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The Association of Cognitive Endophenotypes and Risky Single Nucleotide Polymorphisms of Alzheimer's Disease within the *Alzheimer's Disease Neuroimaging Initiative (ADNI)* Database

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by

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ABSTRACT

Objective: The purpose of this study was to assess the influence of three single nucleotide polymorphisms (SNP) previously associated with Alzheimer's disease on specific domains of cognition, when controlling for Apolipoprotein E gene (*APOE*), in a sample of individuals with Alzheimer's disease. ***Methods****:* The data were drawn from the Alzheimer's Disease Neuroimaging Initiative database, a comprehensive, longitudinal database of controls, persons with mild cognitive impairment, and persons with mild Alzheimer's disease. Each subject has a full neuropsychological assessment, neuroimaging, genetic sequencing, and physical evaluation. For the purposes of this study, individuals were selected based on the presence of the three SNPs of interest: *CR1* (rs3818361_T), *CLU* (rs11136000_T), and PICALM (rs3851179_A). Each SNP was then measured against the available tests of the ADNI neuropsychological battery that measured immediate and long delay memory, semantic fluency, and confrontation naming. ***Results***: Only the *CR1* SNP (rs3818361_T) had significant findings. The presence of the *CR1* SNP associated with lower performance on logical memory recall total score, AVLT immediate recall trials 2 and 4, AVLT delayed recall, and confrontation naming in the 12-month control group. Logical memory and AVLT delayed recall were also negatively associated with *CR1* in the 12-month AD case group. ***Discussion***: These results support previous findings that the CR1 SNP rs3818361_T is a risk factor for cognitive impairment in individuals with and without AD. Such findings

can aid in the earlier detection of Alzheimer's disease, risk for domain specific cognitive impairment, and novel targets for personalized pharmacotherapy.

CHAPTER ONE:

INTRODUCTION

Genetic markers are increasingly becoming an area of interest in the translational science community. This interest stems from the potential that genetic markers have for the early detection of a multitude of diseases and disorders; the use of that information in determining the pathophysiological etiologies; and the potential therapeutic intervention targets of serious, high impact diseases such as Alzheimer's disease (AD) and other forms of dementia (Ramanan & Saykin, 2013). Currently, the greatest known genetic risk factor for sporadic late-onset (after age 65) AD is the *APOE* gene. *APOE* has three allelic variations, ε2, ε3, and ε4, with the ε4 variant conferring the greatest risk for development of AD. However, this polymorphism does not independently predict disease onset in all cases, and can be present in non-demented individuals (Bagyinszky, Youn, An, & Kim, 2014).

Genome Wide Associations Studies (GWAS) have become an effective method of surveying entire genomes for common genetic variants within disease populations. From these studies, various single nucleotide polymorphisms (SNPs) have been identified as having significant association with disease onset and risk. However, these SNPs alone only contribute a small fraction of the overall risk for onset and must be assessed in concert with other variables, such as combined effects of other genetic variants and the influence of environmental factors (Ertekin-Taner, 2011). Because the association of these SNPs with disease is garnered from massive, non-hypothesis

driven association studies, it is important to measure and assess phenotypes that may be affected by these polymorphisms and identify clinical risks that stem from these mutations (Ertekin-Taner, 2011).

Support for Genes of Interest

The European Alzheimer's Disease Initiative (EADI) conducted a GWAS of 537,029 SNPs in a sample of 2032 AD subjects, and 5328 controls and found significant negative association with the complement receptor type 1 (*CR1*) gene and a protective association for the clusterin (also referred to as apolipoprotein J; *CLU*) gene (Lambert et al., 2009). The Genetic and Environmental Risk in Alzheimer's Disease (GERAD) consortium cross validated the findings of the EADI in a sample of 3941 cases, 7848 controls, and analyzed 529,218 SNPs where they found significance for *CLU* as well as the phosphatidylinositol binding clathrin assembly protein (*PICALM*) gene (Harold et al., 2009). In a meta-analysis of EADI, GERAD, and the Cohort for Heart and Aging Research in Genomic Epidemiology (CHARGE; combined case sample of 8371 and control sample of 28,174) the attributable risk for Caucasians in *CR1, CLU*, and *PICALM* were approximated as 4%, 9%, and 9%, respectively (Seshadri et al., 2010). The data on associations of *CR1*, *CLU*, and *PICALM* with AD is variable in different ethnicities. In Chinese samples *CR1* (Zhang et al., 2010) and *CLU* (Yu et al., 2010) have been associated with AD. No association has been found in African Americans, Hispanics, or Arabs, but studies assessing these populations have been regarded as underpowered (Lambert & Amouyel, 2011).

