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ORIGINAL ARTICLE

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Association between Polymorphisms of the *AKT1* Gene Promoter and Risk of the Alzheimer's Disease in a Chinese Han Population with Type 2 Diabetes

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Keywords
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SUMMARY

Aims: Alzheimer's disease (AD) is a multifactor disease that has been reported to have a close association with type 2 diabetes (T2D) where the v-akt murine thymoma viral oncogene homolog 1 (AKT1) plays an important role in the protein synthesis pathways and cell apoptosis processes. Evidence has been shown that AKT1 protein may be related to AD risk among patients with T2D. The aim of this study was to analyze the potential association between single nucleotide polymorphisms of *AKT1* promoter and the risk of AD among patients with T2D. **Methods:** The association between *AKT1* polymorphisms and AD risk in patients with T2D was assessed among 574 consecutive unrelated subjects including 112 AD patients with T2D, 231 patients with AD, and 231 healthy controls in a case–control study. The cognitive function of all subjects was assessed using MMSE. Six single nucleotide polymorphisms with minor allele frequency >0.2 (rs2498786, rs74090038, rs2494750, rs2494751, rs5811155, and rs2494752) in *AKT1* promoter were analyzed by polymerase chain reaction (PCR), and the concentration of AKT1 protein in serum was tested using enzyme-linked immunosorbent assay (ELISA). **Results:** Overall, there was statistically significant difference in *AKT1* rs2498786 polymorphism. The CC frequency of *AKT1* rs2498786 polymorphism in AD with T2D group and AD control group was significantly higher than that in healthy control group ($P_{\text{AD+T2D vs. health}} < 0.0001$, $P_{\text{AD vs. health}} < 0.0001$). However, the difference was not found between AD with T2D group and AD control group. Compared with healthy control group, the plasma levels of AKT1 protein in AD with T2D group ($P_{\text{AD+T2D vs. health}} < 0.0001$) and AD control group ($P_{\text{AD vs. health}} = 0.0003$) decreased significantly. Among genotypes of *AKT1* rs2498786 polymorphism, the AKT1 protein level in GG genotype was significantly higher than that in GC genotype ($P_{\text{GG vs. GC}} < 0.0001$) and CC genotype ($P_{\text{GG vs. CC}} < 0.0001$). **Conclusion:** The study suggests that *AKT1* rs2498786 polymorphism in insulin signaling pathway may be associated with AD risk and different genotypes may affects levels of protein expression. However, the polymorphism is not shown to be exclusive in AD patients with T2D.

Introduction

Alzheimer's disease (AD), also called senile dementia of the Alzheimer type or primary degenerative dementia of the Alzheimer's type, is a degenerative disease of the central nervous system characterized by progressive cognitive impairment and memory damage. The AD includes early-onset, late-onset, and familial AD and, most often, is diagnosed in people over 65 years of age [1]. According to data from the World Alzheimer Report, the number of people with AD is forecast to nearly double every 20 years from 36 million in 2010 to 115 million in 2050, and the costs associated with AD will reach the total of US$604 billion, about 1% of global

GDP [2]. Therefore, it is particularly urgent to gain an insight into the pathogenesis factors of AD in order to discover different possibilities of preventive and effective treatment.

So far, the clue for AD etiology is still essentially unknown and several competing hypotheses exist to try to explain the cause of the disease including cholinergic hypothesis [3], viral hypothesis (herpes simplex virus type 1) [4], and amyloid hypothesis[5, 6]. Recently, presumption about the role that type 2 diabetes (T2D) affecting approximately 6% of the world population (about 300 millions) [7] played in pathogenesis of AD has been suggested. Experimental study showed that glucose metabolism in hippocampal and cortical parts of patients with AD was significantly lower than in the control group [8]. Another study showed that insulin levels and insulin-mediated glucose metabolism in brain fluid of patients with AD were significantly reduced than in normal control group, but intraventricular administration of insulin can not only promote glucose synthesis and metabolism, but also help to improve the scale score of patients with AD [9]. Therefore, it is hypothesized that the mechanism of T2D may affect occurrence of AD, and the hypothesis is evidenced that the insulin PI3K-AKT signaling pathway in pathogenesis of T2D was associated with AD from the molecular point of view [10]. Based on these backgrounds, the abnormality of the *AKT1* gene in PI3K-AKT signaling pathway may be used extensively as a biological marker for onset of AD, as well as a unique model for deciphering the mechanisms.

