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Exploring the Genetic Association of the *ABAT* Gene with Alzheimer's Disease

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Abstract

Accumulating evidence demonstrated that GABAergic dysfunction contributes to the pathogenesis of Alzheimer's disease (AD). The *GABA aminotransferase* (*ABAT*) gene encodes a mitochondrial GABA transaminase and plays key roles in the biogenesis and metabolism of gamma-aminobutyric acid (GABA), which is a major inhibitory neurotransmitter. In this study, we performed an integrative study at the genetic and expression levels to investigate the potential genetic association between the *ABAT* gene and AD. Through re-analyzing data from the currently largest meta-analysis of AD genome-wide association study (GWAS), we identified genetic variants in the 3'-UTR of *ABAT* as the top AD-associated SNPs ($P < 1 \times 10^{-4}$) in this gene. Functional annotation of these AD-associated SNPs indicated that these SNPs are located in the regulatory regions of transcription factors or/and microRNAs. Expression quantitative trait loci (eQTL) analysis and luciferase reporter assay showed that the AD risk alleles of these SNPs were associated with a reduced expression level of *ABAT*. Further analysis of mRNA expression data and single-cell transcriptome data of AD patients showed that *ABAT* reduction in the neuron is an early event during AD development. Overall, our results indicated that *ABAT* genetic variants may be associated with AD through affecting its mRNA expression. An abnormal level of *ABAT* will lead to a disturbance of the GABAergic signal pathway in AD brains.

Keywords Alzheimer's disease · *ABAT* · GABA · Genetic association · Integrative analysis

Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia, which involves a progressive memory loss and cognitive impairment [1–3]. The main pathological hallmarks of AD are extracellular deposition of β-amyloid(Aβ) plaques and neuronal accumulation of neurofibrillary tangles formed by hyperphosphorylated tau protein [3, 4]. The pathogenic factors of AD are complicated, with the genetic effect being an important risk factor for AD [5–7]. Mutations in amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) had been identified as the main pathogenic reason for early-onset familial AD, which only accounts for less than 5% of total AD patients [5–7]. Large-scale genetic studies for the remaining sporadic AD, including genome-wide association study (GWAS) [8, 9], whole-exome sequencing (WES) [10, 11], and whole-genome sequencing (WGS) [12, 13], have been performed in recent years and identified dozens of AD susceptible genes. However, these previous findings could only explain about 33% of the total phenotypic variances, indicating that additional AD-related genes and variants are yet to be identified [5–7, 14].

Dysregulation of the neuronal network involved in learning and memory was recognized as one of the main reasons for cognitive decline in AD patients [15, 16]. Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter, plays important roles in various brain functions including synaptic transmission, neuronal development, relaxation, sleep regulation, and depression [17–19]. An increasing amount of evidence has demonstrated that GABAergic

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dysfunctions, including reduction of GABA receptors [20–22], loss of GABAergic neurons and synapses [23], aberrant GABA production in the reactive astrocytes [24, 25], imbalance between excitatory and inhibitory signals (E/I imbalance) of the central nervous system [16, 26, 27], are early events occurring in the brains of AD patients and animal models. In addition, the GABAergic system was found to be vulnerable to neurotoxicity mediated by AD pathogenic factors, including APP, Aβ, APOE ε4, BACE1, and hyperphosphorylated tau protein [16, 28–31].

GABA aminotransferase (ABAT) is a key mitochondrial enzyme responsible for GABA catabolism and recycling production [32]. GABA is generated from L-glutamate in neurons and is released to bind to the postsynaptic GABA receptors to transmit the inhibitory signals [32, 33]. Excessive GABA from synapses is taken up by astrocytes and is transported into mitochondria by ABAT [32]. In mitochondria, GABA is catalyzed to glutamine through the citric acid cycle; then, glutamine is transported to neurons to participate in a new cycle of GABA production [32, 33]. Therefore, ABAT is a key regulator for GABA biogenesis and metabolism [32]. Mutations in the *ABAT* gene could cause a rare disease, ABAT deficiency (OMIM 613163), which is characterized by encephalopathy, hypotonia, epilepsy, and severe psychomotor retardation [34]. The alterations of *ABAT* expression level and activity had been observed in the brains of AD patients and animal models decades ago [32, 35–37]. Loss of the *ABAT* gene region was identified in a genome-wide copy number variation (CNV) analysis for early-onset familial AD patients [38]. However, the genetic association of *ABAT* with AD has not received much attention in previous studies.

In this study, we performed an integrative analysis using publicly available genetic data, epigenetic data, transcriptome data, and brain expression quantitative trait loci (eQTL) data to investigate the potential role of the *ABAT* gene in AD development. We further performed cellular assays to elucidate the functional effect of the risk alleles. Our results showed that *ABAT* is associated with AD.

Materials and Methods

Description of AD GWAS Dataset

In order to investigate whether the *ABAT* gene is genetically associated with AD, we retrieved data from the largest meta-analysis of AD GWAS [9], which included GWAS data of a total of 71,880 cases and 383,378 controls from four cohorts: the International Genomics of Alzheimer's Project (IGAP) [8], the Psychiatric Genomic Consortium (PGZ-ALZ), the Alzheimer's Disease Sequencing Project (ADSP) [39], and UK Biobank [40]. SNPs located in the – 10 kb~+ 10 kb region of the *ABAT* gene were retrieved and were subjected to subsequent analyses. The linkage disequilibrium (LD) plot of the identified variants was checked in LocusZoom (http://locuszoom.org/) [41].

Description of AD WES Dataset

In order to investigate whether the rare functional variants in the *ABAT* gene are associated with genetic risk of AD, we retrieved data from the ADSP project [10], which included WES data of 5740 late-onset AD cases and 5096 cognitively normal controls of European origin. In addition, we took advantage of the WES data of 107 AD patients and 368 control subjects from the Chinese population in our recent study [11] to further explore the potential functional variants in the *ABAT* gene in the Chinese AD population.

Functional Annotation of *ABAT* Genetic Variants

Functional annotation of variants in the *ABAT* gene was performed using available data from the Encyclopedia of DNA Elements (ENCODE) project [42], which includes the systematically mapped regions of transcription factor association and histone modification. Four types of histone modification (H3K4me3, H3K9ac, H3K4me1, and H3K27ac) in 8 brain tissues (hippocampus, temporal cortex, angular gyrus, caudate nucleus, cingulate gyrus, prefrontal cortex, substantia nigra, and embryonic brain tissue) and 6 cell types (bipolar neuron, neuron, astrocyte, neural cell, radial glial cell, neural stem progenitor cell) were investigated. Over 600 transcription factor ChIP-seq data from various tissues and cell types were also retrieved from the ENCODE [42] database and analyzed. The miRNA dataset miRDB [43, 44] (http://www.mirdb.org/cgi-bin/search.cgi) was applied to investigate whether *ABAT* variants could potentially affect the miRNA-mRNA interactions. The potential pathogenicity of each variant was predicted by using the Combined Annotation Dependent Depletion (CADD) database [45].

eQTL Annotation of the Genetic Variants

The association between genotype and mRNA expression was analyzed by using the available brain eQTL database eMeta [46], which meta-analyzed 1194 brain eQTL data from three eQTL datasets: the Genotype-Tissue Expression project [47, 48] (GTEx: http://www.gtexportal.org/home/), the CommonMind Consortium [49], and the Religious Orders Study and Memory and Aging Project (ROSMAP) [50].

