ATP Binding Cassette Subfamily A Member 2 (ABCA2) Expression and Methylation are Associated with Alzheimer's Disease

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Background: ABCA2 has been genetically linked to Alzheimer's disease (AD) risk, but its mRNA expression and epigenetics in AD have not been investigated.

Material/Method: To explore the diagnosis value of ABCA2 mRNA expression in AD, 2 datasets GES15222 and GSE33000 containing expression profile of brain cortex tissues and 2 datasets GSE63063 (Cohort 1) and GSE63063 (Cohort 2) containing expression profile of blood were downloaded from the NCBI GEO database and analyzed by receiver operating characteristic curve (ROC) analyses and logistic regression. The ABCA2 co-expressed genes were also analyzed by GO annotation to investigate the potential molecular mechanisms.

Results: The analyses results suggested ABCA2 mRNA expression was upregulated significantly in AD compared with controls in all datasets. ROC analysis suggested that ABCA2 was associated with AD in all datasets, which were also proved by univariate and multivariate analyses. Next, the dataset GSE80970 containing methylation profiles of prefrontal cortex tissues from AD patients were downloaded and analyzed. Methylation of 2 of 36 CpG islands in ABCA2 gene with high diagnostic accuracy of AD from controls in ROC analyses were found to be negatively associated with AD risk in univariate analysis. One was still associated with AD risk after adjustment of confounding factors. Additional analyses indicated that ACBA2 mRNA expression could be used to diagnose mild cognitive impairment (MCI) and Huntington's disease (HD) from controls and to distinguish HD from AD, but not AD from MCI. Furthermore, the genes involved in AD during ABCA2 alteration were analyzed by GO analysis.

Conclusions: ABCA2 mRNA expression and methylation is associated AD risk. ABCA2 may be used as a biomarker for AD diagnosis and may be a potential therapeutic target of AD.

MeSH Keywords: **Alzheimer Disease • ATP-Binding Cassette Transporters • Gene Expression Profiling • Methylation**

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Background

Alzheimer's disease (AD), the most common neurodegenerative disease, is defined by short-term memory loss progressing into dementia and neuropathology [1–3]. It has been estimated that 46 million people are affected by dementia syndromes in 2015 and about 60~70% of them are diagnosed as AD [4]. The hippocampus and the surrounding cortical regions are the major sites of AD-related pathology [5]. The early and accurate diagnosis of AD is still a challenge and many patients are misclassified or diagnosed late [6]. AD is irreversible and progressive and there is currently no drug that halts or cures it [7]. Therefore, early and accurate diagnosis of AD, identification of people with risk factors, and early invention are crucial to decrease the socioeconomic burden of AD. However, currently available diagnostic approaches for AD, including brain imaging, are neither suited for mass population screening nor sufficiently cost-effective to be practical [8].

ATP-binding cassette (ABC) transporters are one of the largest protein superfamilies. There are 7 subfamilies, from A to G, of ABC transporters and a total of 49 members have been identified in humans [9,10]. These transporters are located throughout the brain, with a focus at the blood-brain barriers; they facilitate the strictly regulated influx/efflux of various substances, metabolites, and peptides, and hence protect the brain from toxic and harmful compounds [11–13]. ABCA2, a member of the ABCA subfamily, is abundant in the frontal cortex of human AD brains compared to normal controls but was detected at lower concentrations in the parietal, occipital, and cerebellar regions [14]. *In vitro* studies reveal that it regulates the lipid metabolism via modulating the expression and turnover rate of low-density lipoprotein receptor (LDLR) [15,16]. Overexpression of ABCA2 can also enhance the levels of amyloid beta precursor protein (APP) and beta-amyloid, which is a key player in AD progression [17]. ABCA2 has been genetically linked to AD risk. The synonymous SNP rs908832, a C-T polymorphism in exon 14 of the ABCA2 gene, is associated with early-onset and late-onset or sporadic AD, as reported in 2 independent studies [18,19]. A meta-analysis also suggested a significant association between rs908832 and susceptibility to AD (OR=1.55, 95% CI=1.12–2.16) [20].

There have been no studies to date that investigated the mRNA expression and epigenetics of ABCA2 in AD. Here, we compared the mRNA expression of ABCA2 in brain tissues and blood in AD patients with the expression in controls by data mining of datasets from the NCBI GEO databases. The associations of ABCA2 mRNA expression and methylation with AD risk were estimated by receiver operating characteristics curves and logistic regression. In addition, the diagnostic value of ABCA2 mRNA level in distinguishing other neurodegenerative disease, such as Huntington's disease and mild cognitive impairment, from AD or controls were also explored.

