4A6A-rs610932 risk allele had suggestive lower baseline memory scores, but did not have significant association with either slope of memory decline or a significant overall effect.

Given that rate of cognitive decline and its interaction with risk variants may differ between subjects who eventually develop MCI/LOAD vs. those who remain clinically normal, we

also performed secondary analysis, which assessed the association of risk genotypes or scores with rate of change in LMDR separately for these two groups of subjects (Supplementary Tables 3 and 4). The baseline and overall effects of *APOE* on memory remain significant in this secondary analysis ($p=1.79\text{E-}03$ and $3.10\text{E-}4$, respectively) (Supplementary Table 3). Although *APOE* $\epsilon 4$ has trends for faster memory decline in both subjects with last diagnosis of MCI/LOAD ($\beta=-0.74$, $p=0.15$) and those that remain normal ($\beta=-0.44$, $p=0.17$), these terms fail to reach significance.

Assessment of the LOAD GWAS loci on memory decline separately for subjects who eventually developed MCI/LOAD vs. those who remained as normal identified association of two LOAD risk alleles, *ABCA7*-rs3764650-C ($p=0.013$) and *EPHA1*-rs11767557-A ($p=0.050$) with faster rates of decline (Supplementary Table 4). *ABCA7* locus also had nominally significant overall association with memory ($p=0.018$) in these analyses. The effects of the *CLU* and *MS4A6A* loci on baseline memory detected in the primary analyses (Table 3) remain essentially unchanged in these secondary analyses. Risk score including *APOE* retains its significance for effects on baseline memory and overall association with LMDR, however no longer has significant association with faster memory decline in either diagnostic category. *CD33*-locus risk allele has suggestive, though lower rate of memory decline in subjects who remain cognitively normal.

We next evaluated the influence of a single weighted genetic risk score variable, obtained from the 9 LOAD risk GWAS SNPs (Supplementary Table 2), on memory. Although the risk score excluding *APOE* had a negative estimated effect on baseline LMDR, this did not achieve significance. There was no effect of this 9-SNP risk score on the slope of memory change. When *APOE* was included in the risk score, as expected there was significant association with both lower baseline LMDR scores and a negative slope of memory change, with highly significant overall effect ($p=1.21\times 10^{-8}$), similar to that seen for *APOE* alone.

We evaluated the association of the 9 LOAD GWAS SNPs with the risk of progression to MCI/LOAD in our longitudinally followed cohort (Table 4). The primary analysis assessed the time to first diagnosis of MCI/LOAD, regardless of last diagnosis, whereas the sensitivity analysis was censored at last visit for those subjects who had a diagnosis of MCI/LOAD at any point during their assessment but whose last diagnosis was something other than MCI/LOAD. Variants that showed evidence of an association with the risk of progression to MCI/LOAD included the *CLU* locus SNP rs11136000 risk allele (HR=1.10, $p=0.13$; Sensitivity HR=1.14, $p=0.049$) and the *MS4A6A* locus SNP rs610932 risk allele (HR=1.17, $p=0.016$; Sensitivity HR=1.11, $p=0.11$). The only other variant with a nominally significant result was the *PICALM* locus variant rs3851179, which had a protective HR in both analyses (HR=0.85, $p=0.010$; Sensitivity HR=0.82, $p=0.0045$), which is in the opposite direction than would be expected. The weighted risk score for the 9 SNPs did not have a significant HR for progression to MCI/LOAD, in either analysis. When *APOE* $\epsilon 4$ was included in the risk, there was significant association with risk of progression to MCI/LOAD (HR=1.29, $p=1.14\times 10^{-9}$; Sensitivity HR=1.32, $p=5.73\times 10^{-10}$).

C-index estimates which are indicative of the ability of variables to discriminate between subjects who develop MCI/LOAD and those who do not are displayed in Supplementary

Table 5. The c-index was only slightly improved with addition of *APOE* (0.685) relative to a reduced model with only age, gender, education and site (0.674). Similarly, addition of the risk score that included *APOE* (0.684) only barely increased the c-index compared to the reduced model. Inclusion of the other 9 variants individually or the combined risk score excluding *APOE* did not improve the c-index over the reduced model.