Data Mining of mRNA Expression in AD Patients

Alteration of the mRNA level of *ABAT* in AD brains was investigated with the stage I data from the AlzData database (www.alzdata.org) [51], which integrated and normalized the original microarray data of 269 AD brain tissues and 271 control brain tissues from Gene Expression Omnibus (GEO: http://www.ncbi.nlm.nih.gov/sites/GDSbrowser). Furthermore, cell type-specific alteration of the *ABAT* mRNA level in AD was explored by using the available single-cell transcriptomes data of 80,660 single-nucleus from the prefrontal cortex of 48 individuals with varying degrees of AD pathology (https://www.radc.rush.edu/docs/omics.htm) [52].

Luciferase Reporter Assay

A dual-luciferase reporter system (psiCHECK-2, Promega) was used to investigate the effect of *ABAT* variants on its mRNA expression. The following truncated 3'-UTR fragments of *ABAT* containing different alleles of SNPs rs2270288 and rs3743798 were amplified and cloned into the downstream region of the *Renilla* luciferase in the psiCHECK-2 vector with *Pme*I and *Xho*I: (1) rs2270288-G: 1-367 nts of 3'-UTR with rs2270288-G;(2) rs2270288-T: 1-367 nts of 3'-UTR with rs2270288-T;(3) rs2270288-G-rs3743798-G: 1-720 nts of 3'-UTR with rs2270288-G-rs3743798-G;(4) rs2270288-T-rs3743798-A: 1-720 nts of 3'-UTR with rs2270288-T-rs3743798-A. All the constructs were verified by direct sequencing.

The HEK293T and U251 cells were introduced from Kunming Cell Bank, Kunming Institute of Zoology. The HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fishers) supplemented with 10% fetal bovine serum (FBS; Thermo Fishers), whereas the U251 cells were cultured in Roswell Park Memorial Institute 1640 Medium (Thermo Fishers) supplemented with 10% FBS. Cells were seeded to a 24-well plate with a density of 1×10^5 per well 12 h before transfection. psiCHECK-2 vector (500 ng) containing different alleles were transfected into cells in the form of six replicates for each construct by using Lipofectamine 3000 (Thermo Fishers) according to the manufacture's manual. Twenty-four hours after transfection, cells were harvested for luciferase activity assay on an Infinite M1000 Pro multimode microplate reader (30064852; Tecan) following the dual-luciferase reporter assay system technical manual (Promega).

Statistical Analysis

Differences in *ABAT* mRNA level between AD and control brains were quantified by a two-tailed Student's *t* test using the GraphPad Prism software (GraphPad Software, La Jolla, CA, USA). For luciferase reporter analysis, differences of 3'-UTR fragments with reference and risk alleles in regulating the level of *Renilla* luciferase were analyzed by a two-tailed Student's *t* test using the GraphPad Prism software.

Results

Genetic Variants in the 3'-UTR Region of the *ABAT* Gene Were Associated with AD Risk

In order to obtain an unbiased and reliable result for the genetic association analysis, data from a meta-analysis of the available largest sample size of AD cases ($N = 71{,}880$) and controls ($N = 383{,}378$) were retrieved and (re)analyzed [9]. A total of 1041 SNPs spanning the − 10 kb~+ 10 kb region of the *ABAT* gene were identified, among which 117 SNPs were associated with genetic risk of AD ($P < 0.05$, Supplementary Table S1) and 19 SNPs with *P* values less than 0.001 (Table 1). Interestingly, we found that the top associated SNPs ($P < 1 \times 10^{-4}$), including rs1075853, rs2270288, rs3743798, rs3743801, rs1061842, rs62031113, and rs11641192, were all located within or near the 3'-UTR region of the *ABAT* gene. To investigate whether these SNPs are linked with other top SNPs in the adjacent region, we expanded the screened region to − 100 kb~+ 100 kb of rs2270288 and found that the top AD-associated SNP in the expanded region was still rs2270288. Most of the top AD-associated SNPs were linked with rs2270288 ($r^2 > 0.8$) according to the LD plot (Fig. 1).

In order to explore whether there are rare functional variants in the *ABAT* gene to be associated with AD, we retrieved and (re)analyzed the WES data of AD patients in the ADSP project [10] and in our recent study [11]. Two synonymous variants showed a marginal association with AD in the ADSP cohort (Supplementary Table S2, 16:8844347, $P = 0.0457$; 16:8868776, $P = 0.0244$), but the association did not survive correction for multiple testing. No potentially functional rare variant in the *ABAT* gene was identified to be associated with genetic risk of AD in both the ADSP cohort and Chinese cohort (Supplementary Tables S2 and S3), presumably because of insufficiency of the sample size.

Taken together, genetic variants in the 3'-UTR region of the *ABAT* gene, which might have potential regulatory effects on *ABAT* expression, were identified to be significantly associated with AD risk.

mRNA Expression Level of *ABAT* Was Significantly Decreased in Brains of AD Patients at the Early Stage of AD

To explore whether the expression level of the *ABAT* gene is changed in brain tissues of AD patients, mRNA expression profiles of brain tissues from AD patients and controls were

compared. Expression data were retrieved from AlzData webserver (www.alzdata.org) [51], which integrated and normalized the mRNA expression data of 269 AD brain tissues and 271 control brain tissues from four brain regions (entorhinal cortex, hippocampus, temporal cortex, and frontal cortex) from GEO database (https://www.ncbi.nlm.nih.gov/geo). We found a significantly decreased mRNA level of the *ABAT* gene in entorhinal cortex tissues of AD patients compared to controls (Fig. 2a). The entorhinal cortex has been considered as one of the earliest affected brain regions during AD pathology [2]. There were no statistically significant differences in *ABAT* mRNA expression in the hippocampus and frontal cortex tissues of AD patients relative to controls (Fig. 2b, c). The *ABAT* mRNA level was found to be significantly increased in the temporal cortex of AD patients (Fig. 2d).

As ABAT is a key regulator of the inhibitory neurotransmitter, we further investigated whether ABAT showed a cell type-specific change during AD pathological progression. Through analyzing the single-cell transcriptome data of 80,660 single-nucleus from the prefrontal cortex of 48 individuals with varying degrees of AD pathology [52], we found that mRNA expression of *ABAT* was significantly reduced at the early stage of AD in both excitatory and inhibitory neurons, whereas it was significantly increased at the late stage of AD (Table 2).

To sum up, a dynamic change of spatial and temporal expression levels of *ABAT* was observed in AD brain tissues, and the decreased *ABAT* level was an early event during AD progression.

