Late-onset Alzheimer disease risk variants mark brain regulatory loci

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Supplemental data at Neurology.org/ng

ABSTRACT

Objective: To investigate the top late-onset Alzheimer disease (LOAD) risk loci detected or confirmed by the International Genomics of Alzheimer's Project for association with brain gene expression levels to identify variants that influence Alzheimer disease (AD) risk through gene expression regulation.

Methods: Expression levels from the cerebellum (CER) and temporal cortex (TCX) were obtained using Illumina whole-genome cDNA-mediated annealing, selection, extension, and ligation assay (WG-DASL) for ~400 autopsied patients (~200 with AD and ~200 with non-AD pathologies). We tested 12 significant LOAD genome-wide association study (GWAS) index single nucleotide polymorphisms (SNPs) for *cis* association with levels of 34 genes within ±100 kb. We also evaluated brain levels of 14 LOAD GWAS candidate genes for association with 1,899 *cis*-SNPs. Significant associations were validated in a subset of TCX samples using next-generation RNA sequencing (RNAseq).

Results: We identified strong associations of brain *CR1*, *HLA-DRB1*, and *PILRB* levels with LOAD GWAS index SNPs. We also detected other strong *cis*-SNPs for LOAD candidate genes *MEF2C*, *ZCWPW1*, and *SLC24A4*. *MEF2C* and *SLC24A4*, but not *ZCWPW1* *cis*-SNPs, also associate with LOAD risk, independent of the index SNPs. The TCX expression associations could be validated with RNAseq for *CR1*, *HLA-DRB1*, *ZCWPW1*, and *SLC24A4*.

Conclusions: Our results suggest that some LOAD GWAS variants mark brain regulatory loci, nominate genes under regulation by LOAD risk variants, and annotate these variants for their brain regulatory effects. ***Neurol Genet* 2015;1:e15; doi: 10.1212/NXG.0000000000000012**

GLOSSARY

AD = Alzheimer disease; **CER** = cerebellum; **eQTL** = expression quantitative trait loci; **eSNP** = expression single nucleotide polymorphism; **GWAS** = genome-wide association study; **HC** = healthy control; **HD** = Huntington disease; **HLA** = human leukocyte antigen; **IGAP** = International Genomics of Alzheimer's Project; **LD** = linkage disequilibrium; **LOAD** = late-onset Alzheimer disease; **PFC** = prefrontal cortex; **QC** = quality control; **RIN** = RNA integrity number; **RNAseq** = RNA sequencing; **SNP** = single nucleotide polymorphism; **TCX** = temporal cortex; **VC** = visual cortex; **WG-DASL** = whole-genome cDNA-mediated annealing, selection, extension, and ligation assay.

Genome-wide association studies (GWASs) of late-onset Alzheimer disease (LOAD) identified 9 risk loci and confirmed *APOE*.[1–5] The International Genomics of Alzheimer's Project (IGAP) identified 11 additional loci and confirmed 8 of the 9 initial loci.[5] The "disease GWAS" approach does not uncover the identities of the disease risk gene or functional risk variants. Furthermore, some index single nucleotide polymorphisms (SNPs) identified by this approach are near more than 1 gene. It is evident that alternative approaches are needed for the discovery of the disease genes/variants and for uncovering their mechanism of action.[6]

We hypothesize that many LOAD GWAS loci harbor functional variants that influence disease risk by their effects on brain gene expression. We previously determined that some of the most significant (index) LOAD GWAS SNPs and other variants at the top LOAD risk loci associate

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with brain levels of nearby genes.[7] Others also identified strong *cis*-expression SNPs (*cis*-eSNPs) at LOAD risk loci.[8–10] Collectively, these findings strongly suggest the presence of regulatory LOAD risk variants at these loci.

In this study, we comprehensively analyzed LOAD GWAS loci not previously assessed by (1) testing the influence of the index SNPs on brain expression levels of all nearby genes and (2) fine mapping *cis*-eSNPs that associate with brain levels of candidate genes nominated by LOAD GWAS. We annotate these variants for their effects on brain expression, Alzheimer disease (AD) risk, and regulatory potential. Our findings have implications in the search for functional variants and the identity of the AD genes at these loci.

METHODS

Patients and samples. All patients were from the Mayo Clinic Brain Bank and underwent neuropathologic evaluation by Dr. Dennis Dickson. All patients with AD had a Braak score of ≥4.0 and patients without AD had a Braak score of ≤2.5. Many of the patients without AD had unrelated pathologies. All patients with AD had a definite diagnosis according to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria.[11] All patients were part of the published Mayo Clinic LOAD GWAS[12] and expression GWAS.[7,13] RNA samples were isolated from 2 brain regions: cerebellum (CER) (AD n = 197 and non-AD n = 177) and temporal cortex (TCX) (AD n = 202 and non-AD, n = 197), as described previously.[7,13] Three-hundred forty patients had measurements in both CER and TCX. CER was chosen, as it is a relatively unaffected region in AD, whereas TCX is typically one of the first regions affected by AD neuropathology.[14] Choice of both typically unaffected (CER) and affected (TCX) brain regions enables minimization of any confounds on gene expression from AD neuropathology as well as evaluation of disease-relevant brain region, respectively, within the same study. Additional details are provided in table e-1 at Neurology.org/ng.

Standard protocol approvals, registrations, and patient consents. This study was approved by the appropriate institutional review board.