Several molecular studies have investigated the associations of *AKT1* variant with diseases among populations. The findings from Karege et al. [11] suggested polymorphisms of *AKT1* gene appeared to impact the risk for a class of psychiatric symptoms of schizophrenia. Devaney et al. [12] reported that *AKT1* was a risk factor for metabolic syndrome and insulin resistance. Kim et al. [13] showed the *AKT1* polymorphisms could be used as prognostic markers for the patients with early-stage NSCLC. The result from Wang et al. [14] indicated that *AKT1* polymorphisms were associated with susceptibility to pulmonary TB. However, whether the common variants in the *AKT1* are associated with the AD in a Chinese Han population with T2D is unclear so far.

The aims of this study were to explore the relationship of *AKT1* promoter variants to occurrence of AD in patients with T2D and related traits found in a Chinese Han population and to identify the potential mechanisms underlying the associations.

Materials and Methods

Study Population and Characteristics

Consecutive patients were enrolled from the Kangci Hospital of Jiaxing, the Futian People's Hospital of Shenzhen, Shiyan Nursing Home of Shenzhen, and the First Affiliated Hospital of Harbin Medical University. The participants were classified into AD with T2D group and AD control group. T2D was diagnosed based on the diagnostic criteria defined by WHO in 1999 [15] and the American Diabetes Association in 2003 [16], or when the individual was receiving oral hypoglycemic agents or insulin injection therapy at the time of recruitment (fasting plasma glucose $\geq$7.0 mmol/L and/or 2-h plasma glucose $\geq$11.1 mmol/L), probable AD was diagnosed based on criteria consistent with the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) Alzheimer's Criteria extensively updated in 2007 [17]. Meanwhile, unrelated normal controls were recruited from healthy people who were performed physical examination in above hospitals. A written informed consent was obtained from each participant after the study was explained in detail. The study was performed with the approval of the relevant ethical committee and adhered to the tenets of the Declaration of Helsinki.

The demographic and biochemical characteristics were extensively assessed among these groups. The characteristics in our study included age, gender, education, MMSE, ApoE, and AKT protein.

SNP Selection and Genotyping

Candidate SNPs in *AKT1* were selected as follows: (i) SNPs of promoter region, (ii) SNPs from the public literatures and databases, (iii) SNPs that previously were reported to be associated with disease outcomes in epidemiological studies, and (iv) SNPs with a minor allele frequency >0.2. Finally, six tag SNPs (rs2498786, rs74090038, rs2494750, rs2494751, rs5811155, and rs2494752) were selected and genotyped. Genomic DNA was extracted from peripheral blood leukocytes using QIAGEN QIAamp DNA Mini Blood Kit (Germany). A total of 25 ng genomic DNA was amplified in a 20 ul final volume PCR containing 2 × Taq Unicorn Premix and 10 μmol of each primer. The amplification was performed at 94°C for 4 min with an initial denaturation, followed by 20 cycles of 94°C for 20 second, 57°C for 20 second, and 72°C for 20 second and a final extension of 3 min at 72°C. For more PCR fragment, the above PCR product was diluted into 1/100 and was amplified in a 25 μL final volume PCR containing 2 × Taq Unicorn Premix and 10 μmol of each primer again. The amplification was performed at 94°C for 2 min with an initial denaturation, followed by 20 cycles of 94°C for 15 second, 60°C for 15 second, and 72°C for 15 second and a final extension of 3 min at 72°C. Six sets of primers were designed as shown in Appendix Table A1. All amplified PCR products were mixed with formamide containing dextran blue dye, which was subjected to 1.2% agarose gels and visualized by staining with ethidium bromide. The SNPs were detected by the high-throughput sequencing technique using PSTAR-II plus (IDN01-M-P2). APOE genotyping was performed as described previously [18]. Data on AKT1 levels for subjects were determined using established ELISA methods (Yuanye, Shanghai).

Statistical Analysis

In univariate analyses, *Z*-test for quantitative data and chi-square test or Fisher's exact test for qualitative data were used to determine whether there was significant difference in relevant factors between cases and controls. Chi-square test was carried out to assess the deviations from the Hardy–Weinberg equilibrium (HWE) and frequencies of genotype and allele of *AKT1* among cases and controls. Haplotype analyses were conducted using the PHASE 2.0 (University of Washington). The most common haplotype served as the referent, which happened to be the wild-type

allele for all six SNPs. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were estimated to compare cases to controls in association with haplotypes. Logistic regression analysis was used to estimate the association between *AKT1* polymorphisms and AD risk by adjusting for confounding factors. The function of polymorphisms with statistical significance in the *ATK1* gene promoter was predicted by the bioinformatics software AliBaba2.1 (developed by Niels Grabe). All statistical tests were 2-sided, and statistical significance was taken as P value less than 0.05.

