University of Alberta

CLU, CR1 and *PICALM:*

Genotypic Effects on Mild Cognitive Impairment Status and Stability

by

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To my husband, Jesse, who makes me sparkle.

Abstract

Objective: Clinical utility of the Mild Cognitive Impairment (MCI) classification is diminished by uncertainty regarding procedures for detecting preceding transitions from normal aging (NA) and future transitions to Alzheimer's disease (AD). AD genetic markers may clarify underlying neurodegenerative etiology, thereby improving MCI classification. **Method**: Data are from the Victoria Longitudinal Study. We determine if AD-related genotypes [Apolipoprotein E (*APOE*; rs429358, rs7412), Clusterin (*CLU*; rs11136000), Complement Receptor 1 (*CR1;* rs6656401), Phosphatidylinositol Binding Clathrin Assembly Protein (*PICALM;* rs541458)] independently or interactively distinguish (a) MCI (n=101) and NA adults (n=136) at baseline, and (b) longitudinal groups representing two-wave (M=4.5 years) profiles of MCI chronicity and change. **Results**: *CLU* and *APOE* independently predicted baseline MCI. Each gene independently differentiated one combination of longitudinal profiles. The *CR1*(2) x *APOE*(2) interaction differentiated numerous longitudinal profiles. **Discussion**: AD genetic markers are linked with transitions and chronicity involving NA and MCI, and may augment current MCI classification procedures.

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Chapter One: Introduction

Mild Cognitive Impairment (MCI) is a clinical syndrome considered to be a prodrome to Alzheimer's disease (AD). It is now well recognized that the AD diagnosis is preceded by a preclinical period characterized by mild, yet accumulating cognitive deficits, manifesting as a result of increasing AD pathology in the brain (Jack et al., 2010). The MCI diagnosis pertains to individuals with cognitive functioning below that expected based on age and education norms, yet not so affected as to warrant a diagnosis of dementia, or significantly affect social or occupational functioning (Albert et al., 2011). Recent diagnostic guidelines for MCI have been published, outlining the core clinical criteria for MCI (Albert et al., 2011). The basic MCI criteria require (a) below normal cognitive functioning in one or more cognitive domains (typically 1 to 1.5 standard deviations below age and education norms on validated clinical neuropsychological tests), ideally with evidence of (b) a drop in cognitive performance from a previous level (Albert et al., 2011; Winblad et al., 2004). Patients meeting these basic clinical and cognitive criteria for MCI must furthermore exhibit evidence of an etiology consistent with AD pathophysiological processes (Albert et al., 2011).

The importance of the MCI classification is manifold. In clinical settings, identification of individuals with MCI enables lifestyle modification and more aggressive treatment of dementia risk factors (Petersen & Knopman, 2006). Furthermore, the diagnosis enables patients to arrange personal and financial matters before impairment impedes important decision-making capabilities (Petersen & Knopman, 2006). In the future -- as lifestyle, drug and perhaps cognitive training therapies emerge to prevent, delay or reverse the effects of AD -- identification of individuals with as yet early and

perhaps reversible cognitive and neurological changes will be crucial in order to leverage the benefits of new interventions.

There have been multiple specific criteria and classification procedures proposed to identify individuals with MCI-type deficits which vary in their clinical and research utility. Dolcos and colleagues (2012) have used a classification procedure which may be exemplary because it (a) conforms to current clinical and research guidelines which emphasize longitudinal assessment, (b) uses objective, validated, and multi-domain cognitive measures (Albert et al., 2011; Winblad et al., 2004), and (c) has been previously validated in other Victoria Longitudinal Study (VLS) publications (de Frias, Dixon & Strauss, 2009; Dixon et al., 2012; Dixon, Garrett, Lentz, MacDonald, Strauss, & Hultsch, 2007). This multi-stage procedure includes (a) stratification of participants into four age x education subgroups, (b) calculation of within-group mean performance on five cognitive reference measures, and finally (c) classification of participants who score 1 standard deviation or more below their age x education group mean as MCI.

In the future, integrated use of biological markers alongside objective MCI classification procedures may improve the utility of MCI diagnoses by helping to distinguish possible underlying etiologies and at-risk populations (e.g., Risacher et al., 2013). If important clinical considerations such as the particular underlying etiology of MCI-like symptoms can be clarified on a case-by-case basis, overall improvement in the stability of the syndrome, and consequently the prognostic utility of MCI diagnoses may reasonably be expected. Existing biomarker evidence shows progress in several domains: (a) Aβ deposition and biomarkers of brain atrophy (i.e., structural MRI changes), metabolic status (i.e., decreased fluorodeoxyglucose uptake on positron emission

tomography) and tau accumulation (i.e., cerebral spinal fluid tau levels) are all present at abnormal levels in MCI patients (Jack et al., 2010), (b) mass spectrometry-based metabolomic evidence has identified key metabolites in biofluids that can distinguish normal aging from MCI and AD (e.g., Zheng, Dixon & Li, 2012), and (c) AD biomarkers (e.g., T-tau, β-amyloid (Aβ) 42) predict conversion from MCI to AD with high specificity and sensitivity (95% and 83%, respectively; Blom et al., 2009). Reviewers have suggested that genotypic markers may also clarify important etiological considerations (Winblad et al., 2004). The *APOE* gene is strongly associated with MCI (Brainerd, Reyna, Petersen, Smith & Taub, 2011), brain morphological changes (Damoiseaux et al., 2012) and onset of AD (Damoiseaux et al., 2012). MCI patients with the *APOE* ε4 allele have an increased likelihood of transitioning into AD (Blom et al., 2009; Elias-Sonnenschein, Viechtbauer, Ramakers, Verhey, & Visser, 2011). These now well-established findings pertaining to the *APOE* ε4 genotype clearly demonstrate the potential for genetic markers to improve clinical capacity to distinguish among underlying pathophysiological causes for chronic or emerging MCI-like cognitive deterioration.

Recently, *APOE* has been assessed in the context of other aging-related genotypes to determine their independent and interactive associations with MCI status and stability. Dixon and colleagues (2012) assessed AD-related single nucleotide polymorphisms (SNPs) at the *APOE (rs429358, rs7412), BDNF (rs6265)* and *COMT (rs4680)* loci to test (a) baseline associations of the three genotypes with initial cognitive status (MCI or not impaired controls), and (b) longitudinal stability of the provisional baseline classification. The authors observed that only the *APOE* genotype is associated with baseline status, and that neither *BDNF* or *COMT* genotype modify *APOE*'s predictivity for baseline status.

The longitudinal analyses showed that the *APOE* ε4 allele is associated with MCI stability (i.e. chronicity). In addition, the *COMT* genotype is associated with a significant increase in (a) MCI stability (2.6-fold higher likelihood of MCI chronicity in homozygous Val carriers), and (b) transition into MCI (3.94-fold higher likelihood of healthy adults who are homozygous Val carriers transitioning into MCI). These results demonstrate that aging-related genotypes other than *APOE* offer important information on stability and decline characteristics in both healthy older adults and those with early, emerging, and established MCI conditions.

Numerous reviewers have commented on the potential for AD-related genotypes to contribute to understanding current questions regarding AD etiology (Jack et al., 2010; Lambert et al., 2011; Seshadri et al., 2010). By association, it is likely that AD-related genes will also clarify important uncertainties regarding the etiology, and hence stability, of MCI. In recent genome-wide associations studies (GWAS), three SNPs at the Clusterin (*CLU,* rs11136000; otherwise known as Apo J), Complement Receptor 1 (*CR1,* rs6656401), and Phosphatidylinositol Binding Clathrin Assembly Protein (*PICALM,* rs541458) loci were shown to be identified with late onset AD, and these results have subsequently been replicated (Corneveaux et al., 2010; Harold et al., 2009; Lambert et al., 2009; Lambert et al., 2011; Seshadri et al., 2010). *CLU* and the AD-related gene *APOE* have multiple parallel and cooperative roles, including modulation of Aβ toxicity and suppression of Aβ deposition (Harold et al., 2009). Similar to *APOE, CLU* is also likely involved in the clearance of the pathogenic Aβ42 peptide from the brain across the blood brain barrier (Lambert et al., 2009). *CR1* is suspected to be associated with late onset AD due to its influence on Aβ clearance (Lambert et al., 2009). *PICALM* may be

associated with AD as a result of changes in interactions with proteins which function at the synapse (Harold et al., 2009). Deleterious changes occurring at the synapse may be especially important in terms of cognitive outcomes, as it has been shown that changes in synaptic density correlate better with cognitive decline than plaque density in AD patients (Harold et al., 2009).

Relatively few studies have investigated associations between *CLU, CR1* and *PICALM* genotypes and cognitive performance in normal aging. Regarding the *CLU* variant, in a cross-sectional analysis the T allele of the *CLU* SNP was associated with better performance on a cognitive composite comprised of verbal fluency, working memory and episodic memory measures in participants over 90-years of age (Mengel-From, Christensen, McGue, & Christiansen, 2011). In a recent longitudinal study of a heterogeneous sample of patients who had AD, MCI, or were cognitively healthy, the *CLU* T allele was associated with an accelerated rate of decline in global cognition and neurocognitive speed (Sweet et al., 2012), although an earlier GWAS suggested the C variant to be the risk allele (Sweet et al., 2009). This result was not affected by the presence of the *APOE* ε4 allele. Inconsistency in alleles associated with negative cognitive outcomes may have resulted from the heterogeneous clinical sample used in the Sweet and colleagues (2012) study. That is, these seemingly inconsistent findings may reflect the possibility that the *CLU* alleles may differentially affect patients with different cognitive statuses. Results from the Religious Orders Study and the Rush Memory and Aging Project have revealed a risk-increasing effect for the *CR1* A allele, which is manifested in a steeper longitudinal decline in a global cognition composite (Chibnik et al., 2012). This effect persisted after control for the *APOE* ε4 allele. Regarding

PICALM, an earlier midpoint of decline in global cognition was associated with the T allele in a diverse sample of cognitively healthy, AD and MCI patients (Sweet et al., 2012). This result was also not affected by the presence of the *APOE* ε4 allele. To our knowledge, associations with MCI have not been assessed for any of these genes, although this type of analysis represents a natural next step.