Gene expression measures. Total RNA, used in both the array-based Illumina whole-genome cDNA-mediated annealing, selection, extension, and ligation assay (WG-DASL) and next-generation RNA sequencing (RNAseq) (Illumina, San Diego, CA), was isolated from frozen brain tissue using the Ambion RNAqueous kit (Life Technologies, Grand Island, NY) and assessed for RNA quality and quantity using the Agilent RNA 6000 Nano Chip and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Gene expression levels were previously collected for all samples using WG-DASL as described elsewhere[13] and in appendix e-1. We considered only those probes that were expressed in ≥50% of the patients in at least 1 tissue (detection $p < 0.05$). We subsequently collected TCX gene expression measures for a subset of the AD samples using next-generation RNAseq (n = 94, table e-1) on Illumina HiSeq, which were used for validation of significant WG-DASL TCX findings. Further details are provided in appendix e-1.

Genotyping. ***Index SNPs.*** We genotyped 11 novel LOAD risk SNPs (or their proxies) identified by the IGAP consortium[5] and 2 SNPs at additional loci (*CR1* and *CD2AP*) reported by the prior LOAD GWAS,[3,4,15] as they were not assessed in our prior study[7] (table e-2). Eleven SNPs were genotyped using Taqman SNP genotyping assays (Life Technologies), one (rs1476679) was genotyped using a KASP genotyping assay (LGC, Middlesex, UK), and one (rs3818361) was genotyped as part of the Mayo Clinic LOAD GWAS.[12] These SNPs were assessed for association with expression levels of *cis*-genes (±100 kb) with probes that passed our quality control (QC) threshold.

LOAD candidate gene* cis-*eSNPs. We extracted genotypes for a total of 1,899 SNPs from available Mayo Clinic GWAS[12] and HapMap-imputed[13] data that were within ±100 kb of 13 LOAD candidate genes nominated by GWAS,[3–5,15] as well as *PILRB,* which was identified as a candidate LOAD gene in this study. Genotype data were extracted for only those SNPs *in-cis* with candidate genes that had WG-DASL probes that passed our QC thresholds (table e-3). These genotypes were used to test associations of brain levels of LOAD candidate genes with their *cis*-SNPs. Additional genotyping details are provided in appendix e-1.

Statistical analysis. All analyses were run in PLINK[16] using multivariable linear regression assuming an additive model, with the SNP minor allele dosage (0, 1, 2) as the independent variable and adjusting for the following covariates: age at death, sex, number of *APOE* ε4 alleles (0, 1, 2), RNA integrity number (RIN), adjusted RIN value $(RIN_{sample} - MeanRIN)^2$, microarray PCR plate (WG-DASL only), Flowcell (RNAseq only), and diagnosis, when appropriate (AD = 1, non-AD = 0; WG-DASL only). A total of 2,873 tests were conducted, corresponding to a study-wide significant *p* value of 1.74E-05 after Bonferroni correction. This is a highly stringent correction given that multiple probes for the same gene and many tested SNPs in linkage disequilibrium (LD) are not truly independent tests.

For visualization of the top *cis*-SNP/expression associations, expression residuals obtained after adjustment of all covariates were plotted: kernel density plots were generated using the sm.density.compare function of the sm package in R, and box plots were generated using the ggplot package within R. LocusZoom[17] was used to plot results for all HapMap2-imputed eSNPs *in-cis* with the top genes implicated in this study (*cis*-eSNPs).

RegulomeDB. Regulome scores for all SNPs tested, when available, were obtained from the RegulomeDB (http://regulomedb.org/index), where lower numbers represent stronger levels of data supporting the regulatory annotation for an individual SNP.[18]

IGAP LOAD risk association results. Publicly available results from IGAP[5] stage I were used in this study; further details are provided in appendix e-1.

Comparison with other brain gene expression data. We compared the significant *cis*-eSNP results in this study to published brain expression quantitative trait loci (eQTL) data from healthy controls (HCs)[19] and patients with neurodegenerative disease.[20] Results for the former are deposited in http://www.braineac.org/ (referred to as the *Braineac* data set hereforth) and are from 10 brain regions in 134 patients without prominent neuropathology; we compared their CER and TCX *cis*-eSNP data to ours. Results from the latter come from 3 brain regions (dorsolateral prefrontal cortex [PFC], visual cortex [VC], and CER) of 376 patients with LOAD, 194 patients with Huntington disease (HD), and 173 controls without dementia (referred to as LOAD/HD/HC). Details are provided in appendix e-1.

Differential gene expression. We performed differential expression analyses for the 6 genes with significant *cis*-eSNP

Table 1 Significant brain transcript level associations for the index LOAD GWAS SNPs

Locus	Chr	SNP	Pos bp (hg19)	Tested allele	Gene	Method	Probe	TCX eQTL		CER eQTL		LOAD GWAS		Regulome score
								β	p Value	β	p Value	OR	p Value	
CR1	1	rs3818361	207,784,968	A	*CR1*	WG-DASL	ILMN_1742601	0.34	1.59E-07	NA	NA	1.18	3.70E-14	6
						RNAseq	NA	0.72	8.06E-05	NA	NA			
HLA-DRB-5/ HLA-DRB-1	6	rs9271192	32,578,530	C	*HLA-DRB1*	WG-DASL	ILMN_1715169	2.02	7.90E-93	2.12	8.80E-116	1.11	2.90E-12	5
						RNAseq	NA	0.31	3.43E-02	NA	NA			
ZCWPW1	7	rs1476679	100,004,446	C	*PILRB*	WG-DASL	ILMN_1723984	−0.39	2.25E-07	−0.25	4.72E-05	0.91	5.60E-10	1f
						RNAseq	NA	0.06	4.24E-01	NA	NA			