The Top AD-Associated SNPs in the *ABAT* Gene Were Associated with Decreased *ABAT* Expression Level in Brain Tissues

As most of the top AD-associated SNPs in the *ABAT* gene were located in its 3'-UTR region and *ABAT* mRNA level was significantly reduced at the early stage of AD, we further explored whether different alleles of these SNPs could affect the expression level of *ABAT*. Through data mining of the meta-analyzed 1194 brain eQTL data from the eMeta database [46], we found that carriers of AD risk alleles of SNPs rs2270288, rs3743798, rs3743801, and rs1061842 have significantly decreased *ABAT* expression levels in brain tissues (Table 3). Functional annotation of these variants with transcription factor association data [42] showed that SNPs rs3743798, rs3743801, rs7205816, and rs1061842 were located in the binding region of transcription factors (Table 3).

Table 1 Association of *ABAT* SNPs with Alzheimer's disease

SNP[a]	Position	Ref/Alt[b]	Region	Beta	*P* value	PHRED[c]
rs1641024	chr16:8871372	C/T	Intronic	− 0.0087	3.06E-04	4.222
rs1641025	chr16:8871388	C/T	Intronic	− 0.0089	2.47E-04	3.446
rs1641027	chr16:8871432	T/G	Intronic	− 0.0091	1.67E-04	1.834
rs7196462	chr16:8871457	C/G	Intronic	− 0.0090	2.08E-04	1.925
rs1731073	chr16:8874497	G/A	Intronic	0.0084	1.41E-04	0.937
rs1075853	*chr16:8874779*	*T/C*	*Intronic*	*0.0095*	*3.37E-05*	*1.599*
rs2270288	*chr16:8875529*	*G/T*	*3'-UTR*	*− 0.0092*	*2.96E-05*	*14.54*
rs3743798	*chr16:8875799*	*G/A*	*3'-UTR*	*− 0.0088*	*6.07E-05*	*3.247*
rs3743801	*chr16:8876202*	*C/G*	*3'-UTR*	*− 0.0086*	*8.84E-05*	*1.564*
rs7205816	chr16:8876418	A/G	3'-UTR	0.0081	2.90E-04	3.755
rs1061842	*chr16:8876880*	*T/A*	*3'-UTR*	*− 0.0088*	*5.44E-05*	*3.489*
rs62031113	*chr16:8879406*	*G/C*	*Downstream*	*− 0.0087*	*7.41E-05*	*4.497*
rs11641192	*chr16:8879669*	*G/A*	*Intergenic*	*− 0.0086*	*7.71E-05*	*1.120*
rs11641269	chr16:8879824	G/A	Intergenic	− 0.0084	6.90E-04	0.727
rs7196948	chr16:8882170	T/A	Intergenic	− 0.0084	1.13E-04	9.034
rs62031119	chr16:8885445	C/T	Intergenic	− 0.0084	7.02E-04	6.585
rs3743813	chr16:8886128	G/A	Intergenic	− 0.0082	1.71E-04	5.250
rs3743811	chr16:8886409	G/T	Intergenic	− 0.0083	1.43E-04	2.393
rs7192290	chr16:8888030	G/T	Intergenic	0.0073	8.05E-04	4.504

[a] Data were retrieved from a meta-analysis of AD GWAS data, which included a total of 71,880 AD cases and 383,378 controls [9]. Top AD-associated SNPs ($P < 1 \times 10^{-4}$) were marked in italics

[b] *Ref/Alt*, reference allele/alternative allele

[c] The PHRED-like scaled CADD-score: a score greater than 10 indicates that the variant belongs to the top 10% most deleterious substitutions in human genome [45]

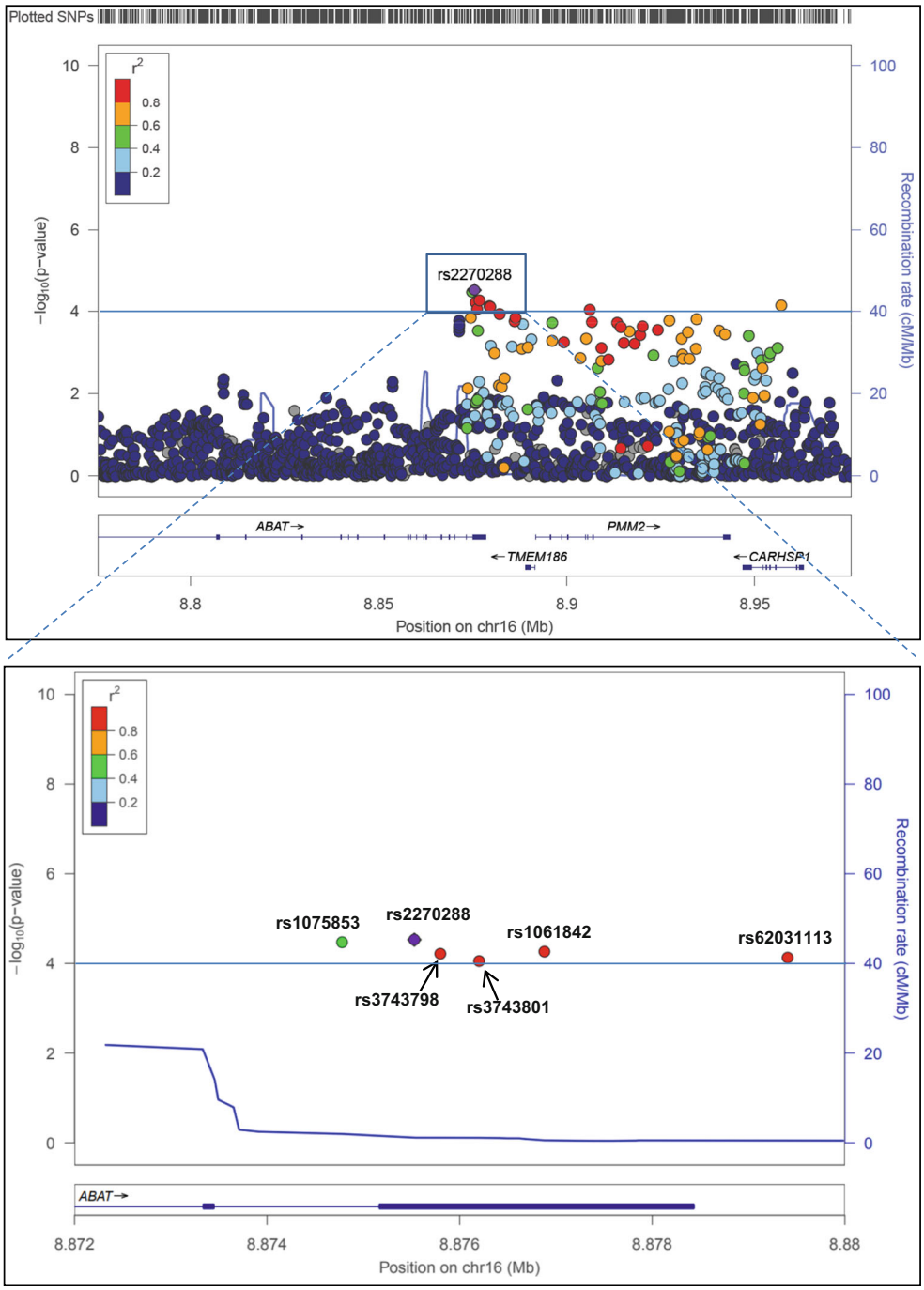

Fig. 1 The linkage disequilibrium (LD) pattern of SNPs in the −100 kb~+100 kb region of rs2270288. The linkage analysis was performed by using LocusZoom (http://locuszoom.org/) [41] based on AD GWAS data from a mate-analysis [9]. The pairwise LD pattern of different SNPs with the most strongly associated SNP rs2270288 was indicated by different colors