Through databases such as the Alzheimer's Disease Neuroimaging Initiative (ADNI), SNPs that have shown association with AD in other GWAS samples are now

being assessed in well controlled, longitudinal cohorts. Of relevance to this study are three SNPs in three genes shown to be present in AD cohorts: *CR1, CLU,* and *PICALM* (Harold, et al., 2009; Lambert, et al., 2009). These genes are of particular relevance, as they are known to associate with risk, or protection from, cognitive decline in carriers of *APOE* ε4, and are implicated in the clearance of Aβ deposits in the central nervous system. Each of these genes and their actions are reviewed below.

CR1

The gene *CR1* is located on chromosome 1 and codes for the complement component (3b/4b) receptor 1, a transmembrane glycoprotein that is implicated in immune and glial-meditated inflammatory response and phagocytosis. The protein CR1 is a cell-surface receptor that has been found to potentially be involved in the clearance of amyloid and apoptotic cells (Wyss-Coray et al., 2002). *CR1* expression in neuronal cells are low, and found most in the choroid plexus, while outside of the central nervous system *CR1* is expressed most frequently in erythrocytes. Erythrocytes, via the CR1 receptor, have been found to effectively sequester peripheral Aβ42 particles and assist in its clearance, a function that is found to be impaired in individuals with AD (Rogers et al., 2006). CR1 is also expressed in the Kolmer cells of the choroid plexus, linking it to CSF exchange with brain cells, and the introduction of leucocytes and CSF antigens, factors that can link AD with immune response to Aβ (Lambert & Amouyel, 2011).

CR1 has been linked to AD through several GWAS studies where the presence of *CR1* risk SNPs in *APOE* ε4 carriers resulted in a greater rate of decline in cognitive function in previously non-demented subjects secondary to increase amyloid deposition in brain cells (Hazrati et al., 2012). However, the influence of various *CR1* alleles

confers different effects on amyloid load. Thambisetty and colleagues (Madhav Thambisetty et al., 2013) found carriers of *CR1* SNP rs3818361_A, considered higher for disease risk, have lower measures of brain amyloid compared to non-carriers. This would seem to be in contradiction to the understood pathophysiology of AD, however as Thambisetty and colleagues note, modest amyloid deposition may be evidence of an adaptive immune response to the onset of disease. In a meta-analysis of current *CR1* risk allele association studies, Luo, et al (2014) tested known studies of *CR1* predictive risk for AD onset. SNPs rs6656401 and rs3818361 were found to be highly significant predictors of AD susceptibility in Caucasians and Asian populations. Specifically of interest to this study, the T allele of rs3818361 conferred a greater risk for AD onset then the C allele (Luo et al., 2014).

CLU

The gene *CLU* is located on chromosome 8, codes for clusterin, and has been associated with the clearance of Aβ (particularly the highly toxic Aβ42), reduction of excessive inflammation and apoptosis, and clearance of neuronal debris (Pedraza et al., 2013). The CLU protein is expressed in great abundance in the central nervous system, and like APOE is an apolipoprotein (DeMattos et al., 2004). Expression levels of *CLU* in mRNA are greater in AD subjects compared to controls (M. Thambisetty et al., 2010). Associations have been assessed between concentrations of clusterin in blood plasma and CSF, and rates of cognitive decline in AD. Thambistetty and colleagues found that higher CSF clusterin concentrations correlated with greater endorhinal cortex and medial temporal lobe atrophy in AD, and to lesser extent, in patients with MCI. Higher blood plasma clusterin levels were also linked to lower Mini

Mental State Exam (MMSE) scores. Patients that had rapidly advancing cognitive impairment also exhibited higher clusterin concentration levels compared to slower advancing patients. There were no differences found between *APOE* ε4 carriers and non-carriers (Thambisetty, 2010).

Allelic variations of rs11136000 confer differing levels of risk and functional consequences. The T allele has had split risk presentations. (Mengel-From et al., 2013) reported a greater rate of cognitive decline for a longitudinal Danish cohort T allele carriers, but subsequently found better cognitive composite score performance for T carriers then non-carriers in a separate Danish cohort, which they attributed to regression to the mean with increased age.