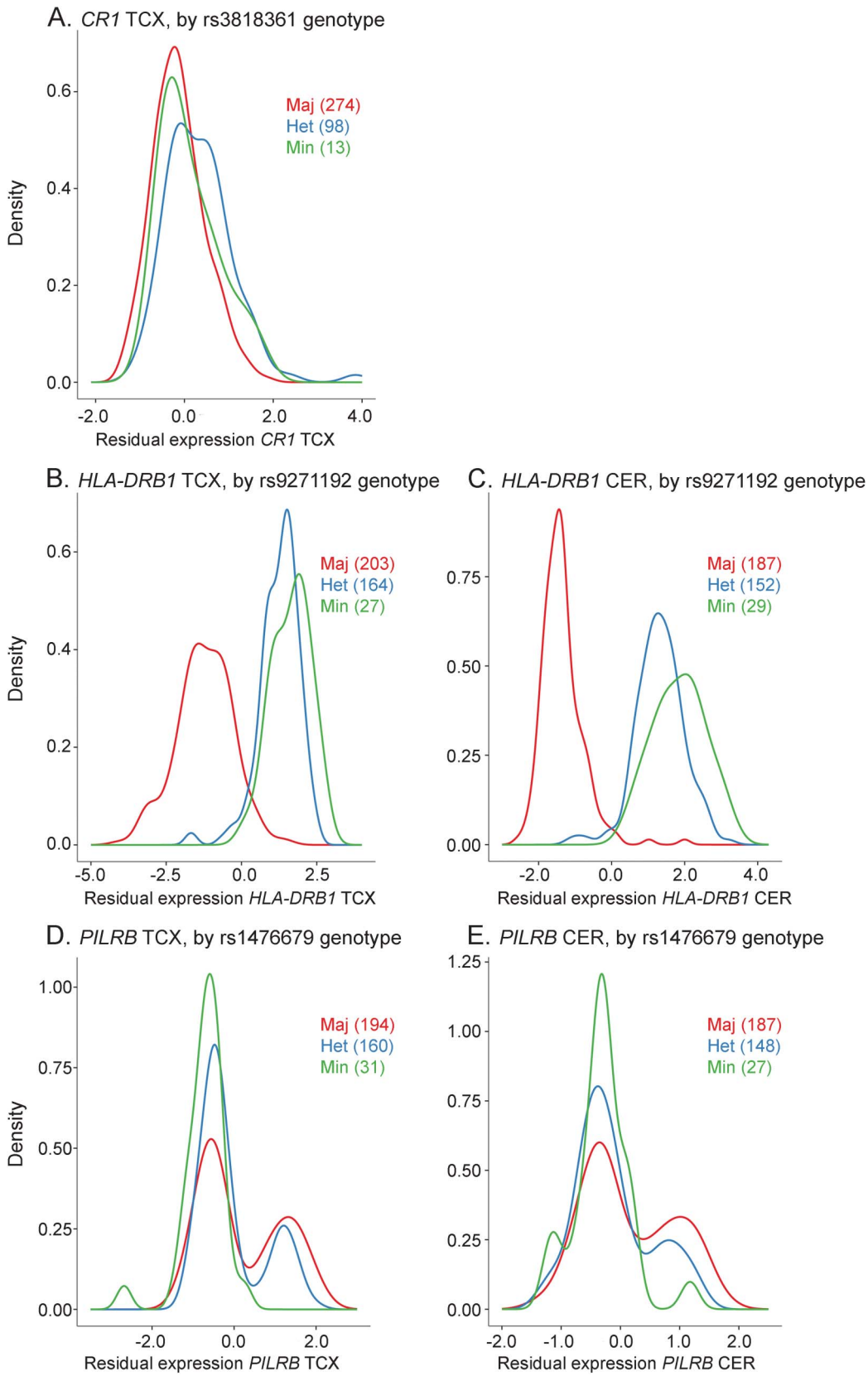
Abbreviations: AD = Alzheimer disease; CER = cerebellum; Chr = chromosome; eQTL = expression quantitative trait loci; GWAS = genome-wide association study; IGAP = International Genomics of Alzheimer's Project; LOAD = late-onset Alzheimer disease; NA = not applicable; OR = odds ratio for the tested allele; TCX = temporal cortex; SNP = single nucleotide polymorphism; WG-DASL = whole-genome cDNA-mediated annealing, selection, extension, and ligation assay.
Results are shown for WG-DASL: AD + non-AD combined cohort (TCX, n = 399, CER, n = 374) and RNAseq (AD, TCX, n = 94). Locus name is given by the LOAD GWAS and is usually the gene name nearest the index SNP. Pos bp: SNP position given in base pairs according to hg19. Tested allele: Results for the eQTL and LOAD GWAS are shown for the same tested alleles. Gene: Transcript levels of the gene tested in the eQTL. Probe: WG-DASL probe measured. TCX: Temporal cortex eQTL results. CER: Cerebellum eQTL results. LOAD GWAS: IGAP Stage I results. β: eQTL effect size for the tested allele. All probes tested had expression levels above background in >50% of the subjects when results were reported for that tissue.

associations identified in this study (*CR1*, *HLA-DRB1*, *PILRB*, *MEF2C*, *ZCWPW1*, and *SLC24A4*). Expression levels were compared between patients with AD and patients without AD in multivariable linear regression analyses adjusting for age at death, sex, RIN, RIN^{2}adjusted (RIN-RINmean)^2, PCR plates as technical variables, and expression levels for 5 transcripts that are specific for 5 cell types in the CNS to account for neuronal loss, gliosis, and/or vascular tissue in the assessed brain regions. The following probes were used as covariates: *ENO2* for neurons (ILMN_1765796), *GFAP* for astrocytes (ILMN_1697176), *CD68* for microglia (ILMN_2267914), *OLIG2* for oligodendrocytes (ILMN_1727567), and *CD34* for endothelial cells (ILMN_1732799).