Results

Baseline Characteristics

The baseline characteristics of all participants in the study are summarized in Table 1. In 574 participants, 112 were AD patients with T2D, 231 were patients with AD, and 231 were healthy controls. The three groups were age- and gender-matched. The mean age was 80.1 years (±8.7 years) for the AD patients with T2D, 79.6 years (±9.9 years) for the patients with AD, and 78.5 (±8.4 years) for the healthy controls. The gender (male/female) ratio was 1:1.3 in the AD patients with T2D, 1:1.1 in the patients with AD, and 1:1.1 in the healthy control. There were no statistical differences in education among three groups. AD patients with T2D displayed higher *APOE ε4+* frequency and lower MMSE scores than patients with AD and healthy controls.

Polymorphisms of *AKT1* Gene and the Risk of AD in Patients with Type 2 Diabetes

The genotype and allele frequencies of six SNPs in the study are shown in Table 2. The deviation from Hardy–Weinberg equilibrium for all the polymorphisms examined was absent in the distributions of genotypes in healthy control group and, however, was found in AD with T2D group and AD control group.

When compared with genotype distributions in healthy control group ($P_{\text{AD+T2D vs. health}} < 0.0001$, $P_{\text{AD vs. health}} < 0.0001$), the CC genotype of *AKT1* rs2498786 in AD with T2D group and AD control group had significantly higher frequency shown by the chi-square test. However, the differences in genotype distribution were not found between AD with T2D group and AD control group ($P_{\text{AD+T2D vs. AD}} = 0.4826$). In addition, significant differences were observed in the frequency of the *AKT1* rs2498786 G→C alleles between AD with T2D group and healthy control group ($P < 0.0001$), and AD control group and healthy control group ($P < 0.0001$), with the frequency of the C allele being higher in AD patients with T2D and patients with AD (Table 2). However, no significant differences were observed among other polymorphisms. After adjustment for confounding factors (age, gender, and *APOE* status), the results still remained robust. In addition, all haplotype analyses are shown in Table 3.

The Level of AKT1 Protein and the Risk of AD in Patients with Type 2 Diabetes

The level of AKT1 protein was measured in AD with T2D group, AD control group, and healthy control group (Table 4). Significantly lower plasma AKT1 level was detected in the AD with T2D group ($P < 0.0001$) and the AD control group ($P = 0.0003$) than that in healthy control group. However, the difference in level of AKT1 protein was not found between AD with T2D group and AD control group ($P = 0.4546$).

The Association of *AKT1* rs2498786 Polymorphism with AKT1 Protein Level

The association between *AKT1* rs2498786 and AKT1 protein level was analyzed among three genotypes (Table 5). Among the genotypes of rs2498786, the AKT1 protein levels in subjects with GC ($P < 0.0001$) and CC ($P < 0.0001$) genotypes were significantly lower than that in the subjects with GG genotype. The genotype-related differences in AKT1 protein levels were also observed in the subgroup of AD with T2D, AD control, and healthy control subjects, and the lowest AKT protein levels were found in subjects involving GC and CC genotypes.

The Function Prediction of *AKT1* rs2498786 Polymorphism

The predicted result from the bioinformatics software showed that the polymorphism C had not binding site with other proteins. However, the substitution of C to G obtained potential ability to combine with 2 transcription factors (ADR1 and Sp1).

Discussion

Although the role of T2D in AD development is not fully elucidated, it has been proposed that it could be responsible for the risk of AD [19]. In T2D individuals, there is a tendency of AD preva-

Table 1 Baseline characteristics of cases with type 2 diabetes and controls

Demographic characteristics	AD with T2D group	AD control group	Healthy control group	$P_{\text{(AD+T2D vs. AD)}}$	$P_{\text{(AD+T2D vs. health)}}$
N	112	231	231		
Age	80.1 ± 8.7	79.6 ± 9.9	78.5 ± 8.4	0.6488	0.1030
Gender (M/F)	48/64	113/118	113/118	0.2916	0.2916
Education	8.6 ± 4.1	8.7 ± 3.6	9.1 ± 3.7	0.8179	0.2583
Apoε4(+)	54	67	39	**0.0005**	**<0.0001**
MMSE scores	16.4 ± 5.2	18.6 ± 3.9	28.3 ± 2.7	**<0.0001**	**<0.0001**

Continuous data were expressed as the means ± SEM.
Bold values represent statistical significance.