4. Discussion

Understanding the influence of the novel LOAD risk GWAS loci variants on cognitive endophenotypes can provide additional information about their plausible mechanism of action. Genetic variants that associate with cognition in a non-time-dependent fashion may suggest static effects, whereas those that associate with rate of cognitive decline may imply a dynamic influence on biological mechanisms underlying cognitive outcomes. We previously evaluated three LOAD risk GWAS SNPs at *CLU*, *CRI* and *PICALM* loci for association with verbal and non-verbal episodic memory scores at last evaluation and identified better memory scores in Caucasian subjects with the protective *CLU* rs11136000 SNP alleles and worse scores in African-American subjects with the risky *CRI* SNP (rs6656401, rs3818361) alleles(27). In the current study, we evaluated the association of nine LOAD risk GWAS loci SNPs for association with memory scores in a longitudinally assessed cohort and identified strong association between *APOE* ϵ 4 with both baseline logical memory and increased rate of memory decline, as expected(10-13). As in our prior study(27), we also identified lower memory scores at baseline associated with the risk allele of the *CLU* locus SNP rs11136000. Although this consistency may be expected given that the cohort from our prior study and the current one largely overlap, the approach used in this study is distinct because we evaluated baseline and longitudinal cognitive change instead of the last memory score as in our prior work. We did not find significant association with rate of memory decline and rs11136000, although the LOAD risk allele had an estimated negative slope of decline. Both our prior(27) and current findings suggest that the *CLU* locus influences cognition, and this effect appears to be static rather than a dynamic effect on the rate of decline. Importantly, the direction of the cognitive associations with the *CLU* locus SNP are congruent with the allelic effects on LOAD risk.

CLU locus SNP rs11136000 was found to associate with cognitive endophenotypes in other studies(19, 20). Fitting a Bayesian model in an initial cohort of 802 subjects, Sweet et al.(19) tested the model in a second cohort of 1,831 subjects who were dementia-free at baseline for association of SNPs at *CLU*, *PICALM* and *CRI* with cognitive endophenotypes. In that study, they found association between *CLU* rs11136000 with rate of global cognitive decline, although the LOAD protective allele was associated with faster decline, which is biologically incongruent. Thambisetty et al.(20) evaluated *CLU* rs11136000 in a cohort of 599 subjects who remained cognitively normal and 95 subjects who converted to MCI or AD in a longitudinal study. There were no significant associations with cognitive decline in the non-converters but significantly greater rate of memory decline was observed in *CLU* rs11136000 risk allele carriers amongst the smaller cohort of converters. Collectively, these studies and our findings support a role for *CLU* locus variants in memory endophenotypes, although the effects on static vs. dynamic memory outcomes and the associating allele need to be firmly established.

Since rates of cognitive decline may differ in subjects who eventually convert to MCI/LOAD vs. those who remain clinically normal, we also assessed the effect of LOAD risk variants on memory decline separately in subjects that pertain to these two “last diagnosis” categories. We determined associations with faster memory decline for the risk alleles *ABCA7*-rs3764650-C and *EPHA1*-rs11767557-A in those subjects who eventually convert to MCI/LOAD. These findings suggest that *ABCA7* and *EPHA1* may have an effect on dynamic memory outcomes. That we can only detect an effect on memory decline in subjects who eventually develop MCI/LOAD may be due to the fact that these subjects are at a later stage in their underlying disease process and therefore are more susceptible to the effects of genetic risk factors. Another possibility is that subjects who are “destined for” MCI/LOAD may have other genetic or environmental risk factors that enhance the effects of the tested genetic risk variants on cognitive decline through interactions.

While separate analysis of different diagnostic categories can be informative, as discussed above, this can also lead to loss of power and therefore significance as we observed for *APOE* ϵ 4 and the risk score including *APOE* for rate of memory decline, although associations with baseline memory and overall effects remained significant. This is likely due to analysis of smaller number of subjects, as well as overlap between effects of *APOE* ϵ 4 allele and MCI/LOAD diagnosis on rate of decline. These results highlight the importance of both total group analyses as well as separate assessments for those with incident MCI/LOAD vs. those that remain clinically normal.

In addition to evaluating memory endophenotypes, we also tested the association of these nine LOAD risk GWAS SNPs for their effects on progression to MCI or LOAD. Genetic variants may influence risk of LOAD by accelerating progression to clinically detectable cognitive decline. Further, genetic risk factors may also continue to influence rate of disease progression after clinical diagnosis of AD or MCI. Alternatively, genetic factors may underlie biological processes that confer a static cognitive disadvantage. Thus, testing the effects of LOAD risk GWAS SNPs for incident LOAD or MCI may yield further information about their mechanism of action. In our study, *CLU* rs11136000 risk allele has an increased hazard ratio for progression to LOAD or MCI. The number of studies evaluating the role of LOAD GWAS SNPs in the rate of progression to MCI/LOAD are yet limited. Rodriguez-Rodriguez et al.(28) investigated the association of eight LOAD risk GWAS loci SNPs with both risk and rate of progression to AD in 297 MCI subjects, 118 of whom were converters. In that study, *CLU* rs11136000 was associated with risk but not with rate of progression from MCI to AD, unlike in our study. That we are not detecting association with rate of memory decline, but find association with rate of progression to disease may seem inconsistent, however may be due to limited power of alleles with modest effect sizes on these tested outcomes. It is also possible that there may be decline in non-memory cognitive domains, which we did not evaluate.