Material and Methods

To explore the diagnostic value of ABCA2 in differentiating Alzheimer's disease patients from controls without related disease, AD gene expression profile data with corresponding clinical data were searched and downloaded from the publicly available GEO database. After reviewing the search results, GES15222 [21], GSE33000 [22], and GSE63063 [23] were finally chosen because these datasets consisted of many subjects. For further analyses, ABCA2 expression data was first normalized with Z-score method. Difference in ABCA2 expression levels between patients with Alzheimer's disease and controls in each dataset was then compared using the Mann-Whitney test. Receiver operating characteristic curve (ROC) analyses were performed to investigate the relationship between ABCA2 expression levels and Alzheimer's disease risk in every dataset. Area under the curves and the corresponding 95% confidence interval (CI) were calculated and compared with 0.5. The optimal cut-off value was calculated by Youden index [24]. ABCA2 expression levels were then divided into low expression and high expression groups by the cut-off values. Associations of ABCA2 with clinical features, age, and sex were determined by Fisher's exact test or chi-square test. Univariate and multiple logistic regression analyses were also performed to explore the correlations of ABCA2 with Alzheimer's disease. In addition, the GSE33000 and GSE63063 datasets contained the expression profile data of patients with other neurodegenerative diseases and Huntington's disease, and mild cognitive impairment, respectively. ABCA2 expression levels among patients with different neurodegenerative diseases and controls were compared using the Kruskal-Wallis test. The associations of ABCA2 with the risk of Huntington's disease or mild cognitive impairment and the diagnostic value of ABCA2 in distinguishing Alzheimer's disease from Huntington's disease or mild cognitive impairment were analyzed by ROC curves and logistic regression. Fisher's exact test, chi-square test, and Kruskal-Wallis test were conducted via GraphPad Prism 6 software. Univariate and multivariate logistic regression were performed using SPSS 19.0 software. All tests were two-sided. $P<0.05$ was considered to be statistically significant.

To investigate the related genes during ABCA2 alteration that might be involved in Alzheimer's disease, GSE33000, which contains the gene expression profile of brain tissue, was chosen for deep analyses. Differently expressed genes (DEGs) between Alzheimer's disease and controls were obtained by the web tool GEO2R in NCBI. The expression correlations of the genes with ABCA2 in GSE33000 were examined by Pearson's correlation analysis. Then, ABCA2 co-expressed genes with Pearson's correlation coefficients above 0.5 (P value was less than 1E-20) were selected and overlapped with the DEGs whose P value was less than 1E-20. The final obtained genes were subjected to GO analysis in the GeneCoDis3 online database (*http://genecodis.cnb.csic.es*).

Table 1. Characteristics of the included datasets.

GEO ID	Year	Country	Tissue source	Control subjects			Alzheimer's disease			Mild cognitive impairment			Huntington's disease		
				N	Age (yr)	F/M	N	Age (yr)	F/M	N	Age (yr)	F/M	N	Age (yr)	F/M
GSE15222	2009	USA	Cortical tissue	187	80.6±10.4	85/102	176	83.6±6.5	88/88		–			–	
GSE33000	2011	USA	Prefrontal cortex	157	63.0±10.2	34/123	310	80.0±9.8	175/135		–		157	56.0±14.7	74/83
GSE63063 (Cohort 1)	2015	UK	Blood	134	75.3±6.0	81/53	139	77.9±6.7	85/54	109	78.2±7.4	65/44		–	
GSE63063 (Cohort 2)	2015	UK	Blood	104	72.4±6.3	62/42	145	75.4±6.6	99/46	80	74.5±6.0	39/41		–	

N – subject number; yr – year; F/M – female/male.

Results

ABCA2 was upregulated in Alzheimer's disease

Four microarray datasets (GES15222 [21], GSE33000 [22], GSE63063 (Cohort 1) [23], and GSE63063 (Cohort 2) [23]) were downloaded from NCBI GEO databases. The characteristics of these datasets are shown in Table 1. Studies of GSE15222 and GSE33000 analyzed the brain transcriptome in postmortem samples, whereas GSE6306 used patient blood as the source of RNA. GSE63063 consisted of 2 independent cohorts. In addition to the Alzheimer's disease patients, GSE3300 also included patients with Huntington's disease and GSE6306 also included patients with mild cognitive impairment. In total, there were 582 controls, 770 cases with Alzheimer's disease, 189 cases with mild cognitive impairment, and 157 cases with Huntington's disease. ABCA2 mRNA profile data were collected from all the datasets and normalized with Z-score method. The difference in ABCA2 mRNA levels was first compared between Alzheimer's disease patients and controls, and the statistical analyses suggested ABCA2 was significantly upregulated in Alzheimer's disease patients in all datasets.

Association of ABCA2 with clinical features, age, and sex, and risk of Alzheimer's disease

Receiver operating characteristics (ROC) curves were used to investigate the diagnostic value of ABCA2 in Alzheimer's (Figure 1). The results suggested that ABCA2 was associated with Alzheimer's disease in all datasets (GSE15222, AUC=0.6575, 95%CI=0.6014–0.7136, P<0.0001; GSE33000, AUC=0.7243, 95%CI=0.6760-0.7727, P<0.0001; GSE63063, Cohort 1, AUC=0.7146, 95%CI=0.6545–0.7750, P<0.0001; GSE63063, Cohort 2, AUC=0.6562, 95%CI=0.5866-0.7258, P<0.0001; GSE63063, Cohort 1+2, AUC=0.6841, 95%CI=0.6388-0.7295, P<0.0001). Then, cut-off values were obtained by calculating the Youden index [24] from the ROC curves and used