Table 2 Genotype and allele frequencies of the AKT1 polymorphism, and their association with risk of AD among AD with T2D group, AD group, and controls group

Reference SNP ID	Genotype and allele	AD with T2D group N (%)	AD control group N (%)	Healthy control group N (%)	$P_{(AD+T2D\ vs.\ AD)}$	$P_{(AD+T2D\ vs.\ health)}$	$P_{(AD\ vs.\ health)}$	P_{HWE}
AKT1 rs2498786	GG	49 (43.7)	110 (47.6)	136 (58.9)	0.4826	**<0.0001**	**<0.0001**	AD with T2D group: **<0.001** AD group: **<0.001** Healthy control group: 0.8851
	GC	34 (30.4)	56 (24.3)	83 (35.9)				
	CC	29 (25.9)	65 (28.1)	12 (5.2)				
	G	132 (58.9)	276 (59.7)	355 (76.8)	0.8391	**<0.0001**	**<0.0001**	
	C	92 (41.1)	186 (40.3)	107 (23.2)				
AKT1 rs74090038	CC	88 (78.6)	180 (77.9)	181 (78.4)	0.1642	0.9635	0.9104	AD with T2D group: 0.2041 AD group: **0.0441** Healthy control group: 0.0651
	CT	24 (21.4)	44 (19.1)	50 (21.6)				
	TT	0 (0)	7 (3.0)	0 (0)				
	C	200 (89.3)	404 (87.4)	412 (89.2)	0.4816	0.9658	0.4127	
	T	24 (10.7)	58 (12.6)	50 (10.8)				
AKT1 rs2494750	CC	14 (12.5)	32 (13.9)	33 (14.3)	0.4643	0.8912	0.4510	AD with T2D group: 0.7484 AD group: **0.0164** Healthy control group: 0.2212
	CG	49 (43.8)	85 (36.8)	97 (42.0)				
	GG	49 (43.8)	114 (49.3)	101 (43.7)				
	C	77 (34.4)	149 (32.3)	163 (35.3)	0.5789	0.8154	0.3301	
	G	147 (65.6)	313 (67.7)	299 (64.7)				
AKT1 rs2494751	AA	14 (12.5)	33 (14.3)	31 (13.4)	0.4201	0.9692	0.3538	AD with T2D group: 0.7484 AD group: **0.0095** Healthy control group: 0.3931
	AG	49 (43.8)	84 (36.4)	99 (42.9)				
	GG	49 (43.8)	114 (49.3)	101 (43.7)				
	A	77 (34.4)	150 (32.5)	161 (34.8)	0.6186	0.9028	0.4438	
	G	147 (65.6)	312 (67.5)	301 (65.2)				
AKT1 rs5811155	TT	14 (12.5)	33 (14.3)	31 (13.4)	0.4201	0.9692	0.3538	AD with T2D group: 0.7484 AD group: **0.0095** Healthy control group: 0.3931
	Tdel	49 (43.8)	84 (36.4)	99 (42.9)				
	deldel	49 (43.8)	114 (49.3)	101 (43.7)				
	T	77 (34.4)	150 (32.5	161 (34.8)	0.6186	0.9028	0.4438	
	del	147 (65.6)	312 (67.5)	301 (65.2)				
AKT1 rs2494752	GG	10 (8.9)	29 (12.5)	25 (10.8)	0.5910	0.8157	0.6498	AD with T2D group: 0.6915 AD group: **0.0266** Healthy control group: 0.3771
	GA	44 (39.3)	84 (36.4)	93 (40.3)				
	AA	58 (51.8)	118 (51.1)	113 (48.9)				
	G	64 (28.6)	142 (30.7)	143 (31.0)	0.5619	0.5241	0.9432	
	A	160 (71.4)	320 (69.3)	319 (69.0)				

$P_{(AD+T2D\ vs\ AD)}$, $P_{(AD+T2D\ vs\ health)}$ and $P_{(AD\ vs\ health)}$ represent the whole comparison among three genotypes in both groups when exploring the genotype difference.