None of these SNPs was located in the region for histone modification (data not shown). We further investigated whether these SNPs would affect microRNA binding motifs. Through searching the microRNA-mRNA interactions in miRNA database miRDB [43, 44], the AD risk alleles rs2270288-T and rs3743798-A were predicted to create new binding sites of microRNAs miR-7113-5P and miR-3130-3P, respectively (Table 3). There was no previous report to show the potential association of these two microRNAs with AD, whereas downregulation of miR-7113-5P was found to be involved in the induction of WNT10B in posttraumatic stress disorder [53].

Based on the above data that AD risk alleles of SNPs in the *ABAT* gene are associated with decreased *ABAT* expression and most of the top associated SNPs in the 3'-UTR region of this gene were linked with each other (Fig. 1), we performed a luciferase reporter assay to investigate whether the 3'-UTR region with different alleles/haplotypes of these AD-associated SNPs could have a regulatory effect on luciferase expression. Four types of the truncated 3'-UTR region containing different alleles of SNPs rs2270288 and rs3743798

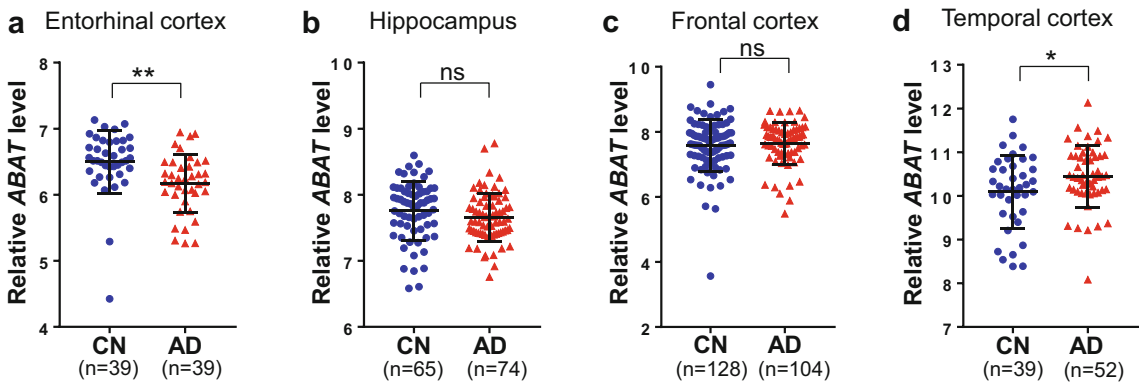

Fig. 2 The mRNA level of the *ABAT* gene was significantly changed in the entorhinal cortex and temporal cortex tissues of AD patients compared to controls. **a**–**d** The mRNA expression data of the *ABAT* gene in entorhinal cortex (**a**), hippocampus (**b**), frontal cortex (**c**), and temporal cortex (**d**) tissues were retrieved from the NCBI Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo) and were normalized and analyzed in the AlzData database (www.alzdata.org) ([51], and references therein). The difference between AD patients and controls was quantified by using a two-tailed Student's *t* test. Shown data are mean ± SD. Ns, not significant; **P* value < 0.05; ***P* value < 0.01

were amplified and cloned into the downstream region of the *Renilla* luciferase in the psiCHECK-2 vector (Fig. 3). For the shorter truncated 3'-UTR region (367 bp), there is no significant difference between rs2270288-G and rs2270288-T in regulating the level of *Renilla* luciferase (Fig. 3). For the longer truncated 3'-UTR region (720 bp), compared with the reference haplotype of rs2270288-G-rs3743798-G, 3'-UTR region with the AD risk haplotype rs2270288-T-rs3743798-A could significantly reduce the expression level of the *Renilla* luciferase in both HEK293T cells and U251 cells (Fig. 3a, b).

Collectively, these results suggested that 3'-UTR variants in the *ABAT* gene may be associated with genetic risk of AD through decreasing *ABAT* expression in the brain.

Discussion

Though GABAergic dysfunctions have been frequently observed in AD patients [16], the genetic association of the related genes in the GABAergic pathway with AD has not received much attention in previous studies. In this study, we performed an integrative analysis to explore the involvement of *ABAT* in contributing genetic risk to AD. We obtained the global evidence to support the association between the *ABAT* gene and AD: (1) multiple genetic variants in the 3'-UTR region of the *ABAT* gene were associated with AD ($P < 1 \times 10^{-4}$) (Table 1 and Fig. 1), whereas the rare functional variants in the coding region of *ABAT* showed no association with AD; (2) the *ABAT* expression level was decreased in neurons and entorhinal cortex and constituted one of the early events during AD development (Fig. 2 and Table 2); (3) functional annotations of these AD-associated SNPs in the *ABAT* gene indicated that these SNPs are located in the regulatory regions of transcription factors or/and microRNAs (Table 3); (4) eQTL analysis and luciferase reporter assays showed that the AD risk alleles of these SNPs were associated with a reduced *ABAT* expression level (Table 3 and Fig. 3). Taken together, these genetic data indicated that variants in the regulatory region of the *ABAT* gene may be associated with AD risk through affecting mRNA expression of *ABAT*. Interestingly, the top AD-associated variants identified in this

Table 2 Cell type-specific changes of *ABAT* during AD pathological progression

Cell type[a]	Pathology vs. no pathology		Early pathology vs. no pathology		Late pathology vs. early pathology	
	P value[b]	log2 fold change	*P* value[b]	log2 fold change	*P* value[b]	log2 fold change
Excitatory neuron	6.14E-71	− 0.198	2.50E-88	− 0.266	1.14E-20	0.194
Inhibitory neuron	6.91E-09	− 0.154	4.37E-19	− 0.287	1.55E-14	0.343
Astrocyte	0.536	0.172	0.52	0.225	0.961	− 0.185
Oligodendrocyte	0.405	0.118	0.821	0.161	0.349	− 0.114
Oligodendrocyte precursor cell	0.848	− 0.057	0.543	− 0.165	0.407	0.306
Microglia	0.989	− 0.246	0.933	− 0.452	0.849	0.433

[a] Single-cell transcriptome data of 80,660 single nucleus from the prefrontal cortex of 48 individuals with varying degrees of AD pathology [52] were retrieved from the Rush Alzheimer's Disease Center (RADC) Research Resource Sharing Hub at https://www.radc.rush.edu/docs/omics.htm