The present study assesses *APOE, CLU, CR1* and *PICALM* genotypes to determine their (a) cross-sectional relationship to MCI status (as compared with normal aging), and (b) longitudinal associations with MCI stability (as compared with normal aging stability and selected status transition groups). It uses procedures and a dataset derived from previous research in the VLS. First, the procedure used by Dolcos and colleagues (2012) to identify and track participants with MCI across two waves is adopted for this study. Second, a VLS dataset based on Dolcos and colleagues (2012) but including only genotyped participants is used for this study (Dixon et al., 2012). Third, *CR1, CLU* and *PICALM* genotypes were assessed to determine if they are independently or interactively associated with MCI status. Specifically, MCI patients and normal aging (NA) adults were followed over a four-year (two-wave) longitudinal period. Based on independent assessments at both waves, participants were classified based on stability/change in cognitive status into four groups: NA-to-NA (i.e., stable normal aging status), or NA-to-MCI (i.e., emerging impairment), or MCI-to-MCI (i.e., impairment chronicity) or MCI-to-NA (i.e., improvement/reversion to normal cognitive status).

Using this process of classification, we focus on four research questions. Our plan follows that established by Dolcos and colleagues (2012) and Dixon and colleagues (2012). Notably, following recent trends in candidate gene studies of aging and

neurodegenerative diseases (e.g., Lindenberger, Nagel, Chicherio, Li, Heekeren, & Bäckman, 2008), Dixon and colleagues investigated both independent and interactional associations with cognitive status and stability. Gene-gene interactions have been increasingly assessed in recent years (e.g., Nagel et al., 2008), as reviewers have suggested that neurocognitive phenotypes may emerge from the action of multiple genes (i.e. polygenicity; Kovas & Plomin, 2006), and the magnification of influences represented by interactions among genetic risk alleles. Indeed, the specific contributions of individual AD-related genes to AD etiology may be small; however, interactions among combinations of risk alleles may nevertheless result in clinically important contributions to detecting disease onset and development. Therefore, we assess both independent genetic associations and gene-gene interactions. The goal for computing gene-gene interactions is to assess if the risk of (a) MCI classification at baseline, or (b) conversion to MCI (NA-to-MCI), or (c) MCI chronicity (MCI-to-MCI), or (d) reversion to normal cognitive status (MCI-to-NA) over the two waves is magnified by the combined presence of risk alleles across two separate loci. Research question one will concentrate on baseline (i.e., W1) MCI status. Specifically, we use W1 cross-sectional data to test if *CLU, CR1* and *PICALM* SNPs uniquely or interactively predict baseline status (i.e., NA or MCI). Research question two assesses if *CLU, CR1* and *PICALM* SNPs interact with *APOE* in two-way combinations to predict W1 baseline status. Research question three focuses on four longitudinal profiles of cognitive status. We test if 2-way combinations of longitudinal profiles may be differentiated based on participant's genotype at the *CLU, CR1* and *PICALM* loci or specific two-way combinations of respective risk alleles. Research question four assesses if these same

longitudinal profiles may be distinguished by 2-way interactions between *CLU*, *CR1* or *PICALM* with *APOE* status.

Chapter Two: Method

2.1 Participants

This research takes place in the context of full, active and continuous human ethics approval from prevailing Institutional Review Boards. Participants provided informed, written consent. Data for this study were taken from recent VLS archives. The VLS is an on-going, multifaceted study of human aging designed to examine cognitive, neuropsychological, health, sensory and biological aspects of aging. Information regarding the VLS (i.e., design, measures and procedures) is available elsewhere (e.g., Dixon & de Frias, 2004). A dataset previously assembled (Dixon et al., 2012) from VLS Samples 1 and 2 was used for the present study. Cross-sectional, Wave 1 (W1) data are assembled from VLS Sample 1 (Wave 5) and Sample 2 (Wave 3). A longitudinal dataset was formed by integrating follow-up assessment waves for each initial cross-sectional wave noted above. Therefore, for the present study, Wave 2 (W2) was composed of VLS Sample 1 (Wave 6) and Sample 2 (Wave 4). All participants were genotyped in 2009-2010. We applied the following exclusionary criteria to W1 data: Participants were excluded based on a history of AD and other dementias, psychiatric disturbance (and medication), and serious episodes of cardio/cerebrovascular disease. Additional exclusionary criteria were applied at W2: Participants were excluded based on Mini Mental Status Exam (MMSE) scores less than 24 and missing data on one or more cognitive classification measures.

Participants were classified into MCI and NA groups based on a procedure used in previous VLS publications (de Frias et al., 2009; Dixon et al., 2012; Dixon et al., 2007; Dolcos, MacDonald, Braslavsky, Camicioli, & Dixon, 2012). The present classifications

were conducted in a previous study (Dixon et al., 2012). In turn, these classifications were produced on the basis of a larger data set (Dolcos et al., 2012) from which only the genotyped participants were used in the present study. An advantage of using the Dolcos and colleagues data set to generate cognitive classifications is that normative groups were larger than would have been available for the genotyped-only subset of data (Dixon et al., 2012). The procedure implemented by Dolcos and colleagues was as follows. Five cognitive reference measures were selected from perceptual speed, inductive reasoning, episodic memory, fluency, and semantic memory domains. Mean performance scores were calculated for separate age x education subgroups. Four age x education subgroups were formed by two serial stratifications of the sample, first into two age subgroups (middle-old, old-old), followed by secondary stratification based on years of education (≤ 12 years, > 13 years). The resulting four subgroups are: middle-old x low education, middle old x high education, old-old x low education, old-old x high education. The age x education subgroup means represent performance norms, which are then used as the basis for cognitive status classification. Participants more than one standard deviation below their age x education group mean on any cognitive reference test were classified as MCI (see de Frias et al., 2009; Dixon et al., 2007). Participants who do not fit the MCI criteria were designated as NA.

Participant demographic data for this study are presented in Table 1. N = 237 participants were available for the W1 analyses, with n = 136 (57.4%) meeting criteria for the NA group (age, M = 73.12, SD = 5.25; years of education, M = 15.21, SD = 2.94; % women = 64%), and n = 101 (42.6%) meeting criteria for the MCI group (age, M = 73.75, SD = 5.55; years of education, M = 14.52, SD = 3.08; 59.4% women). At W2, n =

224 (94.5%) of the original group (i.e., n = 237) returned for follow-up testing. Of the returning participants, n = 6 were removed as a result of Mini Mental Status Exam (MMSE) scores below 24, and n=1 was removed for missing data on tasks used for the MCI classification procedure. This resulted in a final longitudinal sample size of n = 217 (Dixon et al., 2012). Cell sizes for the four longitudinal profile groups as reported in Dixon et al., (2012) are as follows: NA-to-NA, n = 101 (46.5%); NA-to-MCI, n = 25 (11.5%); MCI-to-MCI, n = 68 (31.3%); MCI-to-NA, n = 23 (10.6%).

2.2 Procedure

Previous VLS reports (de Frias et al., 2009; Dixon et al., 2007; Dolcos et al., 2012) have developed and implemented the cognitive reference measures and the classification procedures used in the present study. The following description of the measures and procedures is adopted from Dixon and colleagues (2012).

2.3 Measures

2.3.1 DNA Extraction and Genotyping

Saliva was collected according to standard procedures from Oragene DNA Genotek and stored at room temperature in Oragene® disks until DNA extraction. DNA was manually extracted from 0.8 ml of saliva sample mix using the manufacturer's protocol with adjusted reagent volumes. Briefly, samples were incubated for 2.5 hours at 50°C after inversion. Samples were transferred to a centrifuge tube and mixed with Oragene® purifier, incubated on ice for 10 min, then centrifuged at 15,000g for 5 min to pellet the denatured protein. The supernatant was transferred to a new tube and DNA was precipitated by adding an equal volume of 100% ethanol. The DNA pellet was washed with 70% ethanol, dried and re-suspended with 10 mM Tris, pH 8.0; 1 mM

EDTA buffer. DNA was incubated at 50°C for 1 hour with occasional vortexing followed by incubation at 4°C overnight to ensure complete rehydration before quantification using a NanoDrop® ND-1000 Spectrophotometer (Wilmington, DE).

Genotyping was carried out by using a PCR-RFLP strategy to analyze the allele status for *APOE* (rs429358, rs7412), *CLU* (rs11136000), *CR1* (rs6656401), and *PICALM* (rs541458). Briefly, SNP-containing PCR fragments were amplified in 25 ul of 1X PCR reaction mix containing 25 ng genomic DNA, 12.5 pmol of each specific primer (*APOE* Fwd: 5'-GGCACGGCTGTCCAAGGA-3'; APOE Rev: 5'-GCCCCGGCCTGGTACACTGCC-3'. *CLU* Fwd: 5'-AAAGCAGGCTGCAGACTCC-3'; *CLU* Rev: 5'-AGTGCTGGGATTACAGGTGTC-3'. *CR1* Fwd: 5'-CTCCAGGCTTCCTTCCAGT-3'; *CR1* Rev: 5'-TTAATGATCTCGAGCTGTGACC-3'. PICAM Fwd: 5'-AAACCACAGATGAACTGATGTAACTG-3'; *PICALM* Rev: 5'-GGCATTAGGACCTGCCATC-3') with 6.25 nmol of each dNTP, 1.25U Taq DNA polymerase (NEB), 1.5 mM MgCl2 and 10% DMSO. Reactions were setup in 96-well plates using the QIAgility robotic system (QIAgen) and specific amplicons were amplified using a program consisting of: denaturation step at 95ºC for 2 min; 40 cycles at 94ºC for 30 sec, 56ºC for 30 sec and 72ºC for 1 min before a final extension at 72ºC for 7 min. RFLP analysis was performed after digestion of the PCR amplicons with restriction enzymes (all from NEB) as follows: *APOE*: 16 hours at 37ºC with HhaI; *CLU*: 4 hours at 65oC with Tsp509I; *CR1*: 7 hours at 37ºC with Hpy99I; *PICALM*: 2 hours at 37ºC with HpyCH4IV. RFLP analysis was then performed on a high resolution DNA screening cartridge on a QIAxcel capillary electrophoresis system (QIAgen) using the protocol

OL700. The analysis was confirmed upon migration of the restriction fragments on 10 or 15% acrylamide gels for each SNP.

