Published in final edited form as:
Ann Neurol. 2011 March ; 69(3): 560–569. doi:10.1002/ana.22277.

CR1 is associated with amyloid plaque burden and age-related cognitive decline

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Abstract

OBJECTIVE—Recently, genome-wide association studies have identified three new susceptibility loci for Alzheimer's disease (AD), *CLU*, *CR1*, and *PICALM*. We leveraged available neuropsychological and autopsy data from two cohort studies to investigate whether these loci are associated with cognitive decline and AD neuropathology.

METHODS—The Religious Orders Study (ROS) and Rush Memory and Aging Project (MAP) are longitudinal studies that enroll non-demented subjects and include annual clinical evaluations and brain donation at death. We evaluated *CR1* (rs6656401), *CLU* (rs11136000) and *PICALM* (rs7110631) in 1666 subjects. We evaluated associations between genotypes and rate of change in cognitive function as well as AD-related pathology. Lastly, we used pathway analysis to determine if relationships between SNPs and cognitive decline were mediated through AD pathology.

RESULTS—Among our study cohort, the mean years of follow-up was 7.8 for ROS and 4.3 for MAP. Only the *CR1* locus was associated with both global cognitive decline (p=0.011) and global AD pathology (p=0.025). More specifically, the locus affects the deposition of neuritic amyloid plaque (p=0.009). In a mediation analysis, controlling for amyloid pathology strongly attenuated the effect of the *CR1* locus on cognitive decline.

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INTERPRETATION—We found that common variation at the *CR1* locus has a broad impact on cognition and that this effect is largely mediated by an individual's amyloid plaque burden. We therefore highlight one functional consequence of the *CR1* susceptibility allele and generalize the role of this locus to cognitive aging in the general population.

INTRODUCTION

The 20th century has witnessed a remarkable increase in the number and proportion of older persons, with wide ranging health consequences, since older persons are at risk for chronic conditions that are relatively rare among the young. Alzheimer's disease (AD) in particular is among the most common and debilitating age-related conditions, causing progressive amnesia, dementia and ultimately global cognitive failure and death. Genetic risk factors have long been known to play an important role in the development of AD, but until recently, the only consistently validated susceptibility locus for sporadic late-onset AD was *Apolipoprotein E* (*APOE*), with the ε4 allele conferring an increased risk and the ε2 allele a reduced risk. Using large cohorts of AD cases and controls, two independent genome-wide association studies recently identified three new loci, complement receptor 1 (*CR1*), clusterin (*CLU*), and the phosphatidylinositol-binding clathrin assembly protein (*PICALM*), with substantial evidence for association with AD[1, 2], and these loci have now been independently validated [3].

The majority of studies seeking to identify genetic variation responsible for AD have been case-control studies comparing persons with disease to those without. However, the dichotomous clinical outcome obscures the reality that AD develops slowly over years, and during this time affected individuals typically progress through a phase called mild cognitive impairment [4]. In fact, AD neuropathology, consisting of extracellular amyloid plaques and intracellular neurofibrillary tangles, is commonly found at autopsy in individuals without dementia proximate to death, and a substantial proportion of individuals with mild cognitive impairment meet pathological criteria for AD diagnosis [5, 6]. We and others have therefore proposed that intermediate cognitive and pathological traits might complement AD clinical diagnosis as an outcome phenotype for genetic association studies, in part by overcoming confounding due to phenotypic heterogeneity [7, 8]. Indeed, there is now substantial evidence that the *APOE* locus is associated with age-related cognitive decline as well as the accumulation of AD-related pathology in the general population, including individuals without dementia [9, 10]. Further, in earlier work, the association of *APOE* with cognitive decline was shown to be mediated by an intermediate effect on amyloid burden [10]. It has been proposed that similar to *APOE*, the new AD susceptibility loci, *CR1, CLU*, and *PICALM*, might directly impact the accumulation of amyloid pathology, perhaps through participation in clearance of the A-Beta peptide, which aggregates to form amyloid plaques [1, 2].

