Association Study of Late-Onset Alzheimer's Disease Risk Variants and Memory Decline

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Objective:

To investigate the late-onset Alzheimer's disease (LOAD) risk variants identified from genome-wide association studies (GWAS) for their influence on human memory decline in a longitudinal series.

Background:

The recent large-scale LOAD GWAS [1-5] have reported associations meeting genome-wide significance in or near 11 genes. We and others reported replication of the AD risk association with SNPs near *CLU*, *PICALM*, *CR1*, *ABCA7*, *BIN1*, *MS4A6A*, *EPHA1*, *CD2AP*, *and CD33* [4, 6-8]. Here we evaluate the association of these SNPs with cognitive decline, as this approach may provide valuable information regarding their biological consequence.

Methods:

We evaluated a longitudinal series of 2,594 subjects collected at Mayo Clinic Rochester in Minnesota (MCR, n=2,080) and Mayo Clinic Jacksonville in Florida (MCJ, n=514). Of these subjects, 1,893 were cognitively normal, 359 had mild cognitive impairment (MCI) and 342 had clinical Alzheimer's disease (AD) at baseline (Table 1). We genotyped *APOE*, and the top 9 LOAD GWAS risk SNPs and investigated associations with change in logical memory delayed recall scores (MRLMD) from the Wechsler Memory Scale-Revised, collected longitudinally at 2 or more time points. Linear mixed models were used with intercept and slope as random effects to assess associations adjusting for age, sex, years of education and *APOE* ε4. Analyses were done for all subjects and those who were cognitively normal, at baseline.

Table 1. Description of cohort

Variable	Summary (N=2594)	MCR (N=2080)	MCJ (N=514)
Age at first assessment (years)	77 (49-99)	78 (55-99)	73 (49-91)
Gender (male)	1160 (45%)	984 (47%)	176 (34%)
Education (years)	14 (4-20)	13 (5-20)	16 (4-20)
Diagnosis at first assessment			
Normal	1893 (73%)	1468 (71%)	425 (83%)
MCI	359 (14%)	338 (16%)	21 (4%)
AD	342 (13%)	274 (13%)	68 (13%)
MRLMD at first assessment	16 (1-42)	15 (1-40)	19 (1-42)
APOE (number of e4 alleles)			
0	1860 (72%)	1520 (73%)	340 (67%)
1	666 (26%)	514 (25%)	152 (30%)
2	60 (2%)	45 (2%)	15 (3%)
CLU-rs11136000			
A A	398 (16%)	334 (17%)	64 (13%)
A G	1206 (48%)	956 (48%)	250 (50%)
G G	891 (36%)	706 (35%)	185 (37%)
PICALM-rs3851179			
A A	332 (13%)	268 (13%)	64 (13%)
A G	1173 (47%)	948 (48%)	225 (45%)
G G	984 (40%)	774 (39%)	210 (42%)
CR1-rs3818361			
A A	105 (4%)	82 (4%)	23 (5%)
A G	799 (32%)	628 (31%)	171 (34%)
G G	1605 (64%)	1298 (65%)	307 (61%)
ABCA7-rs3764650			
C C	25 (1%)	20 (1%)	5 (1%)
C A	379 (15%)	288 (14%)	91 (18%)
A A	2146 (84%)	1738 (85%)	408 (81%)
BIN1-rs744373			
G G	190 (8%)	137 (7%)	53 (11%)
G A	987 (40%)	787 (40%)	200 (41%)
A A	1285 (52%)	1049 (53%)	236 (48%)
MS4A6A-rs610932			
A A	457 (18%)	357 (18%)	100 (20%)
A C	1211 (49%)	969 (49%)	242 (49%)
C C	825 (33%)	670 (34%)	155 (31%)
EPHA1-rs11767557			
G G	113 (5%)	93 (5%)	20 (4%)
G A	764 (31%)	604 (31%)	160 (32%)
A A	1564 (64%)	1248 (64%)	316 (64%)
CD2AP-rs9349407			
C C	185 (7%)	144 (7%)	41 (8%)
C G	970 (39%)	773 (39%)	197 (40%)
G G	1321 (53%)	1067 (54%)	254 (52%)
CD33-rs3865444			
A A	237 (9%)	197 (10%)	40 (8%)
A C	1083 (43%)	880 (44%)	203 (41%)
C C	1182 (47%)	935 (46%)	247 (50%)
Risk score	21.1 (0.0-67.2)	21.0 (0.0-67.2)	22.0 (2.3-45.1)