Hardy-Weinberg equilibrium was assessed for the initial cross sectional sample to determine if the genotypic frequencies differ from that expected for any of the four genes. *CLU* and *CR1* genotypic frequencies did not significantly differ from Hardy-Weinberg norms (*CLU*: $\chi^2 = .1634$ (1), p = .69; *CR1*: $\chi^2 = .2494$ (1), p = .62). *PICALM* genotypic frequencies, however, significantly differed from Hardy-Weinberg norms ($\chi^2 = 151.52$ (1), $p < .001$). Observed and expected frequencies for each genotype (as presented in Table 2) illustrate that the present sample includes more homozygous genotypes (i.e., T/T and C/C) than expected. Accordingly, there are less heterozygotes (i.e., participants with the T/C genotype) than expected. For this study, homozygous and heterozygous carriers of the *PICALM* risk allele (i.e., participants with the T/T and T/C genotypes) are grouped together into a single 'at risk' group. This grouping strategy reflects our a priori hypothesis of a dominant effect of the *PICALM* T risk allele, such that participants with the T/T and T/C genotypes are similarly affected in terms of MCI status and longitudinal change. Coding *PICALM* risk alleles in this way is common practice in the literature (e.g., Kok et al., 2011; Mengel-From et al., 2011). Grouping these genotypes in this way may mean that lower than expected frequency of the heterozygous genotype may not substantively affect results, as the lower than expected T/C genotypic frequency is bolstered by the elevated T/T genotypic frequency. When tested for frequencies across the six possible allelic combinations, *APOE* genotypic frequencies significantly differed from Hardy-Weinberg norms ($\chi^2 = 18.77$ (4), $p < .001$). *APOE* genotypes were then clustered according to the presence of the risk ε4 genotype, and Hardy-Weinberg

equilibrium analyses were recomputed using the following genotypic groups: ε4+/ε4+, ε4+/ε4-, ε4-/ε4-. Results of these analyses showed that the frequency distribution was in Hardy-Weinberg equilibrium (χ^2 = .02 (1), p = .89).

2.3.2 Cognitive Reference Measures

To evaluate the participants' cognitive status, we used a standard cognitive reference battery. This battery consisted of indicators of five cognitive domains (i.e., perceptual speed, inductive reasoning, episodic memory, verbal fluency, and semantic memory; see de Frias et al., 2009; Dixon et al., 2007). The psychometric properties of these measures, which are acceptable according to conventional standards, are well-documented elsewhere (Hultsch, Hertzog, Dixon, & Small, 1998). All standardized procedures were followed.

Perceptual Speed. Perceptual processing speed was assessed with the Wechsler Adult Intelligence Scale—Revised Digit Symbol Substitution (DSS) task (Wechsler, 1981). Psychometric characteristics of the DSS are well-established in aging and other populations (e.g., MacDonald, Hultsch, Strauss, & Dixon, 2003). Using a specified coding key, participants were given 90 seconds to transcribe as many symbols as possible into a grid. The number of correctly completed items was used as the final outcome.

Inductive Reasoning. Inductive reasoning was assessed with the Letter Series test (Thurstone, 1962). Participants were presented with 20 strings of letters forming a distinct pattern. The task required inductively deciphering the pattern in the target string and providing the next letter in the string. The outcome was the total number correct.

Episodic Memory. The VLS word recall task, consisting of immediate free recall of two lists of 30 English words selected from the total set of six equivalent lists (Dixon,

Wahlin, Maitland, Hultsch, Hertzog, & Bäckman, 2004) was used. Each list consisted of six words from each of five taxonomic categories (e.g., birds, flowers), typed on a single page in unblocked order. Participants were given 2 minutes to study each list and 5 minutes to write their recall. The outcome was the average number of correctly recalled words from the lists.

Verbal Fluency. Verbal fluency was assessed with the Controlled Associations test from the Educational Testing Service (ETS) kit of factor-referenced cognitive tests (Ekstrom, French, Harman, & Dermen, 1976). The test required the generation of as many synonyms as possible in response to a set of target words within six minutes. The outcome measure was the total number of correct synonyms.

Vocabulary. The 54-item recognition, multiple-choice vocabulary test was composed by concatenating three, 18-item tests from the ETS kit of factor referenced cognitive tests (Ekstrom et al., 1976). Participants were given 15 minutes to complete the task, with the total number of correct items representing the vocabulary score.

2.4 Data Analysis

SPSS version 20.0 statistical software was used to perform all analyses. Hierarchical binary logistic regression was used for all analyses. Chronological age and education (Dixon et al., 2012) and gender (Mengel-From et al., 2011) were used as covariates for all analyses. For all research questions, the covariates of age, education and gender were entered into Block 1; two genetic predictors and their respective two-way interaction term were entered into Block 2. Results for Block 1 omnibus tests (Chi-square test and Nagelkerke R-square) for all research questions are presented in Table 3. Block 1 predictor findings for the covariates are presented in Table 4 for research

questions one and two, and Table 5 for research questions 3 and 4. All analyses assess dominant effects for each gene; therefore, each gene was coded dichotomously based on the presence of the risk allele (1 = risk allele present, 0 = risk allele absent). Following this scheme, the genotypes were coded as follows: *CLU,* C/C and C/T = 1, T/T = 0; *CR1,* A/A and A/G = 1, G/G = 0; *PICALM,* T/T and C/T = 1, C/C = 0; *APOE,* ε4+ = 1, ε4- = 0). Because of uncertainty regarding the effects of the heterozygous ε2/ε4 genotype, genotype for participants bearing this combination of alleles were coded as missing for analyses assessing *APOE* main effects and interactions. Interaction terms are computed by multiplying the dichotomously coded genetic variables. Consequently, participants with genetic risk alleles at both loci are coded "1". Participants without at least one risk allele at both loci are coded "0". Post hoc logistic regression power analyses were conducted using GPower 3.1 software. Mean power to detect main effects and interactions were calculated separately for cross-sectional and longitudinal models. Mean power values were generated by averaging power estimates for (a) cross-sectional main effects (b) cross-sectional interaction terms, (c) longitudinal main effects, and (c) longitudinal interaction terms. Analyses demonstrated modest power to detect cross-sectional main effects and interactions (main effects, M = .68, interactions, M = .86). Power to detect longitudinal main effects and interactions was generally better (main effects M = .83, interactions M = .95).

Logistic regression is used in circumstances wherein researchers aim to predict categorical outcomes (Tabachnick & Fidell, 2007). Binary logistic regression pertains to logistic regression analyses wherein the outcome variable has two distinct levels. Research question 1 analyses assess the predictivity of genotype and gene-gene

interactions for such a binary, categorical outcome: MCI present versus MCI absent (i.e., normal aging). By convention, the categorical outcome variable is coded as 1 if the event of interest occurs (e.g., MCI diagnosis) and 0 if not (e.g., normal aging reference group) (Tabachnick & Fidell, 2007). The goal of logistic regression is to estimate the probability of the event of interest occurring (e.g., MCI diagnosis) in relation to the level of a predictor variable. Odds ratios (ORs) are utilized in logistic regression to express this probability (Larsen, 2008). Parameter estimates, or β-values, in logistic regression models are calculated using maximum likelihood, and represent a log OR which is easily converted to an OR (Larsen, 2008). The resulting OR represents a comparison (i.e., ratio) of the odds of the event of interest happening at different levels of one of the predictor variables, with the influence of all other predictors in the regression equation held constant (Larsen, 2008). ORs above one indicate increased risk of participants' belonging to the affected (e.g., MCI) group when a risk factor is present. Conversely, ORs below one indicate that there is a greater odds of membership in the affected group when the risk factor is absent. Finally, we report 95% confidence intervals (CI) for the OR. The CI refers to a range of odds ratios values within which there is a 95% probability of the true population parameter being found.

For the first research question, W1 baseline data were used to assess *CLU, CR1* and *PICALM* genotypes as predictors of baseline MCI status (i.e., MCI versus NA). Given the association between these genes and AD, we expected baseline MCI status would be predicted by the presence of at least one risk allele (as represented by genotypic main effects). Furthermore, we expected that the odds of MCI would be enhanced by the

combined presence of risk alleles across two loci (as represented by significant 2-way interactions among risk alleles).

For research question two, W1 (baseline) data were used to test genotypic main effects and 2-way interactions involving *APOE*. We assessed the following series of hierarchical logistic regression models: (a) *CLU*(2) x *APOE*(2), (b) *CR1*(2) x *APOE*(2), and (c) *PICALM*(2) x *APOE*(2). As before, we expected that MCI status would be predicted by the presence of at least one risk allele, and the odds of chronic or emerging MCI status across the two waves would be potentiated by the combined presence of *APOE* ε4 plus one other AD risk allele across two loci. Given evidence for overlapping roles in AD pathogenesis, we expected risk alleles at both the *APOE* and *CLU* loci may be especially likely to demonstrate interaction effects.

For the third research question, we used the two-wave longitudinal data and conducted hierarchical logistic regression models. These models assessed (a) independent associations of *PICALM*, *CLU*, and *CR1*, and (b) two-way, gene-gene interactive associations. Specifically, we assessed if genotype and 2-way genotypic interactions may differentiate between pairs of longitudinal change profiles. We select combinations of two change profiles and systematically test if genotype and gene-gene interactions differentiate between the profiles. We focus on specific longitudinal profile combinations in this study: (a) NA-to-NA versus MCI-to-MCI, (b) NA-to-NA versus NA-to-MCI, (c) NA-to-NA versus MCI-to-NA, (d) NA-to-MCI versus MCI-to-MCI, (e) MCI-to-NA versus MCI-to-MCI, and (f) NA-to-MCI versus MCI-to-NA. Subsequently, two additional profile combinations were considered in the case that initial analyses demonstrated genotype and gene-gene interactions do not significantly differ in the

degree to which they distinguish between NA-to-MCI and MCI-to-NA groups. If this combination of profiles cannot be differentiated based on genotype and gene-gene interactions, participants in the two transition groups (NA-to-MCI and MCI-to-NA) were combined to form a single transition group representing individuals demonstrating variability about the MCI cutoff. Analyses were then computed to determine if this transition group can be distinguished from (a) stable, normal aging (i.e., NA-to-NA) older adults and (b) chronic MCI (i.e., MCI-to-MCI) patients. This approach is merited for at least three reasons. First, it has been shown that MCI patients who later revert to normal status have a high propensity towards developing MCI again. For instance, Koepsell and Monsell (2012) showed in a recent study that over half their study participants who reverted to normal status after a prior diagnosis of MCI later developed MCI or dementia within three years. Second, studies have shown that intraindividual variability of cognitive performance in MCI patients may be a cardinal feature of the condition (Dixon et al., 2007), which could result in participants just meeting the MCI criteria at W1 apparently reverting to normal status in W2, and vice versa. Third, recent recommendations for longitudinal follow-up of MCI participants to confirm the diagnosis emphasize that transition into MCI or back to normal status after an earlier MCI diagnosis may be common in newly diagnosed patients, but that the extent to which this occurs can be mitigated by confirming the chronicity of the condition with longitudinal follow-up. The four-year longitudinal follow-up in this study may not be long enough to ensure that all participants with MCI at W1, or who develop MCI at W2, will go on to experience chronic MCI. For these reasons, the transitioning groups in this study may be effectively clustered as a single MCI transition group.