PICALM

The gene *PICALM* encodes for phosphatidylinositol binding clathrin assembly protein. It is primarily expressed in the endothelium of blood vessel walls, and like *CR1* is not found in high concentration in neurons. Thus *PICALM* has been hypothesized to participate in the transport Aβ across the blood brain barrier and into the peripheral blood stream (Lambert & Amouyel, 2011). *PICALM* expression association with AD is only found to be significant in white APOE ε4 carriers (Jun et al., 2010), and these results confirmed in another cohort by Hazrati and colleagues in (2012). The data on *PICALM* risk have been mixed, showing both protective and risky effects for the onset of AD. Meta-analysis of rs3851179 has shown risk for onset of AD in Caucasian populations, but not for African-American, Arab, or Hispanic groups (Jun, et al., 2010), however the association was reported to be moderated by a dose effect presence of APOE ε4 alleles. Of interest to this study, there are data to support the rs3851179 A

allele as a protective factor for the onset of AD. The rs3851179A isoform has been shown to exclude a critical exon necessary for coding of the *PICALM* protein, and comprises much of the association for reducing AD risk compared to other *PICALM* polymorphisms (Parikh, Fardo, & Estus, 2014). The conclusion of Parikh and colleagues for this protective influence is the expression of the rs3851179 A allele modestly increases the efficiency of brain microvasculature through more robust expression of the *PICALM* gene. Parikh, et al went further to say that previous mixed results of *PICALM* association with AD could be attributed to the differential expression of *PICALM* isoforms that were not measured in previous studies. It is also noted that the rs3851179 polymorphism is likely not directly responsible for this effect as it is upstream from the *PICALM* gene, but is rather in LD with a functional SNP not yet identified.
Of interest to this study is the influence of these AD-associated SNPs on the cognitive profile of individuals with AD. Specifically, are these polymorphisms associated with cognitive performance among individuals with AD? Perhaps these changes can be predictive and aid in early diagnosis and targeted treatment planning.

A better understanding of the influence that these genetic polymorphisms have on neuropsychological function may provide insight to more efficient detection methods for serious disorders of cognitive function and the course of progressive diseases such as AD. In this study, three AD-associated SNPs will be assessed for association with standardized measures of memory and language in a cohort of controls and individuals with AD. Results of the study will provide further information for the impact subtle genetic variations may have on the cognitive profile and course of AD.

CHAPTER TWO:

METHODS

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations launched the ADNI in 2003, as a $60 million, 5- year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

Subject demographics

The average age of all subjects was 81, with exception of the *CR1* match baseline group, which was an average of 84. Average education ranged from 14.7 to 15.7 years. Between 52% and 62% of subjects were male sex (summarized in Table 1).

Three SNPs were chosen based on previous publications supporting their association with AD: *CR1* (rs3818361_T)*, CLU* (rs11136000_T), and *PICALM*

(rs3851179_A). Subjects were genotyped using the Human610-Quad BeadChip by Illumina to generate outputs of over 600,000 SNPs (Saykin et al., 2010).

Case subjects were selected based on the presence of the SNPs of interest and a diagnosis of AD. The control group was ADNI subjects without AD with the SNPs of interest. Further, subjects were filtered based on the availability of neuropsychological tests that are clinically relevant to the AD cognitive profile. Subject scores were analyzed at baseline and at the 12-month visit.

After filtering for the desired variables, there were 66, 106, and 95 control subjects at baseline for *CR1*, *CLU*, and *PICALM*, respectively. There were 77, 124, and 110 subjects at 12-month testing. In the case group there were 274, 480, and 432 subjects at baseline, and 249, 434, 390 at 12-month testing.

Psychometrics

Each SNP was measured against the available tests of the ADNI neuropsychological battery that measured immediate and long delay memory, semantic fluency, and confrontation naming; all domains relevant to diagnosis and measure of AD (Karantzoulis & Galvin, 2011). Measures used include Wechsler Memory Scales IV - Logical Memory subtest (Wechsler, 2009), the Auditory-Verbal Learning Test (AVLT; (Rey, 1941), Semantic Fluency tests (animals and vegetable categories), and the Boston Naming Test (Kaplan, Goodglass, & Weintraub, 2001).

