SUPPLEMENTARY MATERIAL

S1. Supplementary narrative

S1.1 The AD-associated variants at the *ABCA7* locus identified in GWAS

GWAS have identified six lead AD-associated lead variants at the *ABCA7* locus in European ancestry populations (Supplementary Table S2).[1-7] Two of these variants (rs3752246 and rs4147929) are essentially co-inherited (R^2 = 0.97). Liu et al.[9] pointed out that the AD-risk allele at rs4147929 is associated with increased *ABCA7* expression in many tissues including certain brain regions in data from UKBEC and GTEx transcriptome projects. However, this allele has no effect on *ABCA7* expression in the hippocampus and cortex, which are critical to AD pathology, in the UKBEC data (exprID t3815416). The allele also has no significant effect on *ABCA7* expression in the hippocampus and cortex after the latest update in the GTEx data (accession phs000424.v8.p2). On the other hand, the AD-risk allele at rs111278892 is associated with decreased *ABCA7* message in the hippocampus in both UKBEC (p = 0.093) and GTEx datasets (p = 0.0034). The rs3752246 variant is a missense mutation, which may affect ABCA7 activity.[10] rs3752246 and rs4147929 are in moderate (R^2 ~ 0.55) linkage disequilibrium with rs4147934, another missense mutation.[11] A protective coding mutation in *ABCA7* has been identified.[12] It is therefore plausible that the risk alleles at rs3752246 and rs4147929 raise AD odds by reducing ABCA7 activity rather than its protein level. This plausibility needs to be further tested in ABCA7 structure-function studies.

S1.2 Identification of the causative genes at the AD-associated loci using chromatin studies

GWAS-identified disease risk variants are in a vast majority of cases non-coding and are assigned to genes based on location proximity on the chromosome and other considerations that the GWAS investigators deem to be appropriate, i.e., the initially assigned gene may not be the true causative gene.[13] One approach to identify the true causative gene is to conduct a

chromatin study to link the risk variants to gene promoters and enhancers. The Encyclopedia of DNA Elements (ENCODE) database contains a large amount of chromatin data for many tissues and cell types.[14] Several brain-specific chromatin studies were recently published.[15-17] Unfortunately, the results are not always in agreement among the studies. For example, a major finding of Nott et al.[15] is that the AD-risk variants at the *BIN1* locus affect a *BIN1* enhancer active only in microglia, but Lu et al.[17] findings point to *SFT2D3* and not *BIN1* as the gene affected by the variants at the *BIN1* locus in neural tissues, and findings from Song et al.[16] suggest that the same *BIN1* enhancer may be active in hippocampal dentate gyrus-like neurons. With respect to *ABCA7*, Nott et al. reported that *ABCA7* does not have a promoter in astrocytes and has an enhancer that is functional only in microglia. Data from ENCODE, however, show that the same promoter-enhancer pair is present in primary astrocytes and neuronal cell lines (Supplementary Figure S4), and Song et al. also reported presence of a functional *ABCA7* promoter in astrocytes. One plausible reason for the discrepant findings is that each study used a different source of human tissue: Nott et al. used tissue resected for treatment of epilepsy in pediatric patients, Lu et al. used postmortem adult samples, and Song et al. derived neural cells from induced pluripotent stem (iPS) cells. In a different potentially more definitive approach, the putative causative gene can be validated by sequencing it and finding coding loss-of-function mutations in it that are associated with the disease.[18] At present most loci with AD-risk variants have not been definitively assigned to a causative gene, and hence the full extent of lipid involvement into AD pathogenesis captured by the AD GWAS remains to be determined.

S1.3 Plausible reasons for the discrepancy between genomics and transcriptomics

Younger (63-78 years of age) individuals with Braak stage II-V neuropathology in our cohort had very low ABCA7 levels, which is in line with the findings from genomics that *ABCA7* loss-of-function alleles are associated with early onset AD and that carriers of *ABCA7* loss-of-function alleles experience selective attrition.[19-21] The AD transcriptome studies have used tissue

from mostly older (>75) individuals.[22,23] In the older (79-93) age group in our cohort, there was no difference in ABCA7 between control/Braak stage I and Braak stage II-V individuals. Given the small size of our 79-93 age group, it is unlikely that we could detect the small increase in *ABCA7* message in AD reported by the transcriptome studies.[22,23] Therefore, the present results do not contradict the findings from transcriptomics either. Nonetheless, the findings from genetics and our 63-78 age group are compelling, and it is more likely that the transcriptome findings came about because of shortcomings in experimental design than that ABCA7 provides protection from AD earlier in life and then becomes an AD risk factor later in life.