For research question 3, we expected that genotype at the *CLU, CR1* and *PICALM* loci, and 2-way gene-gene interactions will differentiate between pairs of longitudinal profiles. As in previous research questions, we expected that the presence of a genotypic risk allele will increase the odds of membership in chronic clinical (i.e., MCI-to-MCI), emerging impairment (i.e., NA-to-MCI), or cognitive impairment instability (i.e., MCI-to-NA) groups. If the MCI-to-NA and NA-to-MCI profiles cannot be differentiated based on genotype or gene-gene interactions, we expect that a transition group formed by these two profiles may be differentiated from (a) stable healthy adults, and (b) adults with chronic MCI.

For the fourth research question, we assessed interactions between *APOE* and *CLU, CR1* and *PICALM* sequentially, using the same combinations of longitudinal cognitive profiles used for research question 3. Similar to research question 3, we expected that the presence of a genotypic risk allele would increase the odds of membership in chronic clinical (i.e., MCI-to-MCI), emerging impairment (i.e., NA-to-MCI), or cognitive instability (i.e., MCI-to-NA) groups. If the MCI-to-NA and NA-to-MCI profiles cannot be differentiated based on genotype or gene-gene interactions, we expected that a transition group formed by combining these two profiles may be differentiated from (a) stable healthy adults, and (b) adults with chronic MCI.

Chapter Three: Results

3.1 Preliminary Checks.

Before conducting the analyses, possible multicollinearity was assessed by correlating genetic predictor variables. Correlations among genetic predictors were uniformly very low (r range = -.075 - .052), indicating no risk of statistical complications due to multicollinearity. Cell sizes for all models are reported in Tables 6 and 7.

3.2 Research Question 1: Genetic Markers of MCI Status and Stability at Baseline.

We assess independent and interactive genotypic predictivity for baseline status (MCI versus NA). The results of the three research question 1 models are presented in Table 8. As can be seen in the table, in the *CLU*(2) x *CR1*(2) model *CLU* genotype significantly differentiated MCI patients from NA controls such that carriers of the *CLU* risk allele were 6.51-fold more likely to belong to the MCI group at baseline compared to the NA reference group (p = .04 (1-tailed), odds ratio 95% confidence interval (CI) .77 to 55.24). Cross-sectional trends emerged for *CR1* (p = .06 (1-tailed), OR 5.84, CI .62 to 54.91) and the *CLU*(2) x *CR1*(2) interaction term (p = .08 (1-tailed), OR .18, CI .02 to 1.86).

3.3 Research Question 2: *APOE* by AD-Gene Associations at Baseline.

Our second research question assesses if genotypic main effects and gene-gene interactions between *CLU, CR1* and *PICALM* genotypes and *APOE* may distinguish between MCI patients and NA adults at baseline. Table 9 shows the 3 models that were tested and the results of these analyses. In the *APOE*(2) x *PICALM*(2) model, *APOE* significantly differentiated between MCI patients and the NA reference group such that

carriers of the *APOE* risk allele were 3.20-fold more likely to experience MCI at baseline (p = .05 (1-tailed), CI .80 to 12.73). No interaction terms with APOE were significant.

3.4 Research Question 3: Genetic Markers of Cognitive Status Stability.

Significant results for research question 3 are presented serially according to the combination of profiles assessed. The results of analyses wherein pertinent cell sizes for main effects and interactions drop below n = 5 are not reported (Tables 6 and 7 present cell size information). Results are presented in Table 10 for the *CLU*(2) x *CR1*(2) model, Table 11 for the *CLU*(2) x *PICALM*(2) model, and Table 12 for the *CR1*(2) x *PICALM*(2) model.

NA-to-NA versus MCI-to-MCI. The *CLU*(2) x *CR1*(2) model showed a significant main effect for *CLU* status (p = .04 (1-tailed). Adults with the *CLU* risk allele were 6.82-fold more likely to belong to the chronic MCI group compared to the stable NA reference group (CI .77 to 60.29). In the same model, trends for *CR1* (p = .06 (1-tailed), OR 6.00, CI .62 to 58.49) and the *CLU*(2) x *CR1*(2) interaction (p = .07 (1-tailed), OR .16, CI .02-1.74) also emerged.

MCI-to-NA versus NA-to-MCI. No significant genetic main effects or gene-gene interactions emerged which significantly differentiated these two longitudinal profiles. Because genotype and gene-gene interactions did not distinguish between these profiles, the profiles are clustered into a single MCI transition group for subsequent analyses.

NA-to-NA versus MCI Transition Group. In the *CR1*(2) x *PICALM*(2) model, a main effect for *CR1* (p = .05) was observed. ORs showed that carriers of the *CR1* risk allele were 7.20-fold more likely to belong to the MCI transition group compared to the stable NA reference group (CI .66 to 78.80). In the same model, trends towards a

PICALM main effect (p = .06 (1-tailed), OR 6.08, CI .66 to 56.13) and a *CR1*(2) x *PICALM*(2) interaction (p = .06 (1-tailed), OR .13, CI .01 to 1.62) emerged. A trend towards a significant interaction also emerged in the *CLU*(2) x *PICALM*(2) model (p = .09 (1-tailed), OR 7.59, CI .40-144.69).

MCI-to-MCI versus MCI Transition Group. In the *CR1*(2) x *PICALM*(2) model, a *PICALM* main effect emerged (p = .04 (1-tailed). For *PICALM* risk allele carriers, the OR of belonging to the chronic MCI group was .13, indicating odds of membership in the chronic MCI group (compared to the MCI transition (reference) group) was smaller in *PICALM* risk allele carriers compared to non-carriers (CI .01 to 1.21). That is, the odds ratio corresponding to membership in the chronic MCI group (compared to the MCI transition group) in non-carriers is 7.69-fold greater than the odds of membership in the chronic MCI group (compared to the MCI transition group) in *PICALM* risk allele carriers. In the same model, trends towards a *CR1* main effect (p = .09 (1-tailed), OR .19, CI .02 to 2.02) and a *PICALM*(2) x *CR1*(2) interaction (p = .06 (1-tailed), OR 7.12, CI .58 to 87.08) emerged. The significant main effect and trends demonstrated in this model show that carriers either the *CR1* or *PICALM* risk alleles tend to demonstrate the transition group phenotype over the chronic MCI phenotype. In contrast, carriers of both risk alleles tend to belong to the chronic MCI group over the MCI transition group.

3.5 Research Question 4: Longitudinal *APOE* Interactions with *CLU, CR1* and *PICALM.*

Significant results for research question 4 are also presented serially according to the combination of profiles assessed. As in research question 3, the results of analyses wherein pertinent cell sizes for main effects and interactions drop below n = 5 are not

reported (Tables 6 and 7 present cell size information). Results are presented in Table 13 for the *CLU*(2) x *APOE*(2) model, Table 14 for the *CR1*(2) x *APOE*(2) model, and Table 15 for the *PICALM*(2) x *APOE*(2) model.

NA-to-NA versus MCI-to-MCI. The *APOE*(2) x *CR1*(2) model demonstrated a significant interaction (p = .05). Carriers of both the *CR1* and *APOE* risk alleles were 3.84-fold more likely to belong to the chronic MCI group compared to the stable NA reference group (CI .81 to 18.17).

NA-to-NA versus NA-to-MCI. A significant *APOE*(2) x *CR1*(2) interaction emerged (p = .02). Carriers of both the *APOE* and *CR1* risk alleles were 17.27-fold more likely to belong to the emerging impairment group (i.e., NA-to-MCI) compared to the stable NA reference group (CI 1.23 to 243.31).

MCI-to-NA versus MCI-to-MCI. No significant genotypic main effects of interactions significantly differentiated between these profiles. However, a trend towards a *PICALM*(2) x *APOE*(2) interaction emerged (p = .08 (1-tailed), OR 6.79, CI .46 to 100.99).

NA-to-MCI versus MCI-to-NA. No genotypic main effects or interactions emerged which significantly differentiated between these longitudinal profiles. Because genotype and gene-gene interactions did not distinguish between these two profiles, the profiles were clustered for subsequent analyses.

NA-to-NA versus MCI Transition Group. The *CR1*(2) x *APOE*(2) model demonstrated a main effect for *APOE* (p = .04), and a significant *APOE*(2) x *CR1*(2) interaction (p = .005). The OR for the *APOE* main effect was .13, indicating carriers of the *APOE* risk allele were seemingly less likely to belong to the transition group

compared to the stable NA reference group (CI .02 to 1.19). However, this *APOE* main effect was modified by a higher order interaction such that carriers of both the *CR1* and *APOE* risk alleles were 24.72-fold more likely to belong to the MCI transition group as compared to the stable NA reference group (CI 2.14 to 286.00).