Wechsler Memory Scales – 4th Edition – Logical Memory

The WMS-IV LM subtests are a measure of immediate and long-delay memory for two stories of different content. The participant is read a story and asked to recall as

many details of the story as they can immediately after the story is read. This procedure is then repeated for a second trial. The participant is then read a different story following the same procedure. These trials function as an assessment of immediate memory recall. A delay of 15-20 minutes is then applied, during which the individual is administered other unrelated tasks. After the delay, the participant is then asked to recall as much of each story as they can without any cues. This is a measure of long-term logical memory retrieval. The delay trial is then followed with a “True or False” forced choice recognition task for details of each story to assess awareness of the content of the original stories with cues (Wechsler, 2009).

Auditory-Verbal Learning Test

The AVLT is a measure of learning, and immediate and long-delay verbal memory, similar in structure to the WMS-IV LM subtests. Participants are read a list of 15 phonemically and semantically dissimilar words and asked to recall as many of words as possible immediately after the list has been read to them. This procedure is repeated over the course five learning trials (Trials 1-5), over which the participant is anticipated to recall more words each subsequent trial. Upon completion of the five learning trials, the participant is then read a new list of 15 words (Trial B) that are semantically similar to the first list. Trial B is intended to serve as a distractor from the content of Trials 1-5. Upon completion of Trial B, the participant is then asked to recall as many words as possible from the first list (Trials 1-5) without the list being read to them again. This serves as a measure of working memory and short-term memory recall. The participant is then administered other tasks during a delay period of 30

minutes. After the delay, the participant is then asked to recall as many words as they can from the first list again (Trial 6). This is a measure of free recall long-term memory. A recognition trial is then administered, with forced choice "Yes or No" responses to assess the participant's awareness of the lists previously read to them (Recognition). This measures long term storage of verbal information and specifically assesses the function of the hippocampus, a structure impaired by AD (Estévez-González, Kulisevsky, Boltes, Otermín, & García-Sánchez, 2003).

Semantic Fluency Test

The semantic fluency test assesses a participant's ability to verbally generate as many words as they can in 60 seconds that fit into a specific semantic category. This study used the standardized categories of Animals and Vegetables. Semantic fluency is a frequently used measure in the assessment of AD severity (Weakley & Schmitter-Edgecombe, 2014); (Bertola et al., 2014).

Boston Naming Test – 2nd Edition.

The BNT assesses confrontational naming, language-based memory retrieval, and knowledge for images of common items that are presented as individual line drawings. The BNT is an effective measure for the differential assessment of aphasia (Williams, Mack, & Henderson, 1989).

Statistical Analysis

Each SNP was tested for association with the chosen cognitive endophenotypes at baseline and 12-month visit. Multivariate linear regression was employed while controlling for sex, age, education, and *APOE* ε4 status

CHAPTER THREE:

RESULTS

CR1 (rs3818361_T)

Results for association of SNP rs3818361_T are summarized in *Table 2*. No significant findings were observed for the baseline measures for either the match or case groups. For the match group at 12-month testing, rs3818361_T associated with lower logical memory immediate recall total score (β = -1.2, p = 0.0023,), AVLT immediate recall Trial 2 (β = -0.5, p = 0.0305,) and Trial 4 (β = -0.6, p = 0.037), AVLT delayed recall total (β = -1.2, p= 0.023,), and confrontation naming spontaneous response total score (β = -1.7, p = 0.047).

Other values that approached significance in the match group include lower scores on the AVLT immediate recall Trial 1 (β = -0.4, p = 0.066) and Trial 5 (β = -0.5, p = 0.0749).

SNP rs3818361_T associated with lower logical memory immediate recall score (β = -0.6, p = 0.027) and AVLT delayed recall total score (β = -0.8, p = 0.0016) in those with AD.

CLU (rs11136000_T)

Results for association of SNP rs11136000_T are summarized in *Table 3.* No significant associations were observed for rs11136000_T. Values that approached significance were observed in the 12-month case group: Trial 3 (β = 0.21, p = 0.101),

Trial 5 (β = 0.25, p = 0.08), and Trial 6 (β = 0.29, p = 0.08) of the AVLT, where better performance was associated with the presence of rs11136000_T in those with AD.