Bold values represent statistical significance.

lence to be higher than in non-T2D, which may be related to the decreased activity of insulin signaling pathway of T2D [10].

The insulin PI3K-AKT signaling pathway is the most discussed topic by many studies because it has been found to prevent excessive accumulation of Aβ protein and abnormal phosphorylation of tau protein that contributed to senile plaques and neurofibrillary tangles in AD by downregulating GSK3 level [20] when exploring the association between T2D and AD risk. Therefore, the insulin PI3K-AKT signaling pathway is considered to be the pathobiochemical basis for the drastic reduction in glucose/energy metabolism in Alzheimer's brain [21].

In our study, we examined whether polymorphic variation of *AKT1* gene encoding AKT1 protein in the insulin PI3K-AKT signaling pathway was associated with genetic risk for AD among T2D population. AKT1 protein is an important serine/threonine protein kinase that plays a key role in mediating the effects of insulin [or insulin-like growth factor (IGF)] regulation of neuronal survival and promoting the survival of a range of cell types in response to various growth factors [22]. Activation of AKT1 is favorable to upstream of Aβ protein and normal phosphorylation of Tau protein that is associated with AD by inhibiting GSK3 and promoting mTORC1. In view of the above-mentioned involvement of AKT1 protein in a wide range of cellular functions in neuronal survival, it is obvious to consider its protective role in various conditions such as nervous system (AKT1 has been shown to phosphorylate both Thr212 and Ser214 in the longest and

Table 3 Haplotype frequencies in the promoter region of *AKT1* gene among three groups

Haplotypes	AD with T2D group n (%)	Control group n (%)	OR (95% CI)	*P*
G-C-C-A-T-G	22 (9.82)	18 (3.9)	1	–
G-C-C-C-del-G	36 (16.07)	88 (19.05)	0.3347 (0.1607–0.6972)	**0.0028**
G-C-C-C-del-A	14 (6.25)	14 (3.03)	0.8182 (0.3108–2.1538)	0.6843
G-C-G-A-T-A	15 (6.7)	9 (1.95)	1.3636 (0.4845–3.8383)	0.5564
G-C-G-C-del-A	4 (1.79)	2 (0.43)	1.6364 (0.2683–9.9796)	0.6836
G-C-G-C-del-A	32 (14.29)	196 (42.42)	0.1336 (0.0646–0.2762)	**<0.0001**
C-C-G-A-T-A	17 (7.59)	50 (10.82)	0.2782 (0.1212–0.6387)	**0.0021**
C-C-G-C-del-A	49 (21.88)	5 (1.08)	8.0182 (2.6394–24.3587)	**<0.0001**
C-T-C-A-T-G	2 (0.89)	12 (2.6)	0.1364 (0.0269–0.6900)	**0.0083**
C-T-G-A-T-A	18 (8.04)	7 (1.52)	2.1039 (0.7199–6.1489)	0.1705
	AD with T2D group n (%)	AD control group n (%)		
G-C-C-A-T-G	22 (9.82)	35 (7.58)	1	–
G-C-C-A-T-A	3 (1.34)	8 (1.73)	0.5966 (0.1428–2.4931)	0.7341
G-C-C-C-T-G	3 (1.34)	1 (0.22)	4.7727 (0.4666–48.8164)	0.2958
G-C-C-C-del-A	36 (16.07)	75 (16.23)	0.7636 (0.3927–1.4850)	0.4262
G-C-C-C-del-A	14 (6.25)	10 (2.16)	2.2273 (0.8435–5.8815)	0.1026
G-C-G-A-T-A	15 (6.7)	49 (10.61)	0.4870 (0.2218–1.0695)	0.0708
G-C-G-C-del-G	4 (1.79)	5 (1.08)	1.2727 (0.3080–5.2592)	0.7304
G-C-G-C-del-A	32 (14.29)	66 (14.29)	0.7713 (0.3907–1.5228)	0.4539
C-C-G-A-T-A	17 (7.59)	1 (0.22)	27.0455 (3.3583–217.8081)	**<0.0001**
C-C-G-C-del-A	49 (21.88)	141 (31.52)	0.5529 (0.2961–1.0324)	0.0610
C-T C-A-T-G	2 (0.89)	15 (3.25)	0.2121 (0.0442–1.0184)	**0.0381**
C-T-G C-del-G	18 (8.04)	17 (3.68)	1.6845 (0.7194–3.9440)	0.2280
	AD control group n (%)	Control group n (%)		
G-C-C-A-T-G	35 (7.58)	18 (3.9)	1	–
G-C-C-A-T-A	8 (1.73)	1 (0.22)	4.1143 (0.4768–35.5041)	0.2533
G-C-C-C-del-G	75 (16.23)	88 (19.05)	0.4383 (0.2296–0.8367)	**0.0113**
G-C-C-C-del-A	10 (2.16)	14 (3.03)	0.3673 (0.1364–0.9894)	**0.0444**
G-C-G-A-T-A	49 (10.61)	9 (1.95)	2.8000 (1.1269–6.9572)	**0.0237**
G-C-G-C-del-A	66 (14.29)	196 (42.42)	0.1732 (0.0919–0.3262)	**<0.0001**
G-T-C-A-T-A	4 (0.87)	10 (2.16)	0.2057 (0.0565–0.7484)	**0.0115**
G-T-G-A-T-A	11 (2.38)	12 (2.6)	0.4714 (0.1741–1.2767)	0.1357
C-C-G-A-T-A	1 (0.22)	50 (10.82)	0.0103 (0.0013–0.0807)	**<0.0001**
C-C-G-C-del-A	141 (30.52)	5 (1.08)	14.5029 (5.0362–41.7640)	**<0.0001**
C-T-C-A-T-G	15 (3.25)	12 (2.6)	0.6429 (0.2490–1.6595)	0.3598
C-T-G-A-T-A	17 (3.68)	7 (1.52)	1.2490 (0.4380–3.5614)	0.6772