MCI-to-MCI versus MCI Transition Group. No genotypic main effects or interactions emerged which significantly differentiated between these longitudinal profiles; however, a number of genotypic main effects and gene-gene interactions emerged at the trend level (see Tables 10, 11 and 12). The *CR1*(2) x *APOE*(2) model demonstrated a trend towards a significant *APOE* main effect (p = .06 (1-tailed), OR 6.26, CI .66 to 59.67), and a trend towards a *CR1*(2) x *APOE*(2) interaction (p = .07 (1-tailed), OR .16, CI .01 to 1.90). In the *PICALM*(2) x *APOE*(2) model, a trend towards a *PICALM* main effect is demonstrated (p = .09 (1-tailed), OR .45, CI .14 to 1.43). In the *CLU*(2) x *APOE*(2) model, a trend emerged towards a *CLU* main effect (p = .06 (1-tailed), OR .17, CI .02 to 1.50) emerged.

Chapter Four: Discussion

Despite the theoretical significance of the MCI phenomenon, reviewers have observed the present clinical utility of MCI could be enhanced by continuing improvements to the validity of present classification procedures (Albert et al., 2011). At the same time, recent GWAS have made progress identifying AD genetic markers which have improved understanding of AD etiology. MCI-type symptoms are considered to be a manifestation of progressive AD etiology. To the extent that evidence for underlying AD pathology can be garnered in MCI patients, improvements may result in the ability to (a) discriminate between normal aging and MCI at the earliest possible point and (b) do so with the highest possible accuracy and validity of MCI classification. These are important clinical and public health goals, as interventions for AD are most likely to be effective in the preclinical period prior to actual AD diagnosis.

The goal of the present study was to determine if newly identified genetic markers of AD bear associations with initial MCI status as well as longitudinal MCI stability and decline profiles. In doing so, this study brings together the (a) need for early interventions to delay the onset of AD with (b) present clinical limitations of the MCI classification and (c) recent developments in the field of AD-related genetic markers. The goals of this study were pursued in a series of four research questions. Overall, we found evidence for both independent and interactive effects of genotype in predicting baseline status and differentiating between longitudinal change profiles. In the future, MCI-genotypic associations may support the use of AD-genetic markers in augmenting current MCI classification procedures.

For research questions one and two, we expected that baseline MCI status would be predicted by the presence of at least one genetic risk allele, and that the odds of baseline MCI would be enhanced by the combined presence of risk alleles across two loci. ORs for main effects indicate that odds of MCI classification may indeed be enhanced by the presence of risk alleles at apolipoprotein related loci, *CLU* (also known as *APOJ*; OR = 6.51; see Table 8) and *APOE* (OR = 3.20; see Table 9). However, contrary to expectation, research question 1 and 2 analyses showed no enhanced risk of MCI at baseline in carriers of two risk alleles, as evidenced by no gene-gene interactions in the cross-sectional analyses. In contrast, longitudinal analyses conducted for research questions 3 and 4 showed gene-gene interactions at the trend and significant level predict numerous cognitive change profiles. The absence of cross-sectional interactions may suggest that interactive effects among AD-related genes are better predictors of MCI chronicity and change (as assessed in the longitudinal analyses) rather than the static MCI classification used in these cross-sectional analyses.

For research question 3, we had three key expectations. First, we expected that genotype at the *CR1, CLU* and *PICALM* loci would independently and interactively distinguish between the six longitudinal profiles (in pairwise comparisons) assessed in this study. Second, we expected that the combined presence of risk alleles across two loci would potentiate the risk of a clinical phenotype. Finally, in the case that the MCI-to-NA and NA-to-MCI profiles could not be distinguished on the basis of genotype or gene-gene interactions, we expected that the MCI transition group (i.e., combined MCI-to-NA and NA-to-MCI group) may be distinguished from stable NA and chronic MCI independently by genotype and interactively by multiplicative gene-gene associations.

Although no significant interactions emerged, these expectations were partially supported by three significant main effects emerging from two separate models: *CLU*(2) x *CR1*(2) and *CR1*(2) x *PICALM*(2).

Analysis of the *CLU*(2) x *CR1*(2) model indicated a main effect for *CLU* in differentiating stable, NA adults from chronic MCI patients (see Table 10). The *CLU* main effect -- which is manifested as a 6.82-fold increase in odds of experiencing chronic MCI in carriers of the *CLU* risk allele -- fits well with current biological evidence that *CLU* may be involved in early, upstream processes leading to AD development. Both *CLU* and *APOE* are thought to be involved in late-onset AD via roles in Aβ clearance (Jack et al., 2010). Processes leading to or supporting Aβ deposition (e.g., problems in Aβ clearance) are considered to be among the most upstream factors initiating AD development (Jack et al., 2010), and therefore may be key processes underpinning MCI-type symptomology. Problems with Aβ clearance in MCI patients may be an early upstream process supporting Aβ deposition and subsequent AD development.

Because genotype and gene-gene interactions did not distinguish between MCI-to-NA and NA-to-MCI profiles -- and these profiles could be conceptually similar -- the profiles were clustered into a single MCI transition group. Clustering these groups corresponds well with current evidence suggesting (a) emerging impairment and clinical reversion may be related, such that (b) the reversion group may represent a sort of provisional clinical group particularly at risk for MCI in the future. Analyses using the MCI transition group were run to determine if genotype or gene-gene interactions distinguished the MCI transition group profile from the stable healthy and chronic MCI

profiles. In accordance with our expectations, 2 significant genotypic main effects from the *CR1*(2) x *PICALM*(2) model emerged which differentiated between the profiles.

A genotypic main effect differentiating between the chronic MCI profile and the combined MCI transition (reference) group emerged for *PICALM* in the *CR1*(2) x *PICALM*(2) model (see Table 12). Using the same model but different longitudinal profile comparison, a main effect differentiating between stable NA adults and the MCI transition group emerged for *CR1* (see Table 12). ORs for these genotypic main effects indicated that carriers of the risk alleles for either the *CR1* (i.e., A) or *PICALM* (i.e., T) polymorphisms were more likely to belong to the transition group rather than the stable NA or chronic MCI group. The propensity for *CR1* and *PICALM* risk allele carriers to belong to the transition group rather than the steady-state, stable NA or chronic MCI groups may suggest that these genotypes are more proximally related to upstream factors (e.g., comorbidities) which foster vulnerabilities for MCI development, rather than MCI status per say. That is, the vulnerability created by these risk alleles may be insufficient to cause the early AD-related changes thought to lead to an MCI diagnosis: they may require the presence of other genetic risk factors (e.g., *APOE,* as evidenced by the multiple *CR1(2) x APOE(2)* interactions discussed below) in order to be reliably associated with chronic MCI. This is supported by results from this study indicating that although carriers of *CR1* and *PICALM* risk genotypes are associated with the transition group, they tend to not be classified as either stable NA or chronic MCI. As information regarding how *CR1* and *PICALM* polymorphisms are associated with early AD pathology emerges, more definitive conclusions as to how *CR1* and *PICALM* genotypes are related to the proximal factors underpinning MCI symptomology may be possible.

For research question 4, we had three key expectations. We expected genotype (including *APOE*) to independently distinguish between the six pairwise comparisons of the longitudinal profiles assessed in this study. Second, we expected that the combined presence of *APOE* and one of the other risk alleles would potentiate the risk of a clinical phenotype. In the absence of significant genotype main effects and gene-gene interactions differentiating between MCI-to-NA and NA-to-MCI profiles, we expected that the MCI transition group may be distinguished from stable NA and chronic MCI by genotype and gene-gene interactions.

Results from research question 4 include one main effect of *APOE* and four significant *APOE*(2) x *CR1*(2) interactions (see Table 14). First, the main effect for *APOE* showed that the *APOE* genotype significantly distinguished the MCI transition group from stable NA adults. However, this effect was qualified by a significant higher order *APOE*(2) x *CR1*(2) interaction for the same comparison. The other three significant *APOE*(2) x *CR1*(2) interactions were detected for the following longitudinal profile combinations: (a) NA-to-NA versus MCI-to-MCI, (b) NA-to-NA versus NA-to-MCI, and (c) NA-to-NA versus MCI transition group. Two of the four results indicated remarkable increased odds of a clinical phenotype (i.e., chronic MCI or emerging impairment) when *APOE* and *CR1* risk alleles are present. The OR pertaining to membership in the stable NA (reference) group compared to the chronic MCI was 3.84. The OR pertaining to membership in the stable NA (reference) group compared to the emerging impairment group was 17.27. The third *APOE*(2) x *CR1*(2) interaction manifested as a 24.72-fold increase in odds of membership in the MCI transition group compared to the stable NA reference group. Two considerations may help to explain the high ORs pertaining to

these *APOE(2)* x *CR1*(2) interactions. First, high ORs for AD in *APOE* ε4 carriers have been explained as a result of excellent AD classification procedures (Corneveaux et al., 2012). The MCI classification procedures utilized in this report conform to current recommendations designed to specifically target individuals with mild to moderate cognitive change whose primary pathophysiology is of the AD-type (Albert et al., 2011). To the extent that these current recommendations are effective in identifying AD pathophysiology in MCI patients, we may reasonably expect to see high odds of clinical change profiles in relation to *APOE* status, a gene highly associated with AD pathology. Second, a recent report has suggested that the effect of AD risk amplification genes, such as *CR1,* may be to shift the emergence of cognitive symptoms of AD earlier in the course of disease pathogenesis such that the development of cognitive symptoms may more closely overlap with early AD changes, including perhaps deposition of Aβ (Jack et al., 2010). *APOE* is thought to be especially related to Aβ clearance. Therefore, correspondence between MCI cognitive deficits and early AD pathological changes may be especially tight in carriers of both *CR1* and *APOE* risk alleles. A result of this high correspondence could reasonably be an increase in odds of emerging impairment, stable impairment and transitioning cognitive health status in carriers of both *CR1* and *APOE* risk alleles. Interestingly, the final *APOE*(2) x *CR1*(2) interaction manifested as a decreased odds of membership in the chronic MCI group compared to the MCI transition group (OR = .16). This result may be related to the fact that *APOE* and *CR1* are likely most related to the earliest stages of pathological change, a point at which variability in cognitive performance (as exemplified by the MCI transition group) may be the cardinal feature of MCI-related cognitive change (Dixon et al., 2007).