Sample median (minimum-maximum) is given for numerical variables. MRLMD: logical memory delayed recall score (plausible range: 0 to 50). Subjects with MRLMD scores of 0 at first assessment were excluded from analyses. Genotype counts (frequencies) for the tested SNPs are shown.

Table 2. Description of SNPs

SNP ID	Nearest Gene	Location	Major Allele	Minor Allele	Control MAF	Risk Allele	OR
rs11136000	*CLU*	Intron 3	G	A	0.41	G	1.22
rs3851179	*PICALM*	88.5 kb 5'	G	A	0.37	G	1.25
rs3818361	*CR1*	Intron 34	G	A	0.20	A	1.15
rs3764650	*ABCA7*	Intron 13	A	C	0.09	C	1.23
rs744373	*BIN1*	29.7 kb 5'	A	G	0.27	G	1.17
rs610932	*MS4A6A*	3' UTR	C	A	0.43	C	1.15
rs11767557	*EPHA1*	3.2 kb 5'	A	G	0.21	A	1.15
rs9349407	*CD2AP*	Intron 1	G	C	0.29	C	1.11
rs3865444	*CD33*	373 bp 5'	C	A	0.32	C	1.09

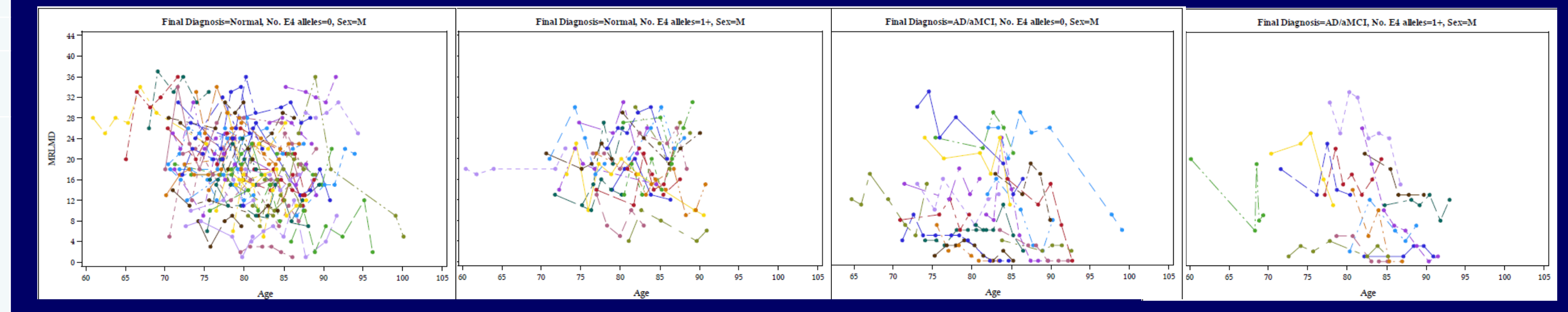
Description of alleles associated with risk at the 9 LOAD GWAS risk loci. Location is relative to the nearest gene, based on the human genome assembly hg18. The minor allele frequency (MAF) and odds ratios (OR) are based on references 6-8. Weighted genetic risk score (Table 5) was calculated based on these odds ratio estimates. $\text{Score}_i = 100*[\Sigma\ (n_{ij} * \log(OR_j))\ ] / [2*\Sigma\ (\log(OR_j))]$ for the i^{th} patient, where: n_{ij}= number of risk alleles for the i^{th} patient and j^{th} SNP; OR_j=odds ratio for the j^{th} SNP.

