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Evaluation of Memory Endophenotypes for Association with *CLU*, *CR1* and *PICALM* variants in African-American and Caucasian Subjects

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Abstract

Background—Genetic variants at the *CLU*, *CR1* and *PICALM* loci associate with risk for late-onset Alzheimer's disease (LOAD) in genome-wide association studies (GWAS). In this study, our aim was to determine whether the LOAD risk variants at these three loci influence memory endophenotypes in African-American and Caucasian subjects.

Methods—We pursued an association study between single nucleotide polymorphism (SNP) genotypes at the *CLU*, *CR1* and *PICALM* loci and memory endophenotypes. We assessed African-American subjects (AA: 44 with LOAD, 224 controls) recruited at Mayo Clinic Florida and Caucasians recruited at Mayo Clinic Minnesota (RS: 372 with LOAD, 1,690 controls) and Florida (JS: 60 with LOAD, 529 controls). SNPs at the LOAD risk loci *CLU* (rs11136000), *CR1* (rs6656401, rs3818361) and *PICALM* (rs3851179) were genotyped and tested for association with Logical Memory immediate recall (LMIR), delayed recall (LMDR) and percent retention (LMPR) and Visual Reproduction (VRIR, VRDR, VRPR) scores from Wechsler Memory Scale-Revised, using multivariable linear regression analysis, adjusting for age-at-exam, sex, education and *APOE* ε4 dosage.

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Results—We identified nominally significant or suggestive associations between the LOAD risky *CR1* variants and worse LMIR scores in the African-Americans (p=0.068 - 0.046, β= −2.7 to −1.2). The LOAD protective *CLU* variant is associated with better logical memory endophenotypes in the Caucasian subjects (p=0.099-0.027, β= 0.31 to 0.93). The *CR1* associations persisted when the control subjects from the African-American series were assessed separately. The *CLU* associations appeared to be driven by one of the Caucasian series (RS) and were also observed when the control subset from RS was analyzed.

Conclusion—These results suggest for the first time that LOAD risk variants at *CR1* may influence memory endophenotypes in African-Americans. Additionally, *CLU* LOAD protective variant may confer enhanced memory in Caucasians. Although these results would not remain significant after stringent corrections for multiple testing, they need to be considered in the context of the LOAD associations, with which they have biological consistency. They also provide estimates for effect sizes on memory endophenotypes that could guide future studies. The detection of memory effects for these variants in clinically normal subjects, implies that these LOAD risk loci might modify memory prior to clinical diagnosis of AD.

BACKGROUND

Recent genome wide association studies of late-onset Alzheimer's disease (LOAD) case-control series identified single nucleotide polymorphisms (SNPs) at nine loci which associate with disease risk[1–5]. Understanding the mechanism of action of these variants or functional variants tagged by these SNPs and determining their contribution to LOAD is an important next step in translating these findings to potential drug targets and predictive biomarkers for this disease. Using endophenotypes as outcomes may be a powerful approach in testing the effects of disease risk variants on quantitative, biologically relevant outcomes[6–9], which may provide valuable information regarding downstream biological consequence of such genetic variation. Further, genetic variants may display stronger association with the intermediate endophenotypes than the disease phenotype, and may be useful in detecting novel disease risk loci. Finally, if disease risk variants influence endophenotypes prior to onset of clinical disease, then these quantitative variables may be used as biomarkers in predicting disease course and onset.

Cognitive measures are proposed as highly relevant endophenotypes for neuropsychiatric conditions[6, 8], including AD[10]. A conceptual model of AD pathogenesis posits that dynamic changes in cognition occur prior to clinical diagnosis of AD[11]. If correct, then risk factors, including genetic variants that influence AD risk should also associate with cognitive endophenotypes and these associations should be detected prior to the diagnosis of dementia. Likewise, genetic factors that influence cognitive endophenotypes should harbor variants that confer AD risk. This model, if accurate, will enable confirmation of candidate AD risk variants for their role in cognition, characterization of their mechanism of action for specific cognitive abilities and may lead to identification of novel genetic risk factors. Indeed, there is ample proof of principle for this model from studies investigating the influence of *APOE*, the strongest known LOAD genetic risk factor, on cognitive endophenotypes, which identified a stronger effect on cognition than disease risk[10] and one that could be detected prior to development of clinical AD[12, 13].