RESULTS ***cis*-Expression association analyses of LOAD GWAS index SNPs.** We evaluated 10 index SNPs reported by the IGAP consortium[5] in addition to 2 other index SNPs at the *CR1* and *CD2AP* loci. Using our WG-DASL brain expression data, we assessed 47 expression probes representing 34 unique genes for *cis* association with the 12 LOAD GWAS index SNPs (table e-2). After study-wide Bonferroni correction, 3 SNPs were significantly associated with *cis*-gene expression levels in TCX (*CR1*, *HLA-DRB1*, and *PILRB*). Of these, *HLA-DRB1* and *PILRB* levels could also be reliably measured in CER and showed significant associations. The TCX associations for *CR1* and *HLA-DRB1* could be validated in the RNAseq data obtained from the smaller subset of AD samples (table 1).

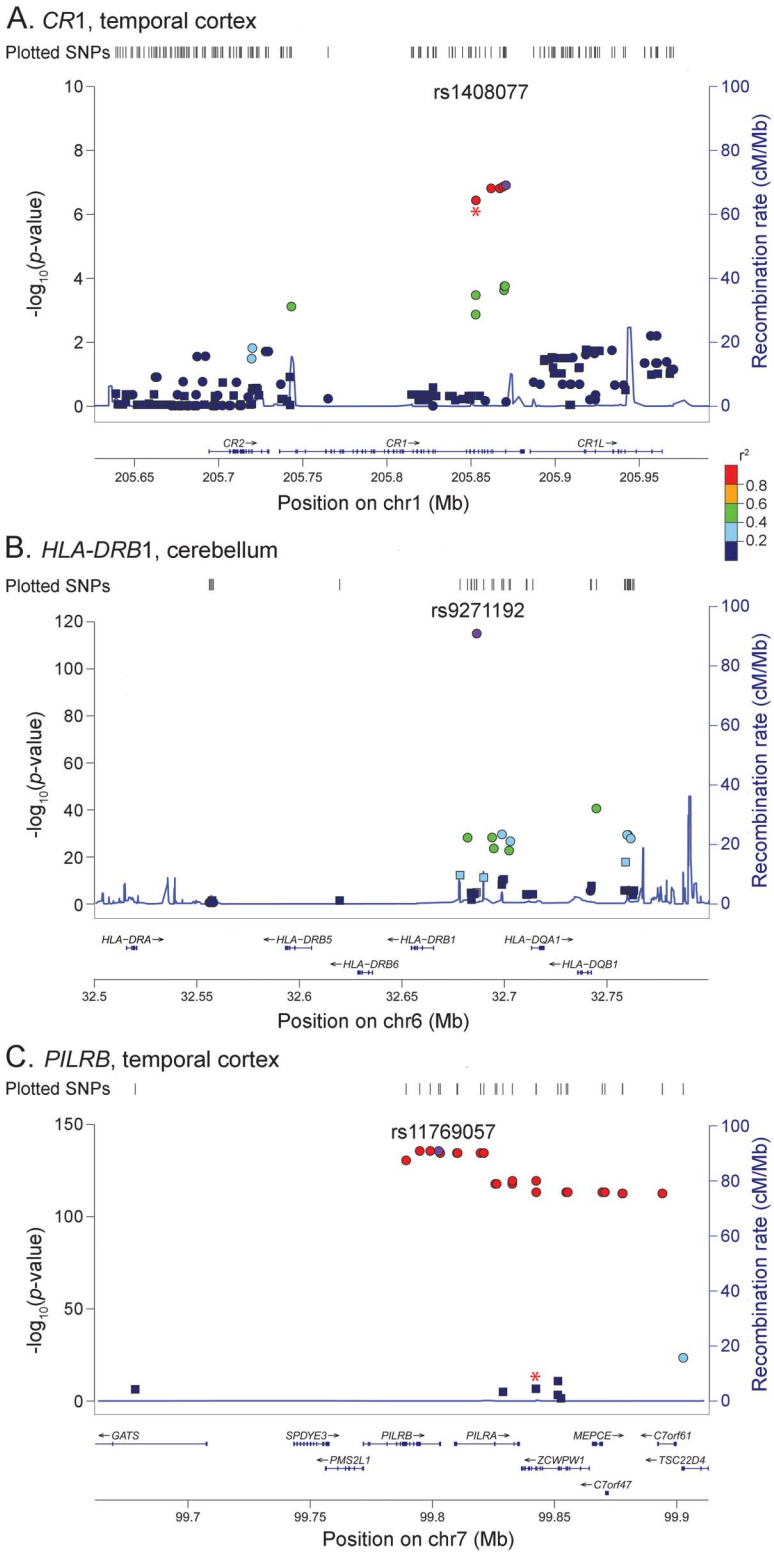
The *CR1* locus LOAD risk allele[4,21,22] for rs3818361 is significantly associated with increased *CR1* messenger RNA levels in TCX when patients with and without AD are assessed together (table 1, figure 1A, figure e-1) and separately (table e-2); this finding is validated in the TCX RNAseq data (table 1). The *CR1* probe did not meet our detection criteria of ≥50% in CER and was not assessed. Fine mapping of the *cis*-eQTL for *CR1* (all *cis*-SNPs within ±100 kb of *CR1*) identified rs1408077 as the strongest *cis*-eSNP (tables e-3 and e-4). LocusZoom[17] plots of the region (figure 2A) demonstrate that rs3818361 and rs1408077 are in strong LD. However, neither these nor other strong *CR1* *cis*-eSNPs, which also associate with LOAD risk, have good Regulome scores, indicating that they are unlikely to be strong functional regulatory variants. This suggests the presence of a yet-undefined regulatory variant(s) in this region tagged by the *CR1* LOAD index and strong *cis*-eSNPs (table e-4). As shown in the kernel density (figure 1A) and box plots (figure e-1), *CR1* levels are lower in rs3818361 major homozygotes compared to heterozygotes but not minor homozygotes. This may be due to imperfect LD between the actual regulatory variant and this SNP. It is also possible that by chance there were more rs3818361 heterozygotes that also harbored the regulatory variant but not as many for the smaller group of minor homozygotes.