Table 3. Association of *APOE* with Memory

	All Subjects				Normal at Baseline			
	Estimated change in MRLMD	95% CI Lower	95% CI Upper	pvalue	Estimated change in MRLMD	95% CI Lower	95% CI Upper	pvalue
Variables	**Effect on Intercept**				**Effect on Intercept**			
APOE (# ε4 alleles)	-1.19	-1.71	-0.68	**5.52E-06**	-0.83	-1.40	-0.26	**4.43E-03**
Site (MCF)	3.93	3.25	4.61	2.32E-29	1.54	0.81	2.28	4.00E-05
Mean Age	-0.23	-0.27	-0.18	1.57E-23	-0.27	-0.32	-0.22	4.95E-30
Male Gender	-1.03	-1.55	-0.51	1.00E-04	-1.13	-1.70	-0.56	9.00E-05
Yrs. Of Education	0.50	0.42	0.59	1.37E-28	0.55	0.45	0.64	1.22E-27
AD or MCI	-8.64	-9.23	-8.05	3.40E-166	NA			
Variables	**Effect on Slope**				**Effect on Slope**			
APOE (# ε4 alleles) x Year	-0.40	-0.51	-0.29	**1.07E-12**	-0.32	-0.45	-0.20	**1.54E-07**
Site (MCF) x Year	0.70	0.56	0.84	4.21E-23	0.81	0.66	0.96	3.52E-27
Mean Age x Year	-0.04	-0.05	-0.03	3.96E-15	-0.03	-0.04	-0.02	1.37E-07
Male Gender x Year	-0.08	-0.19	0.04	1.74E-01	-0.08	-0.21	0.04	1.91E-01
Yrs. Of Education x Year	0.01	-0.01	0.03	3.43E-01	0.01	-0.01	0.03	3.45E-01
Effect of APOE	**8.99E-15**				**2.19E-10**			

Estimated change in logical memory delayed recall (MRLMD), 95% confidence intervals (CI) and P-value results from a linear mixed model (SAS PROC MIXED) with intercept and slope as random effects.

Figure 1. Longitudinal Memory Plots



From cohort of 264 patients normal at 1st diagnosis with 5+ years of follow-up and 5+ measurements

Table 4. Association of LOAD risk SNPs with Memory

		All Subjects				Normal at Baseline			
		Estimated change in MRLMD	95% CI Lower	95% CI Upper	pvalue	Estimated change in MRLMD	95% CI Lower	95% CI Upper	pvalue
CLU-rs11136000 (A-protective)	Intercept	0.40	0.03	0.78	**3.49E-02**	0.51	0.11	0.92	**1.28E-02**
	Slope	0.06	-0.03	0.14	NS	0.04	-0.05	0.13	NS
	Overall				**6.34E-02**				**1.30E-02**
PICALM-rs3851179 (A-protective)	Intercept	0.00	-0.38	0.38	NS	-0.29	-0.70	0.13	NS
	Slope	0.01	-0.08	0.09	NS	-0.01	-0.10	0.08	NS
	Overall				NS				NS
CR1-rs3818361 (A-risky)	Intercept	0.31	-0.14	0.77	NS	0.31	-0.18	0.80	NS
	Slope	0.00	-0.11	0.10	NS	0.02	-0.09	0.13	NS
	Overall				NS				NS
ABCA7-rs3764650 (C-risky)	Intercept	-0.38	-1.02	0.26	NS	-0.44	-1.15	0.27	NS
	Slope	0.00	-0.14	0.13	NS	-0.01	-0.16	0.14	NS
	Overall				NS				NS
BIN1-rs744373 (G-risky)	Intercept	0.09	-0.33	0.50	NS	0.12	-0.33	0.57	NS
	Slope	-0.04	-0.13	0.05	NS	-0.04	-0.14	0.06	NS
	Overall				NS				NS
MS4A6A-rs610932 (A-protective)	Intercept	0.28	-0.09	0.65	NS	0.36	-0.04	0.76	NS
	Slope	0.00	-0.09	0.08	NS	-0.01	-0.10	0.08	NS
	Overall				NS				NS
EPHA1-rs11767557 (G-protective)	Intercept	0.16	-0.30	0.61	NS	0.04	-0.45	0.53	NS
	Slope	0.02	-0.08	0.12	NS	0.03	-0.08	0.13	NS
	Overall				NS				NS
CD2AP-rs9349407 (C-risky)	Intercept	-0.10	-0.52	0.31	NS	-0.32	-0.77	0.13	NS
	Slope	0.00	-0.09	0.09	NS	0.00	-0.09	0.10	NS
	Overall				NS				NS
CD33-rs3865444 (A-protective)	Intercept	0.13	-0.27	0.53	NS	0.21	-0.23	0.64	NS
	Slope	-0.05	-0.13	0.04	NS	-0.06	-0.15	0.03	NS
	Overall				NS				NS

