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An *ABCA1* missense variant decreases cholesterol efflux and confers Alzheimer's disease risk in the Chinese population

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Abstract

Background: Genetic studies have revealed that single-nucleotide polymorphisms (SNPs) of *ABCA1* are associated with Alzheimer's disease (AD) risk. However, their AD-related effects in non-European populations are not well studied. Moreover, the functional implications of these AD-associated SNPs remain unclear.

Objective: We examined the AD associations of *ABCA1* SNPs in the Chinese population and investigated the underlying mechanisms whereby these SNPs modulate AD risk.

Methods: We conducted a genetic analysis in a Hong Kong Chinese AD cohort ($n = 332$ patients with AD, $n = 316$ normal controls). Specifically, we analyzed 6 independent *ABCA1* SNPs reported to be associated with AD risk in populations of European descent. To investigate the effects of these SNPs on ABCA1 protein function and brain molecular phenotypes, we analyzed cholesterol efflux in human glioblastoma cells as well as the associations between the AD risk SNPs and brain transcriptomic profiles, respectively.

Results: The *ABCA1* coding SNP, rs2230806 (p.R219 K), was significantly associated with AD in the Chinese population, specifically in females (odds ratio [95% confidence interval] = 1.65 [1.16–2.33]). Notably, human glioblastoma cells expressing the *ABCA1* R219 K showed a 17% cholesterol efflux reduction ($p < 0.001$). Moreover, *ABCA1* rs2230806 was associated with changes in the expression of oligodendrocyte genes involved in myelination in the brain in females.

Conclusions: We identified a significant AD risk *ABCA1* coding variant in the Chinese population and demonstrated its effects on cholesterol efflux and brain molecular phenotypes. These results shed light on the genetic basis whereby an *ABCA1* genetic variant contributes to AD pathogenesis.

Keywords

ABCA1, Alzheimer's disease, genetics, oligodendrocytes, transcriptomic analysis

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Introduction

Alzheimer's disease (AD), the most common neurodegenerative disorder worldwide, is characterized by the presence of amyloid plaques and neurofibrillary tangles in the brain.[1,2] Genome-wide association studies have identified several AD-associated loci that are involved in lipid metabolism and transportation, including *APOE*, which is the most significant genetic risk locus for AD.[3,4] Besides *APOE*, other AD risk loci involved in lipid regulation include *ABCA1, ABCA7*, and *CLU*.[5] Therefore, the dysregulation of lipid metabolism in the brain has emerged as an important factor in AD pathogenesis.[6]

ABCA1 encodes a membrane protein that plays a key role in mediating cholesterol efflux, which is essential for maintaining lipid homeostasis and supporting neuronal functions.[7] Genetic studies have identified multiple single-nucleotide polymorphisms (SNPs) in the *ABCA1* locus that are associated with AD. These SNPs include both coding variants (e.g., rs2230808, rs2066716, and rs2230806) as well as noncoding variants residing in the *ABCA1* promoter (e.g., rs2422493), 3' untranslated region (3'-UTR) (e.g., rs363717), and introns (e.g., rs2740488).[8–10] However, most genetic studies of *ABCA1* focus on populations of European descent,[8–11] limiting our understanding of the genetic basis of AD in diverse populations. Therefore, it is important to investigate the effects of *ABCA1* SNPs on AD risk in non-European populations, which will provide a more comprehensive understanding of the role of *ABCA1* in AD.

Although several common AD-associated *ABCA1* SNPs have been identified, their specific functional consequences remain largely unknown. Patients with AD have significantly lower ABCA1-mediated cholesterol efflux capacity than normal controls (NCs),[12,13] suggesting that decreased ABCA1-mediated cholesterol efflux contributes to AD pathogenesis. However, it remains unclear how the identified *ABCA1* SNPs modulate ABCA1 function and are involved in AD pathogenesis. Therefore, investigating the regulatory role of AD-associated *ABCA1* SNPs will extend our understanding of the role of *ABCA1* and cholesterol metabolism in AD pathogenesis, providing insights for therapeutic development.

Accordingly, in this study, we investigated the associations between AD and 6 previously reported independent *ABCA1* SNPs—rs363717, rs2230808, rs2066716, rs2230806, rs2740488, and rs2422493—in a Hong Kong Chinese cohort. We showed that a nonsynonymous coding SNP, rs2230806-T (p.R219 K), is associated with increased AD risk exclusively in females. Subsequent meta-analysis in populations of Chinese and European descent revealed that rs2230806-T exerts concordant AD risk effects in females from both populations. Moreover, functional assays demonstrated that the *ABCA1* R219 K variant decreases the cholesterol efflux activity of ABCA1 protein in human glioblastoma cells. Further transcriptomic analysis in female postmortem human brain tissues showed that *ABCA1* rs2230806-T is associated with altered expression levels of genes involved in pathways including myelination and neuronal projection. In addition, single-nucleus RNA sequencing analysis revealed that *ABCA1* rs2230806-T modulates gene expression and pathways specifically in oligodendrocytes. Taken together, our findings identify a female-specific AD risk SNP of *ABCA1* in the Chinese population and demonstrate its role in modulating cholesterol efflux and oligodendrocyte-specific gene expression in the human brain, suggesting the roles of *ABCA1* in AD pathogenesis.

Methods

Hong Kong Chinese Alzheimer's disease cohort

The Hong Kong Chinese AD cohort consisted of 333 patients with AD and 319 NCs. All participants were recruited from the Specialist Outpatient Department at the Prince of Wales Hospital at the Chinese University of Hong Kong. Clinical data included age, sex, and plasma lipid levels. The cognitive performance of all participants (aged ≥ 65 years) was assessed by the Montreal Cognitive Assessment.[14] AD was diagnosed according to the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5).[15] We excluded participants with incomplete phenotype information, resulting in a total of 332 AD cases and 316 NCs for analysis. This study was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee at the Prince of Wales Hospital, the Chinese University of Hong Kong, and the Hong Kong University of Science and Technology. All participants provided written informed consent for both study enrollment and sample collection.

DNA extraction and genotyping

We collected 3 mL whole blood from each participant into K3EDTA tubes (Vacuette) and centrifuged the samples at $2000 \times g$ for 15 min at 4°C. Next, we delivered 420 µL cell pellet (i.e., the lower fraction) from each sample to the Centre for PanorOmic Science (CPOS, Genomics and Bioinformatics Cores, LKS Faculty of Medicine, University of Hong Kong) for DNA extraction. The extraction was conducted automatically by a QIAsymphony SP (Qiagen) using a QIAsymphony DSP DNA Midi Kit (Qiagen). Genomic DNA was eluted with 100 µL Elution Buffer ATE (Qiagen).