Bold values represent statistical significance.

shortest tau isoforms, which may be involved in phosphorylation of tau relevant to AD and other neurodegenerations [23]). Furthermore, AKT1 activity was often affected by the polymorphic variation of *AKT1* gene. Previous studies have shown that polymorphisms of the *AKT1* gene changed AKT expression activity [24, 25]. Subsequently, these findings provide evidence that *AKT1* polymorphisms may lead to variability in the insulin signaling pathway. Based on these data, it is hypothesized in our study that *AKT1* polymorphisms may be potentially associated with the AD in patients with T2D. As is expected, our findings demonstrated a significant association between the *AKT1* rs2498786 polymorphism and AD risk in the AD with T2D group and AD control group compared with healthy control group. The association is possible because the rs2498786 polymorphism is located in promoter in *AKT1* and may have the ability to regulate AKT1 protein expression. In fact, in our study, we found the lower AKT1 protein level in AD with T2D group and AD control group, and the genotype-related differences in AKT1 protein levels in the subgroup of AD with T2D, AD control, and healthy control subjects. Meanwhile, AKT1 mutant modulated reward learning and reward prediction error related to AD development in mice model. Therefore, it is speculated that the aberrant modulation of

Table 4 Plasma levels of AKT1 protein in the AD with T2D group, AD control group, and healthy control group

Protein	AD with T2D group	AD control group	Healthy control group	$P_{(AD+T2D\ vs.\ AD)}$	$P_{(AD+T2D\ vs.\ health)}$	$P_{(AD\ vs.\ health)}$
AKT (μmol/L)	14.6 ± 4.0	14.9 ± 3.2	16.0 ± 3.3	0.4546	**<0.0001**	**0.0003**

Bold values represent statistical significance.

Table 5 Plasma levels of AKT1 protein according to the *AKT1* genotypes

	GG	GC	CC	$P_{(GG\ vs.\ GC)}$	$P_{(GG\ vs.\ CC)}$
Total (N = 574)	295	173	106		
AKT1 (μmol/L)	17.0 ± 3.3	14.2 ± 2.7	12.2 ± 3.8	**<0.0001**	**<0.0001**
AD with T2D group (n = 112)	49	34	29		
AKT1 (μmol/L)	16.9 ± 2.8	13.7 ± 3.3	11.9 ± 3.1	**<0.0001**	**<0.0001**
AD control group (n = 231)	110	56	65		
AKT1 (μmol/L)	16.6 ± 2.2	14.5 ± 3.2	12.4 ± 4.6	**<0.0001**	**<0.0001**
Healthy control group (n = 231)	136	83	12		
AKT1 (μmol/L)	17.3 ± 3.7	14.3 ± 2.5	12.2 ± 4.6	**<0.0001**	**<0.0001**

Bold values represent statistical significance.