Figure 1 **Kernel density plots**



(A) *CR1* TCX, (B) *HLA-DRB1* TCX and (C) CER, (D) *PILRB* (ILMN_1723984) TCX and (E) CER gene expression residuals by relevant index SNP. Distribution of brain gene expression level residuals from all patients obtained after adjustment for all covariates is shown. Green line indicates distribution of gene expression residuals for homozygous minor individuals (Min); blue line indicates the same for heterozygotes (Het); red line indicates the same for major homozygotes (Maj). The number of individuals with each genotype is indicated on the plot (#). CER = cerebellum; SNP = single nucleotide polymorphism; TCX = temporal cortex.

Figure 2 **LocusZoom plots**



(A) *CR1* TCX expression levels *cis*-SNP associations, (B) *HLA-DRB1* CER expression levels *cis*-SNP associations, and (C) *PILRB* (ILMN_1723984) TCX expression levels *cis*-SNP associations. Square points indicate $\beta < 0$ (i.e., minor allele associated with lower expression levels); circle points indicate $\beta > 0$ (i.e., minor allele associated with higher expression levels). Relevant index LOAD GWAS SNP is indicated with a red asterisk below point on plot. LD based on HapMap2 build hg18. r^2 values plotted relative to the most significant *cis*-SNP indicated by purple marker. *HLA-DRB1* locus index LOAD GWAS SNP rs9271192 is the most significant *cis*-SNP. Region displayed = candidate gene ±110 kb. CER = cerebellum; GWAS = genome-wide association study; LOAD = late-onset Alzheimer disease; SNP = single nucleotide polymorphism; TCX = temporal cortex.

Table 2 Significant brain transcript level associations for the candidate genes at the LOAD GWAS loci

Gene	Chr	Method	Probe(s)	No. of *cis*-SNPs[a]	eSNP[b]	Position (hg19)	Tested allele	TCX eQTL		CER eQTL		LOAD GWAS		Regulome score	Full eQTL results[c]
								β	*p* Value	β	*p* Value	OR	*p* Value		
MEF2C	5	WG-DASL	ILMN_1742544	155	rs254776	88,042,649	G	−0.01	7.41E-01	−0.11	2.52E-07	0.97	9.27E-02	6	Table e-10
		RNAseq	NA	NA				0.05	6.54E-01	NA	NA				NA
ZCWPW1	7	WG-DASL	ILMN_1751963	37	rs10241492	99,832,749	G	0.14	4.97E-10	0.07	4.08E-03	1.00	8.56E-01	5	Table e-13
		RNAseq	NA	NA				0.25	2.89E-04	NA	NA				NA
SLC24A4	14	WG-DASL	ILMN_1675391	193	rs7150592	91,776,397	T	0.05	1.98E-02	0.25	9.30E-14	1.04	7.12E-03	7	Table e-11
		WG-DASL	ILMN_2370738	193				0.03	2.08E-01	0.24	5.22E-10				Table e-12
		RNAseq	NA	NA				0.08	1.59E-01	NA	NA				NA

Abbreviations: AD = Alzheimer disease; CER = cerebellum; Chr = chromosome; eQTL = expression quantitative trait loci; GWAS = genome-wide association study; LOAD = late-onset Alzheimer disease; NA = not applicable; OR = odds ratio for the tested allele; TCX = temporal cortex; SNP = single nucleotide polymorphism; WG-DASL = whole-genome cDNA-mediated annealing, selection, extension, and ligation assay. Results are shown for AD + non-AD combined cohort. Gene is the candidate gene nominated by the LOAD GWAS. See also footnotes to table 1 for definitions of the headings.

[a] Number of *cis*-SNPs tested in the WG-DASL eQTL analyses. The *cis*-SNPs had call rates >95% in both eQTL analyses.

[b] Most significant eSNP in either CER or TCX WG-DASL eQTL.