to divide the ABCA2 expression into low expression and high expression groups. Fisher's test analyses indicated that ABCA2 was significantly associated with Alzheimer's disease (Table 2). The associations of ABCA2 with the age and sex of the patients were next estimated, and age was found to be correlated to ABCA2 expression in all datasets except for the combination of both cohorts of GSE63063 (Table 3). No significant association was observed between sex and ABCA2 expression. Then, we performed further analyses via logistic regression to investigate the associations of Alzheimer's disease with age, sex, and especially ABCA2 expression (Table 4). Univariate analysis revealed that ABCA2 was associated with AD risk in all datasets. Age was also found to be associated with AD risk in all datasets and sex was only associated with AD risk in the GSE33000 dataset. After adjusting for the confounding factors and age, we found a significant association of ABCA2 with AD risk in each dataset (GSE15000, OR=2.446, 95%CI=1.359–4.404, P=0.003; GSE33000, OR=2.774, 95%CI=1.770–4.347, P<0.001, GSE63063, Cohort 1, OR=2.391, 95%CI=1.408–4.060, P=0.001; GSE63063, Cohort 2, OR=3.748, 95%CI=2.148–6.542, P<0.001; GSE63063, Cohort 1+2, OR=3.157, 95%CI=2.180–4.572, P<0.001).

As methylation might influence expression of ABCA2, we then investigated the associations of ABCA2 methylation with AD risk. The GSE80970 dataset was downloaded from the GEO database, which contained the methylation profile of prefrontal cortex tissues from 74 AD patients and 68 controls. All the 36 CpG islands within ABCA2 gene were first analyzed by ROC to explore their diagnostic value in AD. However, the AUC of all CpG islands methylation was not significantly different in the ROC analyses (Table 5). Two of the CpG islands, cg13930557 and cg03349123, were significantly different (P values 0.086 and 0.079, respectively). The univariate logistic regression analysis revealed that high methylation level of both the CpG islands was negatively and significantly associated with AD risk (cg13930557, OR=0.465, 95%CI=0.229-0.944, P=0.034; cg03349123, OR=0.393, 95%CI=0.195–0.791,
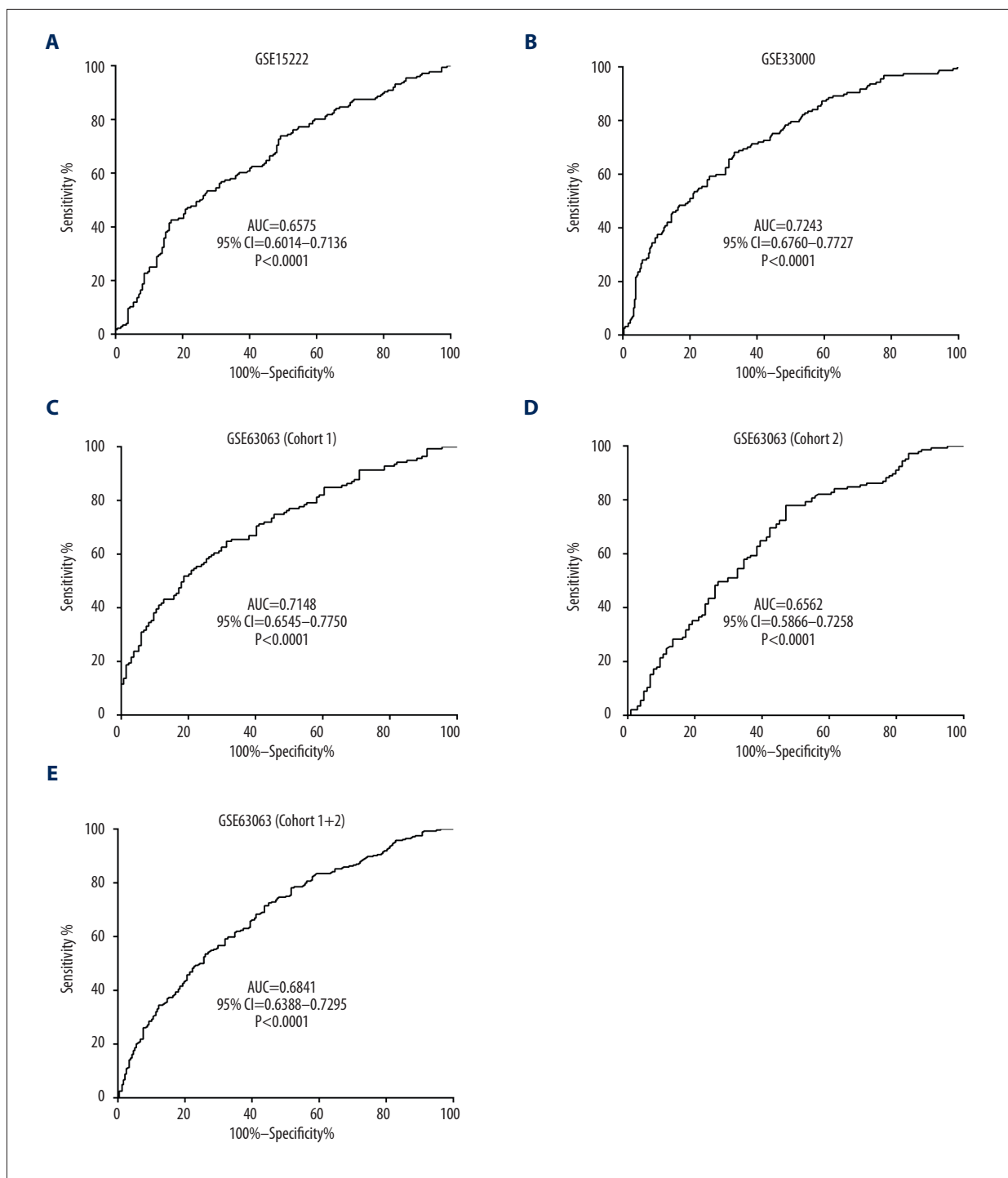
Figure 1. Diagnostic value of ABCA2 in differentiating patients with Alzheimer's disease from control subjects. NCBI GEO datasets GSE15222 (**A**), GSE330000 (**B**), GSE63063 (Cohort 1, **C**), and GSE63063 (Cohort 2, **D**) were downloaded from the online database. ABCA2 mRNA levels in the datasets were normalized with Z-score method. Receiver operating characteristics curves (ROC) were used to investigate the diagnostic value of ABCA2, and its accuracy was indicated by the area under the receiver operating characteristics curve. Integrated analysis of both cohorts of GSE63063 was also conducted (**E**).