Liu et al.[9] summarized publicly available transcriptome evidence that AD have higher *ABCA7* message levels than controls and proposed that ABCA7 increases AD risk. We suggest that the apparent increases in *ABCA7* message in AD may be an artifact of comparing older AD subjects with younger controls without age-matching and cohort segmentation into age groups. Because of select attrition of individuals with low *ABCA7* expression, older homogeneous AD populations may have higher levels of *ABCA7* message by random chance than younger heterogeneous small control populations. Without controlling for age, the difference between AD and control individuals in our 123-subject cohort is much less dramatic (Mann-Whitney p = 0.021). Liu et al. did not seem to control for age, but in three out of four transcriptome data sets analyzed in their report, AD subjects were significantly older than controls (mean ± SD, 80.2 ± 9.3 versus 62.1 ± 10.9, $p < 0.0001$ in HBTRC; 75.4 ± 6.6 versus 72.4 ± 6.3, $p = 0.0003$ in AddNeuroMed; 81.1 ± 9.5 versus 70.8 ± 16.4 in Ciryam et al.; age data could not be readily located for the fourth data set). Tellingly, in these three datasets, smaller p values for the difference in *ABCA7* expression between control and AD subjects were associated with greater differences in the average age between the same control and AD groups (i.e., AD minus control average age; Pearson r = -0.86, $R^2 = 0.73$). This suggests that age differences between control and disease groups affect conclusions about *ABCA7* expression in AD in transcriptome studies.

In addition to lack of controlling for age, *ABCA7* transcript mis-splicing owing to spliceosome derangement in AD[24-26] or failing homeostatic feed back pathways[27] attempting to restore ABCA7 levels may also lead to the apparent increase in ABCA7 message in AD.[28] The increase in *ABCA7* message in AD was found by the transcriptome sequencing studies that compared individuals with Braak and Braak stages I-IV taken as controls against individuals with Braak and Braak stages V/VI taken as cases.[22,23] Thus, loss of ABCA7 protein occurs in early AD pathogenesis and may be part of AD etiology, while increase in *ABCA7* message takes place late in AD pathogenesis and may be part of ensuing neurodegeneration.

Supplementary References

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Supplementary Tables

Supplementary Table S1. Sources of the brain tissue samples used in the study.

Biobank name and location	Brain region	Number of samples	PMI (hours) Avg ± SD Min, max	Braak stages	Neuro-pathology report	Notes
Harvard Brain Tissue Resource Center, McLean Hospital, Belmont, MA	Hippocampus	9	21.9 ± 6.1 5.8, 30.3	Stages I-V	Yes	No ancestry is available
	Parietal cortex (BA-39)	39	21.8 ± 5.7 6.1, 33.7			
The Mount Sinai/JJ Peters VA Medical Center NIH Brain and Tissue Repository, Bronx, NY	Hippocampus	44	9.6 ± 5.5 2.7, 23.9	Unaffected controls and stages I-V	No	
Human Brain and Spinal Fluid Resource Center, Brentwood Biomedical Research Institute, Los Angeles, CA	Hippocampus	21	13.4 ± 4.9 6.8, 26.8	Unaffected controls and stages I-V	Yes	No ancestry is available
University of Miami Brain Endowment Bank, Miami, PA	Hippocampus	6	20.1 ± 8.4 6.8, 27.9	Stages I and II	No	
The Neuropathology Brain Bank at the University of Pittsburgh School of Medicine, Pittsburgh, PA	Hippocampus	4	24.6 ± 2.2 21.8, 26.5	Unaffected controls and stages I and II	Yes	