We conducted genotyping for 333 patients with AD and 319 NCs by TaqMan Assay (i.e., *ABCA1* rs363717, C___2741115_20; *ABCA1* rs2230808, C___2741104_1_; *ABCA1* rs2066716, C___11720781_20; *ABCA1*

rs2230806, C___27410511_1_; *ABCA1* rs12740488, C___1797554_10; and *ABCA1* rs2422493, C___16235101_10; cat. no. 4351374 for all probes; Thermo Fisher Scientific). We performed real-time quantitative PCR using a 7500 Fast and QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems). We stored the results in EDS files and input them into TaqMan Genotyper Software (version 1.7.1, Applied Biosystems) for the joint genotyping of SNPs.

Association analysis of ABCA1 single-nucleotide polymorphisms with Alzheimer's disease

As a quality control step, we first evaluated the Hardy–Weinberg equilibrium of the 6 investigated *ABCA1* SNPs using the *HWExact* function from the HardyWeinberg package in R (version 4.2.2).[16] To examine the additive effects of the *ABCA1* SNPs on AD risk, we conducted logistic regression analysis using the *glm* function in R, adjusting for age and sex. To investigate the sex-specific effects of the SNPs on AD, we performed logistic regression analysis, adjusting for age separately in male and female participants. To control the confounding effects of all tested SNPs, we also conducted multiple logistic regression analysis for all 6 SNPs, adjusting for age. We used the *p.adjust* function from the R stats package with the *fdr* method to adjust *p* values. Statistical power for our study was estimated using the Genetic Association Study (GAS) Power Calculator.[17] Specifically, for rs2230806, we had greater than 95% power to detect an association at a significance level of 0.05 (Supplemental Table 1).

Meta-analysis

To validate the association between *ABCA1* rs2230806-T and AD, we performed meta-analysis under additive, dominant and recessive models using the summary statistics (i.e., odds ratios and 95% confidence intervals) from this study and 14 published studies,[11,18–21] including 1 study that involved sex-stratified analysis (Supplemental Table 6). We converted all summary statistics into *Beta*-values and standard errors, and submitted them to METASOFT (version 2.0.0) for meta-analysis.[22] We calculated statistical significance using the random effects model (RE) and estimated heterogeneity by calculating Cochran's Q and I^2. We used the *p.adjust* function from the R stats package with the *fdr* method to adjust *p*-values.

Interaction analysis between ABCA1 rs2230806-T and APOE genotypes on Alzheimer's disease risk

To investigate the effect of the interaction between *ABCA1* rs2230806-T and *APOE* genotypes on the modulation of AD risk, we conducted logistic regression analysis using the *glm* function in R, adjusting for age and sex in the additive model. To investigate the sex-specific effects of the interaction, we further performed logistic regression analysis on sex-stratified data, adjusting for age. We used the *p.adjust* function from the R stats package with the *fdr* method to adjust *p*-values.

Association analysis of ABCA1 rs2230806-T with plasma biomarkers and lipid levels

To examine the associations of *ABCA1* rs2230806-T with AD pathology status and plasma lipid levels, we measured the plasma levels of phosphorylated tau (i.e., P-tau181 and P-tau271), ratio of amyloid-β 40 to 20 (Aβ42:40), neurofilament light polypeptide (NfL), total cholesterol, high-density cholesterol, low-density cholesterol, and triglyceride in individuals with AD and NCs as described previously (Supplemental Table 2).[23] We subsequently conducted linear regression analysis on plasma biomarkers and lipid profile using the g*lm* function in R, adjusting for age and sex in the dominant or additive model, respectively. To investigate the sex-specific effects of *ABCA1* rs2230806-T, we performed logistic regression analysis on sex-stratified data, adjusting for age. We used the *p.adjust* function from the R stats package with the *fdr* method to adjust *p*-values.

Protein structure simulation

For model simulation and structural analysis, we obtained the sequence of wild-type (WT) *ABCA1* from UniProt (ID: O95477).[24–26] We simulated the protein structure of ABCA1 using SWISS-MODEL and selected the models with the highest Global Model Quality Estimate (GMQE) score.[24] We analyzed the ABCA1 structural changes introduced by the R219 K variant using Missense3D.[25,26]

Plasmids and antibodies

We synthesized WT or R219 K mutant (Mut) human *ABCA1* and subcloned them into the pcDNA3.1 mammalian expression vector (GenScript). The GFP construct was modified from an EGFP construct (Addgene plasmid: 50465) in which the promoter is replaced with the elongation factor 1α short (EFS) promoter. We purchased mouse monoclonal anti-ABCA1 antibody (ab18180) from Abcam, mouse monoclonal anti-α-tubulin antibody (T9026) from Sigma, and rabbit polyclonal anti-GFP antibody (598) from MBL.

Cell culture and transfection

We obtained human glioblastoma U87 cells from the American Type Culture Collection (ATCC) and cultured

them in Eagle's Minimum Essential Medium (Sigma, M5650) supplemented with 10% heat-inactivated fetal bovine serum, 1% Pen-Strep (Gibco, 15140122), and 1% GlutaMAX Supplement (Gibco, 35050061). We then incubated the cells at 37°C in humidified atmosphere with 5% CO_2. We transfected the U87 cells with vector control, *ABCA1* WT plasmid, or *ABCA1* mutant plasmid plus EGFP to label the transfected cells (mixed at a 9:1 mass ratio). For transfection, we seeded U87 cells at 2.5×10^5 cells per 35-mm plate 1 day prior to transfection in antibiotic-free medium. The next day, we transfected the cells using a Lipofectamine 3000 transfection kit (Invitrogen, L3000015) according to the manufacturer's protocol and incubated the cells for 16 h at 37°C. To examine ABCA1 protein levels in transfected U87 cells, we collected the cells, lysed them with 1× RIPA buffer, and performed western blot analysis. We used primary antibodies against ABCA1 (1:1000) and α-tubulin (1:5000). We quantified the intensity of protein bands using ImageJ software (version 1.52p).

Cell-based cholesterol efflux assay

We performed cholesterol efflux experiments using a cell-based cholesterol efflux assay kit (Abcam, ab196985) according to manufacturer's instruction with optimization for the desired condition. Briefly, we seeded the transfected cells in 48-well plates (5×10^4 cells per well) in serum-free RPMI 1640 medium (Gibco, A10491) and maintained them for 4 h at 37 °C. We then incubated the cells with labeling medium (labeling reagent: RPMI medium = 1:27) for 1 h, followed by overnight incubation in equilibration medium. The next day, we incubated the cells in phenol red-free, serum-free RPMI 1640 medium (R&D Systems, M30750) containing 2% human serum (Sigma, H6914) LDL/VLDL-depleted for 8 h. After incubation, we measured the fluorescence (excitation/emission wavelengths: 485/523 nm) in supernatant and cell lysates. We then calculated the cholesterol efflux rate as follows: cholesterol efflux rate = 100% × (fluorescence intensity of supernatant)/ (fluorescence intensity of the supernatant + fluorescence intensity of the cell lysate). Final cholesterol efflux values are expressed as percentage of efflux compared to vector control.