Table 2. ABCA2 mRNA level comparison between patients with Alzheimer's disease and control subjects in included datasets.

GEO ID	Alzheimer's disease			Control dubjects			P-value
	Median	Min	Max	Median	Min	Max	
GSE33000	0.0362	−1.7642	2.4278	−0.5392	−2.1649	2.9482	<0.0001
GSE15222	−0.0446	−1.1870	6.0664	−0.4164	−1.7341	4.7031	<0.0001
GSE63063 (Cohort 1)	0.3607	−1.7120	3.1410	−0.4324	−2.4012	1.7416	<0.0001
GSE63063 (Cohort 2)	0.0362	−1.7642	2.4278	−0.5392	−2.1649	2.9482	<0.0001

Table 3. Associations of ABCA2 expression with AD risk and clinical parameters, age and gender.

Parameters	ABCA2		P-value
	Low	High	
GSE33000			
Disease status			
AD	200	110	0.0001
Ctrl	140	17	
Age			
<75	172	41	0.0005
≥75	168	86	
Gender			
Female	141	68	0.0216
Male	199	59	
GSE15222			
Disease status			
AD	82	94	0.0001
Ctrl	136	51	
Age			
<75	51	16	0.0035
≥75	166	128	
Gender			
Female	103	70	0.9146
Male	115	75	
GSE63063 (Cohort 1)			
Disease status			
AD	49	90	0.0001
Ctrl	92	42	
Age			
<75	66	40	0.0062
≥75	75	92	

Parameters	ABCA2		P-value
	Low	High	
Gender			
Female	84	82	0.7105
Male	57	50	
GSE63063 (Cohort 2)			
Disease status			
AD	32	113	0.0001
Ctrl	55	49	
Age			
<75	50	71	0.0464
≥75	37	91	
Gender			
Female	59	102	0.4885
Male	28	60	
GSE63063 (Cohort 1+2)			
Disease status			
AD	81	203	0.0001
Ctrl	134	104	
Age			
<75	104	123	0.0605
≥75	111	184	
Gender			
Female	139	188	0.4625
Male	76	119	

AD – Alzheimer's disease

Table 4. Associations of ABCA2 with AD risk determined by Univariate and multivariate analysis.

Parameters	Univariate analysis			Multivariate analysis		
	OR	95%CI	P value	OR	95%CI	P value
GSE33000						
Age (≥75 *vs.* <75)	3.078	2.035–4.656	<0.001	17.306	8.835–33.898	<0.001
Gender (Male *vs.* Female)	9.392	5.631–15.456	<0.001	50.328	23.862–106.147	<0.001
ABCA2 (High *vs.* Low)	1.782	1.170–2.715	0.007	2.446	1.359–4.404	0.003
GSE15222						
Age (≥75 *vs.* <75)	4.082	2.198–7.576	<0.001	3.561	1.891–6.708	<0.001
Gender (Male *vs.* Female)	0.833	0.552–1.259	0.386			
ABCA2 (high *vs.* low)	3.057	1.974–4.734	<0.001	2.774	1.770–4.347	<0.001
GSE63063 (Cohort 1)						
Age (≥75 *vs.* <75)	2.738	1.655–4.532	<0.001	3.685	2.202–6.166	<0.001
Gender (Male *vs.* Female)	1.030	0.633–1.675	0.905			
ABCA2 (high *vs.* low)	4.023	2.430–6.662	<0.001	2.391	1.408–4.060	0.001
GSE63063 (Cohort 2)						
Age (≥75 *vs.* <75)	2.152	1.288–3.594	<0.001	1.946	1.136–3.334	0.015
Gender (Male *vs.* Female)	0.686	0.406–1.160	0.159			
ABCA2 (high *vs.* low)	3.964	2.287–6.869	<0.001	3.748	2.148–6.542	<0.001
GSE63063 (Cohort 1+2)						
Age (≥75 *vs.* <75)	2.321	1.629–3.306	<0.001	2.250	1.557–3.250	<0.001
Gender (Male *vs.* Female)	0.818	0.573–1.168	0.269			
ABCA2 (high *vs.* low)	3.229	2.246–4.643	<0.001	3.157	2.180–4.572	<0.001

OR – odds ratio; 95%CI – 95% confidence interval.