MS4A6A-locus rs610932-C risk allele was associated with progression to MCI/LOAD in primary analysis with suggestive results for the sensitivity analysis. This allele also had suggestively lower baseline memory estimates. *PICALM* locus risk allele rs3851179-G had nominally significant association with protective HR estimates in both the primary and sensitivity analyses, which is biologically inconsistent with expected effects based on

LOAD risk estimates. Carriers of this risk allele were previously found by others to have a more rapid rate of clinical decline as determined by changes in the Clinical Dementia Rating-sum of boxes as a quantitative trait in 822 Caucasian subjects with amnesic MCI(29), consistent with effects on LOAD risk. The biologically incongruent protective effect in our cohort of the *PICALM* locus risk allele may be a false positive finding. Alternatively, these opposing effects may be due to the “flip-flop” phenomenon(30) that may ensue when the LD structure between the tested variant and the functional variant(s) differ between studies. Resolution of such findings awaits discovery and testing of putative functional variants at disease risk loci.

To assess the combined influence of genetic risk variants on memory decline and progression to AD or MCI, we utilized a single risk score weighted by the estimated ORs for each of the nine LOAD GWAS variants plus or minus *APOE*. The risk scores which included *APOE* had strong associations with lower baseline memory, increased rate of memory decline and faster rate of progression to MCI/LOAD. The risk scores which lacked *APOE* did not achieve significance for any of the tested outcomes, although they had a negative estimate for baseline memory. These findings are similar to those identified by Verhaaren et al.(31), in a population-based cohort of 5,171 subjects who were nondemented at baseline. That study found only marginal influence of risk scores using 10 GWAS loci variants, on baseline memory and risk of developing AD, despite robust associations when *APOE* was included in the risk score. Likewise, genetic risk scores based on 8 variants did not associate with risk of conversion from 288 MCI subjects to AD in the Rodriguez-Rodriguez et al.(28) study, although faster rate of progression was identified for the second and third tertile of risk score carriers vs. the first tertile.

The GWAS loci variants or the risk scores had no added value over the non-genetic variables of site, gender, age and years of education for discriminating MCI/LOAD converters. Importantly, even *APOE* offered very little additional predictive value. This is similar to findings in two population-based cohorts (Rotterdam and Cardiovascular Health Study), in which the value of adding *APOE* to age and sex variables for predicting progression to AD was minimal and addition of *CLU* and *PICALM* loci SNPs provided essentially no further improvement in prediction(3). Similarly, a 10-SNP risk score had essentially no value for predicting conversion to AD in the Rotterdam cohort(31). It has been suggested that non-genetic variants provide significant predictive power(3), therefore even *APOE* that has strong associations with memory and rate of progression to LOAD or MCI does not contribute significantly to prediction of incident AD above and beyond these non-genetic variables. Despite these findings, it is important to continue to explore these predictive models by including new genetic risk variants as they are uncovered.

Our study has a number of strengths, including a sizable cohort without dementia or MCI at baseline, evaluation of baseline and longitudinal memory associations, in addition to progression to MCI/LOAD with 9 LOAD risk variants both individually and as a single risk score, as well as exploration of their predictive value for MCI/LOAD. The strong associations of *APOE* with memory at baseline, as well as rate of memory decline and progression to MCI/LOAD provide proof of principle for our approach. The biologically congruent memory and incident disease associations with the *CLU* locus SNP provide

additional support for this locus in influencing memory and disease progression in pre-clinical stages of AD. Likewise, the incident MCI/LOAD risk associations with the *MS4A6A*-locus risk allele are supportive for this locus. *PICALM* locus associations for progression to AD needs further assessment due to inconsistent direction of effect compared with the AD risk associating allele.