PICALM (rs3851179_A)

Results for association of SNP rs3851179_A are summarized in *Table 4.* No significant associations were measured for rs3851179_A. Measures that approached significance include higher Logical Memory immediate recall total score at baseline for the match group (β = 0.56, p = 0.09). Marginal significance was also observed for better performance on Trial B (β = 0.26, p = 0.08) of the AVLT for the match group at 12-months.

CHAPTER FOUR:

DISCUSSION

This study approached the hypothesis that genetic polymorphisms associated with risk for AD will associate with cognitive performance consistent with the cognitive profile of the disease. Logical memory, list-learning memory, and confrontation naming were all assessed in non-AD and AD subjects within the ADNI dataset.

Based on these data, the presence of the *CR1* SNP (rs3818361_T) resulted in lower measures of immediate recall memory for list learning and logical memory as well as lower confrontation naming scores in the match group at 12-month testing. The same reduced scores were found in the case group at one year follow up for immediate logical memory, and delayed list recall. This pattern indicates that the presence of rs3818361_T is a risk factor cognitive impairment in non-AD subjects, and to a lesser extent for subjects with AD.

This study provides support for previous findings of Pedraza, et al (2013) for the influence of the rs3818361_T polymorphism of *CR1* on cognitive function, in both cognitively normal older adults and those with AD, when controlling for the influence of *APOE* (Pedraza, et al., 2013). These findings, while supportive, may be underpowered based on a low sample size and influence from other known and unknown polymorphisms in linkage disequilibrium with *CR1*. Replication of this study would

benefit from a greater sample size and further investigation into differential influence of race as a variable.

While the near significant findings for the *CLU* and *PICALM* SNPs are supported by literature indicating the protective effect of these polymorphisms, these values are underpowered and require further analysis in a larger sample to be validated. This is an often-cited concern in genetic association studies insufficient sample size (Naj et al., 2011). However, as noted, *CLU* minor allele rs11136000_T is a known protective factor for AD risk, as it has been associated with increased CLU protein levels in the brain, and more efficient sequestration of Aβ (Ling, Bhongsatiern, Simpson, Fardo, & Estus, 2012). But attempts to find cognitive endophenotypes for this marker have been mixed, often citing concerns for sample size and heterogeneity (Pedraza, et al., 2013).

At the drafting of this manuscript, the *CLU*, *CR1*, and *PICALM* polymorphisms assessed were ranked in the top ten of the AlzGene database for most associated polymorphisms with AD (ranked 3rd, 5th, and 6^{th,} respectively; Bertram, et al., 2007). These SNPs have had mixed results in GWAS-level studies for association with disease presence and phenotype, often modulated by noted factors such as sample size effecting power and ability to detect rare polymorphisms in the general population. As such, the so called “winner’s curse” may be at play with further replication warranted (Xiao & Boehnke, 2009).

Outside of the limitations set by sample size, replication of this study will benefit from more detailed neuropsychological assessment data, specifically assessment of cued recognition for the WMS-IV logical memory subtest and AVLT, as these sub scores aid in determining severity of hippocampus mediated cognitive abilities.

Another consideration for future analysis in larger samples would be grouped analysis of AD and non-AD individuals to increase variance across a continuum of cognitive scores, instead of parsing by diagnostic category. Previous groups (Chibnik et al., 2011; Bennett, De Jager, Leurgans, & Schneider, 2009) that have employed this method have showed greater association with cognitive endophenotypes then in diagnostically separate groups. This method can however cloud the true nature of an association and if the findings are tied to an independent cognitive process or AD mediated pathology.

CHAPTER FIVE:

REFERENCES

Bagyinszky, E., Youn, Y. C., An, S. S., & Kim, S. (2014). The genetics of Alzheimer's disease. *Clin Interv Aging, 9*, 535-551.

Bennett, D. A., De Jager, P. L., Leurgans, S. E., & Schneider, J. A. (2009). Neuropathologic intermediate phenotypes enhance association to Alzheimer susceptibility alleles. *Neurology, 72*(17), 1495-1503.

Bertola, L., Cunha Lima, M. L., Romano-Silva, M. A., de Moraes, E. N., Diniz, B. S., & Malloy-Diniz, L. F. (2014). Impaired generation of new subcategories and switching in a semantic verbal fluency test in older adults with mild cognitive impairment. *Front Aging Neurosci, 6*, 141.