P=0.009; Table 6). Only cg03349123 was found to be a potential negative independent indicator of AD risk (OR=0.436, 95%CI=0.203-0.936, P=0.033).

Diagnostic value of ABCA2 in differentiating AD from Huntington's disease or mild cognitive impairment

The GSE33000 and GSE63063 datasets also contained the data of patients with Huntington's disease and mild cognitive impairment, respectively, and were used to determine the association of ABCA2 with HD risk or MCI risk and the diagnostic value of ABCA2 in distinguishing AD from other neurodegenerative diseases. The differences in ABCA2 mRNA levels among the patients with neurodegenerative diseases and controls were first compared. In GSE33000, the expression of ABCA2 was increased sequentially and significantly in controls, AD patients, and HD patients (Figure 2A). In GSE63063 Cohort 1, Cohort 2, and integrated analyses of both cohorts, ABCA2 expression was higher in MCI patients compared with controls (Figure 2B–2D). However, there was no significant difference

in ABCA2 expression between MCI patients and controls (Figure 2B–2D). Then, ROC curve analyses were performed and the results suggested that ABCA2 could be used to distinguish HD patients from controls, HD from AD, and MCI from controls, but not AD from MCI (Figure 3). As shown in Table 7, univariate and multivariate logistic regression analyses also indicated that ABCA2 was associated with HD compared to controls (OR=20.630, 95%CI=11.273–37.753, P<0.001), with HD compared to AD (OR=6.139, 95%CI=3.421–11.016, P<0.001), and with MCI compared to controls (OR=2828, 95%CI=1.878–4.257, P<0.001), but not with AD compared to MCI.

Analyses of the genes involved in Alzheimer's disease during ABCA2 alteration

GSE33000, consisting of ABCA2 mRNA expression profiles in brain tissues, was chosen to use in analyzing the related genes. First, GEO2R, an online tool in NCBI, was used to obtain the differently expressed genes (DEGs) between Alzheimer's disease patients and controls. Then, Pearson correlation analyses were carried out

Table 5. Diagnosis value of ABCA2 methylaitons in AD demonstrated by receiver operation characteristics curve analyses.

Variables	AUC	95%CI	P-value*	Variables	AUC	95%CI	P-value*
cg13476777	0.577	0.483–0.671	0.113	cg14633421	0.504	0.409–0.600	0.928
cg13795883	0.568	0.473–0.663	0.163	cg15428653	0.489	0.394–0.585	0.825
cg13806966	0.578	0.482–0.673	0.110	cg00785831	0.487	0.391–0.583	0.788
cg13832045	0.520	0.424–0.617	0.677	cg01229519	0.495	0.399–0.592	0.925
cg13845858	0.471	0.376–0.567	0.558	cg02663193	0.489	0.393–0.584	0.816
cg13930557	0.583	0.489–0.678	0.086	cg02820283	0.510	0.414–0.606	0.837
cg13959595	0.481	0.386–0.577	0.701	cg03349123	0.586	0.491–0.680	0.079
cg13970341	0.492	0.396–0.588	0.870	cg03698343	0.455	0.360–0.550	0.357
cg14105458	0.451	0.355–0.546	0.309	cg04723721	0.510	0.413–0.606	0.843
cg14151317	0.471	0.376–0.567	0.558	cg05775779	0.560	0.466–0.655	0.214
cg14173587	0.446	0.351–0.541	0.264	cg06048605	0.527	0.432–0.623	0.579
cg14280283	0.503	0.407–0.598	0.956	cg06400857	0.564	0.469–0.659	0.189
cg14328683	0.464	0.369–0.560	0.462	cg06471656	0.491	0.396–0.586	0.853
cg14341551	0.457	0.362–0.552	0.377	cg08216000	0.512	0.417–0.608	0.800
cg14343544	0.548	0.452–0.643	0.328	cg20850800	0.545	0.448–0.641	0.359
cg14398359	0.467	0.371–0.563	0.500	cg21157707	0.541	0.446–0.636	0.404
cg14485901	0.441	0.346–0.536	0.225	cg21221785	0.492	0.396–0.588	0.870
cg14625975	0.511	0.416–0.607	0.816	cg27558876	0.572	0.477–0.667	0.139

* Null hypothesis: true area=0.5.

Table 6. Associations of ABCA2 methylation with AD risk determined by Univariate and multivariate analysis.