CLU protective allele is associated with better memory scores at mean age of all subjects and at first age at assessment of the cognitively normal subjects at baseline. Other 8 LOAD GWAS SNPs did not have significant association with memory.

Table 5. Association of Risk Score with Memory

		All Subjects				Normal at Baseline			
		Estimated change in MRLMD	95% CI Lower	95% CI Upper	pvalue	Estimated change in MRLMD	95% CI Lower	95% CI Upper	pvalue
Basic model	Intercept	-0.41	-0.81	-0.01	**4.65E-02**	-0.56	-1.00	-0.12	**1.37E-02**
	Slope	0.03	-0.06	0.11	NS	0.07	-0.02	0.16	NS
	Overall				9.49E-02				**2.89E-02**
CLU-adjusted	Intercept	-0.54	-1.04	-0.04	**3.42E-02**	-0.38	-0.92	0.17	NS
	Slope	0.01	-0.10	0.12	NS	0.06	-0.06	0.18	NS
	Overall				9.53E-02				3.03E-01

A weighted risk score calculated from OR estimates of tested SNPs (Table 2) associates with worse memory at mean age of all subjects and at first age at assessment of the cognitively normal control subjects at baseline. This association is significant in all subjects even after controlling for the *CLU* locus SNP.

Summary and Conclusions:

APOE is associated with lower memory scores at mean age in all subjects and at first age at assessment for subjects who are cognitively normal at baseline. *APOE* is also associated with faster memory decline in both groups as expected. The protective *CLU* allele associated with better memory in both groups, but did not associate with slope of memory change. A weighted genetic risk score associated with memory at mean age or baseline, but not with slopes. This association remained significant in all subjects after controlling for the *CLU* genotypes, suggesting a combined effect of the remaining GWAS SNPs (or a subset thereof) on memory. Our results suggest that the use of memory endophenotypes in longitudinal series might be a viable approach in assessing the role of LOAD risk variants in human cognition. These results need to be confirmed in similar series.

References:

1. Harold D et al. *Nat Genet* 2009, 41:1088.
2. Lambert JC et al. *Nat Genet* 2009, 41:1094.
3. Seshadri S et al. *JAMA* 2010, 303:1832.
4. Hollingworth P et al. *Nat Genet* 2011,43:429.
5. Naj AC et al. *Nat Genet* 2011,43:436.
6. Carrasquillo MM et al. *Arch Neurol* 2010, 67:961.
7. Carrasquillo MM et al. *J Alzheimers Dis* 2011, 24:751.
8. Carrasquillo MM et al. *Mol Neurodegener* 2011, 6:54.

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