Intracellular cholesterol staining with filipin III and immunostaining for GFP

We performed intracellular cholesterol detection using a cell-based cholesterol assay kit (Abcam, ab133116) according to manufacturer's instructions. We imaged stained cells using brightfield and fluorescent channels to encompass filipin III signals (excitation/emission wavelengths: 365/415 nm) using a Leica MICA Microhub confocal microscope with a 10× objective.

We performed immunostaining for GFP to include only GFP^+ U87 cells for quantification. First, we blocked U87 cells with 4% goat serum, 1% BSA, and 0.4% Triton X-100 in DPBS for 1 h at room temperature and then incubated them in anti-GFP primary antibody (1:1000) in blocking buffer overnight at 4°C. We then incubated the cells with Cy3-conjugated secondary antibody (Jackson Lab) for 2 h at room temperature. We imaged the GFP signal using MICA and excluded GFP^- cells from analysis. We then quantified the fluorescence results using the SINAP module, which uses deep learning to segment cells from the background, in IN Carta image analysis software (version 2.3.0, Cytiva). We then calculated the filipin fluorescence signal per the total cell area of GFP^+ U87 cells normalized to that of the vector control. We analyzed differences between groups using unpaired *t*-tests.

Association analysis between ABCA1 rs2230806-T and transcriptomic changes

To investigate the additive effect of *ABCA1* rs2230806-T on brain transcriptome, we retrieved genetic and transcriptomic data from cortical regions (i.e., "Frontal Cortex" [BA9] and "Cortex") from postmortem human samples.[27] We first examined the sex-specific association between *ABCA1* rs2230806-T and gene expression in the cortex using the *lm* function in R, adjusting for age, the first 5 principal components, RNA integrity number, and brain region. For Gene Ontology analysis, we submitted the downregulated genes (i.e., $Beta < 0$, $p < 0.05$) and upregulated genes (i.e., $Beta > 0$, $p < 0.05$) associated with *ABCA1* rs2230806-T to the *enrichGO* function from the ClusterProfiler package in R.[28] We used Gene Ontology terms categorized as "biological process" for enrichment analysis.

Single-nucleus RNA sequencing analysis

To investigate the additive effects of *ABCA1* rs2230806-T on gene expression at the single-cell level, we obtained single-nucleus RNA sequencing data from postmortem human frontal cortex samples from a previous study.[29] We identified the differentially expressed genes in oligodendrocytes from females that were associated with *ABCA1* rs2230806-T using the Wilcoxon rank-sum test with the *FindAllMarkers* function (*logfc.threshold = 0*) and MAST in R stratified by sex, adjusting for age, postmortem delay, and the number of detected genes. We set the level of statistical significance for MAST analysis at an adjusted $p < 0.05$.

Data visualization

We used ForestPMPlot to generate forest plots for meta-analysis.[30] We generated bar, box, and volcano plots

Table 1. Associations between ABCA1 single-nucleotide polymorphisms and Alzheimer's disease in the Chinese population.

All participants ($n = 316$ NC, 332 AD)							
SNP	EA	*Beta*[a]	SE	*p*	FDR	EAF (NC)	EAF (AD)
rs363717	C	0.051	0.176	0.771	0.771	0.198	0.200
rs2230808	T	−0.146	0.139	0.293	0.586	0.438	0.429
rs2066716	T	−0.070	0.159	0.660	0.771	0.269	0.285
rs2230806	T	0.354	0.144	**0.014**	0.084	0.362	0.411
rs2740488	C	0.224	0.154	0.147	0.441	0.237	0.262
rs2422493	A	−0.044	0.137	0.751	0.771	0.472	0.482

[a]Estimated effect size. AD: Alzheimer's disease; EA: effect allele; EAF: effect allele frequency; NC: normal control; SE: standard error; SNP: single-nucleotide polymorphism.

Table 2. Sex-specific associations between ABCA1 single-nucleotide polymorphisms and Alzheimer's disease in the Chinese population.

		Male ($n = 129$ NC, 107 AD)						Female ($n = 187$ NC, 225 AD)					
SNP	EA	*Beta*[a]	SE	*p*	FDR	EAF (NC)	EAF (AD)	*Beta*[a]	SE	*p*	FDR	EAF (NC)	EAF (AD)
rs363717	C	0.096	0.315	0.774	0.849	0.205	0.182	0.046	0.213	0.828	0.849	0.193	0.209
rs2230808	T	−0.185	0.234	0.429	0.849	0.419	0.397	−0.118	0.174	0.495	0.849	0.452	0.449
rs2066716	T	−0.069	0.261	0.792	0.849	0.264	0.285	−0.075	0.201	0.708	0.849	0.273	0.284
rs2230806	T	0.048	0.253	0.849	0.849	0.376	0.374	0.498	0.177	**0.005**	0.060	0.353	0.429
rs2740488	C	0.158	0.264	0.548	0.849	0.225	0.248	0.252	0.191	0.187	0.849	0.246	0.269
rs2422493	A	0.116	0.237	0.626	0.849	0.430	0.481	−0.131	0.169	0.439	0.849	0.500	0.482

[a]Estimated effect size. AD: Alzheimer's disease; EA: effect allele; EAF: effect allele frequency; NC: normal control; SE: standard error; SNP: single-nucleotide polymorphism.

using GraphPad Prism (version 9.2.0) and heatmaps using Morpheus.[31] We used the *DotPlot* function from the Seurat package in R to generate dot plots.[32] We also used the *DimPlot* and *FeaturePlot* functions from the Seurat package to generate uniform manifold approximation and projection (UMAP) plots.[32]

Results

Associations of ABCA1 single-nucleotide polymorphisms with Alzheimer's disease in the Chinese population

To determine whether *ABCA1* is associated with AD in the Chinese population, we selected 6 independent common *ABCA1* SNPs (MAF > 5%, $r^2 \leq 0.25$) that have been reported to be associated with AD in previous publications (Supplemental Table 3). Notably, these SNPs were distributed in different exons and introns of *ABAC1*, including the promoter, exons, introns, and 3′-UTR. We subsequently conducted genetic analysis in a Hong Kong Chinese AD cohort ($n = 332$ patients with AD, $n = 316$ NCs; Supplemental Table 4). Among the 6 SNPs, only 1 coding SNP, rs2230806-T (p.R219 K), exerted a significant risk effect on AD in the Hong Kong Chinese AD cohort (*Beta* = 0.354, $p = 0.014$; Table 1). Notably, *ABCA1* rs2230806-T has been reported to exert a female-specific effect on AD risk in a study conducted in a European population.[21] Therefore, we conducted sex-stratified association analysis and observed a significant association between *ABCA1* rs2230806-T and an increased risk of AD only in females (*Beta* = 0.498, $p = 0.005$; Table 2). To further evaluate the independent effect of the 6 investigated *ABCA1* SNPs, we performed multiple logistic regression analysis by simultaneously adjusting for the effects of all 6 SNPs. We showed that only *ABCA1* rs2230806-T exerted a significant effect on AD after multiple testing correction (*Beta* = 0.591, $p = 0.001$; multiple-testing threshold = 0.004; Table 3), indicating that the effect of *ABCA1* rs2230806-T on AD risk is independent of the other SNPs. Taken together, these results reveal a female-specific association between *ABCA1* rs2230806-T and AD in the Chinese population.