Chibnik, L. B., Shulman, J. M., Leurgans, S. E., Schneider, J. A., Wilson, R. S., Tran, D., et al. (2011). CR1 is associated with amyloid plaque burden and age-related cognitive decline. *Ann Neurol, 69*(3), 560-569.

DeMattos, R. B., Cirrito, J. R., Parsadanian, M., May, P. C., O'Dell, M. A., Taylor, J. W., et al. (2004). ApoE and clusterin cooperatively suppress Abeta levels and deposition: evidence that ApoE regulates extracellular Abeta metabolism in vivo. *Neuron, 41*(2), 193-202.

Ertekin-Taner, N. (2011). Gene expression endophenotypes: a novel approach for gene discovery in Alzheimer's disease. *Mol Neurodegener, 6*, 31.

Estévez-González, A., Kulisevsky, J., Boltes, A., Otermín, P., & García-Sánchez, C. (2003). Rey verbal learning test is a useful tool for differential diagnosis in the preclinical phase of Alzheimer's disease: comparison with mild cognitive impairment and normal aging. *Int J Geriatr Psychiatry, 18*, 1021–1028

Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M. L., et al. (2009). Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet, 41*(10), 1088-1093.

Hazrati, L. N., Van Cauwenberghe, C., Brooks, P. L., Brouwers, N., Ghani, M., Sato, C., et al. (2012). Genetic association of CR1 with Alzheimer's disease: a tentative disease mechanism. [Research Support, Non-U.S. Gov't]. *Neurobiol Aging, 33*(12), 2949 e2945-2949 e2912.

Jun, G., Naj, A. C., Beecham, G. W., Wang, L. S., Buros, J., Gallins, P. J., et al. (2010). Meta-analysis confirms CR1, CLU, and PICALM as alzheimer disease risk loci and reveals interactions with APOE genotypes. *Arch Neurol, 67*(12), 1473-1484.

Kaplan, E., Goodglass, H., & Weintraub, S. (2001). Boston Naming Test-2 (BNT-2). Austin, TX: Pro-Ed.

Karantzoulis, S., & Galvin, J. E. (2011). Distinguishing Alzheimer's disease from other major forms of dementia. *Expert Rev Neurother, 11*(11), 1579-1591.
Lambert, J. C., & Amouyel, P. (2011). Genetics of Alzheimer's disease: new evidences for an old hypothesis? [Review]. *Curr Opin Genet Dev, 21*(3), 295-301.
Lambert, J. C., Heath, S., Even, G., Campion, D., Sleegers, K., Hiltunen, M., et al. (2009). Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet, 41*(10), 1094-1099.
Ling, I. F., Bhongsatiern, J., Simpson, J. F., Fardo, D. W., & Estus, S. (2012). Genetics of clusterin isoform expression and Alzheimer's disease risk. *PLoS One, 7*(4), e33923.
Luo, J., Li, S., Qin, X., Song, L., Peng, Q., Chen, S., et al. (2014). Meta-analysis of the association between CR1 polymorphisms and risk of late-onset Alzheimer's disease. *Neurosci Lett, 578*, 165-170.
Mengel-From, J., Thinggaard, M., Lindahl-Jacobsen, R., McGue, M., Christensen, K., & Christiansen, L. (2013). CLU genetic variants and cognitive decline among elderly and oldest old. *PLoS One, 8*(11), e79105.
Naj, A. C., Jun, G., Beecham, G. W., Wang, L. S., Vardarajan, B. N., Buros, J., et al. (2011). Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet, 43*(5), 436-441.
Parikh, I., Fardo, D. W., & Estus, S. (2014). Genetics of PICALM expression and Alzheimer's disease. *PLoS One, 9*(3), e91242.
Pedraza, O., Allen, M., Jennette, K., Carrasquillo, M., Crook, J., Serie, D., et al. (2013). Evaluation of memory endophenotypes for association with CLU, CR1, and PICALM variants in black and white subjects. *Alzheimers Dement*.
Ramanan, V. K., & Saykin, A. J. (2013). Pathways to neurodegeneration: mechanistic insights from GWAS in Alzheimer's disease, Parkinson's disease, and related disorders. *Am J Neurodegener Dis, 2*(3), 145-175.
Rey, A. (1941). L'examen psychologique dans les cas d'encephalopathie traumatique. *Archives de Psychologie, 28*(21).
Rogers, J., Li, R., Mastroeni, D., Grover, A., Leonard, B., Ahern, G., et al. (2006). Peripheral clearance of amyloid beta peptide by complement C3-dependent adherence to erythrocytes. *Neurobiol Aging, 27*(12), 1733-1739.
Saykin, A. J., Shen, L., Foroud, T. M., Potkin, S. G., Swaminathan, S., Kim, S., et al. (2010). Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: Genetics core aims, progress, and plans. *Alzheimers Dement, 6*(3), 265-273.
Seshadri, S., Fitzpatrick, A. L., Ikram, M. A., DeStefano, A. L., Gudnason, V., Boada, M., et al. (2010). Genome-wide analysis of genetic loci associated with Alzheimer disease. *Jama, 303*(18), 1832-1840.
Thambisetty, M., An, Y., Nalls, M., Sojkova, J., Swaminathan, S., Zhou, Y., et al. (2013). Effect of Complement *CR1* on Brain Amyloid Burden During Aging and Its Modification by *APOE* Genotype. *Biological Psychiatry, 73*(5), 422-428.
Thambisetty, M., Simmons, A., Velayudhan, L., Hye, A., Campbell, J., Zhang, Y., et al. (2010). Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. *Arch Gen Psychiatry, 67*(7), 739-748.