Parameters	Univariate analysis			Multivariate analysis		
	OR	95%CI	P value	OR	95%CI	P value
Age (≥85 *vs.* <85)	4.077	2.018–8.237	<0.001			
Gender (Male *vs.* Female)	0.370	0.184–0.745	0.005			
cg13930557 (high *vs.* low)	0.465	0.229–0.944	0.034	0.479	0.223–1.023	0.059
cg03349123 (high vs. low)	0.393	0.195–0.791	0.009	0.436	0.203–0.936	0.033

AD – Alzheimer's disease; OR – odds ratio; 95%CI – 95% confidence interval.

to find the genes co-expressed with ABCA2. The genes derived from these processes overlapped. There were 757 genes remaining, and these were subjected to GO analysis in the GeneCoDis3 online database (*http://genecodis.cnb.csic.es*). The most enriched biological processes of these genes were signal transduction, respiratory electron transport chain, intracellular protein transport, and regulation of transcription. The most enriched KEGG pathways were Huntington's disease, Parkinson's disease, oxidative phosphorylation, cancer, and Alzheimer's disease.

Discussion

ABCA2 has been shown to be genetically linked to AD susceptibility, but whether its expression and methylation are associated with AD risk was not investigated. In the present study, the NCBI GEO database for the datasets containing expression profile of AD disease and controls and 4 datasets – GEO15222, GSE33000, GSE63063 (cohort 1), and GSE63063 (cohort 2) – were derived and downloaded. The sample source
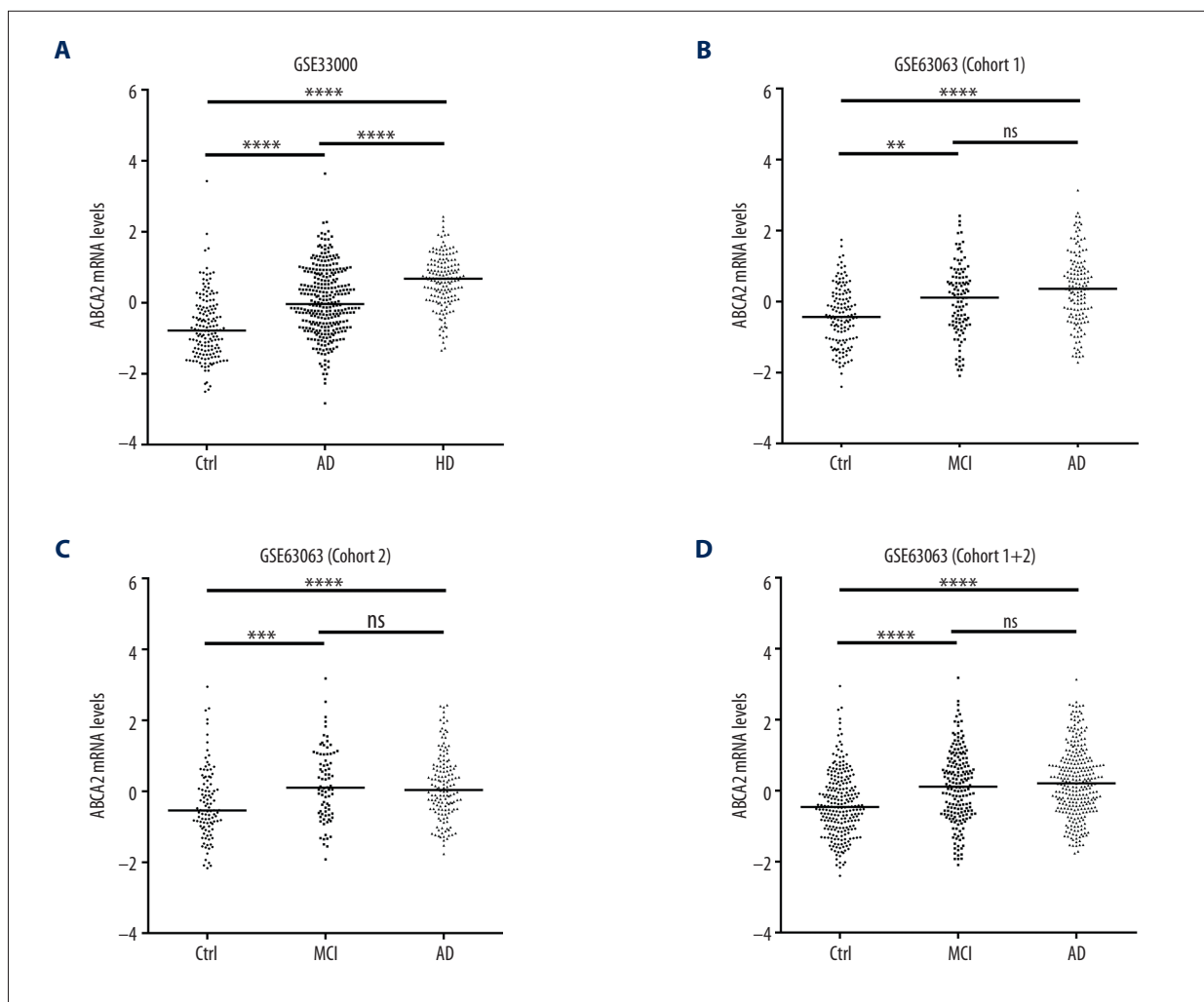
Figure 2. Comparison of ABCA2 levels in AD patients with that in patients with other neurodegenerative diseases, Huntington's disease (HD), and mild cognitive impairment (MCI). Besides AD patients, GSE33000 also included the ABCA2 expression profile of HD patients and GSE63063 also included the data of MCI patients. ABCA2 levels among different types of disease in GSE33000 (**A**), GSE63063 (Cohort 1, **B**), GSE63063 (Cohort 2, **C**), and all patients of GSE63063 (**D**) were compared using nonparametric testing. **, ***, and **** indicate P<0.01, 0.001, and 0.0001, respectively. Ns indicates non-significant.