AKT1 polymorphism on AKT1 protein may affect the insulin signaling pathway leading to AD development. However, the association between the *AKT1* rs2498786 polymorphism and AD was independent of T2D status, which may be explained by the increasing evidence demonstrating that AD has some pathological features in common with T2D, and the two disorders may share a similar etiology [26].

In this study, the genotype-related differences in AKT1 protein levels were also observed in the subgroup of AD with T2D, AD control, and healthy control subjects. Among the examined SNP, the levels of AKT1 protein in subjects with GC and CC genotypes of rs2498786 were significantly lower than that of the subjects with GG genotype, which is speculated that level of AKT1 protein may be relevant to SNP of *AKT1*. Moreover, the findings have the resonance with other studies that demonstrated the attribute of the genotype-related difference in AKT1 protein levels [24, 25].

So far, many publications have shown genetic associations of polymorphisms in and upstream of the *AKT1* gene with human phenotypes. Karege et al. suggested *AKT1* polymorphisms (rs3803300, rs2494732, rs2498804) may be associated with pathogenesis of both schizophrenia and bipolar disorder [11, 24]. Devaney et al. demonstrated that *AKT1* variant (rs1130214) may be used as a marker in the endophenotypes that made up metabolic syndrome [12]. Xiromerisiou et al. showed that variability (rs2498788) within *AKT1* gene had a role as a risk factor for Parkinson's disease [25]. However, most studies mainly focused on regions like exons, introns, and 3′ UTR in *AKT1* gene, and few studies were performed on promoter in *AKT1* gene, especially six polymorphisms of promoter included in our study. We further predicted the function of *AKT1* rs2498786 polymorphism with statistical significance, and the finding from bioinformatics software showed that the substitution of C to G obtained potential ability to combine with 2 transcription factors (ADR1 and Sp1). In fact, Sp1 has been demonstrated to bind to GC boxes of the promoters of several genes expressed in a wide variety of tissues [27, 28], which has the resonance with our predicted findings. The binding phosphorylated Sp1 to regulate the effects of Sp1 on gene expression [29, 30]. For example, Sp1 has been shown to positively regulate of the expression of APP [31], the expression of BACE1 associated with APP cleavage [32], and the expression of tau [33]. Therefore, it is speculated that the potential binding of Sp1 to *AKT1* rs2498786 polymorphism may affect the expression of hallmarks involved in the pathology of AD.

In conclusion, this study is the first report on the relationship of *AKT1* to AD in patients with T2D. Our findings supported the hypothesis that the genetic variant in the *AKT1* rs2498786 may contribute to the occurrence of AD. But the polymorphism may be not exclusive in AD patients with T2D due to no difference in genotype distribution between AD patients with T2D and simple patients with AD. The findings may help to evaluate individual susceptibility and explore the effective measures of disease control and prevention. Regardless, these results need further epidemiological studies to confirm the relationship of molecular mechanism of *AKT1* gene to risk of AD in patients with type 2 diabetes.

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Conflict of Interest

The authors declare no conflict of interest.

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Appendix 1

Table A1 The information on primers of *AKT1* gene in this study

rs number	Primer name	Primer sequence
AKT1	JYDT-SNP-16(90)F	5′ ATTCGTCCCTGACCTGTCTC 3′
rs2498786	JYDT-SNP-16(90)R	5′ AGTTTCCCCGTCTGTAAAGTG 3′
AKT1	JYDT-SNP-17(104)F	5′ GAGGAGGAGCGGTGTCTAGG 3′
rs74090038	JYDT-SNP-17(104)R	5′ CCCAGTGGACTTCGGACTG 3′
AKT1	JYDT-SNP-18(106)F	5′ CGGGTATGGAATGAGTAAGTGG 3′
rs2494750	JYDT-SNP-18(106)R	5′ CGGAGGAACTTCTGGCTAGG 3′
AKT1	JYDT-SNP-19(82)F	5′ GTGACTGCCACCCCGACC 3′
rs2494751	JYDT-SNP-19(82)R	5′ TATCAACTGTGGGCCTCTGG 3′
AKT1	JYDT-SNP-21(105)F	5′ CCCAGCTCAGACTTTGTAACC 3′
rs5811155	JYDT-SNP-21(105)R	5′ CAACCCTTGTGTCAGGTATCC 3′
AKT1	JYDT-SNP-22(103)F	5′ TGGGCTCTGCCATGCAAG 3′
rs2494752	JYDT-SNP-22(103)R	5′ CCACATCCCCAAGCCTCG 3′