Weakley, A., & Schmitter-Edgecombe, M. (2014). Analysis of verbal fluency ability in Alzheimer's disease: the role of clustering, switching and semantic proximities. *Arch Clin Neuropsychol, 29*(3), 256-268.

Wechsler, D. (2009). *Wechsler Memory Scale—Fourth Edition (WMS–IV) technical and interpretive manual*. San Antonio, TX: Pearson.

Williams, B. W., Mack, W., & Henderson, V. W. (1989). Boston naming test in alzheimer's diseaase. *Neuropsychologia, 27*(18), 1073-1079.

Wyss-Coray, T., Yan, F., Lin, A. H., Lambris, J. D., Alexander, J. J., Quigg, R. J., et al. (2002). Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. *Proc Natl Acad Sci U S A, 99*(16), 10837-10842.

Xiao, R., & Boehnke, M. (2009). Quantifying and correcting for the winner's curse in genetic association studies. *Genet Epidemiol, 33*(5), 453-462.

Yu, J. T., Li, L., Zhu, Q. X., Zhang, Q., Zhang, W., Wu, Z. C., et al. (2010). Implication of CLU gene polymorphisms in Chinese patients with Alzheimer's disease. *Clin Chim Acta, 411*(19-20), 1516-1519.

Zhang, Q., Yu, J. T., Zhu, Q. X., Zhang, W., Wu, Z. C., Miao, D., et al. (2010). Complement receptor 1 polymorphisms and risk of late-onset Alzheimer's disease. *Brain Res, 1348*, 216-221.

Table 1 - Subject Demographics					
		n	Average Age	Average Edu	% Male
	CR1 rs3818361				
Match	**Baseline**	**66**	**84**	**14.7**	**62%**
	12-month	**77**	**81**	**14.7**	**59%**
Case	**Baseline**	**274**	**81**	**15.5**	**58%**
	12-month	**249**	**81**	**15.6**	**57%**
	CLU rs11136000				
Match	**Baseline**	**106**	**81**	**15**	**52%**
	12-month	**124**	**81**	**15**	**56%**
Case	**Baseline**	**480**	**81**	**15.7**	**58%**
	12-month	**434**	**81**	**15.7**	**59%**
	PICALM rs3851179				
Match	**Baseline**	**95**	**81**	**15**	**59%**
	12-month	**110**	**81**	**15**	**60%**
Case	**Baseline**	**432**	**81**	**15.6**	**60%**
	12-month	**390**	**81**	**15.7**	**61%**