[b] FDR-adjusted *P* values of two-sided Wilcoxon rank-sum test

Table 3 Functional and eQTL annotations of top AD-associated SNPs in the 3'-UTR region of *ABAT*

SNP	Ref/Alt	eQTL_β (ref)[a]	eQTL_*P* value[a]	TFs/cell type[b]	microRNA (Alt)[c]
rs2270288	G/T	0.13	0.0062	/	hsa-miR-7113-5P
rs3743798	G/A	0.13	0.0049	RBM22 (HepG2)	hsa-miR-3130-3P
rs3743801	C/G	0.15	0.0018	RBM22 (HepG2)	/
rs7205816	A/G	NA	NA	ZNF341 (HEK293) ZNF341 (HEK293)	/
rs1061842	T/A	0.15	0.0019	ZNF341 (HEK293)	/

[a] Data were retrieved from the brain eMeta eQTL summary data (http://cnsgenomics.com/software/smr/#Download) [46]. The *β*-value > 0 indicates that the reference (Ref) allele of the indicated SNP is associated with increased *ABAT* expression level. *NA*, not available

[b] Data were retrieved from the encyclopedia of DNA elements (ENCODE) database (https://www.encodeproject.org/) [42]. "RBM22 (HepG2)" indicates the location of SNP rs3743798 in the binding region of transcription factor (TF) RBM22 in HepG2 cells

[c] Data were retrieved from the miRDB (http://www.mirdb.org/cgi-bin/search.cgi) to investigate whether the alternative (Alt) allele of the indicated SNP might create a new binding site for microRNA [43, 44]. Missing information was marked with "/"

study, including rs2270288, rs3743798, rs3743801, and rs7205816, were reported to be associated with somatosensory evoked potentials in patients with depression [54], which further indicated that the altered *ABAT* expression mediated by genetic variants in the regulatory region of the *ABAT* gene might be an important factor affecting the risk of brain diseases.

It should be mentioned that previous studies concerning the alteration of *ABAT* expression level in AD have not obtained a consistent result. Some studies showed an increased *ABAT* expression level or activity in AD patients [35, 36], whereas others showed the opposite results [32, 37]. These inconsistent results might be explained by relatively small sample size of patients, varied post-mortem interval time of mRNA measurements, and dynamic change of spatial and temporal levels of GABA and *ABAT* in brain tissues. In this study, we took advantage of a more comprehensive dataset (269 AD brain tissues and 271 control tissues) that were retrieved from the GEO database (https://www.ncbi.nlm.nih.gov/geo) and were normalized and analyzed in our previous study [51], to explore the mRNA expression alterations of *ABAT* in AD. We identified a significantly reduced *ABAT* level in the entorhinal cortex tissues of AD patients (Fig. 2a) but observed a significantly increased *ABAT* level in the temporal cortex tissues of AD patients (Fig. 2d). The differential expression patterns of *ABAT* in the entorhinal cortex and temporal cortex might be attributed to different roles of the two brain regions during AD development. The entorhinal cortex is regarded as one of the earliest regions affected in AD [2], while the temporal cortex might be the later affected region. In line with this speculation, analysis of single-cell transcriptome data of 80,660 single-nucleus from the prefrontal cortex of 48 individuals [52] showed that the *ABAT* expression level was significantly reduced at the early stage of AD but was increased at the late stage of AD (Table 2). Due to the important role of ABAT in GABA

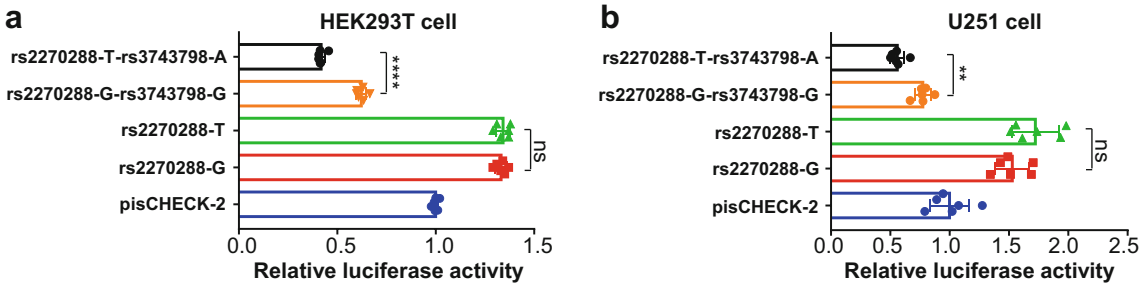

Fig. 3 The AD-associated SNPs in the 3'-UTR of the *ABAT* gene affected gene expression. Four types of luciferase reporters with a truncated 3'-UTR region of *ABAT* contained different combinations of rs2270288 and rs3743798 alleles. Each 3'-UTR fragment was inserted into the downstream region of the *Renilla* luciferase in the psiCHECK-2 vector: (1) rs2270288-G: 1-367 nts of 3'-UTR with rs2270288-G; (2) rs2270288-T: 1-367 nts of 3'-UTR with rs2270288-T; (3) rs2270288-G-rs3743798-G: 1-720 nts of 3'-UTR with rs2270288-G-rs3743798-G; (4) rs2270288-T-rs3743798-A: 1-720 nts of 3'-UTR with rs2270288-T-rs3743798-A. HEK293T cells (**a**) and U251 cells (**b**) were transfected with the indicated luciferase reporters for 24 h before harvest for luciferase analysis. Shown data are mean ± SD. ns, not significant; ***P* value < 0.01; *****P* value < 0.0001. This assay was independently repeated three times and had consistent results

biogenesis and metabolism, the abnormal dynamic level of *ABAT*, either increased or decreased, would cause a disturbance of GABAergic signal pathways and E/I imbalance in the neuronal network of AD brains.

Except for the regulatory effect of GABA, ABAT plays a key role in the maintenance of nucleotide pools in the mitochondria [55]. Mitochondrial dysfunction has been widely reported in AD [56, 57] because of its important role in cellular metabolism and energy production. Therefore, whether the altered *ABAT* level during AD development would affect the risk of AD through affecting the mitochondrial pathway is deserved to be further determined.

The current study has several limitations. First, we did not validate the association of *ABAT* with AD in independent populations. Second, we did not perform experimental assays to further characterize the potential roles of altered ABAT expression in GABAergic signal pathway and mitochondrial pathway, which are all actively involved in AD development.

Taken together, through an integrative analysis of the *ABAT* gene at the genetic and expressional levels, we found that altered *ABAT* level is an early event during AD pathogenesis. Genetic variants in the 3'-UTR region of *ABAT* are associated with AD risk through regulating its mRNA expression. Future functional assays are necessary to explore the potential role of *ABAT* in AD pathogenesis.

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Availability of Data and Material All data generated or analyzed in this study are included in the current manuscript and supplementary materials.

Authors' Contributions Y.-G.Y. and R.B. designed the study; M.X. and D.-F.Z. analyzed the data; R.B. and Q.Z. performed the experiments; R.B., Q.Z., and Y.-G.Y. drafted the manuscript; all authors revised and approved the manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication All authors have seen and approved the manuscript and contributed significantly to this work.