With the identification of additional risk variants from the large LOAD GWAS[1–5], similar studies are beginning to emerge for these novel genetic factors. Chibnik et al. investigated SNPs at the *CLU*, *CR1* and *PICALM* loci and identified significant associations between the LOAD risk allele of *CR1* rs6656401 SNP and faster global cognitive decline, as well as increased amyloid pathology, in a study of two longitudinal, Caucasian cohorts[14]. This group subsequently identified a coding *CR1* variant, rs4844609, in tight linkage disequilibrium (LD) with the LOAD GWAS SNP (rs6656401) and which associated with

faster episodic memory decline, increased AD neuropathology and risk[15]. Barral et al. assessed 2-SNP genotypes at *BIN1*, *CLU*, *CR1* and *PICALM* loci with or without *APOE* for their influence on episodic memory in a Caucasian, family-based sample and identified several genotype patterns with significant association, some of which were also significant in their unaffected subset of subjects[16]. In that study, the combination of the LOAD risky *PICALM* genotypes with either the LOAD protective *CLU*, *CR1* or *BIN1* genotypes associated with worse episodic memory estimates, rendering biologically-consistent interpretations difficult. Hamilton et al. assessed two Scottish cohorts without dementia for 158 SNPs from 11 genes, including *BIN1*, *CLU*, *CR1* and *PICALM*, and detected an interaction between a *BIN1* and an *APP* SNP which influenced Logical Memory scores in the *APOE* ε4 positive subset of one of their cohorts[17]. Finally, Verhaaren et al. constructed a risk score using genotypes of the nine LOAD GWAS variants and assessed their effects on baseline cognitive endophenotypes in a non-demented population-based cohort from the Netherlands[18]. They found only a marginal influence of the joint effect of the LOAD GWAS variants on memory, above *APOE*.

In our study we aimed to assess the influence of the LOAD GWAS variants at the *CLU*, *CR1* and *PICALM* loci on episodic memory in one African-American and two Caucasian series from Mayo Clinic. These variants were the first to be identified from the LOAD GWAS[1, 2] and were therefore the focus of our study. Given that to date all of the studies of cognitive endophenotypes and LOAD GWAS variants are focused on Caucasian subjects, one of our goals was to investigate these effects in a non-Caucasian series. Second, we sought to evaluate six related cognitive scores from two types of episodic memory domains as separate endophenotypes rather than a single combined score. Third, we aimed to replicate any of the previously reported cognitive endophenotype associations or identify new ones in two Caucasian series, for these three LOAD GWAS loci. Our findings support a role for *CR1* and *CLU* loci variants in influencing episodic memory in African-Americans and Caucasians, respectively.

METHODS

Subjects

Study participants were selected from three established Mayo Clinic Alzheimer's disease case-control series. Subjects included Caucasian LOAD patients with an age of diagnosis greater than 60 years and elderly controls older than 60 at the time of testing, as well as African-American LOAD patients who were slightly younger at age of onset, compared to Caucasians (mean=78.9, range=52.2–91.2) and elderly controls (mean=78.7, range = 60.5–96.4) (Table 1). All subjects were diagnosed by a Mayo Clinic neurologist and underwent neuropsychological testing. The African-American case-control series (AA) was collected at Mayo Clinic Florida in Jacksonville, where the cases were participants in the Mayo Clinic Alzheimer's Disease Research Center and the controls were cognitively normal volunteers recruited at local churches and community centers. Two case control series recruited at Mayo Clinic Minnesota in Rochester (RS) and Mayo Clinic Florida in Jacksonville (JS) were composed of North American Caucasian adults. The RS cases and controls were participants in either the Mayo Clinic Alzheimer's Disease Research Center or the population-based Mayo Clinic Study of Aging series[19]. The JS cases were recruited either as part of the Mayo Clinic Alzheimer's Disease Research Center or were clinically diagnosed AD subjects from Mayo Clinic Florida, Department of Neurology. The JS controls were volunteers recruited from retirement communities around Jacksonville, Florida. All controls had a clinical dementia rating (CDR) score of 0 at the most recent time of testing and all LOAD cases had a diagnosis of probable or possible AD according to NINCDS-ADRDA criteria[20]. Additional series details are provided in Table 1. This study was approved by the

Mayo Clinic institutional review board and informed consent was obtained from all participants.

Memory Endophenotypes

Verbal and nonverbal episodic memory were evaluated using phenotypes from the Logical Memory (LM) and Visual Reproduction (VR) subtests from the Wechsler Memory Scale-Revised[21]. Specifically, raw scores from the immediate recall (LMIR, VRIR) and 30-minute delayed recall trials (LMDR, VRDR) were evaluated for association with each single nucleotide polymorphism (SNP). We also evaluated the percent of verbal or nonverbal material retained over the 30-minute interval (LMPR, VRPR). Logical Memory and Visual Reproduction were administered using standardized instructions. All SNPs were assessed for association with these memory endophenotypes measured at each subject's most recent (last or proximal) visit. The descriptive statistics for these memory endophenotypes are depicted in Supplementary Table 1.