Neither cross-sectional or longitudinal models demonstrated an interaction between the *APOE* and *CLU* genotypes (see Tables 9 and 13). This result suggests that the genes may work independently to affect longitudinal MCI status and change despite similarities in biological structure and function (e.g., *APOE* and *CLU* are both apolipoproteins, and are the most abundantly produced apolipoproteins in the central nervous system; Lambert et al., 2009), and evidence of parallel roles in AD pathogenesis.

Several limitations to this study deserve mention. First, for research question 3 analyses cell sizes did not permit assessment of *CLU* and *PICALM* main effects for many of the longitudinal change profile combinations. Despite this general pattern, one profile combination, NA-to-NA versus MCI-to-MCI, facilitated assessment of both main and interaction effects for these genes, and several other profile combinations allowed for assessment of interactive effects only. Additionally, it was possible to assess *PICALM* main effects using two additional profile combinations (NA-to-NA versus MCI transition group, and MCI Transition Group versus MCI-to-MCI; see Table 6). A second related limitation of this study pertains to statistical power. Power analyses illustrate somewhat diminished power to detect (a) cross-sectional main effects and interactions, and (b) longitudinal interactions. Despite this limitation, our study was able to detect a number of significant main effects and interactions, evidencing that the genes assessed in this study may be important predictors of MCI status and change. On this note, identifying more subtle genotypic and gene-gene interactions may be beyond the reach of this study as a result of diminished power; therefore, we do not exclude the possibility of additional, more subtle main and interactive effects existing among these genotypic loci.

A third limitation of this study is that the *PICALM* allelic distribution for this sample was not in Hardy-Weinberg equilibrium (see Table 2). The nature of the departure from the expected frequencies was such that the heterozygous T/C genotype was diminished, and the homozygous T/T and C/C genotypes were increased. For all analyses, genotypes were coded dichotomously such that *PICALM* homozygous T/T carriers were clustered with the heterozygous T/C carriers into a single "risk group". This risk group is about 7.5% smaller than would be expected based on Hardy-Weinberg equilibrium (186 risk allele carriers were observed, whereas Hardy-Weinberg equilibrium specifies a group of about 201 risk allele carriers). Diminished "risk group" cell size means that we may not have been able to detect *PICALM* main effects and interactions existing in the population.

A fourth limitation of this study is the possibility that classification of at least some participants in the emerging impairment or clinical reversion groups may have resulted from measurement unreliability, and not as a result of actual clinical change. On this note, it may be impossible to completely disentangle clinical transition (i.e., NA to MCI or MCI back to NA) from psychometric variability about the MCI cut off. Longitudinal follow-up to determine if individuals with the emerging impairment and reversion profiles develop AD at a greater incidence than the stable normal aging group would help to determine the clinical significance of the emerging impairment and reversion groups, and relevance of the MCI transition group.

Overall, this study supports the use of selected AD genotypic biomarkers alongside current MCI classification procedures. Most prominently, future research on MCI and genetic biomarkers will likely want to consider moderating roles of other

biological markers (e.g., metabolomic and proteomic markers) as well as health (e.g., diabetes and hypertension) and psychiatric comorbidity. For example, depression is considered a modifiable risk factor for AD, and recent evidence has shown that chronic depression increases the risk of transition from NA to MCI (Steenland et al., 2012). In the future, it will be important to determine the degree to which biological health (e.g. metabolic status, blood pressure) and psychiatric factors (such as depression) interact with the genotypic predictivity of MCI status and stability. This research supplemented previous genetic biomarker studies of MCI. To our knowledge, this is the first study to assess longitudinal change in MCI status in relation to genotype at the *CLU, CR1* and *PICALM* loci. While other studies exist assessing genetic biomarkers of MCI, *CLU, CR1* and *PICALM* polymorphisms may be particularly pertinent because of their recently discovered relationship to AD. Evidence from this study showing (in some instances) especially strong associations between MCI and genotype at these loci helps to confirm key roles for these genes in early AD-related pathogenic processes. Using genotypic information for genes involved in early AD-related pathogenesis may help to clarify important clinical and research questions pertaining to MCI stability and change.

Table 1

Sample Demographics by Cognitive Status at Wave 1 and Wave 2

Wave 1	NA	MCI	Wave 2	NA-NA	NA-MCI	MCI-MCI	MCI-NA
N							
W1	136	101	W2	101	25	68	23
Age							
W1	73.12 (5.25)	73.75 (5.55)	W2	73.23 (5.28)	72.64 (5.30)	73.50 (5.40)	73.83 (5.57)
Gender							
W1	64%	59.4%	W2	61.4%	72%	55.9%	65.2%
Education							
W1	15.21 (2.94)	14.52 (3.08)	W2	15.55 (2.96)	13.92 (2.12)	14.32 (3.00)	14.30 (3.13)

Note. W1 = Wave 1; W2 = Wave 2; N = Sample size; NA = Normal Aging; MCI = Mild Cognitive Impairment; Age and education data presented as Average (Standard Deviation).

Table 2

Observed Genotypic Frequencies Compared to Expected Genotypic Frequencies Based on Hardy-Weinberg Equilibrium

SNP	Gene	Genotype	Expected Genotype, n	Observed Genotype, n
rs11136000	*CLU*			
		C/C	88.48	87
		C/T	112.05	115
		T/T	35.47	34
rs6653401	*CR1*			
		A/A	37.84	36
		A/G	113.3	117
		G/G	84.82	83
rs541458	*PICALM*			
		T/T	89.70	105
		T/C	111.59	81
		C/C	34.71	50
rs429358; rs7412	*APOE*			
		ε4/ε4	4.74	5
		ε4/ε3	51.33	48
		ε4/ε2	6.22	9
		ε3/ε2	33.69	21
		ε3/ε3	139.00	147
		ε2/ε2	2.04	7
	APOE (revised)			
		ε4+/ε4+	4.74	5
		ε4+/ε4-	57.55	57
		ε4-/ε4-	174.73	175

Note. SNP = single nucleotide polymorphism; CLU = Clusterin; CR1 = Complement Receptor 1; PICALM = Phosphatidylinositol Binding Clathrin Assembly Protein; APOE = Apolipoprotein E; C = Cytosine; T = Thymine; A = Adenine; G = Guanine; ε4 = APOE epsilon 4 allele; ε3 = APOE epsilon 3 allele; ε2 = APOE epsilon 2 allele; ε4+ = ε4 allele present; ε4- = ε4 allele absent.

Table 3

Block 1 Covariate Omnibus Results: Research Questions 1, 2, 3 and 4

Research Question	Reference Group	Status/ Stability	Chi-Square	Nagelkerke R-Square
1	NA.	MCI	4.21(3) = .24	.02
2	NA	MCI	5.19 (3) = .16	.03
3 and 4	NA-MCI	MCI-MCI	3.11(3) = .38	.05
	NA-NA	NA-MCI	7.56 (3) = .06	.09
	NA-NA	MCI-MCI	7.44 (3) = .06	.06
	MCI-NA	NA-MCI	1.16 (3) = .76	.03
	MCI-NA	MCI-MCI	.86 (3) = .84	.01
	NA-NA	MCI-NA	3.42(3) = .33	.04
	NA-NA	Transition Group	8.45(3) = .04	.08
	Transition Group	MCI-MCI	2.36(3) = .50	.03

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA group; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio.

Table 4

Block 1Covariate Predictor Results: Research Questions 1 and 2

Research Question	Reference Group	Status	Covariate	β	OR	95% CI	p
1	NA	MCI					
			Age	.02	1.02	.97-1.07	.49
			Education	-.08	.93	.85-1.01	.09
			Gender	.27	1.32	.77-2.26	.32
2	NA	MCI					
			Age	.02	1.02	.97-1.08	.43
			Education	-.08	.93	.85-1.01	.09
			Gender	.38	1.46	.84-2.53	.18

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA group; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio.

Table 5

Block 1Covariate Predictor Results: Research Questions 3 and 4

Reference Group	Status/ Stability	Covariate	β	OR	95% CI	p
NA-MCI	MCI-MCI					
		Age	.04	1.04	.95-1.14	.43
		Education	.06	1.06	.89-1.27	.51
		Gender	.71	2.03	.74-5.56	.17
NA-NA	NA-MCI					
		Age	-.03	.97	.89-1.06	.51
		Education	-.21	.81	.68-.96	.02
		Gender	-.33	.72	.27-1.96	.52
NA-NA	MCI-MCI					
		Age	.001	1.00	.94-1.06	.97
		Education	-.14	.87	.78-.97	.01
		Gender	.35	1.42	.74-2.70	.29
MCI-NA	NA-MCI					
		Age	-.05	.95	.86-1.06	.40
		Education	-.06	.94	.75-1.18	.59
		Gender	-.27	.76	.22-2.66	.67
MCI-NA	MCI-MCI					
		Age	-.02	.98	.90-1.07	.70
		Education	-.001	1.00	.85-1.18	.99
		Gender	.44	1.55	.58-4.19	.39
NA-NA	MCI-NA					
		Age	.02	1.02	.94-1.11	.68
		Education	-.14	.87	.75-1.02	.08
		Gender	-.09	.92	.35-2.41	.86
NA-NA	Transition Group					
		Age	-.01	.99	.93-1.06	.84
		Education	-.17	.84	.74-.95	.01
		Gender	-.19	.83	.39-1.77	.63
Transition Group	MCI-MCI					
		Age	.01	1.01	.94-1.08	.81
		Education	.03	1.03	.90-1.18	.70
		Gender	.56	1.75	.80-3.83	.16

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA group; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio.

Table 6

Cell Sizes for Main Effects

Wave	Group (n)	Dichotomous Genetic Variables							
		CLU		CR1		PICALM		APOE	
		C-	C+	A-	A+	T-	T+	ε4-	ε4+
1	NA (136)	23	113	50	86	26	110	106	27
	MCI (100)	11	89	33	67	24	76	69	26
2	NA-NA (101)	21	80	38	63	21	80	80	21
	MCI-MCI (67)	10	57	23	44	17	50	46	22
	NA-MCI (25)	2	23	9	16	3	22	18	7
	MCI-NA (23)	1	22	7	16	4	19	18	5
	Transition Group* (48)	3	45	16	32	7	41	36	12

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA group; CLU = Clusterin; CR1 = Complement Receptor 1; PICALM = Phosphatidylinositol Binding Clathrin Assembly Protein; APOE = Apolipoprotein E; C+ = at least one Cytosine allele present; C- = Cytosine allele absent; T+ = at least one Thymine allele present; T- = Thymine allele absent; A+ = at least one Adenine allele present; A- = Adenine allele absent; G+ = at least one Guanine allele present; G- = Guanine allele absent; ε4+ = at least one ε4 allele present; ε4- = ε4 allele absent.