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Supplementary Table S1. Association of common SNPs in the *ABAT* gene region with genetic risk of AD

rsID	Position	Variant	Beta	Variant type	*P*-value	Gene
rs186562478	16: 8765773	C/T	-0.055511085	intergenic	7.87E-03	*METTL22;ABAT*
rs111966958	16: 8767211	A/G	-0.019034992	intergenic	2.58E-02	*METTL22;ABAT*
rs147854646	16: 8775591	T/C	-0.038593519	intronic	3.55E-02	*ABAT*
rs1273380	16: 8793290	G/C	0.004981242	intronic	4.12E-02	*ABAT*
rs75667650	16: 8794640	T/A	-0.028359466	intronic	3.89E-02	*ABAT*
rs1273381	16: 8795970	A/C	0.005629019	intronic	3.20E-02	*ABAT*
rs79858281	16: 8799403	C/T	-0.01037491	intronic	4.78E-02	*ABAT*
rs189276414	16: 8800095	A/T	-0.061089817	intronic	3.38E-02	*ABAT*
rs1731062	16: 8802752	C/T	0.005037853	intronic	4.56E-02	*ABAT*
rs1640981	16: 8803745	A/G	0.005012899	intronic	4.68E-02	*ABAT*
rs76282974	16: 8804092	C/A	0.01901365	intronic	2.55E-02	*ABAT*
rs1273385	16: 8806421	T/C	0.004968997	intronic	4.93E-02	*ABAT*
rs1060784	16: 8807100	G/T	0.00518994	UTR5	4.15E-02	*ABAT*
rs78272755	16: 8808660	A/G	0.016059165	intronic	5.84E-03	*ABAT*
rs73497685	16: 8808732	T/C	0.016148562	intronic	4.42E-03	*ABAT*
rs140241106	16: 8808741	C/T	0.03028758	intronic	9.55E-03	*ABAT*
rs112522839	16: 8813922	A/G	0.014780297	intronic	1.04E-02	*ABAT*
rs73497697	16: 8814172	C/T	0.013116059	intronic	1.61E-02	*ABAT*
rs73497698	16: 8814227	C/T	0.012906661	intronic	1.77E-02	*ABAT*
rs8052663	16: 8814944	G/C	0.013238989	intronic	1.77E-02	*ABAT*
rs528781428	16: 8818784	T/C	-0.023008999	intronic	3.36E-02	*ABAT*
rs11647162	16: 8819368	G/A	0.013433227	intronic	3.79E-02	*ABAT*
rs11866675	16: 8827489	G/C	-0.048184395	intronic	2.58E-02	*ABAT*
rs1640995	16: 8828533	T/A	0.005302849	intronic	4.35E-02	*ABAT*
rs113864618	16: 8830994	C/T	-0.030559748	intronic	4.67E-02	*ABAT*
rs77060236	16: 8831515	G/C	-0.006527377	intronic	4.93E-02	*ABAT*
rs149675077	16: 8832016	G/A	-0.031868525	intronic	4.40E-02	*ABAT*
rs148441810	16: 8832331	A/T	-0.031765054	intronic	4.47E-02	*ABAT*
rs147065598	16: 8833080	G/C	-0.031387198	intronic	4.04E-02	*ABAT*
rs139857138	16: 8833730	C/T	-0.032346433	intronic	4.14E-02	*ABAT*
rs143419165	16: 8833843	C/T	-0.032193412	intronic	4.21E-02	*ABAT*

rs144177027	16: 8836087	T/A	-0.03347271	intronic	3.43E-02	*ABAT*
rs1965649	16: 8836090	C/G	-0.007801838	intronic	1.23E-02	*ABAT*
rs72770119	16: 8837880	A/G	-0.006934807	intronic	3.18E-02	*ABAT*
rs72770121	16: 8840098	T/G	-0.00808613	intronic	1.78E-02	*ABAT*
rs1078508	16: 8840914	G/A	-0.053183563	intronic	2.43E-02	*ABAT*
rs1078510	16: 8841003	C/A	-0.04680045	intronic	4.55E-02	*ABAT*
rs74008051	16: 8841321	A/G	-0.046800514	intronic	4.55E-02	*ABAT*
rs9936820	16: 8841453	T/G	-0.050996477	intronic	2.93E-02	*ABAT*
rs58127888	16: 8843928	G/A	-0.046708969	intronic	4.46E-02	*ABAT*
rs148036256	16: 8846625	G/A	0.027861162	intronic	3.67E-02	*ABAT*
rs72770141	16: 8849036	C/G	-0.007009808	intronic	2.21E-02	*ABAT*
rs8057162	16: 8851271	T/C	-0.006959942	intronic	2.21E-02	*ABAT*
rs72770147	16: 8851324	A/G	-0.007022927	intronic	2.08E-02	*ABAT*
rs143869798	16: 8852619	C/T	-0.053284058	intronic	2.36E-02	*ABAT*
rs148600174	16: 8852621	A/T	-0.053284058	intronic	2.36E-02	*ABAT*
rs28557212	16: 8852889	T/G	-0.053119817	intronic	2.36E-02	*ABAT*
rs8053030	16: 8853543	T/G	-0.049268061	intronic	3.47E-02	*ABAT*
rs13332999	16: 8853743	C/A	-0.008271724	intronic	6.86E-03	*ABAT*
rs13337024	16: 8853787	A/G	-0.008551536	intronic	5.21E-03	*ABAT*
rs12925086	16: 8866280	T/C	0.004870394	intronic	4.22E-02	*ABAT*
rs12448823	16: 8867408	A/T	0.004859131	intronic	4.14E-02	*ABAT*
rs1273420	16: 8867596	A/G	-0.004660233	intronic	3.32E-02	*ABAT*
rs1641021	16: 8868640	T/C	-0.004619135	intronic	3.45E-02	*ABAT*
rs1641022	16: 8868776	A/C	-0.004488974	exonic	3.99E-02	*ABAT*
rs1641023	16: 8869365	T/C	-0.004524007	intronic	3.85E-02	*ABAT*
rs1641024	16: 8871372	T/C	-0.008721848	intronic	3.06E-04	*ABAT*
rs1641025	16: 8871388	T/C	-0.008850673	intronic	2.47E-04	*ABAT*
rs1641027	16: 8871432	G/T	-0.009114247	intronic	1.67E-04	*ABAT*
rs7196462	16: 8871457	G/C	-0.009007333	intronic	2.08E-04	*ABAT*
rs12445687	16: 8871678	G/A	0.008478897	intronic	4.35E-02	*ABAT*
rs77245798	16: 8872084	T/C	0.009874868	intronic	2.36E-02	*ABAT*
rs542549067	16: 8872645	A/C	-0.049985116	intronic	1.67E-02	*ABAT*
rs117204884	16: 8872822	G/C	0.009805008	intronic	2.59E-02	*ABAT*