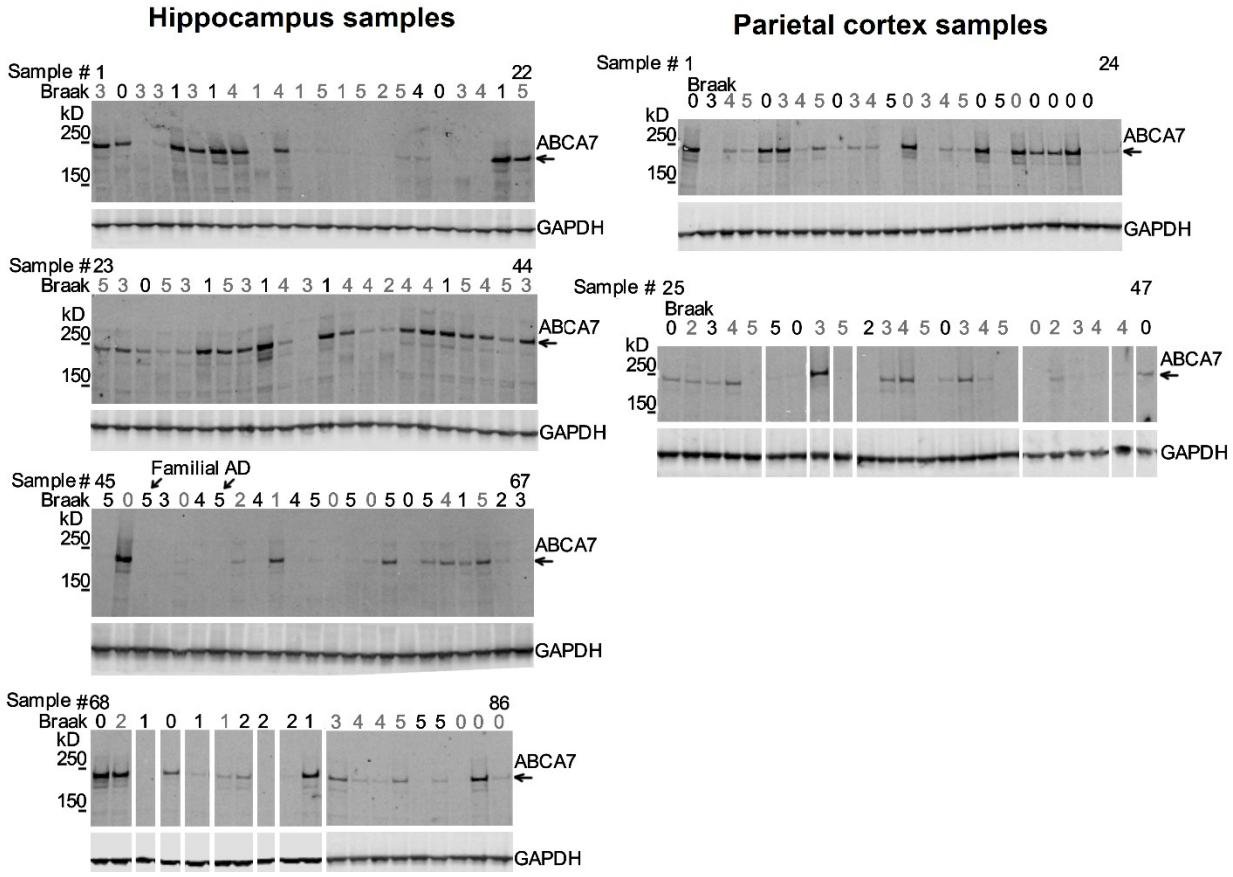
Supplementary Table S2. GWAS lead AD-associated variants identified at the *ABCA7* locus.

Variant	MAF	OR (95% CI)	p	Risk allele	Study	Coding	Analysis type
rs3752231		n/a	4.4 × 10−13	T	Marioni et al.[3]	No	Case-control and AD-by-proxy
rs3752241		n/a	2.9 × 10−8	G	Jansen et al.[1]	Yes, silent	AD-by-proxy phase
	0.18	1.15 (1.11–1.18)	3.1 × 10−16	G	Kunkle et al.[2]	Yes, G[GGT] > A[GCT]	Case-control GWAS
rs3752246	0.19	1.15 (1.09–1.21)	5.8 × 10–7		Naj et al.[6]		Case-control GWAS
rs3764650	0.1	1.23 (1.17–1.28)	5.0 × 10−21	G	Hollingworth et al.[7]	No	Case-control GWAS
		n/a	8.6 × 10−9	A	Jansen et al.[1]	No	Case-control phase
rs4147929	0.19	1.15 (1.11–1.19)	1.1 × 10−15		Lambert et al.[5]		Case-control GWAS
rs111278892	0.16	n/a	7.9 × 10−11	G	Jansen et al.[1]	No	Case-control and AD-by-proxy
rs115550680*	0.07	1.79 (1.47–2.12)	2.2 × 10−9	G	Reitz et al.[4]	No*	Case-control GWAS