of GEO15222 and GE33000 was brain prefrontal cortex tissue and that of GES63063 was blood. Overexpression of ABCA2 was observed in AD compared with controls in all dataset. ROC curves and univariate and multivariate analyses suggested ABCA2 overexpression was associated with AD risk. A methylation profile dataset of AD and controls, GSE80970, was selected to explore the association of ABCA2 methylation with AD risk. Methylation of 2 CpG islands with high diagnostic accuracy in ROC curve analysis was found to be negatively correlated with AD risk. Additional analyses indicated that ACBA2 mRNA expression could be used for diagnosis of other neurodegenerative diseases, mild cognitive impairment (MCI), and Huntington's disease (HD) and to distinguish HD from AD, but not AD from MCI.

The major sites of AD-related pathology in the brain were the hippocampus and the surrounding cortical regions, which were characterized by the increasing accumulation of amyloid beta plaques and tau-related neurofibrillary tangles [5]. The changes in genes in disease sites reflected the "real" regulation of the genes during AD progression; however, sampling from brain tissue is impossible for AD diagnosis. Cerebrospinal fluid (CSF) and blood are alternative sample sources. CSF has contact with extracellular spaces and reflects molecular events; it has been used in several clinical trials for treating AD and Parkinson's disease [25,26]. Compared with CSF, blood samples are easy, non-invasive, and economical to obtain. Although blood RNA regulation has the same mechanisms as the brain, some inconsistencies may exist. Here, the datasets GSE15000 and GSE3300 used brain cortex from postmortem exams as the
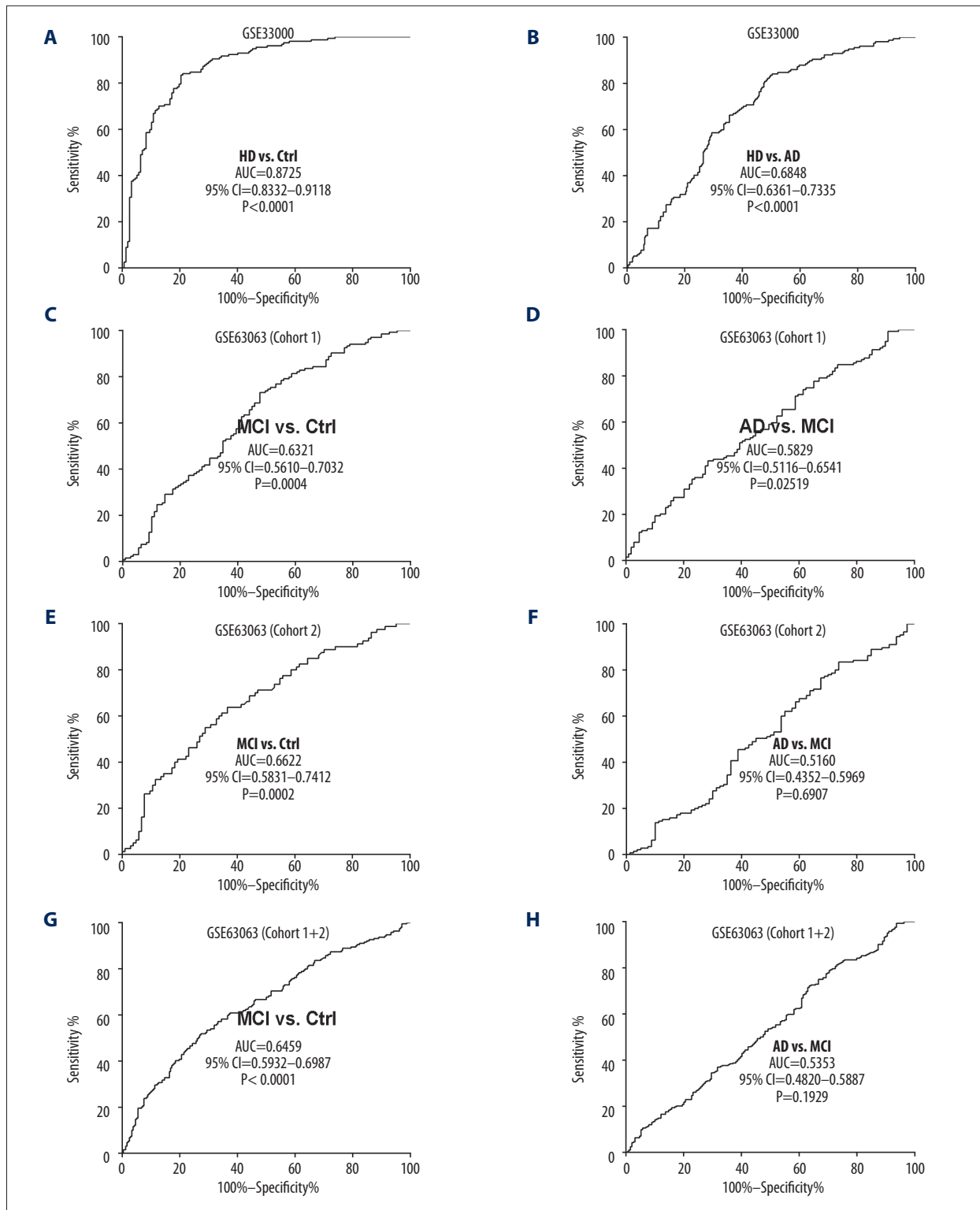
Figure 3. Diagnostic value of ABCA2 in differentiating the patients with Alzheimer's disease from those with other neurodegenerative diseases. Receiver operating characteristics curves (ROC) analyses were performed between HD and controls (**A**), and HD and AD (**B**) in the GSE33000 dataset. Analyses were also performed between MCI and controls in the GSE63063 dataset (**C**, Cohort 1; **E**, Cohort 2; **G**, Cohort 1+2) and between AD and MCI in the GSE63063 dataset (**D**, Cohort 1; **F**, Cohort 2; **H**, Cohort 1+2).

