NIH Public Access
Author Manuscript
Neurobiol Aging. Author manuscript; available in PMC 2013 March 1.

Published in final edited form as:
Neurobiol Aging. 2012 March ; 33(3): 518–521. doi:10.1016/j.neurobiolaging.2010.04.015.

Association of *CLU* and *PICALM* variants with Alzheimer's disease

M.I. Kamboh[a], **R. L. Minster**[a], **F.Y. Demirci**[a], **M. Ganguli**[b], **S.T. DeKosky**[c], **O.L. Lopez**[d], and **M.M. Barmada**[a]

[a]Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA, USA

[b]Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

[d]Department of Neurology, University of Pittsburgh, Pittsburgh, PA, USA

[c]Office of the Dean and Department of Neurology, University of Virginia School of Medicine, Charlottesville, VA, USA

Abstract

Two recent large genome-wide association studies have reported significant associations in the *CLU* (*APOJ*), *CR1* and *PICALM* genes. In order to replicate these findings, we examined 7 single nucleotide polymorphisms (SNPs) most significantly implicated by these studies in a large case-control sample comprising of 2,707 individuals. Principle components analysis revealed no population substructure in our sample. While no association was observed with *CR1* SNPs (P=0.30–0.457), a trend of association was seen with the *PICALM* (P=0.071–0.086) and *CLU* (P=0.148–0.258) SNPs. A meta-analysis of three studies revealed significant associations with all three genes. Our data from an independent and large case-control sample suggest that these gene regions should be followed up by comprehensive resequencing to find functional variants.

Keywords

Alzheimer's disease; Genetics; Association

1. Introduction

The genetics of late-onset Alzheimer's disease (LOAD) is complex with the possible involvement of several genes. Although *APOE* has emerged as the strongest susceptibility marker for LOAD, it accounts for <30% of the disease risk (Slooter et al., 1998). In order to identify the remaining genes for LOAD, efforts have been focused on conducting genome-wide association (GWA) studies (Coon et al., 2007; Grupe et al., 2007; Li et al., 2008; Reinman et al., 2007; Abraham et al., 2008; Beecham et al., 2009; Bertram et al., 2008; Carrasquillo et al., 2009). However, with the exception of the *APOE* region, no other significant associations were replicated across these GWA studies. This highlights the difficulties in identifying the remaining LOAD genes which are thought to make a relatively

© 2010 Elsevier Inc. All rights reserved

Correspondence author at: M. Ilyas Kamboh, PhD, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261, USA Tel: 412-624-3066; Fax: 412-383-7844; kamboh@pitt.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

small contribution to the overall risk of disease and they can only be discovered using well powered case-control samples. Two recent large GWA studies have identified significant association of LOAD with single nucleotide polymorphisms (SNPs) in the clusterin (*CLU,* a.k.a. *APOJ*), complement component receptor 1 (*CR1)* and phosphatidylinositol-binding clathrin assembly protein (*PICALM)* genes (Harold et al., 2009; Lambert et al., 2009). We set out to replicate these findings in a relatively large case-control sample of 2,707 individuals from a single geographical location in Western Pennsylvania.

2. Materials and Methods

Late-onset AD cases were Caucasian Americans *(n*=1,348; mean age-at-onset [AAO] 72.6 ± 6.4 [S.D.] years; 65.6% women; 22.8% autopsy confirmed) recruited by the University of Pittsburgh Alzheimer's Disease Research Center, all of whom met NINCDS-ADRDA criteria for probable or definite AD. Controls were non-demented Caucasian Americans, age 60 or above (*n*=1,359; mean age 74.7 ± 6.5 years; 60.8% women). The study was approved by the University of Pittsburgh Institutional Review Board.

Genotyping was performed using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA). Each 384-well plate used for genotyping comprised both cases and controls. The *E*2*/*E*3*/*E*4APOE* polymorphism was determined either as described previously (Kamboh et al., 1995) or using TaqMan genotyping assays. Allele and genotype frequencies were calculated by the direct counting method. Odds ratios (ORs) were adjusted for the effects of age (AAO in cases), gender and *APOE* genotype. Association with AAO was tested with ANOVA in the LOAD sample using gender and *APOE* genotype as covariates. A meta-analysis was performed by combining our genotyping data for the 7 SNPs examined with the available genotype data for the corresponding SNPs from the two published GWA studies (Harold et al., 2009; Lambert et al., 2009). A fixed-effects (inverse variance) meta-analysis (Kazeem and Farrall, 2005) was performed using the catmap 1.6 (Nicodemus, 2008) package in R 2.7.2 (R Development Core Team, 2007). Odds ratios and confidence intervals and their P-values were calculated as well as the P-values from a formal test of the homogeneity of the various studies' ORs.