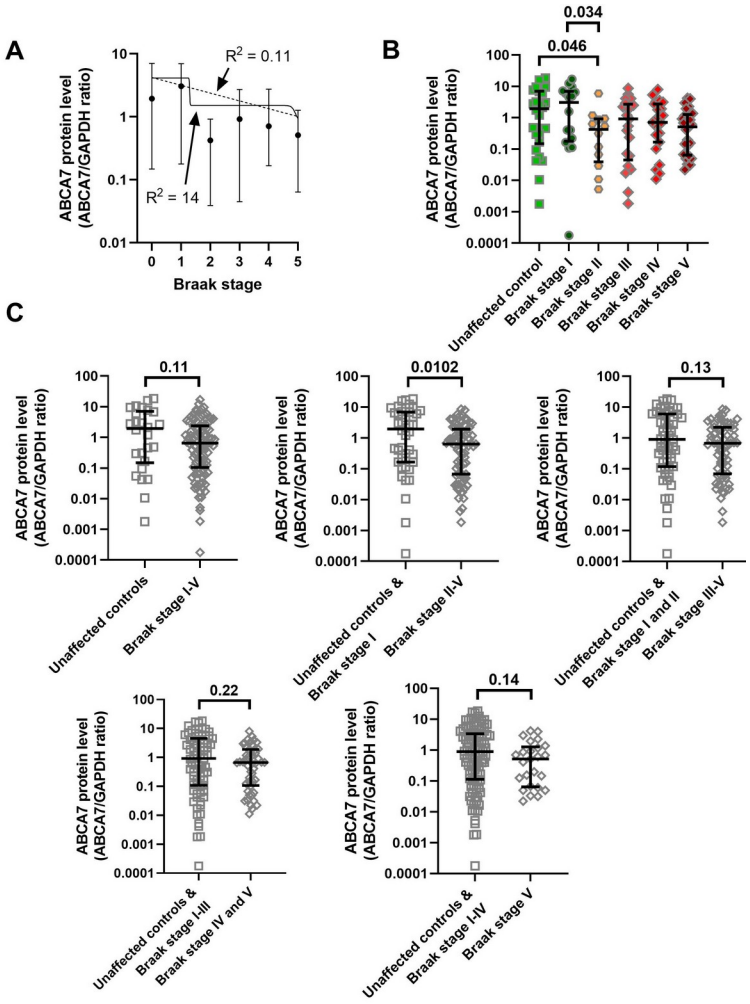
* - Reitz et al[4] study was conducted in an African ancestry population, the lead variants rs115550680 is in a tight linkage disequilibrium (R^2 = 0.94, MAF 0.07 for both) with the deletion loss-of-function variant rs142076058 identified by Cukier et al[9]