The lack of associations with the other variants and the genetic risk score may be a reflection of small effect sizes of the tested SNPs, assessment of marker polymorphisms rather than the functional variants, the sensitivity of the memory measure used to the earliest effect of neuropathology, potential survival bias in our elderly cohort where some of the variants may have stronger effects on cognitive or clinical decline earlier on, or the incompleteness of the tested models as there are clearly additional genetic and non-genetic variables to be uncovered that may improve the models, including those for prediction. The approaches used in our study can generalize to other genetic variants that are expected to emerge from AD risk variant discovery efforts and therefore provide valuable information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of subjects included in the analyses

a. Median follow-up refers to that for LMDR assessments for subjects in the cognitive decline analyses and is the time to first MCI/AD or last follow-up for those in the time to MCI/AD analyses. MCR: Mayo Clinic Rochester in Minnesota, MCJ: Mayo Clinic Jacksonville in Florida. LMDR: Logical Memory Delayed Recall scores from the Wechsler Memory Scale-revised.

Variable	Cognitive Decline Analysis			Time to MCI/AD Analysis		
	Overall (N=2262)	MCR (N=1800)	MCJ (N=462)	Overall (N=2674)	MCR (N=2228)	MCJ (N=446)
Median age at 1 st assessment (range), years	77 (49-98)	78 (55-98)	72 (49-91)	77 (48-98)	78 (55-98)	74 (48-94)
Male gender - n (%)	991 (44)	842 (47)	149 (32)	1187 (44)	1034 (46)	153 (34)
Median years of education (range)	14 (4-20)	13 (5-20)	16 (4-20)	14 (4-20)	13 (5-20)	16 (4-20)
Last diagnosis - n (%)						
Normal	1881 (83)	1467 (82)	414 (90)	2166 (81)	1778 (80)	388 (87)
MCI	252 (11)	236 (13)	16 (3)	347 (13)	332 (15)	15 (3)
AD	129 (6)	97 (5)	32 (7)	132 (5)	101 (5)	31 (7)
Other	-	-	-	29 (1)	17 (1)	12 (3)
Median follow-up (range), months ^a	45.9 (8.9-214.1)	44.6 (8.9-214.1)	69.1 (10.2-190.4)	61.7 (0.1-269.3)	60.7 (8.0-269.3)	66.8 (0.1-245.3)
APOE ε4 alleles - n (%)						
0	1681 (74)	1361 (76)	320 (69)	1991 (74)	1679 (75)	312 (70)
1	544 (24)	412 (23)	132 (29)	636 (24)	512 (23)	124 (28)
2	37 (2)	27 (2)	10 (2)	47 (2)	37 (2)	10 (2)
Median LMDR at 1 st assessment (range)	17 (1-42)	16 (1-40)	20 (1-42)	-	-	-

Effect of APOE on logical memory (LMDR)

Coefficients for the estimated change in LMDR, 95% confidence intervals (CI) and P-values are shown for all tested variables. A likelihood ratio test comparing the full model to a reduced model omitting APOE and the interaction of APOE with time was performed to evaluate the overall effect of APOE on LMDR.

Variables	Coefficient (95% CI)	P-value
Baseline (intercept)		
APOE (no. ε4 alleles)	17.66 (17.17 to 18.15)	
MCJ site	-0.88 (-1.45 to -0.30)	2.78E-03
Baseline age (5 yrs)	1.60 (0.86 to 2.33)	2.00E-05
Male gender	-1.35 (-1.58 to -1.12)	3.84E-30
Yrs. of education (5 yrs)	-1.07 (-1.63 to -0.50)	2.30E-04
Yrs. of education (5 yrs)	2.73 (2.24 to 3.21)	6.75E-28
5-Year Change (slope)		
APOE (no. ε4 alleles)	0.79 (0.26 to 1.31)	3.18E-03
MCJ site	-1.43 (-2.04 to -0.83)	3.71E-06
Baseline age (5 yrs)	4.17 (3.44 to 4.89)	6.91E-29
Male gender	-0.65 (-0.88 to -0.41)	1.01E-07
Yrs. of education (5 yrs)	-0.28 (-0.89 to 0.33)	0.37
Yrs. of education (5 yrs)	0.29 (-0.24 to 0.82)	0.28
Overall effect of APOE		3.88E-09

Table 3

Effect of LOAD risk GWAS loci SNPs on logical memory

Coefficients for the estimated change in LMDR, 95% confidence intervals (CI) and P-values are shown for all tested variables and are interpreted as either the estimated change in baseline LMDR or the estimated change in LMDR over a 5-year period for a. each additional copy of the risk allele or b. 1 standard deviation increase in the risk score. Each model was adjusted for site, baseline age, gender, years of education and *APOE* ϵ 4, c. except for the model testing the risk score which includes *APOE* ϵ 4. Interactions with time were included for each variable in the model. Likelihood ratio tests were performed to evaluate the overall effect of each SNP or risk score on LMDR.

