

Association Study of Late-Onset Alzheimer's Disease Risk Variants and Risk for Posterior Cortical Atrophy



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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 association with posterior cortical atrophy (PCA).

Background:

PCA is characterized by visual-spatial impairment, relative sparing of memory and focal involvement of the parieto-occipital cortices on neuroimaging and neuropathology. Although the most common etiopathology of PCA is Alzheimer's disease (AD), there are no well-powered genetic association studies of this atypical AD subtype.

Therefore, here we evaluate the association of the 11 most significant single nucleotide polymorphisms (SNPs) from the published LOAD GWAS loci (*APOE*, *CLU*, *CR1*, *PICALM*, *BIN1*, *EXOC3L2*, *ABCA7*, *MS4A6A/MS4A4E*, *CD2AP*, *CD33* and *EPHA1*) [refs. 1-5] with the risk of PCA.

Methods:

We assessed a cohort of 135 subjects with clinical and/or neuropathologic diagnosis of PCA collected at Mayo Clinic Florida vs. 2,523 cognitively normal elderly controls collected at Mayo Clinic Florida and Mayo Clinic Minnesota. The cohort description is shown below in **Table 1**:

Group	N	Female (%)	<i>APOE</i> ε4 copies 0/1/2 (%) ^a	Mean Age±SD (Range) ^b
PCA	135	91 (67%)	56/59/20 (41%/44%/15%)	68.8±13.2 (42-99)
Control	2,523	1431 (56%)	1935/554/34 (77%/22%/1%)	81.8±6.1 (61-105)
p		0.01 ^c	< 0.0001 ^c	< 0.0001 ^d

Table 1. Subject demographics: a. The number of subjects with no, one or two copies of *APOE* ε4 allele (percentage of subjects); b. Age at onset is used for subjects recruited by clinical diagnosis and age at death is used for autopsied samples; c) P values for chi-square test; d) P value for two-sided, unpaired t-test.

Genotyping was performed as previously described using Taqman assays [ref 6]. Minimum detectable odds ratios were estimated for an additive effect, using allele frequencies estimated in the controls and effect size estimates published for LOAD risk, for 80% power and α=0.05. We used multivariable logistic regression in PLINK to test for association with risk of PCA, adjusting for age, sex and *APOE* ε4 dose. We also obtained empirical p values using 10,000 random permutations in PLINK [ref. 7] to account for multiple testing. Finally, the difference in effect sizes between PCA and LOAD subjects were tested using polytomous logistic regression analyses in SAS for the SNPs with nominally significant associations in PCA (effect size estimates for LOAD were obtained using a cohort of 696 subjects with diagnosis of LOAD who were compared against the same control group utilized for the PCA risk estimates).

Results:

- As shown in **Table 2** below, the *APOE* ε4-tagging SNP, rs429358 significantly associated with risk of PCA (OR=4.74, p=4.9x10⁻¹⁹). There were two SNPs that showed nominally significant association with risk for PCA, rs11136000 at *CLU* (p=0.017) and rs744373 at the *BIN1* locus (p=0.032). Importantly, the minor alleles of these variants had effects that were in the same direction as that for LOAD risk, with a protective OR estimate for the *CLU* SNP (OR=0.68, 95% CI=0.49-0.93) and increased risk with the *BIN1* locus SNP (OR=1.42, 95% CI=1.03-1.96). No other SNPs had nominal significance with the additive model, although the LOAD protective *APOE* ε2-tagging SNP rs7412 had a suggestive protective effect (p=0.073) in our PCA cohort (OR=0.46, 95% CI=0.20-1.07).
- Observed and expected genotype frequencies in PCA cases and controls as well as HWE p-values for all SNPs tested are shown in the 5 rightmost columns in **Table 2**. Upon review of the genotype frequencies for the tested SNPs in PCA vs. control subjects, we determined that there were significantly less heterozygote subjects for the *ABCA7* SNP, rs3764650, than expected in the PCA cohort, leading to deviation from Hardy-Weinberg equilibrium (HWE p=0.005). Given this observation, the *ABCA7* rs3764650 was tested using a recessive model, which yielded nominally significant association (p=0.049) between *ABCA7* rs3764650 and PCA, with the minor allele conferring risk (OR=5.06). The direction of association for this SNP is also consistent with that for LOAD, although the published OR estimate for LOAD risk (OR=1.23) is based on an additive model.
- We compared the effect sizes of the variants with nominal significance in PCA to the ORs estimated for LOAD subjects in our cohort and determined that they were not statistically different, except for *BIN1*, which has a stronger risk effect in PCA (OR=1.42, 95% CI=1.03-1.96) compared to estimates in our LOAD subjects (OR=1.05, 95%CI=0.91-1.21).
- Our power calculations based on known OR estimates from LOAD studies and minor allele frequencies in control subjects revealed the smallest effect sizes that would be detectable in our relatively modest PCA sample size of 135 subjects (**Table 2**, column named "Detectable OR in PCA"). As would be predicted from these calculations, only *APOE* ε4 and *CLU* rs11136000 SNPs can be detected in this sample, and *APOE* ε2 and *BIN1* rs744373 are slightly below 80% power for detection. Given the multiple testing problem, we obtained empirical p values correcting for multiple comparisons. Only *APOE* ε4 rs429358 achieved significance with corrected empirical p value <0.0001, with marginal significance for *APOE* ε2 rs7412 (p=0.05).