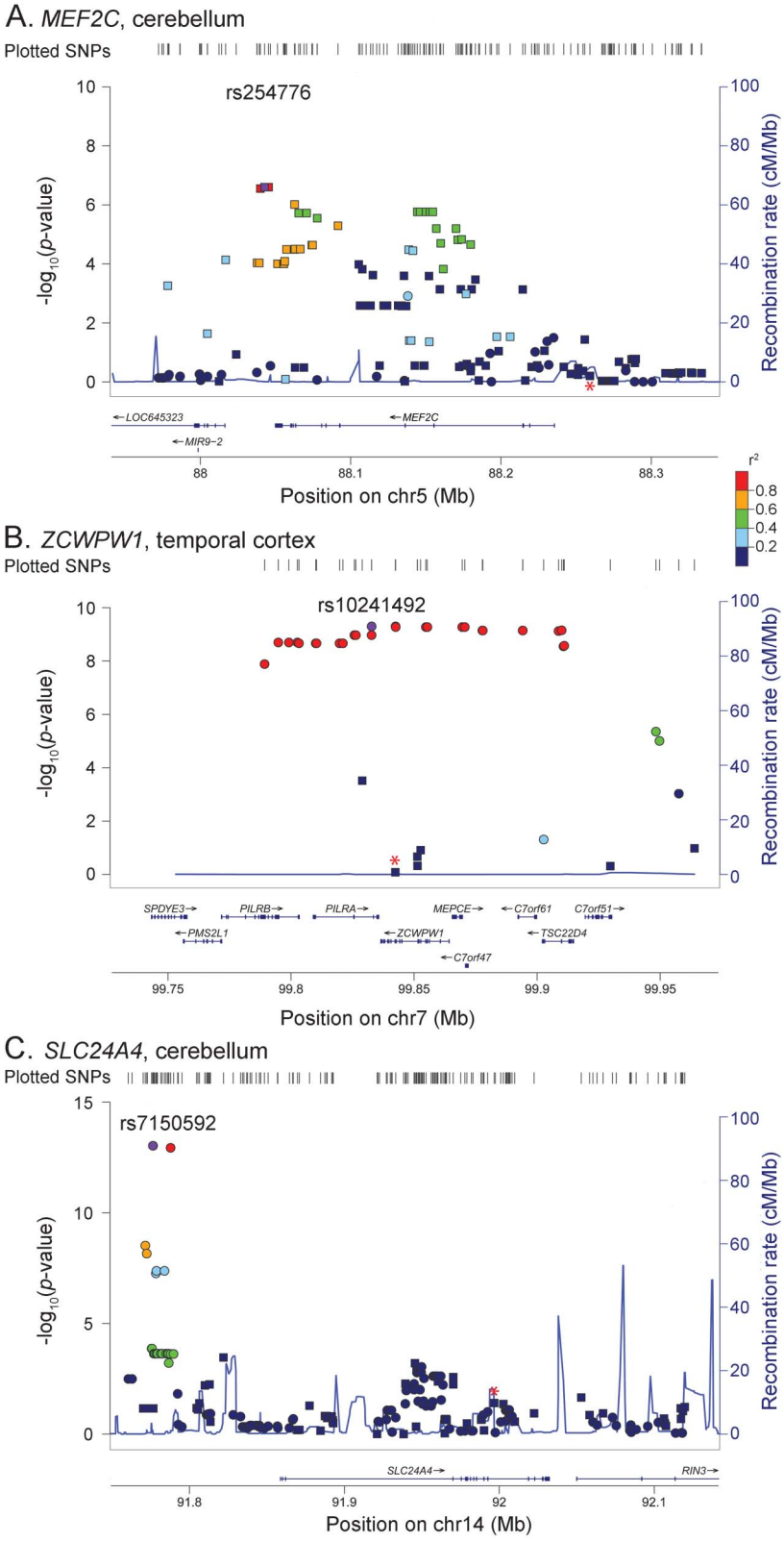
[c] Supplementary table that has WG-DASL results of all *cis*-SNPs.

The LOAD risk allele for the IGAP index SNP rs9271192 is significantly associated with increased levels of *HLA-DRB1* in both TCX and CER in all patients with AD and patients without AD (table 1; figure 1, B and C; figure e-2; table e-2). RNAseq TCX results validate the WG-DASL TCX findings with the same direction of effect (table 1). Both kernel density (figure 1, B and C) and box plots (figure e-2) depict the clear dissociation of *HLA-DRB1* levels that are lower in major homozygotes compared to minor allele carriers. Fine mapping of the eQTL for *HLA-DRB1* reveals rs9271192 to be the strongest *cis*-eSNP (tables e-3 and e-5, figure 2B), although this SNP has a high (poor) Regulome score. We identify 1 *HLA-DRB1 cis*-eSNP, rs2516049, with a low Regulome score, significant *HLA-DRB1* brain expression association, and nominally significant association with LOAD risk (table e-5).

Finally, the LOAD protective allele of rs1476679 at the *ZCWPW1* locus is significantly associated with decreased brain expression levels of the proximal gene *PILRB* in TCX when patients with and without AD are assessed together (table 1; figure 1, D and E; figure e-3). This SNP reaches nominal significance in all but AD CER but was not validated by RNAseq in the subset of TCX AD samples (table 1). Although rs1476679 is closest to *ZCWPW1*, it had no association with brain levels of this gene. It is interesting that this index SNP has a Regulome score of 1f,[18] suggesting that this variant itself may be functional. Based on the multimodal distribution of *PILRB* expression (figure 1, D and E), it appears that a subset of major homozygotes and heterozygotes have higher brain *PILRB* levels. It is possible that the risky major allele of rs1476679 may have a regulatory role in increasing brain *PILRB* levels but that other genetic and/or nongenetic factors also influence levels of this gene, thus leading to this multimodal distribution. Indeed, fine mapping of this eQTL (tables e-6 to e-9, figure 2C) indicates 2 distinct groups of variants. One group is in strong LD with the index SNP rs1476679, the minor allele of which has significant associations with lower brain *PILRB* levels and reduced LOAD risk. The other group is in strong LD with the most significant *PILRB cis*-SNP rs11769057 (table e-3) and has more significant associations with higher *PILRB* levels but no association with LOAD risk. Both groups have variants with good Regulome scores and may represent 2 distinct haplotypes.

We also identified nominally significant *cis* associations of the *ZCWPW1* locus SNP rs1476679 with reduced *PILRA* levels; the *CELF1* locus SNP rs7933019 (proxy for rs10838725) with brain *C1QTNF4*, *MTCH2*, and *RAPSN* levels; the *FERMT2* locus SNP rs17125944 with *FERMT2* levels; and the *CASS4* locus SNP rs7274581 with *C20orf43* and *CASS4* levels (table e-2).

Figure 3 LocusZoom plots



(A) *MEF2C* CER expression levels *cis*-SNP associations, (B) *ZCWPW1* TCX expression levels *cis*-SNP associations, and (C) *SLC24A4* cerebellum expression levels *cis*-SNP associations. See figure 2 legend for description of other features. CER = cerebellum; SNP = single nucleotide polymorphism; TCX = temporal cortex.