3. Results and Discussion

As expected, the presence of *APOE*4* was associated with LOAD risk (OR=4.41; 95% confidence interval [CI] (4.05–4.80). We examined a total of 7 SNPs (3 in *CLU*, 2 each in *CR1* and *PICALM* genes) most significantly implicated by either one or both GWA studies (Table 1). All SNPs were in Hardy-Weinberg equilibrium in both cases and controls, except for rs2279590 in *CLU* that deviated slightly from Hardy-Weinberg equilibrium in LOAD cases (*P*=0.034). Population stratification analysis by principle components was undertaken in PLINK (Purcell et al., 2007) and SpectralGEM (Lee et al., 2009) software, both of which demonstrated an even distribution of cases and controls across all dimensions, suggesting no anticipated biases from population substructure.

We did not observe an association of either *CR1* SNP individually or in two-site haplotype analysis with AD risk. On the other hand, two of the *CLU* SNPs (rs1136000 and rs2279590) and both *PICALM* SNPs, although not achieving statistical significance at the α=5% level, showed a trend of association similar to that reported by both GWA studies (Harold et al., 2009; Lambert et al., 2009). The trend of association was more evident for the *PICALM* SNPs (*P*=0.071 – 0.086) than the two *CLU* SNPs (*P*=0.148 – 0.258). Three-site *CLU* and two-site *PICALM* haplotype analyses did not provide improved significant signals (data not shown). The absence of statistically significant signals in our sample could be due to a lack of power because the reported effects sizes of these SNPs are extremely small and were

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

detected using several thousand cases and controls. However, our sample had 80% power to detect ORs of 1.18–1.22 for the risk alleles and 0.83–0.85 for the protective alleles of the 7 SNPs examined, which is comparable to the reported ORs in the original reports (Harold et al., 2009; Lambert et al., 2009). Despite the lack of statistically significant signals, the similarity of the directional effects observed in our sample to those reported in the two GWA studies suggests that *PICALM* and perhaps *CLU* could be relevant to AD, although the effects are small. For this reason, we performed a meta-analysis by combining our genotyping data for the 7 SNPs to those reported in the two GWA studies (Table 2). For the *CLU*/rs11136000, *PICALM*/rs541458 and *PICALM*/rs3851179 SNPs, actual genotyping counts were available for all three studies (ours, Lambert et al. and Harold et al.). However, for the other SNPs, genotypes were available from two studies (ours and Lambert et al.). As shown in Table 2, the combined genotype data helped to improve the published associations for *CLU*/rs11136000 (from $P = 10^{-9}$ to 10^{-16}), *CR1*/rs3818361 (from $P = 10^{-9}$ to 10^{-13}) and both *PICALM* SNPs (from $P = 10^{-8}$ to 10^{-9}). These findings warrant comprehensive resequencing of these gene regions to identify functional SNPs (common, rare or both) because the reported significant SNPs with weak effect sizes are present either in introns (*CLU* and *CR1*) or in the 5' flanking region (*PICALM*) and they could be in linkage disequilibrium with functional SNPs either in these or nearby genes.

In addition to the AD risk, we also examined the association of 7 SNPs with AAO. Although *CR1* SNPs demonstrated no association with AD risk in our sample, they revealed association with AAO that was stronger for rs3818361 (P=0.0097). The homozygosity of the less common allele rs3818361 was associated with lower AAO than the other two genotypes (70.75 ± 7.5 years vs. 72.32 ± 6.6 and 72.86 ± 6.2 years). This AAO association needs to be confirmed in other independent samples, as it is not clear if this was present in the two GWA studies (Harold et al., 2009; Lambert et al., 2009).

In summary, our data from an independent and large case-control sample in conjunction with two published GWA studies suggest that the *PICALM*, *CLU* and *CR1* gene regions should be followed-up by comprehensive resequencing to find functional variants. While the relatively small statistical effects suggest that these loci are not major risk factors for LOAD, identification of functional variants in these loci and their detailed characterization may help to broaden our understanding about the underlying mechanism(s) of LOAD.