The Religious Orders Study (ROS) and Rush Memory and Aging Project (MAP) are prospectively following more than 2300 subjects without dementia at baseline, and include longitudinal clinical evaluation and brain donation at death. In this study, we leverage the wealth of neuropsychiatric and neuropathologic phenotypes available in ROS and MAP to assess whether these loci, like *APOE*, more generally influence age-related cognitive decline. Finally, we perform a functional genetic dissection to evaluate whether associations of these loci with cognition are mediated by an effect on AD neuropathology.

SUBJECTS/MATERIALS AND METHODS

Subjects

Subjects are participants from two longitudinal follow-up studies.

The *Religious Order Study* (ROS), started in 1994, enrolled Catholic priests, nuns and brothers, aged 53 or older from about 40 groups in 12 states. Since January 1994, 1132 participants completed their baseline evaluation, of which 1001 were non-Hispanic white. The follow-up rate of survivors of exceeds 90% as does the autopsy rate (481 autopsies of 511 deaths, of whom 457 were non-Hispanic white) Participants were free of known dementia at enrollment agreed to annual clinical evaluations and signed both an informed consent and an Anatomic Gift Act donating their brains at time of death [11]. More detailed description of the ROS can be found in previously published literature [11].

The *Rush Memory and Aging Project* (MAP), started in 1997, enrolled older men and women from assisted living facilities in the Chicagoland area with no evidence on dementia at baseline. Since October 1997, 1285 participants completed their baseline evaluation of which 1118 were non-Hispanic white. The follow-up rate of survivors of exceeds 90%, and the autopsy rate exceeds 80% (336 autopsies of 411 deaths, of whom 320 were non-Hispanic white). Similar to ROS, participants agreed to annual clinical evaluations and signed both an informed consent and an Anatomic Gift Act donating their brains, spinal cords and selected nerves and muscles to Rush investigators at the time of death [11, 12]. More detailed descriptions of these studies can be found in previously published literature[11, 12]. Both ROS and MAP were approved by the Institutional Review Board of Rush University Medical Center.

Genotyping

DNA was extracted from whole blood, lymphocytes or frozen post-mortem brain tissue. We used the SNPs outlined in recent publications [1, 2] *CR1* (rs6656401) and *CLU* (rs136000). For *PICALM*, the published SNP (rs3851179) did not pass quality control in our dataset, so we used a SNP proxy, rs7110631, r^2=0.75 with rs3851179 in HapMap subjects of European ancestry (http://hapmap.ncbi.nlm.nih.gov/). Genotype data for rs6666401 (*CR1)*, rs7110631 *(PICALM)* and rs11136000 (*CLU)* were extracted from a genome-wide dataset generated on the Affymetrix Genechip 6.0 platform at the Broad Institute's Center for Genotyping (n=1204) or the Translational Genomics Research Institute (n=674). These two sets of data underwent the same quality control (QC) analysis in parallel, and QC'ed genotypes were pooled. Only self-declared non-Hispanic Caucasians were genotyped to minimize population heterogeneity. The PLINK toolkit (http://pngu.mgh.harvard.edu/~purcell/plink/) was used to implement our QC pipeline: We applied its standard quality control pipeline for subjects (genotype success rate >95%, genotype-derived gender concordant with reported gender, excess inter/intra-heterozygosity) and for SNPs (HWE $p > 1\times10^{-6}$; MAF > 0.01, genotype call rate > 0.95; misshap test $> 1\times10^{-9}$) to these data. In a second step, EIGENSTRAT [13] was used to identify and remove population outliers using its default parameters. At the conclusion of the QC pipeline, 1666 individuals were available for analysis, among those 28 (2%) subjects were missing a *CR1* genotype, 10 (0.6%) were missing a *PICALM* genotype and 19 (1%) were missing a *CLU* genotype. All 3 SNPs had allele frequencies which satisfied Hardy-Weinberg equilibrium in our data (p >0.001).

Cognitive outcomes

Our analyses focus on two outcome measures of cognition, episodic memory and global cognition. Episodic memory is measured as a composite score of 7 instruments, word memory list and word list recognition from CERAD; immediate and delayed recall of Story A from the logical memory subset of the Wechsler Memory Scale-Revised; and immediate and delayed recall of the East Boston Story. The global cognition score was a composite of 19 cognitive tests, measuring 5 domains of cognitive function (episodic memory, visuospatial ability, perceptual speed, semantic memory and working memory) and is described in detail in previously published literature [11, 12, 14-25].