LOAD GWAS candidate genes *cis*-expression analyses. We fine mapped and annotated the SNPs within ±100 kb of the candidate LOAD GWAS genes that we had not previously assessed[7] to identify strong *cis*-eSNPs with LOAD risk association and functional evidence of regulatory potential. The most significant *cis*-eSNPs for each gene are summarized in table e-3, with detailed findings depicted in tables e-4 to e-24. *MEF2C*, *ZCWPW1*, and *SLC24A4* had *cis*-eSNPs that achieved study-wide significance in at least 1 tissue region. These variants were different than the LOAD GWAS index SNPs (table 2, table e-3).

Seventeen *cis*-eSNPs were associated with *MEF2C* CER but not TCX levels (table e-10). The most significant, rs254776, associates with lower CER levels and has a suggestive association with lower LOAD risk (table 2). This variant is in poor LD with the index LOAD GWAS SNP at this locus, rs190982 ($r^2 = 0.047$; $D' = 0.305$) (figure 3A), and has a high Regulome score.

SLC24A4 *cis*-eSNPs achieved study-wide significant association with CER gene levels, many of which also had nominally significant TCX level associations (tables e-11 and e-12). The top *SLC24A4* *cis*-eSNP rs7150592 is significantly associated with increased *SLC24A4* CER levels and suggestively associated with higher TCX levels and increased LOAD risk.[5] TCX RNAseq validation also reveals suggestive association with higher levels. This variant is not in LD with the LOAD index SNP at this locus, rs10498633 ($r^2 = 0.001$; $D' = 0.053$; figure 3C). None of the strong *SLC24A4* *cis*-eSNPs have good Regulome scores.

ZCWPW1 *cis*-eSNP associations were strongest in TCX, with RNAseq validation and nominally significant CER associations (table e-13). Fine mapping of *ZCWPW1* variants revealed many strong *cis*-eSNPs, of which rs10241492 was the most significant (table 2, table e-13). None of the strong *ZCWPW1* *cis*-eSNPs have any LOAD risk association. The most significant *ZCWPW1* *cis*-eSNP rs10241492 has weak LD with the LOAD index SNP rs1476679, as measured by r^2 ($r^2 = 0.092$; $D' = 1.0$) (figure 3B). These *ZCWPW1* *cis*-eSNPs associate with higher levels of this gene and belong to the same group of *cis*-eSNPs that also associate with higher levels of *PILRB* but not with LOAD risk.

Comparison with other brain gene expression data. We compared the *cis*-eSNP associations identified in our study to HC brain gene expression data[19] deposited in the *Braineac* Web site and data obtained in brains from patients with LOAD, patients with HD, and HCs.[20] Of the significant *cis*-eSNP associations highlighted in tables 1 and 2 of our study, all but *HLA-DRB1*/rs9271192 were present in *Braineac* (table e-25), whereas only *CR1*/rs3818361 was present in the LOAD/HD/HC data set (table e-26).

There were nominally significant ($p < 0.05$) associations with *cis*-eSNPs for *CR1*, *PILRB*, and *SLCA24A4* and suggestive results ($p < 0.2$) for *MEF2C* and *ZCWPW1* in *Braineac* (table e-25). It is important that the direction of association for the minor *cis*-eSNP allele is consistent with our findings. The *CR1*/rs3818361 association is highly significant in the VC data from the LOAD/HD/HC data set.

To compare *cis*-eSNPs for the other genes highlighted in our study with the LOAD/HD/HC data, we evaluated significant *cis*-eSNPs from this study[20] that also existed in our data set (tables e-4 to e-13). We were able to make comparisons for all except *SLC24A4 cis*-eSNPs, as none existed in the LOAD/HD/HC study. *Cis*-eSNPs for *CR1*, *HLA-DRB1*, *ZCWPW1*, and *PILRB* were highly significant in both data sets (table e-26). The common *MEF2C* cis-eSNP rs770463 had nominal significance in our study and was highly significant in the LOAD/HD/HC study.[20]

Differential gene expression. TCX *CR1* levels were nominally significantly higher in patients with AD compared to patients without AD (table e-27). While this finding would not remain significant after Bonferroni corrections for the 6 genes evaluated, this trend is consistent with expectations based on the highlighted *cis*-eSNP results (table 1), i.e., rs3818361 minor allele is associated with both increased AD risk (higher frequency in patients with AD) and greater TCX *CR1* levels. TCX *SLC24A4* levels are significantly higher in patients with AD, again consistent with the direction of AD risk and *SLC24A4* associations of rs7150592. The trends for higher *HLA-DRB1* and *PILRB* in CER are consistent with the directions of associations of their respective *cis*-eSNPs, but these trends were not observed for TCX. None of the other differential gene expression analyses were significant or suggestive.

DISCUSSION In this study, we identified strong brain gene expression associations for the index LOAD GWAS variants near *CR1*, *HLA-DRB1*, and *PILRB*. We also detected strong *cis*-eSNPs for *MEF2C* and *SLC24A4*, some of which also associate with LOAD risk, independent of the top LOAD risk SNPs at these loci. Finally, we determined that despite harboring very strong brain *cis*-eSNPs, *ZCWPW1* is unlikely to be the affected LOAD risk gene at this locus, given the lack of any LOAD risk for the strong *ZCWPW1 cis*-eSNPs. These findings can have immediate mechanistic implications for each of the eQTL identified in this study.

The association of the index *CR1* locus SNPs with both higher TCX levels of this gene and LOAD risk suggests that higher levels of complement receptor 1 in the brain may have adverse effects, although in reality the relationship of *CR1* expression and LOAD risk is likely to be more complex.[23–26] This SNP was also found to associate with PFC *CR1* levels in a study of patients with neurodegenerative disease[20] and also with higher TCX *CR1* levels in a study of HCs,[19] both data sets that are independent from this study. In addition to the LOAD GWAS index SNPs, a copy number variation resulting in a long CR1 isoform[23] and a CR1 coding variant[26] has also been implicated in LOAD risk at this locus. Given these and our results reported here, joint investigation of these coding variants and *CR1* regulatory variants is warranted.