Table 7. Associations of ABCA2 with HD risk and MCI risk determined by Univariate and multivariate analysis.

Parameters	Univariate analysis			Multivariate analysis		
	OR	95%CI	P value	OR	95%CI	P-value
GSE33000 (HD vs. Ctrl)						
Age (≥75 vs. <75)	0.938	0.464–1.894	0.858			
Gender (Male vs. Female)	0.310	0.191–0.507	<0.001	0.283	0.149–0.537	<0.001
ABCA2 (high vs. low)	20.000	11.111–35.714	<0.001	20.630	11.273–37.753	<0.001
GSE33000 (HD vs. AD)						
Age (≥75 vs. <75)	0.038	0.022–0.067	<0.001	0.035	0.019–0.063	<0.001
Gender (Male vs. Female)	0.688	0.468–1.011	0.057			
ABCA2 (high vs. low)	5.208	3.215–8.475	<0.001	6.139	3.421–11.016	<0.001
GSE63063 (Cohort 1+2, MCI vs. Ctrl)						
Age (≥75 vs. <75)	2.045	1.385–3.021	<0.001	2.007	1.342–3.002	<0.001
Gender (Male vs. Female)	0.813	0.5521196	0.293			
ABCA2 (high vs. low)	2.045	1.385–3.021	<0.001	2.828	1.878–4.257	<0.001
GSE63063 (Cohort 1+2, AD vs. MCI)						
Age (≥75 vs. <75)	1.134	0.773–1.664	0.521			
Gender (Male vs. Female)	0.665	0.457–0.969	0.033			
ABCA2 (high vs. low)	1.103	0.760–1.600	0.606			

AD – Alzheimer's disease; HD – Huntington's disease; MCI – mild cognitive impairment; OR – odds ratio; 95%CI – 95% confidence interval.

sample source and GSE63063 used peripheral blood as the sample source. Both studies of GSE15222 and GSE33000 analyzed the brain transcriptome in postmortem samples, whereas GSE6306 used patient blood as the source of RNA. The associations of ABCA2 mRNA expression with AD in brain tissues and blood were consistent, indicating ABCA2 in blood reflected ABCA2 alteration in brains and could be used as a biomarker for diagnosis of AD.

RNA expression is under genetic, epigenetic, and environmental control [27–30]. Although ABCA2 SNP rs908832 was identified to be associated with AD susceptibility, the mutation is a synonymous mutation, meaning that the transition of U to C does not change. We speculate that ABCA2 expression is correlated with its methylation. We found the CpG cg03349123 methylation in ABCA2 was negatively associated with AD risk, consistent with ABCA2 mRNA results, because the transcriptional activity is usually downregulated by the methylation of the gene. This speculation needs to be verified in further studies.

Several shortcomings exist in the present study. First, the patients included in the datasets were mainly white and the

effects of ethnicity could not be analyzed. Second, although the methylation of ABCA2 was associated with susceptibility to AD, its correlations with transcriptional activity of ABCA2 were not verified. Third, the sample sizes of the patients with Huntington's disease and mild cognitive impairment were relative small. Further well-designed studies with larger sample sizes and more diverse ethnic composition should be performed to verify our conclusions.

Conclusions

ABCA2 mRNA expression and methylation is associated with AD risk. The results of ABCA2 mRNA expression in brain cortex in diagnosis of AD were consistent with that in blood. ABCA2 may be used for early diagnosis of AD and is as a potential therapeutic target of AD.

Conflict of interest

None.

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