Genotyping

Genotypes for the four SNPs, rs11136000 (*CLU*), rs3818361 (*CR1*), rs6656401 (*CR1*) and rs3851179 (*PICALM*) were obtained by one of two approaches. Subjects from all series were genotyped for rs6656401 using Taqman® technology. A subset of the subjects from the RS (198 LOADs and 667 controls) and JS (33 LOADs and 230 controls) series were participants in the Mayo Clinic LOAD GWAS[22]. These subjects had genotypes for the rs11136000, rs3818361 and rs3851179 SNPs that were extracted from the GWAS genotypes. The remaining subjects from the RS (352 LOADs and 1,797 controls) and JS (33 LOADs and 427 controls), as well as all African-American subjects were genotyped for these three SNPs using Taqman® assays. The descriptive statistics for the genotyped SNPs are shown in Supplementary Table 2.

Statistical analyses

Each of the four SNPs were tested for association with the six memory endophenotypes obtained at the most recent visit for each subject, using multivariate linear regression analysis implemented in PLINK[23]. An additive model was employed for each SNP, where the dosage effect of each minor allele was evaluated, while controlling for the covariates, which were sex, age-at-examination, years of education, and number of APOE ε4 alleles for all analyses. When both LOADs and controls (All) were analyzed together an additional term for diagnosis was also included (LOAD = 1, control = 0). When both Caucasian series were analyzed together a further term was included in the model for series (JS=1, RS=0). In all analyses with the African-American series, we also included a reading score, the Reading subtest from the Wide-Range Achievement Test-3[24], as a proxy for quality of education[25, 26].

Two sided, unpaired t-tests assuming unequal variances were conducted to compare age at test, years of education (Table 1) and cognitive scores (Supplementary Table 1) of the largest RS control series with each of the other series, using StatsDirect v2.7.8. Chi-squared test was used to compare APOE4 dose and gender between the RS controls and each of the other series (Table 1).

Power calculations were done for the Caucasian and African-American series separately, for sample sizes of 2,500 and 250, respectively, as approximations of the largest sample sizes for each ethnic group, and for 2,000 and 500, reflecting sample sizes for the Caucasian RS and JS series, respectively. Minor allele frequencies were chosen to reflect those of the four tested SNPs for each ethnic group and standard effect sizes for a range of 0.05–1 were utilized. Standardized effect sizes refer to the average increase (or decrease) in memory

score with an increase in one copy of the minor allele divided by the standard deviation of the memory score. Power estimates were based on simulations with linear regression and additive effect for minor allele using $\alpha<0.05$. Given the six memory endophenotypes tested with four SNPs, an uncorrected p value of 0.002 is required to achieve significance (24 tests, p required = 0.05/24 = 0.002), assuming completely independent tests. Since the memory endophenotypes are expected to correlate with each other; the two CR1 SNPs are in LD and given our study needs to be considered in the context of the prior LOAD GWAS findings, we focused on an $\alpha<0.05$ for our power calculations and results.

Box plots for the cognitive scores vs. SNP genotypes, which were generated using the functionalities in R. Box plots, represent the residuals after accounting for the effects of covariates on the cognitive scores.

RESULTS

We had 268 African-American (44 LOAD, 224 controls) and a total of 2,651 Caucasian (432 LOAD, 2,219 controls) subjects with episodic memory endophenotypes (Table 1, Supplementary Table 1). Based on our power estimates, assuming a sample size of 250 African-Americans, minor allele frequencies (MAF) of 0.01, 0.1 or 0.4 (Supplementary Table 2), we expect to have 10%, 51% and 90% power, respectively, to detect a standardized effect size of 0.3 at $\alpha=0.05$. For 2,500 or 2,000 Caucasians and MAF of 0.2 or 0.4, we can expect >99% power to detect a standardized effect size >0.2 (Supplementary Table 3). Under the same assumptions, a sample size of 500 will yield 72% or 87% power respectively for MAF of 0.2 or 0.4.

The APOE ε4 positive genotype frequencies were higher in the LOAD subjects in all three series (AA, RS, JS), as expected (Table 1). The genotype frequencies for APOE fall within the expected range for the different series[27]. The mean education was lowest in the AA series, followed by RS and JS series. Comparison of education years for each group, compared to the largest group, RS control subjects, revealed marginal to significant differences for lower education in the AA series and RS LOAD subjects and higher education for JS series. The years of education was also slightly lower in the LOAD subjects vs. controls from within the two Caucasian series, but not the African-American series. There were fewer male than female participants across all series and diagnoses, with lowest male frequencies in the AA controls (23%) and LOADs (25%) and highest in the RS controls (48%). Compared to the RS controls, RS LOAD subjects were slightly older and all other groups were somewhat younger.

