Neurobiology of Aging 36 (2015) 60–67

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Late-onset Alzheimer's risk variants in memory decline, incident mild cognitive impairment, and Alzheimer's disease

Minerva M. Carrasquillo [a], Julia E. Crook [b], Otto Pedraza [c], Colleen S. Thomas [b], V. Shane Pankratz [d], Mariet Allen [a], Thuy Nguyen [a], Kimberly G. Malphrus [a], Li Ma [a], Gina D. Bisceglio [a], Rosebud O. Roberts [e,f], John A. Lucas [c], Glenn E. Smith [g], Robert J. Ivnik [g], Mary M. Machulda [g], Neill R. Graff-Radford [h], Ronald C. Petersen [f], Steven G. Younkin [a], Nilüfer Ertekin-Taner [a,h,*]

[a] Department of Neuroscience, Mayo Clinic, Jacksonville, FL, USA
[b] Department of Health Sciences Research, Mayo Clinic, Jacksonville, FL, USA
[c] Department of Psychiatry and Psychology, Mayo Clinic, Jacksonville, FL, USA
[d] Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA
[e] Department of Health Sciences Research, Division of Epidemiology, Mayo Clinic, Rochester, MN, USA
[f] Department of Neurology, Mayo Clinic, Rochester, MN, USA
[g] Department of Psychiatry and Psychology, Mayo Clinic, Rochester, MN, USA
[h] Department of Neurology, Mayo Clinic, Jacksonville, FL, USA

ARTICLE INFO

Article history:
Received 23 April 2014
Received in revised form 4 July 2014
Accepted 28 July 2014
Available online 4 August 2014

Keywords:
Alzheimer's disease
Memory
Mild cognitive impairment
Genetic risk
Association
Cognitive decline

ABSTRACT

We tested association of nine late-onset Alzheimer's disease (LOAD) risk variants from genome-wide association studies (GWAS) with memory and progression to mild cognitive impairment (MCI) or LOAD (MCI/LOAD) in older Caucasians, cognitively normal at baseline and longitudinally evaluated at Mayo Clinic Rochester and Jacksonville (n>2000). Each variant was tested both individually and collectively using a weighted risk score. *APOE*-e4 associated with worse baseline memory and increased decline with highly significant overall effect on memory. *CLU*-rs11136000-G associated with worse baseline memory and incident MCI/LOAD. *MS4A6A*-rs610932-C associated with increased incident MCI/LOAD and suggestively with lower baseline memory. *ABCA7*-rs3764650-C and *EPHA1*-rs11767557-A associated with increased rates of memory decline in subjects with a final diagnosis of MCI/LOAD. *PICALM*-rs3851179-G had an unexpected protective effect on incident MCI/LOAD. Only *APOE*-inclusive risk scores associated with worse memory and incident MCI/LOAD. The collective influence of the nine top LOAD GWAS variants on memory decline and progression to MCI/LOAD appears limited. Discovery of biologically functional variants at these loci may uncover stronger effects on memory and incident disease.

1. Introduction

Genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) at 20 genetic loci in addition to apolipoprotein E (*APOE*) ε4, that are associated with late-onset Alzheimer's disease (LOAD) risk in large case-control series (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009, 2013; Naj et al., 2011; Seshadri et al., 2010). These 20 SNPs are unlikely to be functional variants, but are rather markers that tag the biologically functional genetic variation at these loci (Ferrari et al., 2012). 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 Alzheimer's disease (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.

* Corresponding author at: Mayo Clinic Florida, 4500 San Pablo Road, Birdsall 3, Jacksonville, FL 32224. Tel.: +1 904 953 7103; fax: +1 904 953 7370.
E-mail address: taner.nilufer@mayo.edu (N. Ertekin-Taner).

0197-4580/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2014.07.042

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 before the clinical diagnosis of mild cognitive impairment (MCI) and certainly AD (Jack et al., 2013; Sperling et al., 2011). Genetic variants that influence cognitive decline in these preclinical stages of AD may serve as predictive factors for this disease. Indeed, *APOE* ε4, which is the strongest, common genetic risk factor for LOAD, associates with cognitive decline before the diagnosis of MCI/AD (Bennett et al., 2009; Caselli et al., 2004, 2007, 2009). Consistent with the model of clinical progression of AD from preclinical cognitive decline to MCI, and then AD (Sperling et al., 2011), *APOE* ε4 is also associated with increased incidence of MCI (Luck et al., 2010), AD (Aggarwal et al., 2005), or dementia (Fitzpatrick et al., 2004).

Studies that evaluate the influence of the LOAD risk GWAS loci variants on cognitive endophenotypes are emerging. *CR1* locus variant rs6656401 (Chibnik et al., 2011), and a coding variant in linkage disequilibrium with it (Keenan et al., 2012) were associated with episodic memory decline in a longitudinal cohort of >1600 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 (Sweet et al., 2012) in 1831 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 (Thambisetty et al., 2013).

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, 9 loci were reported from LOAD GWAS (Harold et al., 2009; Hollingworth et al., 2011; Naj et al., 2011; Seshadri et al., 2010). In our study, we evaluate a longitudinally followed cohort of >2000 elderly, Caucasian subjects who were cognitively normal at baseline for association of memory decline and with incident MCI/LOAD with these 9 LOAD GWAS variants. We also investigate their ability to discriminate between subjects that develop MCI/LOAD from those who 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 the Mayo Clinic Rochester, Minnesota (MCR), or the Mayo Clinic Jacksonville, Florida (MCJ). Incident MCI was diagnosed according to Petersen criteria (Petersen, 2011) and clinically possible or probable AD was determined according to National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria (McKhann et al., 1984). All subjects underwent ≥2 clinical evaluations. The 30-minute delayed recall scores (LMDR) from the Wechsler Memory Scale-Revised (Wechsler, 1987) Logical Memory subtest were used as the cognitive endophenotypes. For the analyses assessing progression to MCI/LOAD, a total of 2674 subjects were evaluated. For the memory analysis (n = 2262), patients were excluded if they had <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 the Mayo Clinic's Institutional Review Board.

2.2. Genotyping

The most significant LOAD risk GWAS SNPs from 9 loci (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011; Seshadri et al., 2010) near *CLU, PICALM, CR1, ABCA7, BIN1, MS4A6A, EPHA1, CD2AP,* and *CD33,*in addition to 2 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 1 with and 1 without *APOE* ε4, were calculated based on previously reported odds ratio estimates from large AD risk GWAS (Hollingworth et al., 2011) or their follow-up studies (Carrasquillo et al., 2010, 2011a, 2011b), according to the following formula: $\text{Score}_i = \Sigma\,(n_{ij} * \log(\text{OR}_j))$ for the ith patient, where: n_{ij} = number of risk alleles for the ith patient and jth SNP; OR_j = odds ratio for the jth SNP. The contribution of each variant to the risk score is shown in Supplementary Table 2. Those subjects missing ≥2 SNPs were excluded from the risk score analyses. If a subject was missing only 1 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, the 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 (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009, 2013; Naj et al., 2011; Seshadri et al., 2010). 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 versus 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 2 "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, and years of education, with or without adjustment for the number of *APOE* ε4 alleles, as described. The primary endpoint for these analyses was the time to first diagnosis of MCI/LOAD; those subjects who did not develop