Summary measures were created as previously described [12, 15, 23, 24] by converting each test into a Z score using the mean and SD from baseline evaluation of all participants and averaging the measure for each of the cognitive areas. Summary measures are beneficial for limiting the influence of outliers. For our analysis at least half of the composite scores must be present for the summary measure to be valid.

Pathology outcomes

Brain autopsies were performed for nearly all cases at 12 pre-determined sites across the United States including Rush University in Chicago. Autopsy procedures are described in detail in previously published literature [11, 26, 27]. We classified persons as having pathologic AD based on intermediate or high likelihood of AD by National Institute on Aging (NIA)-Reagan criteria using CERAD estimates of neuritic plaque density and Braak staging of neurofibrillary pathology [28-30], as previously described [11]. The quantitative composite AD pathology score was based on counts of neuritic plaques and neurofibrillary tangles as previously described [31, 32] We standardized the raw counts by dividing each person's count by the standard deviation for that particular count and formed a global pathology summary score by averaging the standardized scores. In addition, separate summary measures of neurofibrillary tangles and neuritic and diffuse plaques were created in a similar fashion.

Statistical Analysis

Demographic characteristics of each cohort are described using means and standard deviations for continuous variables and frequency and proportions for categorical variables, including the SNPs of interest. Demographics and genotypic frequencies were compared across studies using 2-independent sample t-tests and χ^2 tests. General linear mixed models adjusting for age at enrollment, years of education and sex were used to compare the rate of cognitive decline between genotypes. For all three SNPs we initially used an additive model which examines the affect of each additional risk allele. Since this is a follow-up study to replicate previous findings by Harold et. al.[1] and Lambert et. al[2], we imposed a nominal threshold of $p<0.05$ for significance in the primary analyses for association with global cognitive decline and global pathology in which the default additive model was tested. If a relationship was found at $p<0.10$, we further examined the relationship using a dominant model comparing the homozygote for the non-risk allele to the combined heterozygote and homozygote for risk allele, or using a recessive model comparing the combined homozygote for the non-risk allele and the heterozygote to the homozygote for the risk allele. We used these mixed models to plot the paths of change in each cognitive measure by genotype for our significant findings. Additional models were also run with further adjustment for *APOE ε4* allele. As a secondary analysis, logistic regression models adjusted for age at baseline, sex and education were used to test if the 3 SNPs were associated with any clinical diagnosis of AD after baseline.

Linear regression and analysis of covariance (ANCOVA) models adjusted for age at death, years of education and sex were used to assess the relationship between pathology measures and genotypes within the deceased participants. First, a linear regression model was used and if a significant relationship was found at $p <0.10$, an ANCOVA model was run where the adjusted mean pathology score is compared between each genotype, the p-value testing if at least one mean is different. Lastly, two causal pathway analyses using Cooper's Local Causal Discovery algorithm [33] are performed by repeating the mixed models adjusting AD pathology measures in order to determine if the relationships between SNP and cognitive decline is mediated by pathology. All results were initially stratified by study and then combined. An interaction term was used to examine possible effect modification over time and determine whether effect sizes vary across study. All tests were performed using SAS software, version 9.2 (SAS Institute, Cary, NC).

RESULTS

Demographics

Genotype data on 791 participants in ROS was available, and 326 (41%) of these subjects had neuropathologic data. Genotype data on 875 participants in MAP was available, and 227 (26%) of these subjects had neuropathology data. The mean (±SD) age at enrollment for ROS and MAP participants were 76 (±7) and 81 (±7) years respectively, and the mean (±SD) time of follow-up for ROS and MAP were 8 (±5) and 4 (±3) years respectively. Although ROS and MAP differ significantly in demographic information, they do not differ in allele frequencies at the three SNPs considered in this analysis ($p>0.05$ for all SNPs) and none of the interaction analyses were significant (all ps > 0.01, with 21 tests performed), Because of this, we combined the two studies to maximize the statistical power of our primary analyses, however we also present the results from stratified analyses in supplemental tables 1 and 2. In addition, when including a term for study in the models we saw no change in our results.. Thus, we removed the study term from the final models. This approach has been used previously [11] and is possible because of the extensive overlap in the information collected prospectively from the two cohorts and of their management by a single team of investigators. Demographic information on participants is presented in table 1. Within the combined cohort, 404 (24%) of the subjects fulfilled criteria for a diagnosis of AD dementia at some point during the follow-up time.