Validation of the female-specific association between ABCA1 rs2230806-T and Alzheimer's disease

To validate the association between *ABCA1* rs2230806-T and AD, we performed meta-analysis using summary statistics from the present study and other published AD genetic

Table 3. Independent effects of ABCA1 single-nucleotide polymorphisms on Alzheimer's disease in the Chinese population stratified by sex.

		Male ($n = 129$ NC, 107 AD)						Female ($n = 187$ NC, 225 AD)					
SNP	EA	*Beta*[a]	SE	*p*	FDR	EAF (NC)	EAF (AD)	*Beta*[a]	SE	*p*	FDR	EAF (NC)	EAF (AD)
rs363717	C	0.128	0.340	0.708	0.708	0.205	0.182	0.134	0.232	0.563	0.695	0.193	0.209
rs2230808	T	−0.337	0.283	0.234	0.569	0.419	0.397	−0.411	0.230	0.074	0.416	0.452	0.449
rs2066716	T	−0.264	0.334	0.430	0.695	0.264	0.285	−0.421	0.259	0.104	0.416	0.273	0.284
rs2230806	T	0.134	0.276	0.628	0.695	0.376	0.374	0.591	0.186	**0.001**	**0.012**	0.353	0.429
rs2740488	C	0.144	0.270	0.593	0.695	0.225	0.248	0.233	0.197	0.237	0.569	0.246	0.269
rs2422493	A	0.113	0.238	0.637	0.695	0.430	0.481	−0.122	0.173	0.482	0.695	0.500	0.482

[a]Estimated effect size. Multiple logistic regression analysis. AD: Alzheimer's disease; EA: effect allele; EAF: effect allele frequency; NC: normal control; SE: standard error; SNP: single-nucleotide polymorphism.

studies of the 6 *ABCA1* variants (Supplemental Tables 5 and 6). Specifically, we found that the effect of *ABCA1* rs2230806-T on AD risk is highly heterogeneous across different studies in both populations of Chinese ($I^2 = 80.21\%$, $p = 0.358$) and European descent ($I^2 = 61.17\%$, $p = 0.502$) before sex stratification (Figure 1(a), Supplemental Table 7). However, in females, there was a consistently stronger AD risk effect of *ABCA1* rs2230806-T in both populations (*Beta* = 0.537, $p = 6.98 \times 10^{-7}$; Figure 1(b), Supplemental Table 7), suggesting that *ABCA1* rs2230806-T exerts a similar AD risk effect across both populations in a female-specific manner. Given that the major AD risk factor, *APOE*, is involved in ABCA1-mediated cholesterol transport, we performed interaction analysis between *APOE* genotype and rs2230806-T to investigate whether rs2230806-T interacts with *APOE* to modulate AD risk. While the interaction between rs2230806-T and *APOE* genotype in the modulation of AD risk was marginally significant in females (*APOE*-ϵ2: *Beta* = −0.876, $p = 0.072$; *APOE*-ϵ4: *Beta* = 0.824, $p = 0.069$; Supplemental Table 8), these results further suggest a female-specific association of rs2230806-T with AD.

Effect of the ABCA1 R219 K variant on plasma ATN biomarkers of Alzheimer's disease

To further examine the effect of *ABCA1* rs2230806-T on AD pathology, we investigated its association with plasma ATN biomarkers of AD (i.e., P-tau, Aβ42:40, and NfL; Supplemental Table 9) in the Chinese population. Notably, *ABCA1* rs2230806-T was associated with an increased plasma level of P-tau181 in NCs (total: *Beta* = 0.465, $p = 0.002$; female: *Beta* = 0.458, $p = 0.035$; Figure 1(c)), supporting the association of *ABCA1* rs2230806-T with AD pathology.

Effect of the ABCA1 R219 K variant on ABCA1 protein function

Given that rs2230806-T (p.R219 K) is a nonsynonymous SNP that results in an amino acid change from arginine to lysine in ABCA1 protein, it may confer disease risk by regulating the activity or function of ABCA1. ABCA1 protein comprises 2261 amino acids that form 2 extracellular domains (i.e., ECD1 and ECD2), 2 transmembrane domains (i.e., TMD1 and TMD2), and 2 nucleotide binding domains (i.e., NBD1 and NBD2) in the intracellular domain.[33] The *ABCA1* R219 K variant is in the ECD1 region (Figure 2(a)), where it binds to apolipoproteins to facilitate cholesterol efflux.[33,34] The *ABCA1* R219 K variant was predicted to result in a salt bridge breakage, which may lead to the destabilization of the structure of ECD1 (Supplemental Figure 1), suggesting that structural alteration could have downstream effects on the cholesterol efflux function of the protein.

Accordingly, we examined the effect of the *ABCA1* R219 K variant on cholesterol efflux, which is a key function of ABCA1 protein. In the central nervous system, astrocytes express the most *ABCA1* (Supplemental Figure 2) and serve as a primary cell source for cholesterol synthesis and cholesterol efflux in the brain.[35] Therefore, to examine the effect of *ABCA1* R219 K on cholesterol efflux, we overexpressed WT *ABCA1* or the R219 K mutant in human glioblastoma U87 cells. We observed similar expression levels of the WT and R219 K mutant ABCA1 proteins in U87 cells (Supplemental Figures 3 and 4), suggesting that the *ABCA1* R219 K variant does not affect the expression of ABCA1 protein. We performed cholesterol efflux assays using fluorescently labeled cholesterol and showed that cells overexpressing the *ABCA1* R219 K mutant exhibited a 17% reduction in cholesterol efflux compared to cells overexpressing *ABCA1*-WT ($p < 0.001$; Figure 2(b)). This finding suggests that the *ABCA1* R219 K variant impairs ABCA1 function by affecting its cholesterol efflux activity. Concordantly, in *ABCA1* R219K-overexpressing cells, we observed a 20% higher intracellular level of cholesterol than *ABCA1* WT-overexpressing cells ($p < 0.001$) (Figure 2(c), d). This result suggests accumulation of cholesterol within the cells, which aligns with the reduced cholesterol efflux observed above. Notably, we showed that *ABCA1* rs2230806-T (p.R219 K) was associated with a lower total cholesterol level in the plasma of

