Novel late-onset Alzheimer's disease loci variants associate with brain gene expression

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Abstract

Background: Recent genome-wide association studies (GWAS) of late-onset Alzheimer's disease (LOAD) identified nine novel risk loci. Discovery of functional variants within genes at these loci is required to confirm their role in AD. Single nucleotide polymorphisms that influence gene expression (eSNPs) constitute an important class of functional variants. We therefore investigated the influence of the novel LOAD risk loci on human brain gene expression.

Methods: We measured gene expression levels in the cerebellum and temporal cortex of autopsied AD subjects and those with other brain pathologies (~400 total subjects). To determine whether any of the novel LOAD risk variants are eSNPs, we tested their cis-association with expression of six nearby LOAD candidate genes detectable in human brain (ABCA7, BIN1, CLU, MS4A4A, MS4A6A, PICALM) and an additional 13 genes ±100kb of these SNPs. Secondly, to identify additional eSNPs that influence brain gene expression levels of the above six novel candidate LOAD genes, we identified SNPs ±100kb of their location and tested for cis-associations.

Results: CLU rs11136000 (p=7.81E-04) and MS4A4A rs2304933/rs2304935 (p=1.48E-04 -1.86E-04) significantly influence temporal cortex expression levels of these genes. The LOAD-protective CLU and risky MS4A4A locus alleles associate with higher brain mRNA levels of these genes. There are other cis-variants that significantly influence brain expression of CLU and ABCA7 (p=3.53E-05 - 9.09E-09), some of which also associate with AD risk (p=2.64E-02 - 6.25E-05).

Conclusions: CLU and MS4A4A eSNPs may at least partly explain the LOAD risk association at these loci. CLU and ABCA7 may harbor additional strong eSNPs. These results have implications in the search for functional variants at the novel LOAD risk loci.

Aims

1) To determine if any of the new LOAD susceptibility SNPs from recent GWAS influence brain gene expression in cis.

2) To determine if any of the new candidate LOAD genes have any other SNPs that influence their expression in the brain.

Methods

Subjects: RNA was isolated from Cerebellum (CER) and Temporal Cortex (TCX) brain tissue sample selected from the Mayo Clinic Brain Bank (Dr. Dennis Dickson). CER: 197 pathologic AD cases and 177 non-ADs. TCX: 198 pathologic AD cases and 193 non-ADs.

Transcriptome Measurements: Whole-genome DASL (24,526 probes, NCBI Ref Seq, Build 36.2). Designed for partially degraded fresh frozen and FFPE tissues

GWAS Platform: Illumina Hap300 (313,330 SNPs) (Carrasquillo et al., Nature Genetics, 2009). Use cisSNPs (±100kb of tested transcript)

Candidate LOAD SNPs: LOAD GWAS identified SNPs at nine novel loci. Eight of these novel LOAD risk SNPs and/or their proxies were evaluated for influence on gene expression; a total of eighteen SNPs (67 cisSNP/probe expression associations in TCX and 60 in CER) were evaluated.

Candidate LOAD Genes: LOAD GWAS identified eight novel LOAD candidate genes and one gene cluster. Eleven DASL probes for seven of these genes (*ABCA7, BIN1, CLU, CD2AP, MS4A4A, MS4A6A* and *PICALM*) passed QC criteria. Two-hundred and seven SNPs identified *in cis* with these probes were evaluated for association with gene expression.

Statistical Analysis: Multivariable linear regression analysis in PLINK. Corrected for AD diagnosis, age at death, gender, RIN and *APOE* ε4 genotype.

Results

- The minor allele (T) of the LOAD susceptibility SNP rs11136000, which associates with decreased risk for LOAD, is associated with increased expression of *CLU* in the TCX beta= 0.17, P=7.81E-04. (Table 1). This SNP is also highlighted in Fig.2.
- Two proxies (D'>0.9) for *MS4A4E* locus LOAD risk SNP rs670139, associate with increased expression of *MS4A4A* mRNA in the TCX (Table 1) The location of these SNPs relative to the *MS4A4A* gene is highlighted in Fig.3.
- The expression in brain of two LOAD candidate genes *ABCA7* and *CLU,* appears to be influenced by multiple eSNPs (Figs 1 and 2). Analysis of these variants for association with LOAD in a large case control series identified significant association for some of these variants (data not shown).