Cognitive Decline

For the *CR1* locus, we see a significantly faster rate of decline in global cognition with each additional risk allele ($rs6656401^{A}$) (p=0.011). In secondary analyses, this association with the *CR1* locus is still present if we control for *APOE e4* (p=0.02) (Table 2). The effect of the $rs6656401^{A}$ risk allele may be dominant as this model returns a p=0.0008 for the rate of global cognitive decline. In other secondary analyses, we explored the components of our global cognitive measure and found that the *CR1* locus is associated with a faster decline not only in global cognition but also in episodic memory (p=0.003), semantic memory (p=0.05), perceptual speed (p=0.002), and visuospatial ability (p=0.03), (Supplemental table 3). To illustrate these differences in cognitive trajectory, we plotted the paths of change in global cognition during the first ten years of observation: we used the dominant model that best fits our data to compare subjects with $rs6656401^{AA/AG}$ genotypes to those with a $rs6656401^{GG}$ genotype (Figure 1). We can see that, although there is a decline for all subjects, those with any $rs6656401^{A}$ allele have a steeper decline in global cognition when compared to subjects of the $rs6656401^{GG}$ genotype. Similar figures showing a genotypic model and stratified by study are presented in supplementary material (Supplementary Figures 1 and 2). In addition, we saw a 1.4 (95%CI (1.1 – 1.8), p=0.006) increased odds of being diagnosed with clinical AD for those with any $rs6656401^{A}$ allele as compared to subjects with the $rs6656401^{GG}$ genotype. (Supplementary table 4)

For the *PICALM* locus, we saw suggestive evidence of association in the additive model for global cognition with a faster rate of decline for each additional risk allele ($rs7110631^{G}$) (p=0.10). In secondary analyses, the recessive model returns a p=0.03 for global cognition (Table 2). One interesting finding is in the stratified analyses (Supplementary Table 1). The effect we see at the *PICALM* locus is being driven primarily by the MAP cohort, Adjustment for *APOE e4* genotype did not change the results in our combined analyses, however it does affect the MAP specific analysis. For MAP alone we see a significant recessive relationship for $rs7110631^{GG}$ vs. $rs7110631^{CC/CG}$ with a p = 0.009 (Supplementary table 1).

We see no significant relationship between the rate of change in global cognitive performance and the *CLU* locus. Adjustment for *APOE e4* genotype did not change the results of the *CLU* loci (Table 2).

Pathology

We followed up our investigations of cognitive decline with an analysis of traits derived from our autopsy material (n=553): There is an association between the rs6656401^{A} risk allele in the *CR1* locus and greater AD-related neuropathologic burden on autopsy using our default additive model for the effect of the risk allele (p=0.03) (Table 3). In this case, the small sample size precludes a powerful assessment of the minor (risk) allele homozygote class of subjects (rs6656401^{AA}). Nonetheless, we observe a trend in global neuropathology scores when examining each genotype individually in secondary analyses, with the lowest score seen in rs6656401^{GG} class and increasing scores for each additional risk allele (Table 3). When breaking down the global pathology score into its components, we see that the significant relationship between the *CR1* locus and pathology is driven by an effect on diffuse and neuritic plaques (p=0.03 and p=0.01 respectively) but not neurofibrillary tangles (p=0.37). The *APOE e4* allele does not modify the associations presented above (data not shown).

On the other hand, no significant differences were seen in analyses of our measure of global pathologic burden with the *CLU* and the *PICALM* loci (p=0.98 and p=0.34 respectively); this continues to hold true when controlling for the *APOE e4* allele (data not shown). Exploratory analyses of the components of the global pathology score revealed no association between either loci and measures of amyloid deposition or neurofibrillary tangles (data not shown).