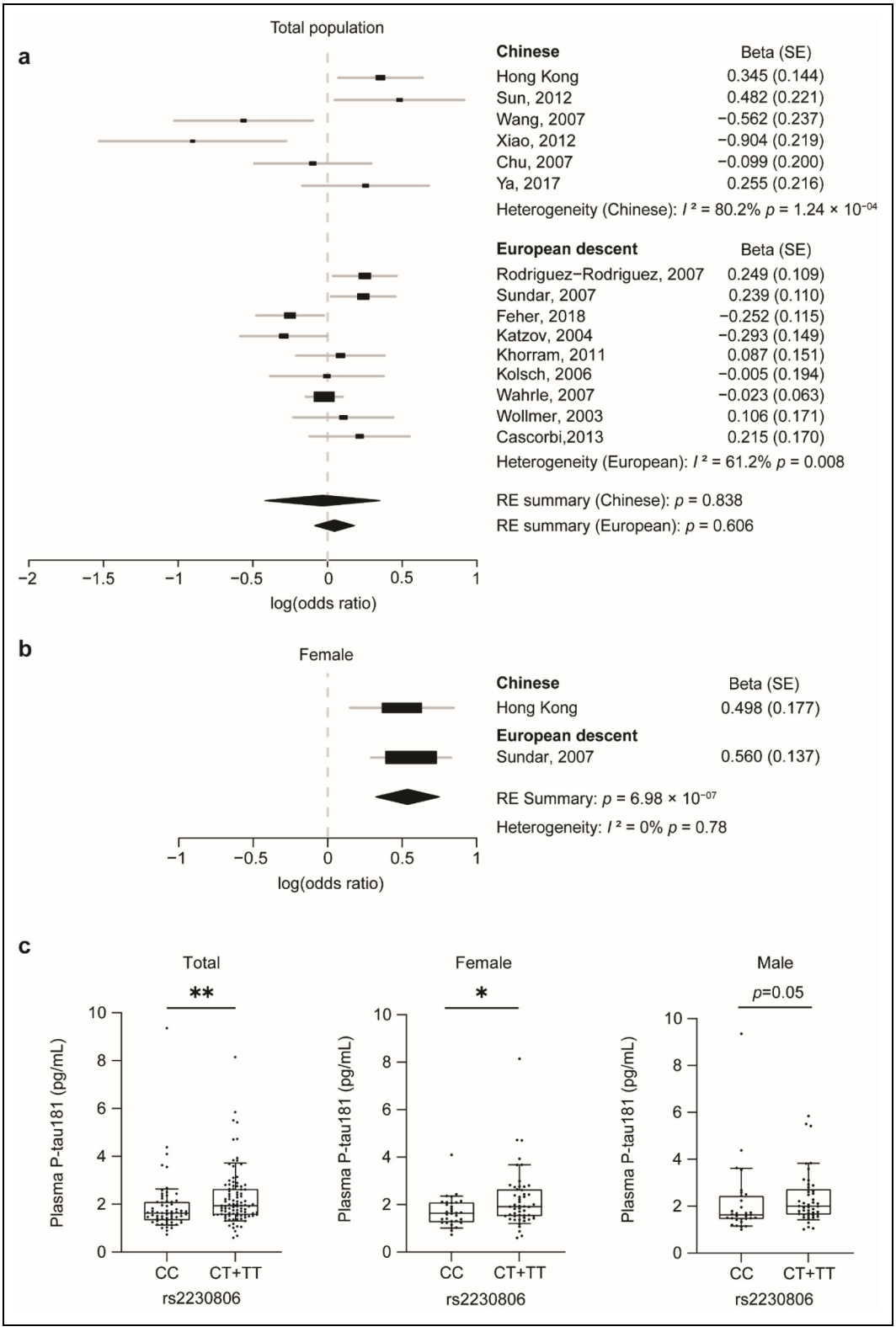

Figure 1. Meta-analysis of *ABCA1* rs2230806-T and Alzheimer's disease and association between *ABCA1* rs2230806-T and P-tau181. (a, b) Meta-analysis of AD genetics studies of *ABCA1* in populations of Chinese and European descent (a) without sex stratification and (b) in females only. Boxes and diamonds represent the effect sizes in independent studies and meta-analysis, respectively. Lines through boxes indicate 95% confidence intervals, and box size indicates weight in meta-analysis. (c) Association between *ABCA1* rs2230806 and P-tau181 plasma level in normal controls of the total population, females, and males (*$p < 0.05$, **$p < 0.01$). AD: Alzheimer's disease; I^2: variation across studies; RE: random effects model; SE: standard error.