Acknowledgments

The study was supported by U.S. National Institute on Aging grants AG030653, AG005133, and AG023651. We thank Dr. Jean-Charles Lambert for providing the actual genotyping counts from his study for our meta-analysis.

References

Abraham R, Moskvina V, Sims R, Hollingworth P, Morgan A, Georgieva L, Dowzell K, Cichon S, Hillmer AM, O'Donovan MC, Williams J, Owen MJ, Kirov G. A genome-wide association study for late-onset Alzheimer's disease using DNA pooling. BMC Med Genomics. 2008; 1:44. [PubMed: 18823527]

Beecham GW, Martin ER, Li YJ, Slifer MA, Gilbert JR, Haines JL, Pericak-Vance MA. Genome-wide association study implicates a chromosome 12 risk locus for late-onset Alzheimer disease. Am J Hum Genet. 2009; 84(1):35–43. [PubMed: 19118814]

Bertram L, Lange C, Mullin K, Parkinson M, Hsiao M, Hogan MF, Schjeide BM, Hooli B, Divito J, Ionita I, Jiang H, Laird N, Moscarillo T, Ohlsen KL, Elliott K, Wang X, Hu-Lince D, Ryder M, Murphy A, Wagner SL, Blacker D, Becker KD, Tanzi RE. Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. Am J Hum Genet. 2008; 83(5):623–632. [PubMed: 18976728]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Carrasquillo MM, Zou F, Pankratz VS, Wilcox SL, Ma L, Walker LP, Younkin SG, Younkin CS, Younkin LH, Bisceglio GD, Ertekin-Taner N, Crook JE, Dickson DW, Petersen RC, Graff-Radford NR, Younkin SG. Genetic variation in PCDH11X is associated with susceptibility to late-onset Alzheimer's disease. Nat Genet. 2009; 41(2):192–198. [PubMed: 19136949]

Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Lince DH, Zismann VL, Beach TG, Leung D, Bryden L, Halperin RF, Marlowe L, Kaleem M, Walker DG, Ravid R, Heward CB, Rogers J, Papassotiropoulos A, Reiman EM, Hardy J, Stephan DA. A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. J Clin Psychiatry. 2007; 68(4):613–618. [PubMed: 17474819]

Grupe A, Abraham R, Li Y, Rowland C, Hollingworth P, Morgan A, Jehu L, Segurado R, Stone D, Schadt E, Karnoub M, Nowotny P, Tacey K, Catanese J, Sninsky J, Brayne C, Rubinsztein D, Gill M, Lawlor B, Lovestone S, Holmans P, O'Donovan M, Morris JC, Thal L, Goate A, Owen MJ, Williams J. Evidence for novel susceptibility genes for late-onset Alzheimer's disease from a genome-wide association study of putative functional variants. Hum Mol Genet. 2007; 16(8):865–873. [PubMed: 17317784]

Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, Jones N, Thomas C, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Hardy J, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schürmann B, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Hüll M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Carrasquillo MM, Pankratz VS, Younkin SG, Holmans PA, O'Donovan M, Owen MJ, Williams J. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet. 2009; 41(10):1088–1093. [PubMed: 19734902]

Kamboh MI, Aston CE, Hamman RF. The relationship of APOE polymorphism and cholesterol levels in normoglycemic and diabetic subjects in a biethnic population from the San Luis Valley, Colorado. Atherosclerosis. 1995; 112(2):145–159. [PubMed: 7772075]

Kazeem GR, Farrall M. Integrating case-control and TDT studies. Ann Hum Genet. 2005; 69(3):329–335. [PubMed: 15845037]

Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr C, Pasquier F, Fiévet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck A, Helisalmi S, Porcellini E, Hanon O, European Alzheimer's Disease Initiative Investigators. de Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossù P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Blanché H, Dartigues JF, Tzourio C, Gut I, Van Broeckhoven C, Alpérovitch A, Lathrop M, Amouyel P. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet. 2009; 41(10):1094–1099. [PubMed: 19734903]

Lee AB, Luca D, Klei L, Devlin B, Roeder K. Discovering genetic ancestry using spectral graph theory. Genet Epidemiol. 2009 Epub ahead of print.