Pathway Analyses

Having independent associations of the *CR1* loci with cognitive decline and AD-related neuropathology, we explored whether these associations were consistent with our putative causal chain linking genetic variation and cognitive dysfunction. For the pathway analyses we examined cognitive decline as a function of the *CR1* locus and measures of AD pathology in the same model. The results for *CR1* are presented in Table 4. For this analysis we focus on the slope estimates of cognitive decline and whether controlling for global pathology attenuates the magnitude of the estimate of the risk allele's effect on the rate of global cognitive decline. The slope estimate (Standard Error) for the association of *CR1* with the rate of global cognitive performance drops from −0.030 (0.016) in the model without global pathology to −0.017 (0.015), (a reduction of 43% in effect size), when we adjust for global pathology. A similar pattern is seen with our estimate of episodic memory decline which drops from −0.025 (0.018) to −0.008 (0.017) after controlling for global pathology. This represents a 64% decrease in effect size for episodic memory decline (supplementary table 5). The decline in the estimates suggests that global pathology is in the pathway between rs6656401^{A}, the *CR1* risk allele, and decline in global cognitive function. Looking at the components of our global pathology measure, we see that the effect of the rs6656401^{A} risk allele in the *CR1* locus on cognitive performance is primarily mediated through an effect on neuritic plaques (37% decrease in effect size) with a more modest effect of diffuse plaques (20% decrease in effect size). In our dataset, neurofibrillary tangles have no effect on the correlation between rs6656401^{A} and cognitive decline (Table 4).

DISCUSSION

Three novel susceptibility loci have recently been proposed for AD, but additional studies are needed to confirm these findings and clarify how the risk alleles within these loci exert

their effect. Here, we demonstrate that the risk allele within the *CR1* locus is associated with both a greater burden of AD-related neuropathology and a faster rate of cognitive decline in an aging population that is non-demented at baseline. More specifically, our results suggest that the *CR1* locus has an effect on almost all cognitive domains tested: its effect is not limited to episodic memory. Furthermore, our modeling suggests that this contribution to global cognitive dysfunction is mediated in large part (43% for global cognition and 60% for episodic memory) by an enhanced burden of AD-related neuropathology, particularly amyloid plaques. Nonetheless, our measure of global neuropathology does not fully capture the effect of the *CR1* locus on cognitive function, suggesting that it may also have effects on different, as yet unrecognized mechanisms, or that measures of neuropathology that are molecularly specific may have stronger effects than those seen with histochemistry. Our results are therefore consistent with experiments *in vitro* and in model organisms that suggest a role for *CR1* in clearance of amyloid plaques [34]. These results also establish clear hypotheses relating to the functional consequences of the *CR1* risk allele that can now be explored further in a number of systems and raises the intriguing possibility that this variant may influence the success of different immunotherapies now being tested in AD.

The fact that we have performed these analyses in two large prospective cohorts of subjects that are non-demented on enrollment also generalizes the role of *CR1*: this locus which was discovered in an AD susceptibility scan may have a broader role in affecting cognitive function in older age. Indeed, we demonstrate that it does not have a predilection for affecting episodic memory; instead, it affects almost all of the cognitive domains that we have tested. It may therefore have a broader role in aging-related neurodegenerative processes. This is also supported by the persistence of the association if we restrict the analysis to cognitive assessments that are obtained from subjects that do not have a diagnosis of AD (supplementary table4).

The other two loci that we tested in this study showed no significant evidence of association with either cognitive function or global pathology in our primary analyses. However, the *PICALM* locus displays suggestive evidence of association with cognitive decline. Given our sample size, we can exclude a strong effect of the *PICALM* and *CLU* loci on our outcome measures, but it remains possible that more modest effects exist. These results are consistent with the findings by Guerreiro et al[35]. which showed no common coding variants at the *CLU* loci associated with AD. Neither the *CLU* nor the *PICALM* variant are likely to be the causal allele; they are merely the best surrogate marker tested so far. Thus, it is possible the true causal variant in these or the *CR1* locus could have much stronger effects on our outcomes of interest. The *PICALM* analysis was also limited by the unavailability of the reported SNP (rs3851179) in our dataset; instead, we used a surrogate marker, rs7110631, which has an r^2 of 0.75 with rs3851179 in subjects of European ancestry in HapMap (http://hapmap.ncbi.nlm.nih.gov/).