Compared to the controls, the LOAD subjects had lower (worse) scores for all memory endophenotypes as expected (Supplementary Table 1). When the largest RS controls were used as the comparison group, the African-American controls had lower and JS controls had higher cognitive scores, in the same order as years of education.

Our main analyses were confined to the largest possible African-American (i.e. LOAD cases + controls) and Caucasian (i.e. RS and JS combined with LOAD cases + controls) series. Table 1 depicts the results of these main analyses and Figure 1 contains the forest plots for the effect sizes of all SNPs tested for each memory endophenotype. In the analysis of the AA series, minor alleles of the *CR1* locus SNPs rs6656401 and rs3818361 were both associated with worse LMIR scores ($\beta= -2.7$, p=0.068 and $\beta= -1.2$, p=0.046), respectively (Table 2, Supplementary Figure 1). The associations with the LMDR scores also showed similar trends for these two SNPs in the AA series ($\beta= -0.80$ to -2.43), however these results did not achieve significance (p=0.125–0.211). Similarly, although the LMPR scores for both SNPs had lower score estimates, these findings were not significant. As a secondary

analysis, we also assessed the subset of AA subjects who were clinically non-demented, separately and determined that LMIR scores were also lower in the carriers of the *CR1* risk allele who are controls (Supplementary Table 4, Supplementary Figure 2).

Only about a third of the AA subjects who obtained the Logical Memory test also completed Visual Reproduction. Therefore, although the results from the latter are also shown, it is not possible to do a direct comparison for the two groups of memory endophenotypes in this series (Table 2). For the same reason, although there is a significant association with better VRPR scores for the *CR1* rs3818361 SNP in all AA subjects (Table 2) and a marginal trend in their control subset (Supplementary Table 4), given the small sample sizes for these tests (n=57–88) and the absence of consistent results from other memory endophenotypes or the *CR1* rs6656401 SNP, the validity of these VRPR endophenotype findings are questionable.

When the four SNPs for the three LOAD GWAS loci were assessed for association with memory endophenotypes in the Caucasian subjects, the LOAD-protective *CLU* rs11136000 minor allele showed association with higher (better) scores for the LMDR test (β=0.45, p=0.027) and similar trends for the immediate recall (β=0.31, p=0.099) and percent retention (β=0.93, p=0.081) endophenotypes (Table 2, Supplementary Figure 3). As a secondary analysis we assessed each Caucasian series (Supplementary Table 5) and control subjects (Supplementary Table 6), separately. In the RS series (LOAD + control), the LMIR and LMDR scores were significantly higher in the *CLU* rs11136000 LOAD-protective minor allele carriers, with suggestive trends for LMPR (Supplementary Table 5, Supplementary Figure 3), though no suggestive or significant results were observed for the JS series. The same trends were also observed for the control subset from the RS series with significant LMDR and suggestive LMIR and LMPR associations with *CLU* rs11136000 (Supplementary Table 6, Supplementary Figure 3). Again, no memory endophenotype associations were observed for the JS series.

The forest plots of the effect sizes for each SNP and memory endophenotype is depicted for the Caucasians (red) and African-Americans (blue) in Figure 1. The suggestive or significant *CR1* SNPs in the African-American series, demonstrate trends in the opposite direction for the Caucasians. Similarly, *CLU* rs11136000 SNP with memory-enhancing associations in the Caucasians have non-significant trends in the opposite direction for the African-Americans. These results could be due to heterogeneity between these cohorts due to different functional variants, environmental effects, gene-gene, gene-environment interactions, inaccurate effect sizes due to low power or a combination of these factors. These results highlight the importance of assessing different ethnic groups, as there may be common as well as different disease variants that influence such populations.

DISCUSSION

In this study we assessed associations between four SNPs from three loci previously identified by the first large LOAD GWAS[1, 2] and episodic memory endophenotypes. The underlying hypothesis is that genetic variants that confer risk of LOAD, which is typically characterized by memory dysfunction, will also influence cognitive endophenotypes. We tested six memory endophenotypes and four SNPs. If these tests were completely independent, our results would not hold up to multiple testing (24 tests, p required = 0.05/24 = 0.002). The memory endophenotypes, however are expected to correlate with each other. Furthermore, *CR1* 6656401 and rs3818361 are in LD, but were tested individually to allow for comparison with the available LOAD association studies which use one or the other. Given these aspects and the fact that our study needs to be considered in the context of the prior evidence for these three loci in LOAD risk, we focused on those associations that have nominal significance at p≤0.05. Although we did not find significant associations besides

CR1 in the AA and *CLU* in the Caucasian series, this could be due to low power, testing of markers and not the functional variant, series-specific differences (locus or allelic heterogeneity) and/or uncaptured environmental or gene-gene interaction effects. The AA series composed of at most 260 subjects have 20–60% power to detect the *CR1* cognition association (Supplementary Table 3). Our Caucasian series of ~2,500 subjects have 98% power to detect the *CLU* cognition association,. Nonetheless, given the "winner's curse" phenomenon[28], larger sample sizes are likely required to detect these effects in future studies. The results from our studies can be utilized to guide future, larger studies to test the memory effects for *CR1* and *CLU* suggested by our paper.