Previous eQTL studies also identified associations with expression levels of *HLA-DRB1*, *HLA-DRB5*, and other members of the human leukocyte antigen (HLA) family.[5,19,27,28] Collectively, our findings and the published results highlight the role of strong regulatory variants in the HLA region for human disease. Given the highly polymorphic nature of this region, the identity of the functional regulatory variants may prove difficult to discern. The high sequence variability in this region may also pose technical problems with respect to artifactual associations of gene expression levels arising from probes that may harbor variants.[29,30] Nevertheless, our validation of the findings in TCX using the alternative RNAseq approach and strong eQTL in this region identified by others[20] effectively argues against a false-positive expression association secondary to a probe-binding artifact.

Our findings at the *ZCWPW1/PILRB* locus highlight the potential utility of the combined gene expression and disease risk association approaches in discerning the plausible risk gene at the disease locus. We detected strong *cis* associations of the protective index SNP rs1476679 with lower brain *PILRB* levels but not *ZCWPW1* levels. *PILRB* association was also noted in other eQTL studies.[5,19,20] Although this association did not validate in our smaller subset of AD TCX with RNAseq, this may be a reflection of the smaller sample size (n = 94) or the tissue and group analyzed. Notably, WG-DASL association was weakest in AD samples. This may also be due to the presence of multiple regulatory variants that influence brain levels of *PILRB*, where large enough sample sizes are required to discern the effects of each of these variants.

Indeed, further mapping of the *ZCWPW1/PILRB* locus identified 2 distinct sets of *cis*-eSNPs: one group that includes the protective LOAD index SNP rs1476679, which associates with lower brain levels of *PILRB*, and one group that consists of strong *cis*-eSNPs that associate with increased levels of *PILRB* but do not associate with LOAD risk. Although these findings require further investigations through mechanistic studies, they may imply that moderate reductions in *PILRB* may have implications for LOAD risk, whereas there may be a ceiling effect for increased levels of this gene that precludes any functional

consequences on disease risk. These future studies need to incorporate investigations of both *PILRA* and *PILRB*, whose expression may be coregulated.

The *cis*-eSNPs that associate with higher brain *PILRB* levels but not LOAD risk also influence higher brain *ZCWPW1* levels. It is possible that this locus harbors an enhancer element with effects on expression levels of nearby genes, including *PILRB* and *ZCWPW1*, without any effects on LOAD risk. These findings emphasize the importance of detailed fine mapping of disease loci that may enable dissection of the most plausible disease risk genes and location of the functional variants.

Finally, *MEF2C* and *SLC24A4* were found to have strong *cis*-eSNPs, some of which also had nominally significant associations with LOAD risk.[5] These results may imply the presence of regulatory LOAD risk variants that are distinct from the index SNPs, as we previously identified for *CLU* and *ABCA7*.[7] For a detailed discussion for each of the genes see appendix e-2.

Consistent findings between our data and 2 other brain eQTL data sets[19,20] highlight the authenticity of our results. Although a prior study failed to identify significant brain gene expression associations[31] for some of the LOAD risk variants previously identified,[3] including *CR1* highlighted in our study, this may represent a false-negative in the context of findings reported here and by others. Many of the *cis*-eSNPs highlighted in our study appear to influence gene expression in patients with AD, patients with other neurologic diseases, and HCs. In addition, many but not all of the significant *cis*-eSNPs in our current and previous studies[7,13] have effects on gene expression in tissue affected and unaffected by disease neuropathology. These findings suggest that many disease-associated common regulatory variants influence gene expression across disease phenotypes and tissue regions; however, they do not rule out disease- and tissue-specific effects for other *cis*-eSNPs.

Finally, the comparison of gene expression levels between patients with vs without AD in our study revealed significant differential gene expression for *CR1* and *SLC24A4* and suggestive trends for *HLA-DRB1* and *PILRB* that are consistent with the direction of associations of their respective *cis*-eSNPs. Lack of significant differential expression for the other transcripts could be due to lack of significant differences in the frequency of functional regulatory variants between AD and non-AD groups in our study, especially given that our non-AD group includes patients with other neurodegenerative diseases; a low signal:noise ratio for differential expression given multiple potential confounders despite our best efforts for correction; or a combination of these factors.

The strengths of our study are the size of the cohort for the brain expression analyses, detailed fine mapping and annotation approaches, built-in replication with 2 different brain regions, 2 distinct study populations, additional validation through the alternative RNASeq approach, and comparison to 2 public eQTL results. Despite these strengths, it is possible that some of the convergent LOAD risk and expression associations are coincidental. Other weaknesses include gene expression measurements in tissue rather than specific cell types, focusing only on SNPs rather than copy number variants, and lack of a true control group without any neurodegenerative pathology. We tried to address these weaknesses by assessing public eQTL data from both HCs[19] and patients with neurodegenerative disease[20] and by investigating multiple brain regions from our data and others. In summary, the collective evidence from this and other studies[7–10,13] implicates gene expression regulation as a key mechanism of function for some LOAD risk variants, which mark brain regulatory loci.

AUTHOR CONTRIBUTIONS

M.A. and N.E.-T.: drafting/revising the manuscript for content; study concept; data acquisition; and analysis or interpretation of data. M.K., M.M.C., A.K., L. Manly, J.D.B., C.W., D.S., X.W., J.S., F.Z., H.S.C., C.Y., J.C., C.M., T.N., L. Ma, K.M., and S.L.: data acquisition; analysis or interpretation of data. R.C.P., N.R.G.-R., Y.W.A., D.W.D., and S.G.Y.: drafting/revising the manuscript for content.

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Late-onset Alzheimer disease risk variants mark brain regulatory loci

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