In modeling the causal chain of events, we took our analysis further and showed that, when controlling for global pathology, the strength of the relationship between *CR1* and cognitive decline decreases. This suggests that global pathology plays an important role in the causal pathway between *CR1* and cognitive decline. When limiting our analysis to only those with autopsy data - thus decreasing our sample size - we found only borderline statistical significance with global cognition that is probably due to limitations in statistical power. Nonetheless, we feel that because (a) we show a significant relationship between *CR1* and the cognition measure when using the larger sample, and (b) the magnitude of the beta estimates clearly decreased when controlling for global pathology that this shows convincing evidence supporting a model in which global pathology mediates the effects of *CR1* on cognition. Further work in model organisms will be necessary to test the hypothesis

that alteration of CR1 function can yield to cognitive dysfunction that is mediated by an effect on amyloid pathology.

Our main analyses considered 3 loci and 2 outcomes, resulting in 6 different tests. Since our primary goal was to replicate previous findings by Lambert et. al[2]. and Harold et. al[1]., we used a nominal level of significance of 0.05 and did not correct for multiple comparisons. We note, however, that our primary finding that CR1 is significantly associated with decline in global cognition under the dominant model still holds when comparing our resulting p-value to a Bonferroni corrected p-value of 0.008 (=0.05/6).

This study has several strengths. First, both cohorts are rich in data, with high rates of follow-up and autopsy thus decreasing bias. Second, the analysis was performed on a prospective cohort of older adults, all of whom were cognitively normal at the time of enrollment; while this design may reduce our power to detect susceptibility genes associated with an earlier age at dementia onset due to differential survivor bias, it permits us to assess associations with cognitive decline and neuropathology unrelated to the subject selection biases in retrospective case-control studies. Third, the standardized nature of both the clinical and pathology procedures produced very similar and reliable measures between the cohorts and allowed us to combine the data and increase our statistical power. Fourth, this study complements and supports findings from a study of an independent group of neuropathologically verified cases and controls, which found associations between *CR1* (as well as *CLU* and *PICALM*) in susceptibility to late-onset AD [3]. Finally, we illustrate the advantages of detailed intermediate phenotypes in understanding the functional consequences of common variants associated with AD. In particular, we provide new insights about the neuropathological features underlying the association between *CR1*, age-related cognitive decline, and the susceptibility to late-onset AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the participants in the Religious Orders Study and the Memory and Aging Project and the staff of the Rush AD Center and the Broad Institute. This work is supported by NIH grants R01 AG30146, R01 AG179917, R01 AG15819, P30 AG10161 and K08 AG034290.

AJM is supported by NIA grant R01 AG034504 and the Johnnie B Byrd Institute. JAH would like to thank the Verum foundation. MJH, JC and EMR are supported by grants from Kronos Life Sciences Laboratories, the National Institute on Aging (Arizona Alzheimer's Disease Center P30 AG19610, RO1 AG023193, Mayo Clinic Alzheimer's Disease Center P50 AG16574 and Intramural Research Program), the National Alzheimer's Coordinating Center (U01 AG016976), and the state of Arizona. Additional funding was obtained from the NIH Neuroscience Blueprint (U24NS051872), the ENDGAME Consortium (UO1HL084744), a National Institute on Aging grant to Carl Cotman (University of California, Irvine, P50 AG23173), National Institute on Aging (K01AG024079) and the state of Arizona.

FUNDING: This work is supported by NIH grants R01 AG30146, R01 AG179917, R01 AG15819 and P30 AG10161.