rs76613993	16: 8872942	G/A	0.00877586	intronic	4.13E-02	*ABAT*
rs75520478	16: 8872972	T/G	0.008520124	intronic	4.75E-02	*ABAT*
rs12447235	16: 8873332	G/A	0.008815819	intronic	4.02E-02	*ABAT*
rs1079348	16: 8873456	C/T	0.013796798	intronic	1.75E-02	*ABAT*
rs45545237	16: 8873576	G/C	0.005319058	intronic	4.96E-02	*ABAT*
rs1079350	16: 8873652	A/G	0.014274106	intronic	7.49E-03	*ABAT*
rs75511680	16: 8874327	C/G	0.009221711	intronic	3.53E-02	*ABAT*
rs1731073	16: 8874497	A/G	0.008359727	intronic	1.41E-04	*ABAT*
rs1075853	16: 8874779	C/T	0.009497536	intronic	3.37E-05	*ABAT*
rs2270288	16: 8875529	T/G	-0.009182665	UTR3	2.96E-05	*ABAT*
rs737695	16: 8875545	C/G	0.005010381	UTR3	3.42E-02	*ABAT*
rs1731071	16: 8875763	C/T	0.005521639	UTR3	1.73E-02	*ABAT*
rs3743798	16: 8875799	A/G	-0.008810057	UTR3	6.07E-05	*ABAT*
rs1641031	16: 8875858	C/A	0.005567964	UTR3	1.65E-02	*ABAT*
rs1641032	16: 8875861	G/A	0.005613342	UTR3	1.57E-02	*ABAT*
rs3743801	16: 8876202	G/C	-0.008592005	UTR3	8.84E-05	*ABAT*
rs4985000	16: 8876277	G/C	0.005696064	UTR3	1.38E-02	*ABAT*
rs7205816	16: 8876418	G/A	0.00806108	UTR3	2.90E-04	*ABAT*
rs17566580	16: 8876620	G/A	0.01092404	UTR3	1.25E-02	*ABAT*
rs7201586	16: 8876692	T/C	0.006907735	UTR3	5.07E-03	*ABAT*
rs1061842	16: 8876880	A/T	-0.008828309	UTR3	5.44E-05	*ABAT*
rs11648372	16: 8877401	G/A	0.00588438	UTR3	1.09E-02	*ABAT*
rs12597124	16: 8877854	C/G	0.006003939	UTR3	9.35E-03	*ABAT*
rs193136026	16: 8878639	A/C	-0.053051808	downstream	3.77E-02	*ABAT*
rs7203550	16: 8878865	C/G	0.0053456	downstream	3.27E-02	*ABAT*
rs113875073	16: 8878956	C/T	-0.045235486	downstream	2.82E-02	*ABAT*
rs12918957	16: 8879046	C/T	0.0058015	downstream	1.76E-02	*ABAT*
rs62031113	16: 8879406	C/G	-0.00865784	downstream	7.41E-05	*ABAT*
rs73501459	16: 8879596	A/G	0.010553022	intergenic	1.43E-02	*ABAT;TMEM186*
rs11641192	16: 8879669	A/G	-0.008637054	intergenic	7.71E-05	*ABAT;TMEM186*
rs11641269	16: 8879824	A/G	-0.00844124	intergenic	6.90E-04	*ABAT;TMEM186*
rs78185493	16: 8879954	A/G	0.010444247	intergenic	1.53E-02	*ABAT;TMEM186*
rs73501462	16: 8880643	T/C	0.010402711	intergenic	1.56E-02	*ABAT;TMEM186*

rs10163445	16: 8880755	A/G	0.007160336	intergenic	1.01E-03	*ABAT;TMEM186*
rs73501464	16: 8880866	T/C	0.010480527	intergenic	1.49E-02	*ABAT;TMEM186*
rs73501467	16: 8880989	G/A	0.010211163	intergenic	1.67E-02	*ABAT;TMEM186*
rs75050913	16: 8881120	A/G	0.016489361	intergenic	4.15E-02	*ABAT;TMEM186*
rs12446135	16: 8881807	A/G	0.010636966	intergenic	1.39E-02	*ABAT;TMEM186*
rs7204765	16: 8881967	C/A	0.006321303	intergenic	6.22E-03	*ABAT;TMEM186*
rs12445150	16: 8882155	C/T	0.010151977	intergenic	1.67E-02	*ABAT;TMEM186*
rs7196948	16: 8882170	A/T	-0.008412748	intergenic	1.13E-04	*ABAT;TMEM186*
rs1865806	16: 8882652	C/T	0.005884456	intergenic	6.75E-03	*ABAT;TMEM186*
rs1865805	16: 8882839	A/G	0.010371369	intergenic	1.50E-02	*ABAT;TMEM186*
rs7192702	16: 8882888	C/A	0.005761968	intergenic	1.82E-02	*ABAT;TMEM186*
rs2091632	16: 8883437	C/A	0.006272011	intergenic	4.15E-03	*ABAT;TMEM186*
rs13335327	16: 8884036	C/T	0.005757779	intergenic	1.82E-02	*ABAT;TMEM186*
rs13338256	16: 8884341	C/G	0.005934634	intergenic	1.50E-02	*ABAT;TMEM186*
rs61167351	16: 8885022	A/C	0.005884532	intergenic	2.77E-02	*ABAT;TMEM186*
rs62031119	16: 8885445	T/C	-0.008359325	intergenic	7.02E-04	*ABAT;TMEM186*
rs72766359	16: 8885792	A/G	0.005655987	intergenic	3.54E-02	*ABAT;TMEM186*
rs3743813	16: 8886128	A/G	-0.008177351	intergenic	1.71E-04	*ABAT;TMEM186*
rs3743811	16: 8886409	T/G	-0.008276422	intergenic	1.43E-04	*ABAT;TMEM186*
rs7192290	16: 8888030	T/G	0.007258744	intergenic	8.05E-04	*ABAT;TMEM186*

Note: Data were retrieved from the largest meta-analysis of AD genome-wide association study (Jansen et al. 2019. Nat Genet 51: 404-13)

Supplementary Table S2. Rare variants in the *ABAT* gene in 5,740 late-onset AD cases and 5,096 cognitively normal controls in the ADSP project