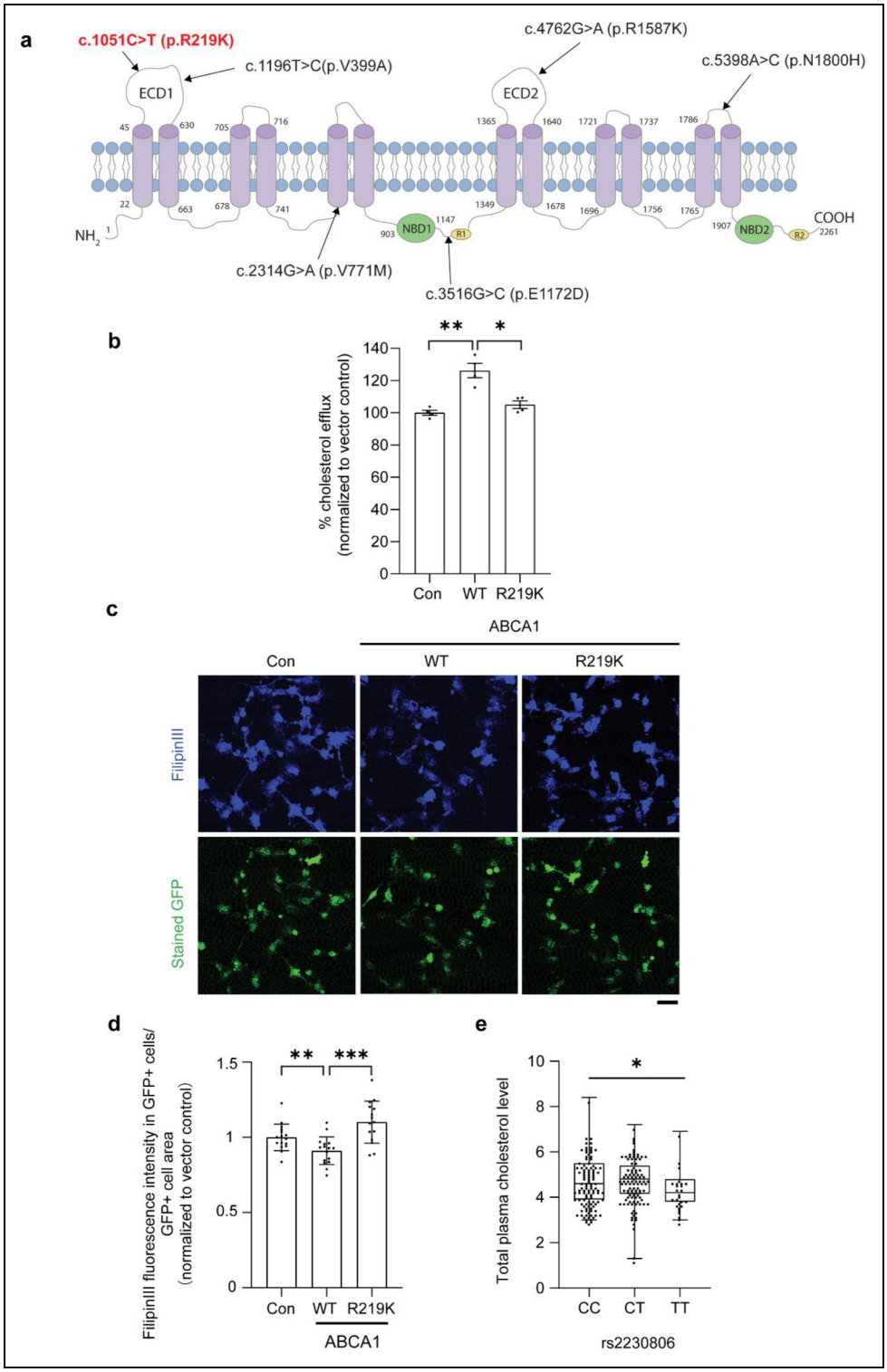

Figure 2. *ABCA1* rs2230806-T (p.R219 K) alters the function of ABCA1 protein. (a) Topological model of the protein domain structure of ABCA1 and location of Alzheimer's disease-associated coding single-nucleotide polymorphisms. (b) WT *ABCA1* or its R219 K mutant was overexpressed in U87 cells to examine their effects on cholesterol efflux rate ($n = 4$ wells per condition from 2 experiments; unpaired *t*-test; $^{*}p < 0.05$, $^{**}p < 0.01$). (c) Representative images of cholesterol staining with filipin III in GFP^{+} U87 cells. Scale bar = 100 μm. (d) Filipin III fluorescence intensity per area of GFP^{+} cells normalized to the vector control ($n = 15$–16 wells per condition from 4 experiments; unpaired *t*-test; $^{**}p < 0.01$, $^{***}p < 0.001$). (e) Association between *ABCA1* rs2230806-T and total plasma cholesterol level in normal controls ($^{*}p < 0.05$). ECD: extracellular domain; NBD: nucleotide-binding domain; R1 and R2: regulatory domains; WT: wild-type.

NCs (Figure 2(e), Supplemental Table 10), further suggesting that the *ABCA1* R219 K variant causes dysregulation of cholesterol efflux in humans. Together, these results indicate that the *ABCA1* R219 K variant affects the protein function of ABCA1, resulting in decreased cholesterol efflux.

Female-specific effect of ABCA1 rs2230806-T on molecular phenotypes in the human brain

To understand the effects of *ABCA1* rs2230806-T in the human brain, we examined its association with transcriptomic changes in the cerebral cortex of postmortem human brains.[27] While *ABCA1* rs2230806-T did not affect *ABCA1* gene expression in the brain (Supplemental Table 11), it was associated with changes in the expression levels of other genes in the brain (Figure 3(a)). Notably, compared to males, females had twice as many differentially expressed genes associated with *ABCA1* rs2230806-T in the brain (Figure 3(b)), suggesting a stronger effect of *ABCA1* rs2230806-T on transcriptomic changes in the brain in females. Gene Ontology analysis showed that the upregulated genes in females were mainly involved in pathways such as "myelination" (e.g., *MAG*, *MYRF*, and *PLLP*; false discovery rate [FDR] = 1.07×10^{-6}; Figure 3(c), Supplemental Table 12) and "negative regulation of neuron projection development" (e.g., *MAG* and *LPAR1*; FDR = 1.89×10^{-2}; Figure 3(c), Supplemental Table 12). Meanwhile, the downregulated genes in females were involved in pathways such as "unfolded protein response" (e.g., *HSPH1* and *DNAJB5*; FDR = 1.55×10^{-4}; Figure 3(c), Supplemental Table 12) and "histone modification" (e.g., *KDM6B* and *BRPF1*; FDR = 1.55×10^{-4}; Figure 3(c), Supplemental Table 12). The enrichment of these pathways was not observed in males (Figure 3(d), Supplemental Figure 5, Supplemental Table 13), indicating that *ABCA1* rs2230806-T modulates gene expression and pathways in the brain in a sex-specific manner.

As the differentially expressed genes associated with *ABCA1* rs2230806-T were mostly enriched in oligodendrocytes in the brain in females (Supplemental Figure 6), *ABCA1* rs2230806-T may exert stronger regulatory effects in oligodendrocytes. Therefore, to examine the cell-type-specific effects of *ABCA1* rs2230806-T on gene regulation in females, we examined the gene expression changes in oligodendrocytes of *ABCA1* rs2230806-T carriers using single-nucleus RNA sequencing data (Figure 4(a)).[29] Accordingly, *ABCA1* rs2230806-T was associated with the upregulation of genes involved in "gliogenesis," "axon development," "regulation of neuron projection development," and "myelination" in females (Figure 4(b)). Specifically, the oligodendrocyte- and myelin-related genes were upregulated in *ABCA1* rs2230806-T carriers (Figure 4(c)).[29] Together, these results suggest that *ABCA1* rs2230806-T modulates oligodendrocyte-specific gene expression and myelin-associated pathways in the female human brain.

Discussion

Here, we identified that a common *ABCA1* coding variant, rs2230806-T (p.R219 K), is associated with 1.65-fold increased AD risk in female individuals in the Chinese population. While *ABCA1* is important for cholesterol efflux in brain cells, we showed that the *ABCA1* R219 K variant impairs the cholesterol efflux function of ABCA1 protein. As rs2230806-T is associated with the dysregulation of myelin-related gene expression in the human brain, the disrupted protein function of ABCA1 (i.e., cholesterol efflux) may contribute to the process of myelination dysfunction. Hence, our study elucidates the role of an *ABCA1* coding variant in the alteration of ABCA1 protein function and modulation of myelination in the brain. Thus, these findings advance our understanding of the roles of *ABCA1* and cholesterol metabolism in AD.

The disruption of cholesterol metabolism in the brain is suggested to be involved in the pathogenesis of AD,[36–38] potentially by leading to Aβ accumulation and impaired synaptic function.[39] *ABCA1,* a key player in cholesterol transport, mediates cholesterol efflux from astrocytes to neurons in the brain,[7] highlighting its significance in AD. Notably, various coding variants of *ABCA1* have been associated with AD (Figure 2(a), Supplemental Table 14), suggesting that the dysregulation of ABCA1 protein function can contribute to AD pathogenesis. This study shed light on the potential role of ABCA1 in AD by demonstrating that a common AD risk variant of *ABCA1*, R219 K, affects the cholesterol transport function of ABCA1 in human glioblastoma cells, resulting in a decrease in ABCA1-mediated cholesterol efflux (Table 1, Figure 2(e)). Consistently, a rare loss-of-function ABCA1 variant, N1800H, has shown to reduce cholesterol efflux and is associated with a 4-fold increase in AD risk in the European populations (Figure 2(a)).[40] Yet, a previous functional study does not report a similar effect of R219 K on cholesterol efflux,[41] this discrepancy could be attributed to the differences in cell types (i.e., HEK293 T versus U87 cells) and cholesterol acceptors (i.e., human apoA-I versus human serum, which includes multiple apolipoproteins) used. Since the causal relationship between plasma cholesterol levels, particularly HDL-cholesterol, and AD risk remains unclear (Supplemental Table 15), further investigation is required to understand the mechanisms underlying the involvement of ABCA1-mediated cholesterol efflux in AD pathogenesis.