Table 7

Cell Sizes for Interactive Effects

Wave	Group (n)	Genetic Interaction Terms											
		CLU(2)xCR1(2)		CLU(2)xPICALM(2)		CR1(2)xPICALM(2)		APOE(2)xCLU(2)		APOE(2)xCR1(2)		APOE(2)xPICALM(2)	
		-	+	-	+	-	+	-	+	-	+	-	+
1													
	NA (136)	65	71	45	91	66	70	113	21	117	19	111	23
	MCI (100)	43	57	32	68	47	53	74	22	76	21	78	17
2													
	NA-NA (101)	51	50	39	62	50	51	86	15	90	11	85	16
	MCI-MCI (67)	32	35	24	43	31	35	50	18	52	16	51	17
	NA-MCI (25)	11	14	4	21	12	13	19	6	19	6	18	7
	MCI-NA (23)	8	15	5	18	10	13	19	4	18	5	20	3
	Transition Group (48)	19	29	9	39	22	26	38	10	37	11	38	10

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA group; CLU = Clusterin; CR1 = Complement Receptor 1; PICALM = Phosphatidylinositol Binding Clathrin Assembly Protein; APOE = Apolipoprotein E; (-) = risk allele(s) are not present for both genes; (+) = at least one risk allele for each gene is present.

Table 8

Results for Research Question 1

Model	Predictors	β	OR	95% CI	p
1	CLU	1.87	6.51	.77-55.24	.04
	CR1	1.77	5.84	.62-54.91	.06
	CLU(2) x CR1(2)	-1.69	.18	.02-1.86	.08
2	CLU	.12	1.12	.22-5.74	.44
	PICALM	-.73	.48	.09-2.72	.20
	CLU(2) x PICALM(2)	.51	1.67	.26-10.77	.30
3	CR1	-.01	.99	.31-3.13	.49
	PICALM	-.48	.62	.22-1.74	.18
	CR1(2) x PICALM(2)	.29	1.34	.36-4.97	.33

Note. CLU = Clusterin; CR1 = Complement Receptor 1; PICALM = Phosphatidylinositol Binding Clathrin Assembly Protein; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio; the NA group was the reference group for each of the research question 1 models.

Table 9

Results for Research Question 2

Model	Predictors	β	OR	95% CI	p
1	CLU	.65	1.92	.70-5.25	.10
	APOE	.63	1.88	.38-9.32	.22
	CLU(2) x APOE(2)	-.24	.79	.14-4.52	.40
2	CR1	-.08	.93	.49-1.76	.41
	APOE	-.21	.81	.23-2.81	.37
	CR1(2) x APOE(2)	.82	2.27	.53-9.74	.14
3	PICALM	-.13	.88	.41-1.88	.37
	APOE	1.16	3.20	.80-12.73	.05
	PICALM(2) x APOE(2)	-1.01	.37	.08-1.75	.11

Note. CLU = Clusterin; CR1 = Complement Receptor 1; PICALM = Phosphatidylinositol Binding Clathrin Assembly Protein; APOE = Apolipoprotein E; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio; the NA group was the reference group for each of the research question 2 models.

Table 10

Research Question 3 Results: CLU(2) x CR1(2) Model

Predictors	Reference Group	Status/Stability	β	OR	95% CI	p
CLU	NA-NA	MCI-NA	19.76	3.81×10^8	-- [a]	.50
	NA-NA	NA-MCI	20.15	5.62×10^8	-- [a]	.50
	NA-MCI	MCI-MCI	-19.84	-- [b]	-- [a]	.50
	MCI-NA	MCI-MCI	-19.80	-- [b]	-- [a]	.50
	NA-NA	MCI-MCI	1.92	6.82	.77-60.29	.04
	MCI-NA	NA-MCI	-.58	.56	.04-8.03	.34
	NA-NA	Transition Group	20.66	9.37×10^8	-- [a]	.50
	Transition Group	MCI-MCI	-20.54	-- [b]	-- [a]	.50
CR1	NA-NA	MCI-NA	18.65	1.25×10^8	-- [a]	.50
	NA-NA	NA-MCI	19.34	2.50×10^8	-- [a]	.50
	NA-MCI	MCI-MCI	-19.11	-- [b]	-- [a]	.50
	MCI-NA	MCI-MCI	-18.72	-- [b]	-- [a]	.50
	NA-NA	MCI-MCI	1.79	6.00	.62-58.49	.06
	MCI-NA	NA-MCI	-.34	.71	.21-2.46	.30
	NA-NA	Transition Group	19.76	3.83×10^8	-- [a]	.50
	Transition Group	MCI-MCI	-19.64	-- [b]	-- [a]	.50
CLU(2) x CR1(2)	NA-NA	MCI-NA	-18.29	-- [b]	-- [a]	.50
	NA-NA	NA-MCI	-19.5	-- [b]	-- [a]	.50
	NA-MCI	MCI-MCI	19.18	2.14×10^8	-- [a]	.50
	MCI-NA	MCI-MCI	18.39	9.71×10^7	-- [a]	.50
	NA-NA	MCI-MCI	-1.83	.16	.02-1.74	.07
	MCI-NA	NA-MCI	-- [c]	-- [c]	-- [c]	-- [c]
	NA-NA	Transition Group	-19.69	-- [b]	-- [a]	.50
	Transition Group	MCI-MCI	19.53	3.02×10^8	-- [a]	.50

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA group; CLU = Clusterin; CR1 = Complement Receptor 1; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio.
[a]Diminished cell sizes precluded calculation of CI.
[b]Diminished cell sizes precluded calculation of OR.
[c]Diminished cell sizes precluded inclusion of interaction term in the model assessing this longitudinal profile comparison; β, OR, 95% CI and p-value could not be generated.

Table 11

Research Question 3 Results: CLU(2) x PICALM(2) Model

Predictors	Reference Group	Status/Stability	β	OR	95% CI	p
CLU	NA-NA	MCI-NA	19.60	3.26 x 10^8	-- [b]	.50
	NA-NA	NA-MCI	-1.18	.31	.02-4.92	.20
	NA-MCI	MCI-MCI	.46	1.59	.10-25.53	.37
	MCI-NA	MCI-MCI	-20.19	-- [c]	-- [b]	.50
	NA-NA	MCI-MCI	-.26	.77	.13-4.58	.39
	MCI-NA	NA-MCI	-22.09	-- [c]	-- [b]	.50
	NA-NA	Transition Group	-.15	.86	.07-10.52	.45
	Transition Group	MCI-MCI	-.55	.58	.05-7.09	.34
PICALM	NA-NA	MCI-NA	18.07	7.01 x 10^7	-- [b]	.50
	NA-NA	NA-MCI	-2.18	.11	.01-2.60	.09 [a]
	NA-MCI	MCI-MCI	.59	1.80	.08-41.04	.36
	MCI-NA	MCI-MCI	-19.47	-- [c]	-- [b]	.50
	NA-NA	MCI-MCI	-1.15	.32	.05-2.05	.11
	MCI-NA	NA-MCI	-21.59	-- [c]	-- [b]	.50
	NA-NA	Transition Group	-1.41	.24	.02-3.81	.16
	Transition Group	MCI-MCI	-.05	.95	.06-15.32	.49
CLU(2) x PICALM(2)	NA-NA	MCI-NA	-17.82	-- [c]	-- [b]	.50
	NA-NA	NA-MCI	3.17	23.81	.71-796.73	.04 [a]
	NA-MCI	MCI-MCI	-1.60	.20	.01-6.93	.19
	MCI-NA	MCI-MCI	19.17	2.11 x 10^8	-- [b]	.50
	NA-NA	MCI-MCI	.99	2.68	.35-20.81	.17
	MCI-NA	NA-MCI	22.55	6.21 x 10^9	-- [b]	.50
	NA-NA	Transition Group	2.03	7.59	.40-144.69	.09
	Transition Group	MCI-MCI	-.59	.55	.03-11.01	.35

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA groups; CLU = Clusterin; PICALM = Phosphatidylinositol Binding Clathrin Assembly Protein; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio.

[a]Diminished cell sizes precluded interpretation of these significant and trend effects.

[b]Diminished cell sizes precluded calculation of CI.

[c]Diminished cell sizes precluded calculation of OR.

Table 12

Research Question 3 Results: CR1(2) x PICALM(2) Model

Predictors	Reference Group	Status/Stability	β	OR	95% CI	p
CR1	NA-NA	MCI-NA	1.38	3.97	.30-52.88	.15
	NA-NA	NA-MCI	20.05	5.09×10^8	-- [a]	.50
	NA-MCI	MCI-MCI	-20.00	-- [b]	-- [a]	.50
	MCI-NA	MCI-MCI	-.99	.37	.03-4.40	.22
	NA-NA	MCI-MCI	-.07	.94	.25-3.56	.46
	MCI-NA	NA-MCI	21.80	2.92×10^9	-- [a]	.50
	NA-NA	Transition Group	1.97	7.20	.66-78.80	.05
	Transition Group	MCI-MCI	-1.64	.19	.02-2.02	.09
PICALM	NA-NA	MCI-NA	.85	2.34	.23-23.42	.24
	NA-NA	NA-MCI	20.06	5.13×10^8	-- [a]	.50
	NA-MCI	MCI-MCI	-20.52	-- [b]	-- [a]	.50
	MCI-NA	MCI-MCI	-1.13	.32	.03-3.22	.17
	NA-NA	MCI-MCI	-.57	.57	.18-1.82	.17
	MCI-NA	NA-MCI	22.26	4.64×10^9	-- [a]	.50
	NA-NA	Transition Group	1.81	6.08	.66-56.13	.06
	Transition Group	MCI-MCI	-2.03	.13	.01-1.21	.04
CR1(2) x PICALM(2)	NA-NA	MCI-NA	-1.11	.33	.02-5.27	.22
	NA-NA	NA-MCI	-20.37	-- [b]	-- [a]	.50
	NA-MCI	MCI-MCI	20.53	8.24×10^8	-- [a]	.50
	MCI-NA	MCI-MCI	1.05	2.85	.19-43.37	.23
	NA-NA	MCI-MCI	.35	1.42	.31-6.61	.33
	MCI-NA	NA-MCI	-22.31	-- [b]	-- [a]	.50
	NA-NA	Transition Group	-2.04	.13	.01-1.62	.06
	Transition Group	MCI-MCI	1.96	7.12	.58-87.08	.06

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA groups; CR1 = Complement Receptor 1; PICALM = Phosphatidylinositol Binding Clathrin Assembly Protein; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio.