An important novel aspect of our study is the simultaneous investigation of this hypothesis in both Caucasian and African-American subjects. To our knowledge, this is the first study that shows associations between LOAD risk variants at the *CR1* locus and memory endophenotypes in African-American subjects. The risky allele of both *CR1* rs6656401 and rs3818361 variants were associated with lower (worse) memory scores, especially for immediate recall phenotypes. Although verbal delayed recall and percent retention phenotypes did not attain statistical significance, their direction of association with the *CR1* variants is consistent with the immediate recall results. The association was stronger in the combined subjects, but was also significant or suggestive even when non-demented subjects were assessed separately. These findings are consistent with the biological expectation from a risk variant as well as a prior report, which identified faster global cognitive decline with the risky allele of the *CR1* rs6656401 SNP in Caucasian cohorts[14]. Our results suggest that the *CR1* locus variants may influence memory endophenotypes in a way that can be detected prior to clinical diagnosis of dementia, consistent with the prior report on *CR1*[14] as well as studies on the influence of *APOE* on cognition in non-demented series[12, 13]. Given the small size of our African-American cohort, these findings require replication. Nevertheless, they suggest that the cognitive effect of the *CR1* locus previously obtained in Caucasian subjects may generalize to other ethnic groups. Though we did not identify significant associations with *CLU* or *PICALM* variants, this could be secondary to our small sample size in the African-American series and should be re-investigated in larger cohorts.

We also evaluated two Caucasian series, collectively composed of ~2,500 subjects. We identified higher (better) episodic memory scores for the *CLU* rs11136000 LOAD protective variant in the RS series. This association is biologically congruent and adds to the various lines of evidence for a protective role of *CLU* in AD. Functional evidence suggests multiple potentially protective roles in LOAD for clusterin, encoded by *CLU*, including Aβ clearance, mitigation of excess inflammation and apoptosis, and clearing of neuronal debris[29]. In an expression GWAS of brain transcript levels, our group recently determined that the *CLU* rs11136000 protective allele associates with higher brain *CLU* levels[30], a finding also corroborated by others[31]. Thus, an emerging hypothesis is that this SNP, or more likely a functional variant(s) that is in linkage disequilibrium (LD) with it increases brain *CLU* levels, which confers a protective effect on cognition that can be detected prior to onset of clinical dementia, and ultimately decreases LOAD risk. It will be important to replicate the effect of this SNP on cognition. We observed this effect in the RS, but not the JS series. A potential reason for this discrepancy may be that the JS series is ~0.3 times the size of the RS series. Other differences are that the RS series is older, has lower education and worse memory scores compared to the JS series. If the cognitive effects of this variant become more pronounced with aging and in the context of unfavorable environmental effects, such as lower education, then it could have been easier to capture in the RS than JS series. We also note the differences between the recruitment strategies for the subjects in the Caucasian RS and JS series and the African-Americans, which could have contributed to the heterogeneity in the findings from these series.

For both the *CR1* results in the AA and *CLU* results in the Caucasian series, the episodic memory associations were stronger in the LOAD cases and controls combined group than controls-only analyses. This improvement cannot be solely attributed to increased sample sizes, as LOAD subjects constitute less than a quarter of the controls. Likewise, this is unlikely to be an effect of the LOAD diagnosis itself, as we have controlled for this in our model. If we postulate that memory endophenotypes represent a continuum, with LOAD subjects representing the lower end of the spectrum, and that genetic variants influence cognition throughout this spectrum, then inclusion of LOAD subjects will increase the variance of these quantitative endophenotypes and the relative contribution of the genetic variants to this variance, thereby increasing the power to detect the association in the combined group of LOAD and control subjects. Indeed, studies by others on *APOE*[10] and *CR1*[14] showed stronger association with combined LOAD and control series compared to controls alone. The fact that an association can be detected for controls only in our study and others, albeit weaker, suggests that these genetic variants indeed influence cognition and that the memory associations are not a mere reflection of LOAD risk association only.