As cholesterol is a major component of myelin in the brain, it is essential for myelination.[42] Patients with AD have lower myelin content in the brain than NCs,[43]

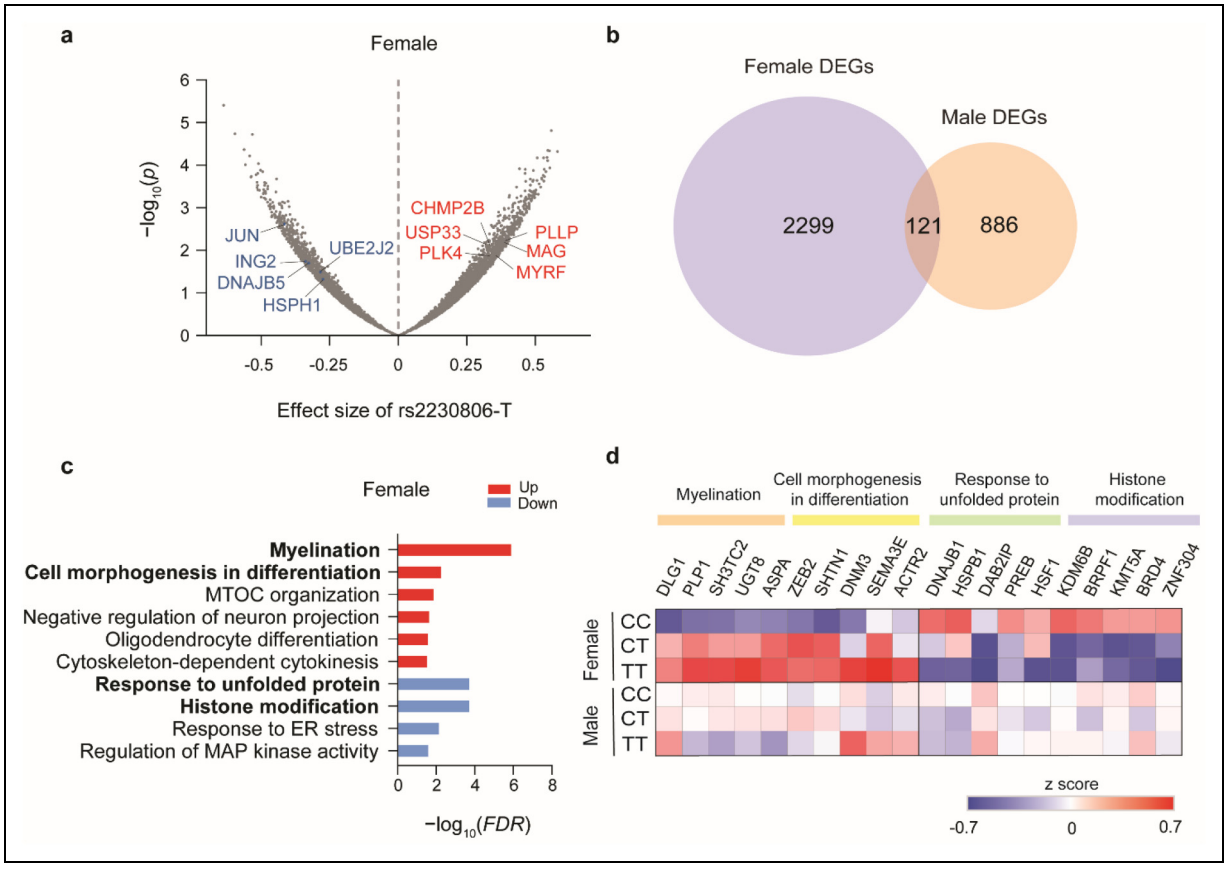

Figure 3. Sex-specific associations between *ABCA1* rs2230806-T and transcriptomic alterations in the human cerebral cortex. (a) Volcano plot showing the associations between *ABCA1* rs2230806-T and gene expression in the cerebral cortex in females. (b) Venn diagram showing the numbers of rs2230806-associated DEGs in male and female cerebral cortices. (c) Gene Ontology analysis showing the associations between *ABCA1* rs2230806-T and biological pathways in the cerebral cortex in females. (d) Heatmap showing the expressions of the 5 rs2230806-associated DEGs that were most strongly associated with the top 2 up- and downregulated pathways in the cerebral cortex in females (bolded terms in (c)). DEG, differentially expressed gene; ER: endoplasmic reticulum; FDR, false discovery rate; MTOC: microtubule-organizing center, OPC: oligodendrocyte progenitor cell.

suggesting a role of myelination in AD. Of note, ABCA1 plays a role in the regulation of myelination; when its function is disrupted in ABCA1-deficient mice, myelination is reduced.[44] Moreover, the upregulation of *ABCA1* expression in AD mouse models can promote remyelination and improve cognitive deficits.[45,46] Notably, we found that the presence of *ABCA1* rs2230806-T, which affects ABCA1 protein function, upregulates the expression of myelin-related genes (e.g., *DLG1* and *PLP1*) in the female human brain (Figure 3), suggesting that *ABCA1* plays a role in AD pathogenesis by modulating myelination. These genes are key regulators of myelin composition and integrity,[47,48] which are important for efficient conduction velocity in axons.[49] Dysregulation of myelin integrity increases Aβ deposition,[50] thereby contributing to AD pathogenesis.

In this study, the R219K-associated differentially expressed genes enriched in oligodendrocytes and the myelination pathway was specific to females (Figures 3 and 4; Supplemental Table 12), suggesting that the female-specific effect of R219 K on AD risk identified in this study could be attributed to the molecular differences in the myelin-related pathways in oligodendrocytes between sexes. Oligodendrocytes are the myelinating cells in the central nervous system that support the integrity of axons.[51] A recent study shows a decreased transcriptional response specifically in oligodendrocytes in females with AD.[52] This sex-specific effect may be attributable to the different cellular characteristics of oligodendrocytes between females and males, with oligodendrocytes in females having a shorter lifespan and lower myelination capacity than those in males,[53,54] which might make them more susceptible to dysregulated cholesterol transport. Together, the decreased cholesterol efflux activity of the ABCA1 protein due to the R219 K variant may have stronger adverse effects on oligodendrocyte functions and myelination in females, potentially contributing to a higher AD risk. Therefore, this study highlights the importance of considering sex differences in genetic studies of AD as well as the significance of investigating the regulatory roles of *ABCA1* on molecular and cellular phenotypes in oligodendrocytes in the context of AD.