MAF – minor allele frequency; OR – odds ratio; CI – confidence interval

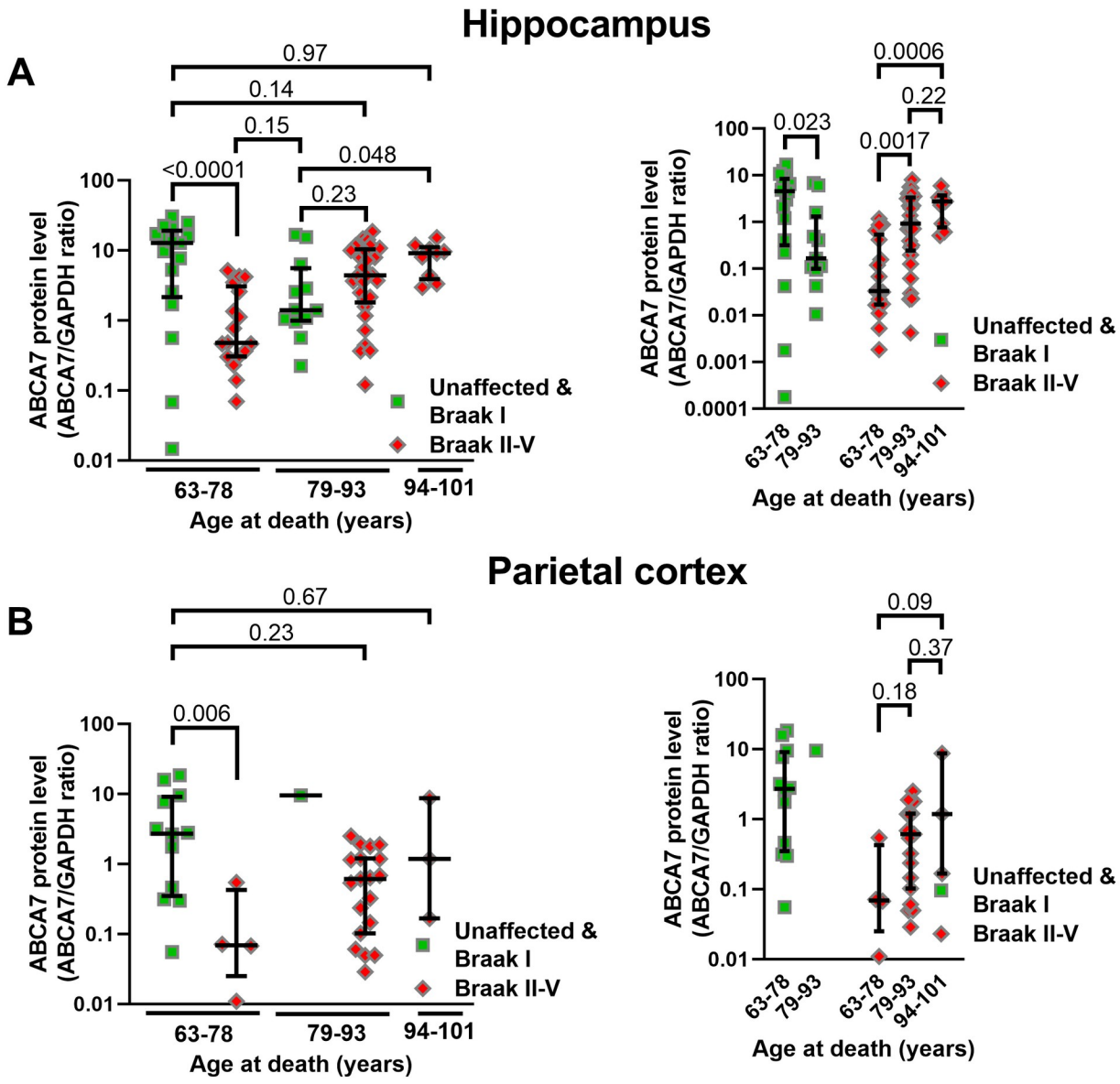
Supplementary Figures



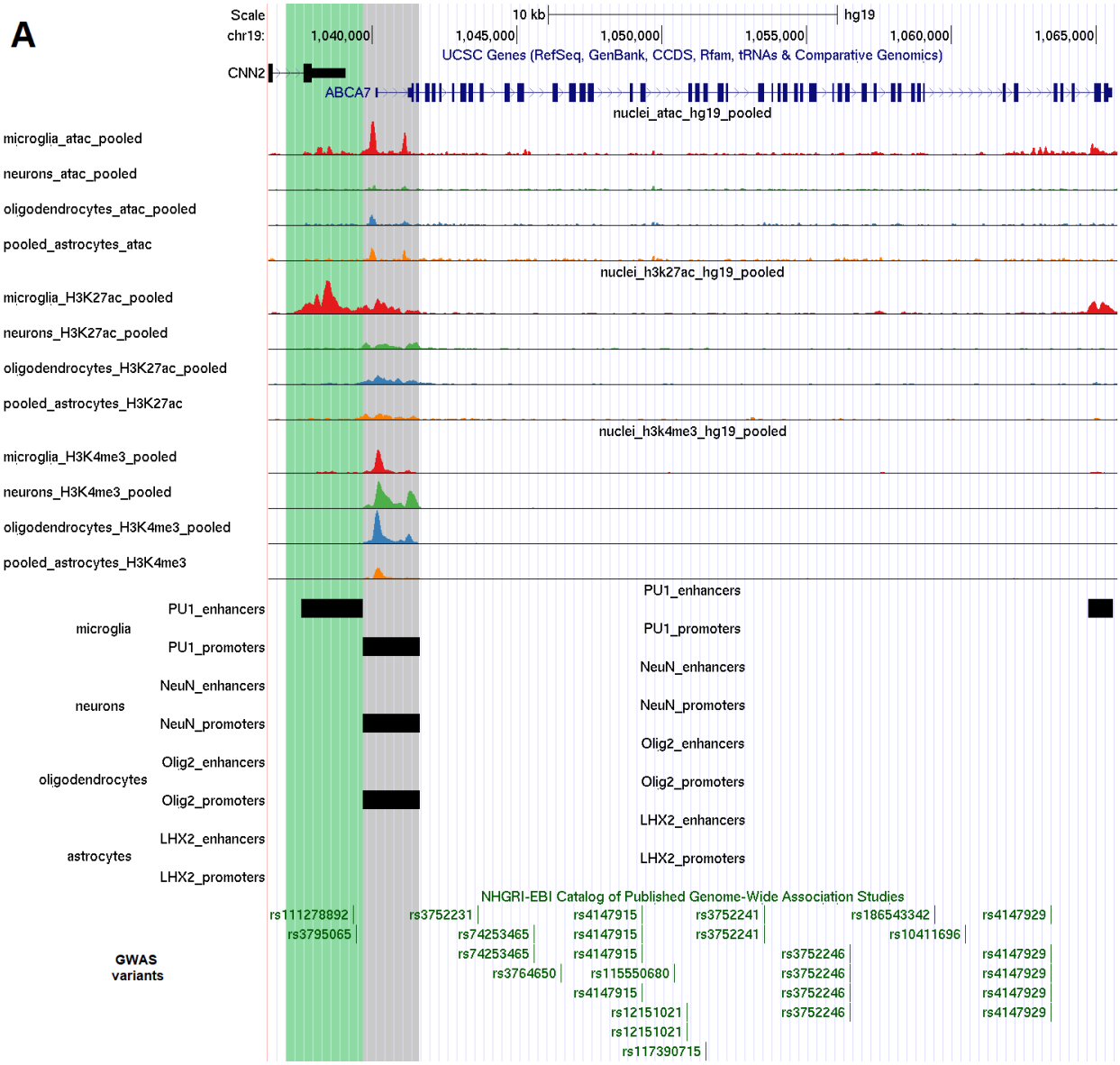
Supplementary Figure S1. ABCA7 levels in the hippocampus and parietal cortex. Left panel, ABCA7 in hippocampus of 86 individuals (84 who died after the age of 60 and two who died before the age 50 with familial AD and were excluded from the study cohort). Right panel, ABCA7 in the parietal cortex of 47 individuals. Eight individuals were represented by a hippocampus and parietal cortex sample. For these individuals, measurements from the hippocampus were used in the study. The total number of unique individuals involved in the study was 123: 84 represented by the hippocampus and 39 represented by the parietal cortex. Braak stages of the surveyed individuals are shown above each lane. For individuals <78 years of age, the Braak stage number is shown in black, and for those >78, the number is shown in gray.



Supplementary Figure S2. Analyses of the relationship between ABCA7 level and Braak stage AD neuropathology. A, A biphasic sigmoidal line provided a better fit to the relationship between ABCA7 level and Braak stage in the 123-subject cohort. No *p* values are calculated for curve fitting. B, There was no overall significant difference among unaffected controls and individuals with Braak stages I-V in the Kruskal-Wallis test ($H = 8.39$, $p = 0.14$). Nonetheless, unaffected controls and Braak stage I subjects had higher ABCA7 level than Braak stage II subjects in *post hoc* multiple comparison (false discovery rate 0.05). C, Only when unaffected controls and Braak stage I individuals were combined in one group and Braak stage II-V individuals were combined into the other group, there was a significant difference between the two groups (Mann-Whitney test).