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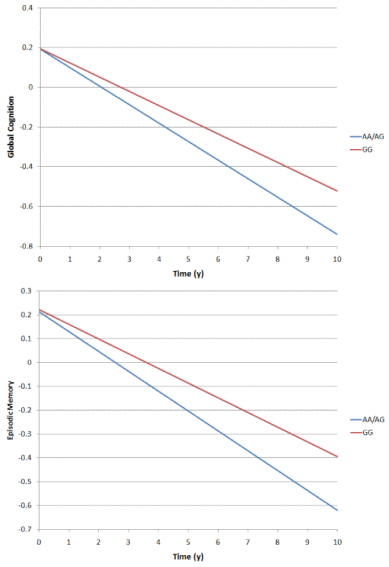

Figure 1. Average paths of 10 year change in global cognition and episodic memory for typical persons for CR1 (rs6656401) using the dominant model

a. Global Cognition (p=0.0008)

b. Episodic Memory (p=0.003)

Ann Neurol. Author manuscript; available in PMC 2012 March 9.

Table 1

Demographics, genotypes, cognitive and pathology information[1] for subjects in the ROS and MAP studies

	ROS (n=791)	MAP (n=875)	p-value		ROS (n=326)	MAP (n=227)	p-value
Age at enrollment	75.5 ± 7.3	81.0 ± 6.7	<0.0001	**Age at enrollment**	79.8 ± 6.9	84.4 ± 5.7	<0.0001
Education (years)	18.1 ± 3.4	14.3 ±3.2	<0.0001	**Education (years)**	18.0 ± 3.4	14.5 ± 2.8	<0.0001
Male Sex	269 (34%)	240 (27%)	0.004	**Male Sex**	124 (38%)	83 (37%)	0.725
Deceased	326 (41%)	227 (26%)	<0.0001	**Age at death**	87.0 ± 6.9	88.8 ± 5.6	0.0008
Years of follow-up	7.8 ±4.5	4.3 ± 2.6	<0.0001	**Years of follow-up**	6.7 ± 3.8	3.8 ± 2.6	<0.0001
Clinical diagnosis of AD	218 (28%)	186 (21%)	0.0001	**Pathology diagnosis of AD**	135 (41%)	85 (37%)	0.349
Genotypes				**Genotypes**			
CLU (rs11136000)				**CLU (rs11136000)**			
TT	122 (16%)	141 (16%)	0.152	**TT**	37 (12%)	35 (15%)	0.393
CT	358 (46%)	437 (50%)		**CT**	167 (54%)	123 (54%)	
CC	296 (38%)	293 (34%)		**CC**	107 (34%)	69 (30%)	
PICALM (rs7110631)				**PICALM (rs7110631)**			
CC	63 (8%)	87 (10%)	0.105	**CC**	29 (9%)	17 (8%)	0.529
CG	352 (45%)	401 (46%)		**CG**	151 (47%)	104 (47%)	
GG	373 (47%)	380 (44%)		**GG**	143 (44%)	101 (46%)	
CR1 (rs6656401)				**CR1 (rs6656401)**			
AA	28 (4%)	27 (3%)	0.215	**AA**	7 (2%)	2 (1%)	0.193
AG	261 (33%)	254 (30%)		**AG**	112 (35%)	64 (29%)	
GG	493 (63%)	575 (67%)		**GG**	203 (63%)	152 (70%)	
Cognition at baseline				**Pathology**			
Global Cognition	0.14 (0.60)	−0.05 (0.64)	<0.0001	**Global AD Pathology**	0.74 (0.39)	0.770 (0.39)	0.201
Episodic Memory	0.16 (0.71)	−0.11 (0.83)	<0.0001	**Diffuse Plaques**	0.75 (0.52)	0.62 (0.51)	0.004
Semantic Memory	0.12 (0.72)	0.02 (0.71)	0.004	**Neuritic Plaques**	0.73 (0.54)	0.69 (0.54)	0.334

	ROS (n=791)	MAP (n=875)	p-value		ROS (n=326)	MAP (n=227)	p-value
Working Memory	0.07 (0.79)	0.03 (0.73)	0.247	**Neurofibrillary Tangles**	0.59 (0.37)	0.61 (0.42)	0.594
Perceptual Speed	0.20 (0.88)	−0.08 (0.88)	<0.0001				
Visuospatial Ability	0.11 (0.80)	0.05 (0.79)	0.117				