Table 1

						LOAD		CER		TX	
CHR	SNP	Proxy	r2	D'	Symbol	OR	P	BETA	P	BETA	P
8	rs11136000	NA	NA	NA	CLU	0.84	1.40E-09	-0.04	0.10	0.17	7.81E-04
11	rs670139	rs2304933	0.62	0.91	MS4A4A	1.09	1.40E-09	0.02	0.51	0.22	1.48E-04
		rs2304935	0.62	0.91				0.02	0.51	0.22	1.86E-04

Table 1. Significant expression level associations for the top AD locus SNPs in the TCX. Results shown maintain significance following bonferonni correction for multiple tests.

Table 2

				CER		TCX	
CHR	SNP	BP	Symbol	BETA	P	BETA	P
19	rs7247087	982,212	ABCA7	0.18	**1.03E-07**	0.14	**3.53E-05**
2	rs905656	127,636,090	BIN1	-0.05	0.001	-0.03	0.111
8	rs894019	27,550,531	CLU	0.01	0.800	0.28	**9.00E-09**
6	rs871654	47,788,409	CD2AP	0.03	0.084	0.00	0.944
11	rs668134	59,756,902	MS4A4A	-0.27	3.88E-04	-0.23	0.037
11	rs668134	59,756,902	MS4A6A	-0.34	0.006	-0.16	0.140
11	rs475639	85,367,433	PICALM	-0.08	0.002	0.03	0.298

Table 2. Most significant cis-SNP/probe associations for each of the seven LOAD candidate genes tested.

Figure 1

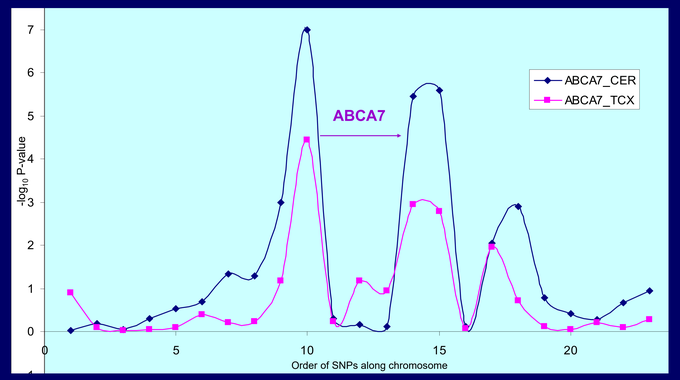


Figure 2

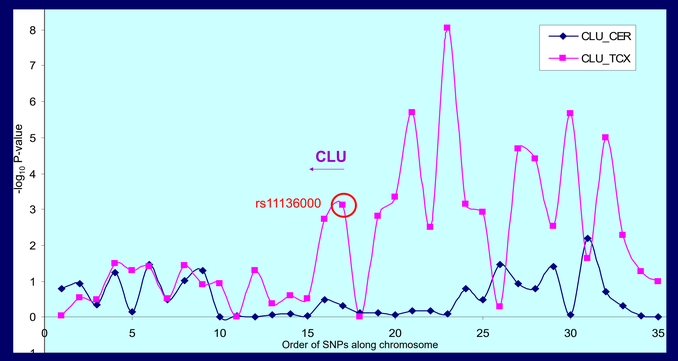
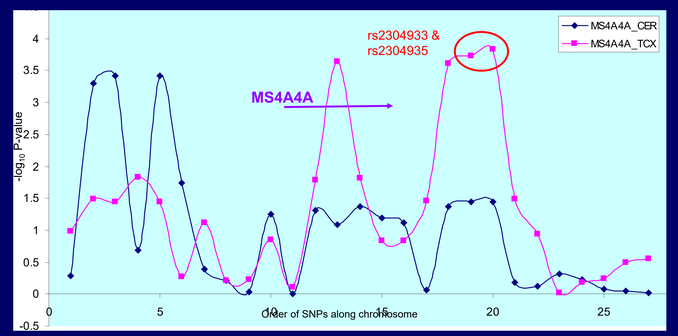


Figure 3



Conclusions

- Alteration of expression of the *CLU* and *MS4A4A* genes may at least partly explain the LOAD risk association for two recently identified LOAD SNPs: *CLU* rs1136000 which is associated with reduced risk of LOAD and increased expression of the *CLU* gene in the Temporal Cortex and *MS4A4E* rs670139 (proxies) which is associated with increased risk of LOAD and increased expression of the *MS4A4A* gene in the Temporal Cortex.
- Novel LOAD candidate genes *CLU* and *ABCA7* may harbor powerful eSNPs.
- These results have implications in the search for functional variants at the novel LOAD risk loci.