Li H, Wetten S, Li L, St Jean PL, Upmanyu R, Surh L, Hosford D, Barnes MR, Briley JD, Borrie M, Coletta N, Delisle R, Dhalla D, Ehm MG, Feldman HH, Fornazzari L, Gauthier S, Goodgame N, Guzman D, Hammond S, Hollingworth P, Hsiung GY, Johnson J, Kelly DD, Keren R, Kertesz A, King KS, Lovestone S, Loy-English I, Matthews PM, Owen MJ, Plumpton M, Pryse-Phillips W, Prinjha RK, Richardson JC, Saunders A, Slater AJ, St George-Hyslop PH, Stinnett SW, Swartz JE, Taylor RL, Wherrett J, Williams J, Yarnall DP, Gibson RA, Irizarry MC, Middleton LT, Roses AD. Candidate single-nucleotide polymorphisms from a genome-wide association study of Alzheimer disease. Arch Neurol. 2008; 65(1):45–53. [PubMed: 17998437]

Nicodemus KK. Catmap: case-control and TDT meta-analysis package. BMC Bioinformatics. 2008; 9:130. [PubMed: 18307795]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly M, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3):559–575. [PubMed: 17701901]

R Development Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; Vienna, Austria: 2007.

Reiman EM, Webster JA, Myers AJ, Hardy J, Dunckley T, Zismann VL, Joshipura KD, Pearson JV, Hu-Lince D, Huentelman MJ, Craig DW, Coon KD, Liang WS, Herbert RH, Beach T, Rohrer KC, Zhao AS, Leung D, Bryden L, Marlowe L, Kaleem M, Mastroeni D, Grover A, Heward CB, Ravid R, Rogers J, Hutton ML, Melquist S, Petersen RC, Alexander GE, Caselli RJ, Kukull W, Papassotiropoulos A, Stephan DA. GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers. Neuron. 2007; 54(5):713–720. [PubMed: 17553421]

Slooter AJ, Cruts M, Kalmijn S, Hofman A, Breteler MM, Van Broeckhoven C, van Duijn CM. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. Arch Neurol. 1998; 55(7):964–968. [PubMed: 9678314]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 1

Association of SNPs in the *CLU* (*APOJ*), *PICALM* and *CR1* genes with Alzheimer's disease

	N		MAF		Association Test	
Gene/SNP#	**Cases**	**Controls**	**Cases**	**Controls**	**OR (95% CI)**	***P* value***
CLU (APOJ)						
rs11136000	1,344	1,350	0.365	0.380	0.931 (0.873 – 0.992)	0.258
rs2279590	1,297	1,323	0.372	0.390	0.911 (0.854 – 0.972)	0.148
rs9331888	1,322	1,323	0.308	0.307	1.015 (0.949 – 1.085)	0.826
PICALM						
rs541458	1,322	1,338	0.289	0.316	0.890 (0.832 – 0.953)	0.087
rs3851179	1,328	1,337	0.335	0.360	0.889 (0.833 – 0.949)	0.071
CR1						
rs6656401	1,348	1,359	0.220	0.210	1.079 (1.003 – 1.162)	0.30
rs3818361	1,336	1,352	0.228	0.217	1.056 (0.981 – 1.137)	0.457

**P* values and ORs (odds ratios) using additive logistic regression models and adjusted for age (age-at-onset in cases), gender and *APOE* (*E*4* vs. non-*E*4* status).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

Meta-analysis based on combined genotyping data from Lambert et al., Harold et al., and the present study

	N		Association Test		Heterogeneity Test
	Cases	**Controls**	**OR (95% CI)**	***P*-value***	***P*-value**
CLU (APOJ)					
rs11136000	11,154	17,786	0.862 (0.832–0.894)	4.44×10^{-16}	0.490
rs2279590	6,925	9,748	0.873 (0.835–0.913)	3.07×10^{-9}	0.241
rs9331888	7,209	9,831	1.114 (1.063–1.168)	6.76×10^{-6}	0.058
PICALM					
rs541458	7,288	14,509	0.876 (0.838–0.915)	3.48×10^{-9}	0.674
rs3851179	7,294	14,508	0.880 (0.844–0.918)	3.35×10^{-9}	0.080
CR1					
rs6656401	7,253	9,885	1.178 (1.117–1.244)	2.29×10^{-9}	0.093
rs3818361	7,171	9,770	1.217 (1.154–1.283)	5.20×10^{-13}	0.026

* Inverse variance fixed-effects P-value