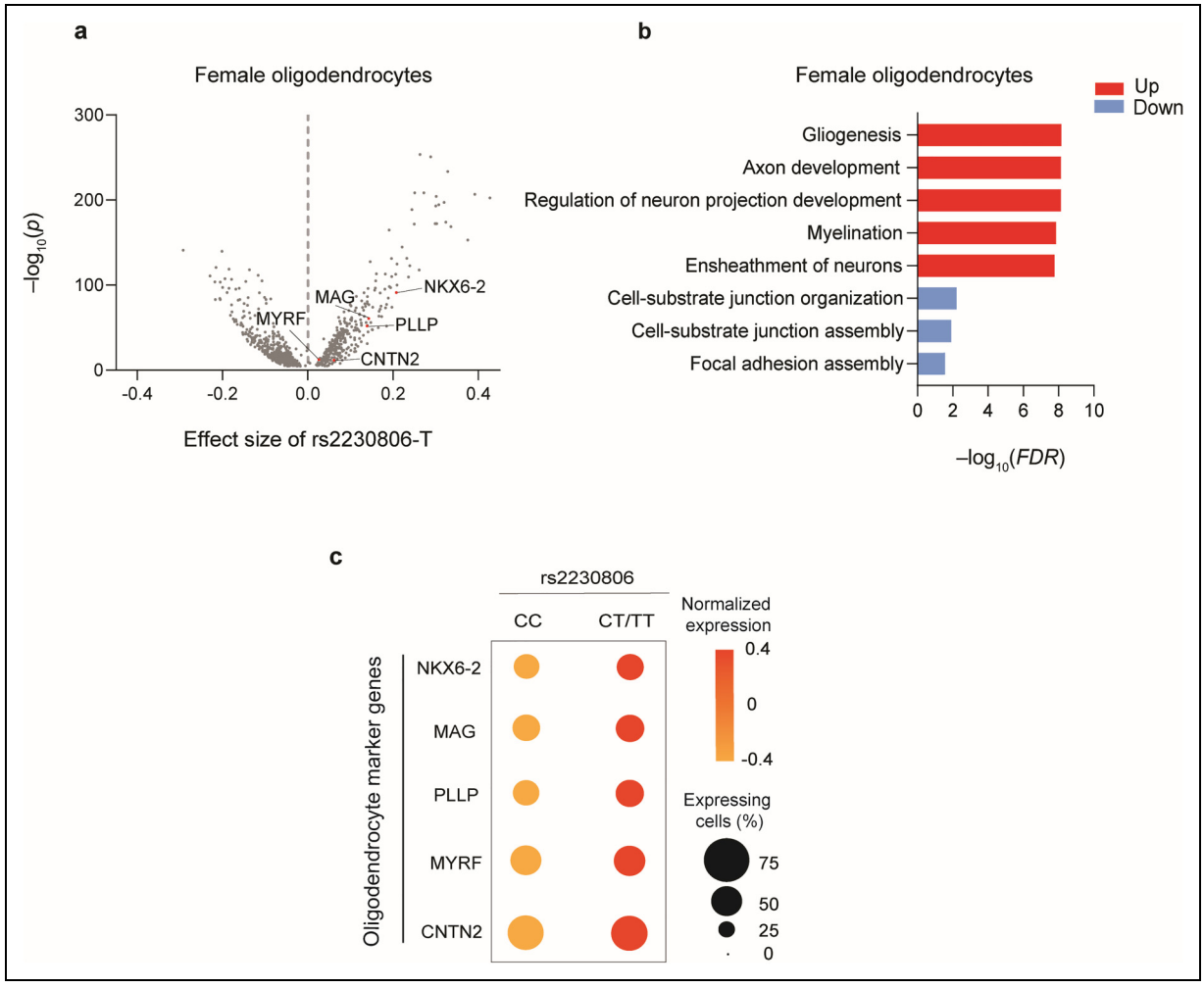

Figure 4. Associations between *ABCA1* rs2230806-T and transcriptomic alterations in oligodendrocytes from the cerebral cortex in females. (a) Volcano plot showing the associations between *ABCA1* rs2230806-T and gene expression in oligodendrocytes. (b) Gene Ontology analysis showing the associations between *ABCA1* rs2230806-T and biological pathways in oligodendrocytes. (c) Dot plot showing the expression levels of key oligodendrocyte/myelin-related marker genes in oligodendrocytes. FDR: false discovery rate.

There are some limitations in this study. First, we identified rs2230806 as being significantly associated with AD risk with a statistical power of over 95% (Supplemental Table 1). However, 2 variants tested in this study (i.e., rs363717 and rs2740488) have small effect sizes in populations of European descent, limiting statistical power in this study. Additional genetic data from a larger AD cohort are needed to confirm the effects of these variants on AD in the Chinese population. Second, we showed that the rs2230806-T variant is associated with the expression levels of myelin-related genes in females by genotype–expression association analysis. Further investigation is required to verify the changes of myelination in rs2230806-T carriers.

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Ethical considerations

This study was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee at the Prince of Wales Hospital, the Chinese University of Hong Kong, and the Hong Kong University of Science and Technology.

Consent to participate

All participants provided written informed consent for both study enrollment and sample collection.

Author contributions

Sze Kei Liu (Conceptualization; Data curation; Formal analysis; Writing – original draft; Writing – review & editing); Han Cao (Conceptualization; Data curation; Writing – original draft; Writing – review & editing); Xin Yang (Data curation; Formal analysis); Xiaopu Zhou (Conceptualization; Writing – review & editing); Yu Chen (Conceptualization; Funding acquisition; Writing – review & editing); Wing-Yu Fu (Formal analysis; Writing – review & editing); San Yuen Chan (Data curation; Formal analysis; Writing – review & editing); Fanny C F Ip (Resources; Writing – review & editing); Kin Y Mok (Conceptualization; Supervision; Writing – review & editing); Vincent C T Mok (Resources; Writing – review & editing); Timothy C Y Kwok (Resources; Writing – review & editing); John Hardy (Supervision; Writing – review & editing); Amy K Y Fu (Conceptualization; Project administration; Supervision; Writing – review & editing); Nancy Yuk-Yu Ip (Conceptualization; Funding acquisition; Investigation; Methodology; Resources; Supervision; Writing – review & editing).

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability statement

The data and results of this study are provided in the Supplemental Material. For raw genetic data, the consent form signed by each participant states that the research content will remain private under the supervision of the hospital and research team. Therefore, these data will be made available and shared only in the context of a formal collaboration; applications for data sharing and project collaboration will be processed and reviewed by a Review Panel hosted at the Hong Kong University of Science and Technology. Researchers may contact sklneuros-ci@ust.hk for further details on project collaboration and the sharing of the data from this study.

Supplemental material

Supplemental material for this article is available online.

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