SNP ID	Chr	Nearest Gene	Location	Major Allele	Minor Allele	Control MAF	PCA MAF	AD risk OR ^b	Detectable OR in PCA	PCA risk OR (95% CI)	PCA risk P-value	Group	Genotype Counts (22/12/11)	Genotype Frequencies			Expected Hets ^e	H-W p
														22	12	11		
rs429358 ^a	19	<i>APOE</i>	Exon 4	T	C	0.12	0.37	4.36	1.63	4.74 (3.37-6.67)	4.90x10 ⁻¹⁹	PCA	20/59/56	14.8%	43.7%	41.5%	46.4%	0.58
												Control	34/554/1938	1.3%	21.9%	76.7%	21.6%	0.46
rs11136000	8	<i>CLU</i>	Intron 3	G	A	0.41	0.34	0.82	0.69	0.68 (0.49-0.93)	0.017	PCA	12/67/55	9.0%	50.0%	41.0%	44.9%	0.25
												Control	416/1165/843	17.2%	48.1%	34.8%	48.5%	0.71
rs744373	2	<i>BIN1</i>	29.7 kb 5'	A	G	0.27	0.36	1.17	1.47	1.42 (1.03-1.96)	0.032	PCA	19/58/58	14.1%	43.0%	43.0%	45.8%	0.46
												Control	175/958/1264	7.3%	40.0%	52.7%	39.7%	0.76
rs7412	19	<i>APOE</i>	Exon 4	C	T	0.08	0.03	0.25	0.42	0.46 (0.20-1.07)	0.073	PCA	0/8/126	0.0%	6.0%	94.0%	5.8%	1.00
												Control	17/372/2135	0.7%	14.7%	84.6%	14.8%	0.79
rs3764650	19	<i>ABCA7</i>	Intron 13	A	C	0.08	0.08	1.23	1.78	5.06 (1.01-25.4)	0.049 ^d	PCA	4/14/114	3.0%	10.6%	86.4%	15.3%	0.005
										0.98 (0.57-1.70)		0.946	Control	20/346/2120	0.8%	13.9%	85.3%	14.3%
rs9349407	6	<i>CD2AP</i>	Intron 1	G	C	0.27	0.25	1.11	1.48	0.72 (0.50-1.05)	0.089	PCA	10/47/77	7.5%	35.1%	57.5%	37.5%	0.49
												Control	169/956/1296	7.0%	39.5%	53.5%	39.2%	0.72
rs3851179	11	<i>PICALM</i>	88.5 kb 5'	G	A	0.37	0.41	0.8	0.68	1.23 (0.90-1.68)	0.19	PCA	21/70/44	15.6%	51.9%	32.6%	48.6%	0.48
												Control	320/1137/965	13.2%	46.9%	39.8%	46.5%	0.63
rs3818361	1	<i>CR1</i>	Intron 34	G	A	0.19	0.23	1.15	1.52	1.19 (0.82-1.71)	0.36	PCA	6/49/79	4.5%	36.6%	59.0%	35.2%	0.81
												Control	99/746/1589	4.1%	30.6%	65.3%	31.3%	0.33
rs610932	11	<i>MS4A6A</i>	3' UTR	C	A	0.43	0.43	0.87	0.69	0.88 (0.64-1.20)	0.404	PCA	22/70/41	16.5%	52.6%	30.8%	49.0%	0.48
												Control	463/1179/783	19.1%	48.6%	32.3%	49.1%	0.62
rs3865444	19	<i>CD33</i>	373 bp 5'	C	A	0.32	0.30	0.92	0.66	0.88 (0.64-1.21)	0.436	PCA	15/51/69	11.1%	37.8%	51.1%	42.0%	0.30
												Control	254/1046/1140	10.4%	42.9%	46.7%	43.4%	0.54
rs11767557	7	<i>EPHA1</i>	3.2 kb 5'	A	G	0.20	0.19	0.87	0.61	0.93 (0.63-1.38)	0.726	PCA	3/44/87	2.2%	32.8%	64.9%	30.4%	0.57
												Control	108/751/1518	4.5%	31.6%	63.9%	32.4%	0.23

Table 2. Association of LOAD risk SNPs with PCA. Results of multivariable logistic regression analysis testing for association of LOAD risk SNPs with PCA. All analyses included *APOE* ε4 dosage, age and sex as covariates, except for a. where *APOE* was not included as a covariate. b. Odds ratio (OR) estimates for AD risk for these SNPs were taken from published studies [refs. 4, 6,8-11]. c. Minimum detectable OR at 80% power and α=0.05. d. An additive model was used for the minor allele except for the *ABCA7* SNP, where a recessive model was also tested. Chr: chromosome. Location: location of the SNP with respect to the nearest gene. MAF: minor allele frequency. Minor and major alleles, counts and frequencies for minor homozygote (22), heterozygote (12) and major homozygote genotypes (11) are shown for PCA and control groups, separately. e. Expected heterozygote frequencies. H-W p: Hardy-Weinberg p-value.

Conclusions:

These preliminary results in a small PCA cohort suggest that some genetic risk factors for this syndrome may overlap with those for AD, which is expected given their common neuropathology despite distinct regional distributions.

The risk of *APOE* ε4 is demonstrated unequivocally in this cohort of 135 PCA subjects. We also identified nominally significant associations for *CLU* and *BIN1* loci, with protective and risk effects on PCA, respectively, for the minor alleles of the tested variants which is consistent with the direction of their effect in LOAD. Under a recessive model, the minor allele of *ABCA7* rs3764650 was nominally significant, with an effect that is also in the same direction as that for LOAD risk.

While the significance of PCA association for the *CLU*, *BIN1* and *ABCA7* variants would not survive multiple testing, the directions of their ORs that are consistent with those for LOAD, effect size estimates that are either indistinguishable from or greater than those in LOAD, and strong *a priori* rationale for being tested in PCA lend further support to these findings.

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