A shortcoming of our study is that we focused on memory endophenotypes at a single time point, the last available exam, rather than assessing longitudinal outcomes. We opted for a cross-sectional analysis to maximize our sample size, as not all subjects had longitudinal testing and many subjects, especially from the AA cohort, had a single cognitive assessment. We focused on the last available examination as the most accurate assessment of each individual's latest cognitive state corresponding to their diagnosis at the time and also to increase our ability to capture memory endophenotype changes secondary to genetic factors, based on the premise that such changes become more pronounced with aging. Indeed, in the longitudinal Chibnik et al. study, the cognitive score differences between the different *CR1* genotypes are much smaller at baseline, compared to last examination[14]. This may be the reason that assessment of baseline cognitive endophenotype associations in a large population-based cohort from the Netherlands revealed only marginal associations with LOAD GWAS variants[18]. It may be that in the absence of longitudinal data on many subjects within cohorts, using the last available examination, while carefully controlling for age and education, is the next, most powerful approach to capture associations with cognitive phenotypes.

Contrasting the asssociations obtained from the Logical Memory (LM) versus Visual Reproduction (VR) tests in our Caucasian series, we found a significant association between LM phenotypes and the *CLU* SNP, but no significant findings for any phenotype from VR. This may suggest that episodic memory as captured by LM is either more susceptible to genetic variation than VR or that it is a more sensitive test in detecting more subtle cognitive changes. Alternatively, *CLU* variants may have a role in verbal but not nonverbal memory.

In summary, we provide here, for the first time, evidence of an effect of the LOAD risky *CR1* locus on memory endophenotypes in African-Americans. We also demonstrate better memory endophenotypes for the LOAD protective *CLU* variant rs11136000 in a Caucasian series. These findings provide further support for the role of these loci in LOAD through mechanisms that influence cognition prior to development of clinical dementia. These results have implications for the utility of genetic variants and cognitive endophenotypes in studies of the mechanism of action of these factors as well as their potential future application in disease prediction paradigms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Systematic Review

In this study, our aim was to determine whether the LOAD risk variants at three genome-wide association study (GWAS) loci for late-onset Alzheimer's disease (LOAD) influence memory endophenotypes in African-American and Caucasian subjects. We reviewed the literature for the novel LOAD GWAS loci and memory endophenotype associations, and detected several papers which identified associations in Caucasian cohorts for some of these loci.

Interpretations

Our study identifies for the first time associations between LOAD-risky *CR1* variants and worse Logical Memory scores in African-Americans. We also describe associations between the LOAD-protective *CLU* variant and better Logical Memory scores in Caucasians. The memory effects for both loci could be detected in both the entire cohorts which include both LOAD cases and controls, and also in just the control subsets.

Future Directions

These findings need to be confirmed in well-powered additional studies and also tested with longitudinal memory endophenotypes.