[1] n=553 deceased subjects who passed the genotyping quality control

Table 2

Relationship between genes and change in global cognition over time in the 1666 ROS and MAP subjects

		Model 1[1]		Model 2[2]	
	n	Global Cognition	p-value	Global Cognition	p-value
CLU (rs11136000)					
Additive		−0.002 (0.005)	0.737	0.001 (0.005)	0.837
PICALM (rs7110631)					
Additive		−0.008 (0.005)	0.099	−0.008 (0.005)	0.103
CC/CG	903	0 (ref)		0 (ref)	
Recessive, GG	753	−0.014 (0.006)	0.034	−0.014 (0.007)	0.033
CR1 (rs6656401)					
Additive		−0.015 (0.006)	0.011	−0.014 (0.006)	0.022
GG	1068	0 (ref)		0 (ref)	
Dominant, AG/AA	570	−0.023 (0.007)	0.0008	−0.021 (0.007)	0.002

[1]Model 1: adjusted for age at baseline, sex and years of education

[2]Model 2: adjusted for age at baseline, sex, years of education and *APOE ε4*

Table 3

Association between loci and global pathology and its components among 553 deceased subjects[1]

	Global Pathology[2]		Diffuse Plaques[2]		Neuritic Plaques[2]		Neurofibrillary Tangles[2]	
	estimate (SE)	**p-value**	**estimate (SE)**	**p-value**	**estimate (SE)**	**p-value**	**estimate (SE)**	**p-value**
***CLU* (rs11136000)**								
β (SE)	−0.001 (0.026)	*0.975*	0.023 (0.035)	*0.513*	0.017 (0.035)	*0.642*	−0.089 (0.083)	*0.280*
***PICALM* (rs7110631)**								
β (SE)	0.025 (0.026)	*0.343*	0.009 (0.035)	*0.790*	0.039 (0.036)	*0.281*	0.076 (0.085)	*0.372*
***CR1* (rs6656401)**								
β (SE)	0.073 (0.032)	*0.025*	0.095 (0.043)	*0.029*	0.117 (0.044)	*0.009*	0.093 (0.104)	*0.373*
Adjusted Mean (SE)								
GG (n=355)	0.701 (0.397)	*0.079*	0.664 (0.524)	*0.082*	0.681 (0.542)	*0.029*	0.588 (0.394)	*0.591*
AG (n=176)	0.766 (0.381)		0.743 (0.505)		0.784 (0.519)		0.618 (0.396)	
AA (n=9)	0.895 (0.377)		0.961 (0.641)		1.010 (0.520)		0.561 (0.259)	

[1] adjusted for age at death, sex and years of education.

[2] Measures are square root of global pathology, diffuse and neuritic plaques and neurofibrillary tangles.

Table 4

Pathway analysis for global pathology, plaques and tangles affect on the association between *CR1* and global cognition among the 553 deceased subjects

CR1 (rs6656401)		Global Cognition	
Model 1	**n**	**Model 1[1]**	**p-value**
Additive		−0.026 (0.015)	0.084
GG	355	0 (ref)	
Dominant, AG/AA	185	−0.030 (0.016)	0.064

Global Pathology & Neurofibrillary Tangles	n	Model 1 + Global Pathology[1]	p-value	Model 1 + Neurofibrillary Tangles[2]	p-value
Additive		−0.010 (0.014)	0.458	−0.023 (0.014)	0.097
GG	355	0 (ref)		0 (ref)	
Dominant, AG/AA	185	−0.017 (0.015)	0.266	−0.027 (0.015)	0.077

Plaques	n	Model 1 + Diffuse Plaques[2]	p-value	Model 1 + Neuritic Plaques[2]	p-value
Additive		−0.020 (0.014)	0.176	−0.015 (0.014)	0.281
GG	355	0 (ref)		0 (ref)	
Dominant, AG/AA	185	−0.024 (0.015)	0.126	−0.019 (0.015)	0.206

[1] Model 1: adjusted for age at death, sex and years of education

[2] Model 1 + pathology measure: adjusted for age at death, sex, years of education and square root of pathology measure