[a]Diminished cell sizes precluded calculation of CI.

[b]Diminished cell sizes precluded calculation of OR.

Table 13

Research Question 4 Results: CLU(2) x APOE(2) Model

Predictors	Reference Group	Status/Stability	β	OR	95% CI	p
CLU	NA-NA	MCI-NA	19.97	4.71×10^8	-- [b]	.50
	NA-NA	NA-MCI	1.50	4.47	.51-38.98	.09 [a]
	NA-MCI	MCI-MCI	-1.04	.35	.04-3.23	.18
	MCI-NA	MCI-MCI	-20.49	-- [c]	-- [b]	.50
	NA-NA	MCI-MCI	.54	1.71	.60-4.91	.16
	MCI-NA	NA-MCI	-21.35	-- [c]	-- [b]	.50
	NA-NA	Transition Group	2.18	8.82	1.07-72.59	.02 [a]
	Transition Group	MCI-MCI	-1.77	.17	.02-1.50	.06
APOE	NA-NA	MCI-NA	19.38	2.60×10^8	-- [b]	.50
	NA-NA	NA-MCI	.93	2.53	.12-51.29	.27
	NA-MCI	MCI-MCI	-.51	.60	.03-13.09	.37
	MCI-NA	MCI-MCI	-19.90	-- [c]	-- [b]	.50
	NA-NA	MCI-MCI	.57	1.76	.34-9.00	.25
	MCI-NA	NA-MCI	-21.59	-- [c]	-- [b]	.50
	NA-NA	Transition Group	1.60	4.97	.36-69.48	.12
	Transition Group	MCI-MCI	-1.13	.32	.02-4.99	.21
CLU(2) x APOE(2)	NA-NA	MCI-NA	-19.30	-- [c]	-- [b]	.50
	NA-NA	NA-MCI	-.49	.62	.03-15.23	.31
	NA-MCI	MCI-MCI	.74	2.09	.08-55.12	.33
	MCI-NA	MCI-MCI	20.63	9.07×10^8	-- [b]	.50
	NA-NA	MCI-MCI	.08	1.08	.17-6.66	.47
	MCI-NA	NA-MCI	22.08	3.90×10^9	-- [b]	.50
	NA-NA	Transition Group	-1.33	.26	.02-4.29	.17
	Transition Group	MCI-MCI	1.60	4.97	.28-88.76	.14

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA groups; CLU = Clusterin; APOE = Apolipoprotein E; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio.
[a]Diminished cell sizes precluded interpretation of these significant and trend effects.
[b]Diminished cell sizes precluded calculation of CI.
[c]Diminished cell sizes precluded calculation of OR.

Table 14

Research Question 4 Results: CR1(2) x APOE(2) Model

Predictors	Reference Group	Status/Stability	β	OR	95% CI	p
CR1	NA-NA	MCI-NA	-.15	.87	.28-2.64	.40
	NA-NA	NA-MCI	-.64	.53	.17-1.60	.13
	NA-MCI	MCI-MCI	.48	1.61	.50-5.17	.21
	MCI-NA	MCI-MCI	.12	1.13	.36-3.54	.42
	NA-NA	MCI-MCI	-.17	.84	.39-1.84	.33
	MCI-NA	NA-MCI	-.25	.78	.20-2.98	.36
	NA-NA	Transition Group	-.39	.68	.29-1.59	.18
	Transition Group	MCI-MCI	.26	1.30	.52-3.25	.29
APOE	NA-NA	MCI-NA	-20.05	-- [b]	-- [a]	.50
	NA-NA	NA-MCI	-1.48	.23	.02-2.17	.10
	NA-MCI	MCI-MCI	1.25	3.49	.34-35.73	.15
	MCI-NA	MCI-MCI	20.57	8.55×10^8	-- [a]	.50
	NA-NA	MCI-MCI	-.25	.78	.23-2.64	.34
	MCI-NA	NA-MCI	21.15	1.52×10^9	-- [a]	.50
	NA-NA	Transition Group	-2.01	.13	.02-1.19	.04
	Transition Group	MCI-MCI	1.84	6.26	.66-59.67	.06
CR1(2) x APOE(2)	NA-NA	MCI-NA	21.19	1.59×10^9	-- [a]	.50
	NA-NA	NA-MCI	2.85	17.27	1.23-243.31	.02
	NA-MCI	MCI-MCI	-1.47	.23	.02-3.28	.14
	MCI-NA	MCI-MCI	-20.47	-- [b]	-- [a]	.50
	NA-NA	MCI-MCI	1.35	3.84	.81-18.17	.05
	MCI-NA	NA-MCI	-20.93	-- [b]	-- [a]	.50
	NA-NA	Transition Group	3.21	24.72	2.14-286.00	.005
	Transition Group	MCI-MCI	-1.83	.16	.01-1.9	.07

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA groups; CR1 = Complement Receptor 1; APOE = Apolipoprotein E; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio.

[a]Diminished cell sizes precluded calculation of CI.

[b]Diminished cell sizes precluded calculation of OR.

Table 15

Research Question 4 Results: PICALM(2) x APOE(2) Model

Predictors	Reference Group	Status/Stability	β	OR	95% CI	p
PICALM	NA-NA	MCI-NA	.74	2.09	.42-10.40	.18
	NA-NA	NA-MCI	.11	1.12	.28-4.49	.44
	NA-MCI	MCI-MCI	-.49	.61	.15-2.57	.25
	MCI-NA	MCI-MCI	-1.03	.36	.07-1.81	.11
	NA-NA	MCI-MCI	-.46	.63	.26-1.54	.16
	MCI-NA	NA-MCI	-.43	.65	.08-5.09	.34
	NA-NA	Transition Group	.49	1.64	.53-5.07	.20
	Transition Group	MCI-MCI	-.81	.45	.14-1.43	.09
APOE	NA-NA	MCI-NA	1.48	4.39	.43-44.44	.11
	NA-NA	NA-MCI	-19.29	-- [b]	-- [a]	.50
	NA-MCI	MCI-MCI	19.67	3.49×10^8	-- [a]	.50
	MCI-NA	MCI-MCI	-.91	.40	.04-3.92	.22
	NA-NA	MCI-MCI	.24	1.27	.28-5.71	.38
	MCI-NA	NA-MCI	-21.47	-- [b]	-- [a]	.50
	NA-NA	Transition Group	.66	1.94	.26-14.60	.26
	Transition Group	MCI-MCI	-.10	.90	.12-6.61	.46
PICALM(2) x APOE(2)	NA-NA	MCI-NA	-1.81	.16	.01-2.55	.10
	NA-NA	NA-MCI	19.86	4.23×10^8	-- [a]	.50
	NA-MCI	MCI-MCI	-19.60	-- [b]	-- [a]	.50
	MCI-NA	MCI-MCI	1.92	6.79	.46-100.99	.08
	NA-NA	MCI-MCI	.46	1.58	.28-8.91	.30
	MCI-NA	NA-MCI	22.31	4.87×10^9	-- [a]	.50
	NA-NA	Transition Group	-.47	.62	.07-5.92	.34
	Transition Group	MCI-MCI	.60	1.82	.20-16.81	.30

Note. NA = normal aging; MCI = mild cognitive impairment; Transition Group = combined NA-MCI and MCI-NA groups; PICALM = Phosphatidylinositol Binding Clathrin Assembly Protein; APOE = Apolipoprotein E; β = coefficient; OR = odds ratio; 95% CI = 95% confidence interval for odds ratio.

[a]Diminished cell sizes precluded calculation of CI.

[b]Diminished cell sizes precluded calculation of OR.

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Glossary

A+ = Carrier of the Complement Receptor 1 Alzheimer's disease risk allele.

A- = Non-carrier of the Complement Receptor 1 Alzheimer's disease risk allele.

Aβ = β amyloid; one of two key pathological markers of Alzheimer's disease.

AD = Alzheimer's disease; a type of dementia characterized by the presence of β amyloid plaques and neurofibrillary tangles in the brain.

APOE = Apolipoprotein E; a gene associated with Alzheimer's disease.

APO J = Apolipoprotein J; an alternate name for the gene Clusterin.

BDNF = Brain Derived Neurotrophic Factor; a gene associated with Alzheimer's disease.

C+ = Carrier of the Clusterin Alzheimer's disease risk allele.

C- = Non carrier of the Clusterin Alzheimer's disease risk allele.

CLU = Clusterin; a gene associated with Alzheimer's disease.

COMT = Catechol-o-methyltransferase; a gene associated with Alzheimer's disease.

CR1 = Complement Receptor 1; a gene associated with Alzheimer's disease.

ε 4+ = Carrier of the APOE risk allele.

ε 4- = Non-carrier of the APOE risk allele.

GWAS = Genome wide association study; a research method used for detecting associations between genotype and a specific trait or condition.

MCI = mild cognitive impairment; a clinical condition considered to be prodromal to Alzheimer's disease.

NA = normal aging.

PCR = polymerase chain reaction; a method of generating numerous copies of a sequence of DNA.

PICALM = Phosphatidylinositol Binding Clathrin Assembly Protein; a gene associated with Alzheimer's disease.

SNPs = Single nucleotide polymorphisms; in the population, variation in a genomic sequence wherein one nucleotide has been replaced by another.

T+ = Carrier of the PICALM Alzheimer's disease risk allele.

T- = Non-carrier of the PICALM Alzheimer's disease risk allele.

VLS = Victoria Longitudinal Study; a large-scale study of human aging.

W1 = Measurement wave 1.

W2 = Measurement wave 2