Table 2 - CR1 Association Study Results

	Trait	Match - Baseline			Match 12-Month			Case - Baseline			Case 12-Month		
		n	Beta (SE)	P-value	*n*	Beta (SE)	P-value	*n*	Beta (SE)	P-value	*n*	Beta (SE)	P-value
CR1 rs3818361	LIMMTOTAL	66	-0.2586	0.5077	77	-1.2083	0.0023	274	-0.1993	0.3407	249	-0.5617	0.0265
	LDELTOTAL	66	-0.0801	0.7515	77	-0.2640	0.2666	274	-0.0948	0.6452	249	-0.1490	0.5969
	AVTOT1				77	-0.3727	0.0656				249	-0.0311	0.7989
	AVTOT2				77	-0.5235	0.0305				249	0.0175	0.9038
	AVTOT3				77	-0.3830	0.1221				249	-0.1559	0.3393
	AVTOT4				77	-0.5718	0.0370				249	-0.2362	0.1750
	AVTOT5				77	-0.4885	0.0749				249	-0.1482	0.4132
	AVTOT6				77	-0.2215	0.3107				249	0.0235	0.9117
	AVTOTB				77	-0.1788	0.3264				249	0.0283	0.8144
	AVDEL30MIN				77	-0.1080	0.4479				249	0.0699	0.7474
	AVDELTOT				77	-1.2017	0.0230				249	-0.8181	0.0016
	CATANIMSC				77	-0.5428	0.3863				249	0.1885	0.6127
	CATVEGESC				77	-0.3543	0.4287				249	0.1259	0.6383
	BNTSPONT				77	-1.7033	0.0470				249	-0.2599	0.4860

Table 3 - CLU Association Study Results

	Trait	Match - Baseline			Match 12-Month			Case - Baseline			Case 12-Month		
		n	Beta (SE)	P-value	*n*	Beta (SE)	P-value	*n*	Beta (SE)	P-value	*n*	Beta (SE)	P-value
CLU rs11136000	LIMMTOTAL	106	-0.0575	0.8689	124	0.2565	0.4516	480	-0.0363	0.8297	434	0.0528	0.7955
	LDELTOTAL	106	-0.0751	0.7392	124	0.0915	0.6511	480	0.0924	0.5778	434	-0.0727	0.7478
	AVTOT1				124	0.2093	0.2250				434	-0.0489	0.6181
	AVTOT2				124	0.2563	0.2147				434	0.0832	0.4752
	AVTOT3				124	0.2051	0.3311				434	0.2143	0.1014
	AVTOT4				124	0.1527	0.5145				434	0.1576	0.2597
	AVTOT5				124	0.1204	0.6071				434	0.2527	0.0820
	AVTOT6				124	0.1939	0.2963				434	0.2904	0.0873
	AVTOTB				124	-0.0656	0.6722				434	-0.1137	0.2401
	AVDEL30MIN				124	0.0360	0.7664				434	0.2125	0.2222
	AVDELTOT				124	0.4709	0.2972				434	0.2886	0.1670
	CATANIMSC				124	0.1761	0.7411				434	-0.0113	0.9698
	CATVEGESC				124	-0.0562	0.8827				434	0.1663	0.4392
	BNTSPONT				124	0.9504	0.1937				434	0.0676	0.8215

Table 4 - PICALM Association Study Results

	Trait	Match - Baseline			Match 12-Month			Case - Baseline			Case 12-Month		
		n	Beta (SE)	P-value	*n*	Beta (SE)	P-value	*n*	Beta (SE)	P-value	*n*	Beta (SE)	P-value
PICALM rs3851179	LIMMTOTAL	95	0.5633	0.0980	110	0.3780	0.2500	432	0.1462	0.4006	390	0.2445	0.2439
	LDELTOTAL	95	0.1177	0.5950	110	0.1368	0.4834	432	-0.0565	0.7415	390	0.1003	0.6673
	AVTOT1				110	0.0714	0.6685				390	-0.0605	0.5499
	AVTOT2				110	0.1327	0.5061				390	0.1124	0.3493
	AVTOT3				110	0.1980	0.3309				390	0.0660	0.6251
	AVTOT4				110	0.3440	0.1273				390	0.0365	0.8005
	AVTOT5				110	0.1643	0.4669				390	0.1045	0.4859
	AVTOT6				110	0.0384	0.8308				390	-0.0762	0.6638
	AVTOTB				110	0.2570	0.0848				390	0.1462	0.1427
	AVDEL30MIN				110	0.0295	0.8009				390	-0.1299	0.4696
	AVDELTOT				110	-0.2353	0.5898				390	-0.1807	0.4016
	CATANIMSC				110	-0.0252	0.9610				390	0.0464	0.8805
	CATVEGESC				110	-0.1800	0.6244				390	0.0488	0.8257
	BNTSPONT				110	0.2450	0.7290				390	0.2544	0.4101