Variant	Variant type	Frequency (AD)	Frequency (CN)	*P*-value	Odds ration
16:8839881:G:C	nonsynonymous	0.0001	0	0.3656	NA
16:8839896:G:A	nonsynonymous	0.0001	0	0.3655	NA
16:8839897:T:C	nonsynonymous	0	0.0001	0.2690	0
16:8839910:T:A	stopgain	0	0.0001	0.2690	0
16:8839916:G:A	synonymous	0.0013	0.0011	0.6147	1.2280
16:8839931:G:T	synonymous	0	0.0001	0.2691	0
16:8839945:C:T	nonsynonymous	0	0.0001	0.2690	0
16:8839954:A:G	synonymous	0.3823	0.3942	0.0805	0.9513
16:8839958:G:A	intronic	0.0001	0	0.3655	NA
16:8841960:T:C	intronic	0.3898	0.4013	0.0900	0.9531
16:8841982:G:T	synonymous	0	0.0001	0.2687	0
16:8841986:A:G	nonsynonymous	0	0.0001	0.2687	0
16:8844270:T:A	intronic	0	0.0001	0.2687	0
16:8844291:G:T	nonsynonymous	0.0001	0	0.3659	NA
16:8844308:T:G	nonsynonymous	0	0.0001	0.2688	0
16:8844317:G:C	nonsynonymous	0	0.0001	0.2688	0
16:8844347:C:T	synonymous	0.0006	0.0015	0.0457	0.4085
16:8844389:C:T	synonymous	0.1097	0.1097	0.9975	0.9999
16:8844393:A:G	nonsynonymous	0.0001	0	0.3659	NA
16:8851609:C:G	intronic	0	0.0001	0.2688	0
16:8851628:G:A	nonsynonymous	0.0001	0	0.3658	NA
16:8851662:C:T	nonsynonymous	0.0001	0	0.3658	NA
16:8857952:C:T	synonymous	0.0004	0.0007	0.3543	0.5850
16:8857967:G:A	synonymous	0.0002	0.0003	0.4996	0.5450
16:8858005:C:T	nonsynonymous	0.0002	0	0.2015	NA
16:8858006:G:T	synonymous	0.0001	0	0.3661	NA
16:8858590:C:A	intronic	0.0001	0	0.3659	NA
16:8858632:C:T	nonsynonymous	0.0001	0	0.3659	NA
16:8858633:C:T	synonymous	0	0.0001	0.2687	0
16:8858640:T:G	nonsynonymous	0.0002	0	0.2009	NA
16:8858685:C:T	nonsynonymous	0.0001	0	0.3656	NA

16:8858695:T:C	intronic	0	0.0001	0.2688	0
16:8860085:G:C	nonsynonymous	0.0001	0	0.3657	NA
16:8860098:G:A	nonsynonymous	0	0.0001	0.2688	0
16:8860112:G:A	synonymous	0.0001	0	0.3658	NA
16:8860130:G:A	intronic	0.0097	0.0107	0.4710	0.9058
16:8862087:T:C	nonsynonymous	0.0036	0.0027	0.2624	1.3220
16:8862091:C:A	synonymous	0.0003	0.0002	0.8227	1.2270
16:8862678:T:A	intronic	0.0001	0	0.3661	NA
16:8862679:C:T	intronic	0.0001	0	0.3659	NA
16:8862697:C:T	nonsynonymous	0.0001	0	0.3657	NA
16:8862698:G:A	synonymous	0.0002	0.0002	0.8402	0.8177
16:8862700:A:G	nonsynonymous	0	0.0001	0.2689	0
16:8862707:A:T	nonsynonymous	0	0.0001	0.2688	0
16:8862747:A:G	nonsynonymous	0	0.0001	0.2687	0
16:8862785:G:C	nonsynonymous	0.0001	0	0.3659	NA
16:8862789:G:C	nonsynonymous	0	0.0001	0.2687	0
16:8862803:A:T	nonsynonymous	0.0001	0	0.3659	NA
16:8862827:A:G	synonymous	0.0001	0	0.3659	NA
16:8862837:C:A	intronic	0.0002	0.0001	0.6850	1.6350
16:8866631:A:G	intronic	0.0001	0.0002	0.4503	0.4088
16:8866633:C:G	intronic	0	0.0001	0.2687	0
16:8866660:T:C	synonymous	0	0.0001	0.2688	0
16:8866678:G:A	synonymous	0.0001	0	0.3658	NA
16:8866693:C:T	synonymous	0.0001	0	0.3658	NA
16:8866700:C:G	nonsynonymous	0	0.0001	0.2688	0
16:8866737:A:G	nonsynonymous	0.0001	0	0.3658	NA
16:8866748:C:T	nonsynonymous	0.0001	0	0.3659	NA
16:8866749:G:A	nonsynonymous	0.0002	0	0.2010	NA
16:8866765:C:T	synonymous	0.0001	0	0.3659	NA
16:8866775:G:A	splicing	0	0.0001	0.2687	0
16:8868776:C:A	synonymous	0.4038	0.4193	0.0244	0.9380
16:8868782:C:T	synonymous	0	0.0001	0.2688	0
16:8868839:A:T	synonymous	0	0.0001	0.2688	0

16:8868894:G:C	nonsynonymous	0.0001	0	0.3658	NA
16:8868900:T:C	nonsynonymous	0	0.0001	0.2688	0
16:8870225:C:T	synonymous	0.0011	0.0008	0.5235	1.3310
16:8870230:G:T	synonymous	0.0001	0	0.3657	NA
16:8870235:C:T	nonsynonymous	0	0.0001	0.2689	0
16:8870264:A:G	nonsynonymous	0	0.0002	0.1177	0
16:8870280:G:A	nonsynonymous	0.0002	0	0.2011	NA
16:8870319:C:A	nonsynonymous	0	0.0001	0.2689	0
16:8870332:A:T	synonymous	0	0.0002	0.1176	0
16:8870351:A:C	intronic	0.0106	0.0095	0.4195	1.1210
16:8870354:C:T	intronic	0	0.0001	0.2669	0
16:8873332:A:G	intronic	0.0672	0.0710	0.2724	0.9419
16:8873340:G:A	nonsynonymous	0.0001	0.0001	0.8866	0.8176
16:8873350:G:T	nonsynonymous	0	0.0001	0.2689	0
16:8873373:G:A	nonsynonymous	0	0.0001	0.2688	0
16:8873392:C:T	synonymous	0	0.0001	0.2689	0
16:8873412:T:C	nonsynonymous	0	0.0001	0.2687	0
16:8873415:G:A	nonsynonymous	0	0.0001	0.2682	0
16:8873431:A:T	nonsynonymous	0	0.0001	0.2673	0
16:8875159:C:T	intronic	0.0001	0	0.3659	NA
16:8875208:G:A	nonsynonymous	0.0001	0	0.3658	NA
16:8875214:C:T	nonsynonymous	0.0002	0	0.2009	NA

Note: Data were retrieved from the whole-exome sequencing data of the ADSP project (Bis et al. 2020. Mol Psychiatry 25: 1859-1875).

Supplementary Table S3. Rare variants in the *ABAT* gene in 107 AD patients and 368 control subjects from Chinese populations

Vriant	Ref/Alt	Variant type	AD patient	Control	*P*-value	Odds ratio
16:8839954	A/G	nonsynonymous	116/96	438/298	0.2355	0.8221
16:8829579	C/T	5'UTR	1/211	0/160	1	2.2766
16:8878273	T/C	3'UTR	5/195	21/715	1	0.8730
16:8870340	A/T	nonsynonymous	1/209	0/160	1	2.2983
16:8866651	T/G	nonsynonymous	1/211	0/160	1	2.2766

Note: Data were retrieved from the whole-exome sequencing data of Chinese AD patients in our recent study (Zhang et al. 2019. Natl Sci Rev 6: 257-274 and references therein).