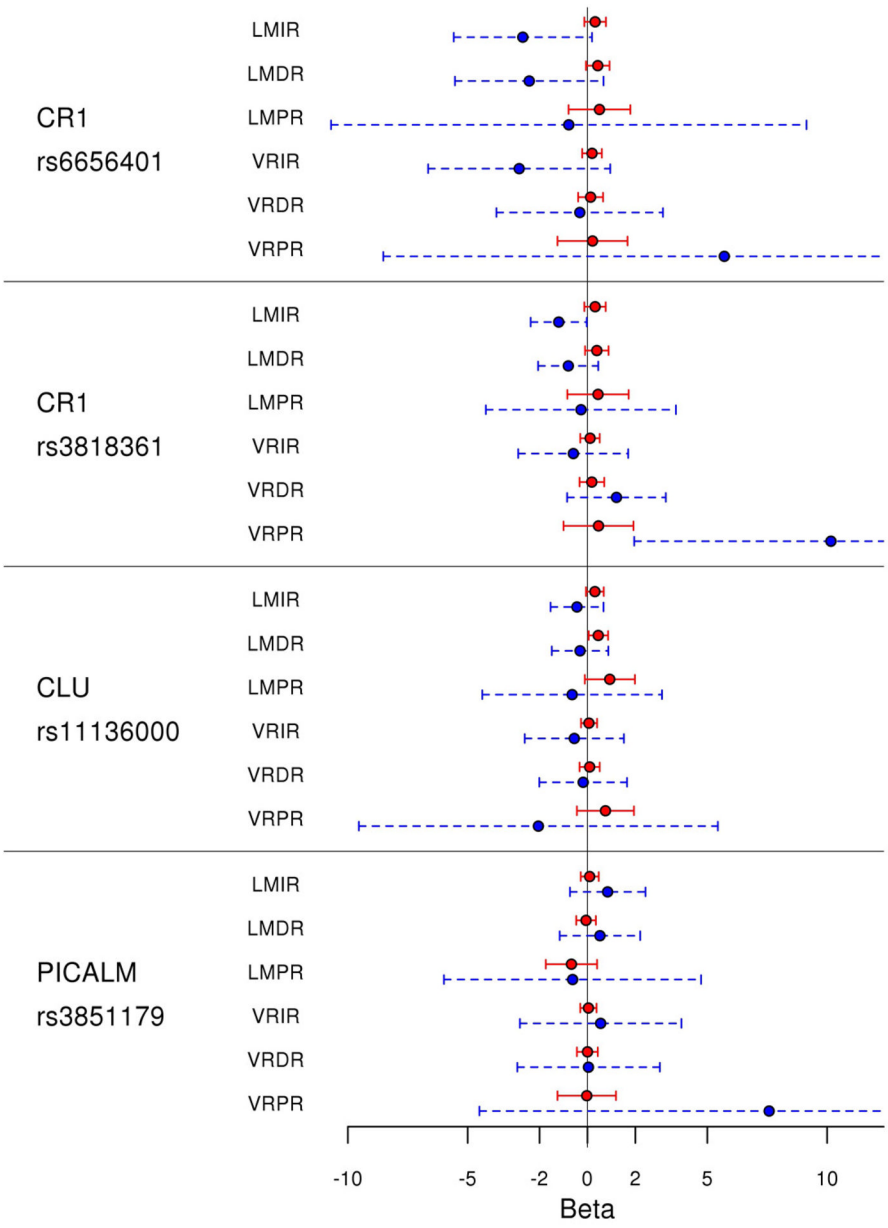

Figure 1. Forest plot of effect size for CLU, CR1 and PICALM loci variants and memory endophenotypes

The circles represent the β coefficient of variation from the multivariable linear regression analysis results shown in Table 2. The lines represent the 95% confidence intervals (CI). The blue figures denote the effects for the African-Americans and the red figures are for the Caucasians. Some of the confidence intervals in the African-American group are truncated due to large CI.

Table 1

Demographic characteristics of the series. Number of subjects (N); number and percentage of male participants; number and percentage of subjects with 0 (ApoE X/X), 1 (ApoE X/4) or 2 (ApoE 4/4) copies of the APOE ε4 allele, mean age at last Logical Memory test (standard deviation =SD) and mean number of years of education are shown. More subjects were tested for Logical Memory than Visual Reproduction, therefore the mean age at former test was used. Two-sided unpaired t-test assuming unequal variances was used to test significance of difference between at test or education years for each group against the largest RS control group. Differences in gender and *APOE* 4 dosage between the RS control and other groups was determined by chi-squared test.

Series (Diagnosis)	N	Male (%)	p (Male)	ApoE X/X (%)	ApoE X/4 (%)	ApoE 4/4 (%)	p (ApoE)	Mean Age at Test (SD, Range)	p (Mean Age)	Mean Yrs Education (SD, Range)	p (Education yrs)
African- Americans (AD)	44	11 (25%)	0.0043	11 (25%)	21 (48%)	12 (27%)	<0.0001	78.9 (7.8, 52.2–91.2)	0.0046	12.8 (2.6, 4–20)	0.074
African-Americans (CON)	224	51 (23%)	<0.0001	153 (68%)	65 (29%)	6 (3%)	0.014	78.7 (7.0, 60.5–96.4)	<0.0001	12.6 (3.4, 2–20)	<0.0001
RS (AD)	372	145 (39%)	0.0021	183 (49%)	163 (44%)	26 (7%)	<0.0001	83.2 (6.9, 61.6–101.1)	0.0439	13.3 (3.0, 7–20)	0.0019
RS (Controls)	1690	810 (48%)	NA	1295 (77%)	373 (22%)	22 (1%)	NA	82.4 (5.5, 65.9–100.2)	NA	13.8 (2.9, 5–20)	NA
JS (AD)	60	27 (45%)	0.7529	15 (25%)	38 (63%)	7 (12%)	<0.0001	79.7 (6.1, 62.2–94.9)	0.0011	14.5 (3.0, 8–20)	0.0861
JS (Control)	529	178 (34%)	<0.0001	377 (71%)	138 (26%)	14 (3%)	0.012	78.9 (6.5, 61.6–95.9)	<0.0001	15.1 (2.6, 4–20)	<0.0001

Table 2

Association between *CLU, CR1* and *PICALM* loci variants and memory endophenotypes in an African-American and Caucasian series

Six episodic memory endophenotypes [Logical Memory immediate recall (LMIR), delayed recall (LMDR), and percent retention (LMPR), and Visual Reproduction immediate recall (VRIR), delayed recall (VRDR) and percent retention (VRPR) from the Wechsler Memory Scale-Revised] were used to assess association with four SNPs at three LOAD GWAS loci, using multivariable linear regression analysis assuming an additive model, and correcting for age-at-testing, sex, APOE ε4 dosage, education years and reading score. The number of subjects for each test (N), β coefficients of variation for each copy of the minor allele, standard error (SE) and 95% confidence interval of this effect (95%CI) and p values of association are depicted. Tests were done for the combined non-demented control and LOAD subjects, and include a covariate for LOAD. Caucasian series from Mayo Clinic Minnesota in Rochester (RS) and Mayo Clinic Florida in Jacksonville (JS) were analyzed. P values <0.1 are shown in bold and those <0.05 are shown in bold, red. The minor alleles tested for cognitive score associations and their known effects on LOAD risk are also shown.