Supplementary Figure S3. Analysis of the relationship between ABCA7 and Braak stage and age separately in the hippocampus and parietal cortex. A,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ABCA7
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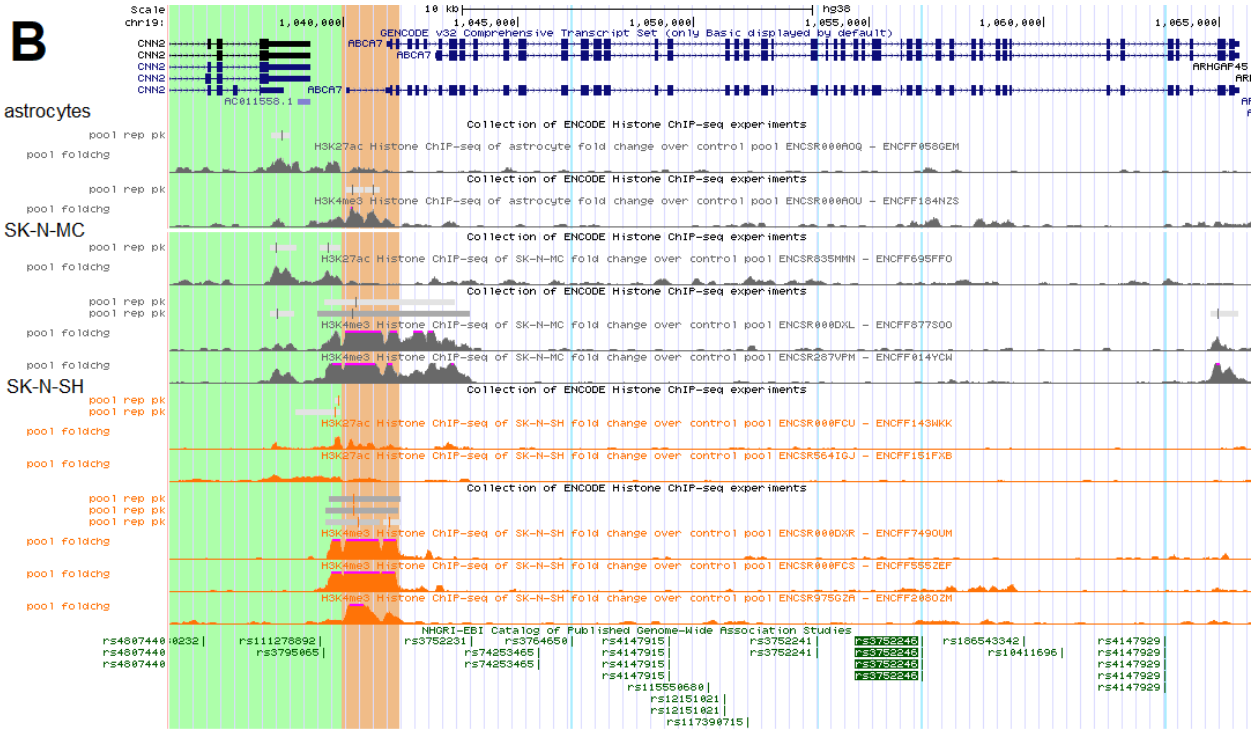

Supplementary Figure S4. Comparison of the findings from Nott et al. and from ENCODE regarding the location of *ABCA7 cis*-acting regulatory elements. A, Findings from the work of Nott et al. The gray and green areas show the locations of the *ABCA7* promoter and enhancer, respectively. Promoters in the study of Nott et al. are defined as regions bound by histone H3 with tri-methylation at the 4th lysine (H3K4me3) and histone H3 acetylated at lysine 27 (H3K27ac). Enhancers are defined as regions bound only by H3K27ac. Nott et al. used brain tissue dissected from pediatric epilepsy patients. Nuclei were obtained from tissue and separated into microglia, neuron, oligodendrocyte and astrocyte nuclei using fluorescent-activated nuclei sorting. B, Data from the ENCODE project for the same *ABCA7* region and the same histone marks. The promoter and enhancer are shown in orange and green, respectively. Presence of H3K3me3 and H3K27ac at the promoter in human adult astrocytes and SK-N-MC and SK-N-SH human neuronal cells is clearly discernible. Presence of H3K27ac at the enhancer is also notable. ENCODE used primary astrocytes from Lonza for this experiment.