Variable Type	Risk Variable	Tested Effects	Coefficient (95% CI)	P-value	Overall p-value
Nearest gene SNP ID (risk allele) ^a	<i>CLU</i> rs11136000 (G)	Baseline 5-year change	-0.51 (-0.92 to -0.110) -0.23 (-0.68 to 0.22)	0.012 0.32	0.012
	<i>PICALM</i> rs3851179 (G)	Baseline 5-year change	0.30 (-0.12 to 0.71) 0.05 (-0.41 to 0.50)	0.16 0.83	0.32
	<i>CRI</i> rs3818361 (A)	Baseline 5-year change	0.31 (-0.18 to 0.80) 0.04 (-0.50 to 0.58)	0.22 0.88	0.42
	<i>ABCA7</i> rs3764650 (C)	Baseline 5-year change	-0.42 (-1.13 to 0.28) -0.12 (-0.85 to 0.62)	0.24 0.76	0.41
	<i>BIN1</i> rs744373 (G)	Baseline 5-year change	0.14 (-0.31 to 0.59) -0.23 (-0.72 to 0.26)	0.55 0.36	0.60
	<i>MS4A6A</i> rs610932 (C)	Baseline 5-year change	-0.35 (-0.75 to 0.05) 0.11 (-0.33 to 0.56)	0.08 0.63	0.22
	<i>EPHA1</i> rs11767557 (A)	Baseline 5-year change	-0.07 (-0.56 to 0.42) -0.15 (-0.67 to 0.38)	0.77 0.58	0.78
	<i>CD2AP</i> rs9349407 (C)	Baseline 5-year change	-0.34 (-0.78 to 0.11) -0.01 (-0.49 to 0.47)	0.14 0.97	0.31
	<i>CD33</i> rs3865444 (C)	Baseline 5-year change	-0.20 (-0.63 to 0.23) 0.32 (-0.15 to 0.78)	0.37 0.18	0.34
	Risk score 1 with 9 SNPs	Baseline 5-year change	-0.19 (-0.48 to 0.10) -0.08 (-0.39 to 0.23)	s0.20 0.63	0.31
Risk score ^b	Risk score 2 ^c with 9 SNPs & <i>APOE</i>	Baseline 5-year change	-0.50 (-0.78 to -0.21) -0.64 (-0.95 to -0.34)	6.70E-04 4.00E-05	1.21E-08

Table 4
Effect of LOAD risk GWAS loci SNPs on risk of progression to MCI or LOAD

Hazard ratios (HR) and 95% confidence intervals (CI) were obtained for the genetic variants. Hazard ratios correspond to a. an additional risk allele or b. 1 standard deviation increase in the risk score. All models were adjusted for site, gender, age, years of education and *APOE* $\epsilon 4$, c. except for the models testing the effect of *APOE* $\epsilon 4$.

Variable Type	SNP ID (risk allele) or risk score	Time-to-MCI/LOAD analysis			Sensitivity analysis		
		HR	95% CI	P-value	HR	95% CI	P-value
Nearest gene - SNP ID (risk allele) ^a	<i>APOE</i> rs429358 ($\epsilon 4$) ^c	1.71	1.46-1.99	1.09E-10	1.80	1.52-2.11	4.98E-11
	<i>CLU</i> rs11136000 (G)	1.10	0.97-1.24	0.13	1.14	1.00-1.30	0.049
	<i>PICALM</i> rs3851179 (G)	0.85	0.75-0.96	0.010	0.82	0.72-0.94	4.54E-03
	<i>CRI</i> rs3818361 (A)	1.11	0.95-1.28	0.19	1.12	0.95-1.32	0.16
	<i>ABCA7</i> rs3764650 (C)	1.05	0.85-1.27	0.67	1.07	0.85-1.31	0.57
	<i>BIN1</i> rs744373 (G)	1.08	0.94-1.24	0.27	1.05	0.90-1.22	0.54
	<i>MS4A6A</i> rs610932 (C)	1.17	1.03-1.32	0.016	1.11	0.98-1.28	0.11
	<i>EPHA1</i> rs11767557 (A)	0.99	0.86-1.14	0.84	0.97	0.83-1.13	0.68
	<i>CD2AP</i> rs9349407 (C)	1.02	0.89-1.17	0.76	1.04	0.90-1.21	0.57
	<i>CD33</i> rs3865444 (C)	1.00	0.88-1.14	0.98	0.95	0.83-1.10	0.51
Risk score ^b	Risk score with 9 SNPs	1.03	0.95-1.13	0.43	1.02	0.93-1.11	0.75
	Risk score with 9 SNPs & <i>APOE</i> ^c	1.29	1.19-1.39	1.14E-09	1.32	1.21-1.43	5.73E-10