	Trait	African American All Subjects				RS-JS-ALL			
		N	Beta (SE)	95% CI	P-value	N	Beta (SE)	95% CI	P-value
CR1 rs6656401 Tested Allele=A (risky)	LMIR	261	−2.7 (1.47)	−5.58 to 0.19	0.068	2,587	0.32 (0.23)	−0.13 – 0.77	0.168
	LMDR	260	−2.43 (1.58)	−5.53 to 0.67	0.125	2,583	0.43 (0.25)	−0.06 – 0.92	0.088
	LMPR	260	−0.78 (5.06)	−10.7 to 9.14	0.878	2,583	0.5 (0.66)	−0.79 – 1.79	0.448
	VRIR	92	−2.85 (1.94)	−6.65 to 0.95	0.145	2,518	0.19 (0.21)	−0.22 – 0.6	0.358
	VRDR	92	−0.32 (1.77)	−3.80 to 3.15	0.855	2,516	0.13 (0.27)	−0.39 – 0.65	0.628
	VRPR	92	5.71 (7.26)	−8.52 to 19.93	0.434	2,516	0.21 (0.75)	−1.25 – 1.67	0.777
CR1 rs3818361 Tested Allele=A (risky)	LMIR	261	−1.2 (0.60)	−2.37 to −0.03	0.046	2,568	0.32 (0.23)	−0.13 – 0.76	0.167
	LMDR	260	−0.80 (0.64)	−2.06 to 0.45	0.211	2,564	0.39 (0.25)	−0.1 – 0.88	0.119
	LMPR	260	−0.27 (2.03)	−4.24 to 3.70	0.892	2,564	0.44 (0.65)	−0.84 – 1.72	0.502
	VRIR	88	−0.59 (1.17)	−2.89 to 1.70	0.613	2,502	0.11 (0.21)	−0.3 – 0.51	0.601
	VRDR	88	1.21 (1.05)	−0.85 to 3.28	0.252	2,500	0.18 (0.26)	−0.33 – 0.7	0.487
	VRPR	88	10.16 (4.19)	1.95 to 18.36	0.018	2,500	0.46 (0.74)	−1 – 1.92	0.534
CLU rs11136000 Tested Allele=C (risky)	LMIR	267	−0.44 (0.56)	−1.54 to 0.67	0.439	2,556	0.31 (0.19)	−0.06 – 0.68	0.099
	LMDR	266	−0.31 (0.60)	−1.49 to 0.87	0.608	2,552	0.45 (0.21)	0.05 – 0.86	0.027
	LMPR	266	−0.64 (1.92)	−4.39 to 3.11	0.738	2,552	0.93 (0.53)	−0.11 – 1.98	0.081
	VRIR	94	−0.55 (1.06)	−2.62 to 1.52	0.603	2,490	0.06 (0.17)	−0.27 – 0.4	0.701
	VRDR	94	−0.18 (0.93)	−2.01 to 1.65	0.847	2,488	0.09 (0.22)	−0.33 – 0.51	0.681
	VRPR	94	−2.05 (3.82)	−9.54 to 5.44	0.593	2,488	0.75 (0.61)	−0.44 – 1.94	0.215
PICALM rs3851179 Tested Allele=A (protective)	LMIR	267	0.84 (0.80)	−0.73 to 2.42	0.297	2,551	0.09 (0.19)	−0.28 – 0.47	0.626

	Trait	African American All Subjects				RS-JS-ALL			
		N	Beta (SE)	95% CI	P-value	N	Beta (SE)	95% CI	P-value
	LMDR	266	0.52 (0.86)	−1.16 to 2.20	0.547	2,547	−0.06 (0.21)	−0.47 – 0.35	0.767
	LMPR	266	−0.62 (2.74)	−5.99 to 4.74	0.820	2,547	−0.67 (0.55)	−1.74 – 0.4	0.223
	VRIR	94	0.55 (1.72)	−2.82 to 3.92	0.749	2,485	0.04 (0.17)	−0.3 – 0.38	0.824
	VRDR	94	0.04 (1.52)	−2.93 to 3.02	0.978	2,483	0 (0.22)	−0.44 – 0.43	0.989
	VRPR	94	7.59 (6.17)	−4.51 to 19.68	0.222	2,483	−0.03 (0.62)	−1.25 – 1.19	0.966