Original Article
Glycogen synthase kinase-3β (GSK-3β) rs334558 polymorphism is not associated with mild cognitive impairment in Chinese Han type 2 diabetic patients

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Abstract: *Background and objective:* Activation of glycogen synthase kinase-3β (GSK-3β) increases the risk of insulin resistance and type 2 diabetes mellitus (T2DM). Considering the association between GSK-3β rs334558 polymorphism and Alzheimer's disease, we aimed to investigate the association between GSK-3β rs334558 polymorphism and mild cognitive impairment (MCI) in T2DM patients. *Methods:* This case-control study was performed to evaluate the association between GSK-3β rs334558 polymorphism and MCI in the recruited 88 Chinese Han T2DM patients, 51 of which satisfied the MCI diagnostic criteria and 37 matched individuals with healthy cognition as the control. *Results:* Genotype and allele distributions of GSK-3β rs334558 polymorphism in the MCI patients were not significantly different from those in healthy-cognition controls (χ^2=4.377, df=2, P=0.112 and χ^2=0.031, df=1, P=0.859, respectively). There was no significant difference in the serum GSK-3β concentration between the two groups (16.40 ± 16.61 ng/ml *vs.* 18.63 ± 16.07 ng/ml, P>0.05). Nor difference was found between the two groups in terms of GSK-3β genotypes (CC, TC and TT, all P>0.05). Neuropsychological test scores were not significantly different between genotypic subgroups in either the MCI group or control group (all P>0.05). *Conclusions:* Our findings failed to identify the association between the GSK-3β rs334558 polymorphism and diabetic MCI. GSK-3β rs334558 polymorphism might not be a stratification marker to predicate the disease risk in China.

Keywords: Glycogen synthase kinase-3β, polymorphism, mild cognitive impairment, type 2 diabetes mellitus

Introduction

Patients with diabetes have an increased risk of developing mild cognitive impairment (MCI) [1, 2], which is considered as a transitional stage between normal cognition and dementia. Diabetes contributes to MCI via a variety of ways, e.g. impaired insulin signaling. Nevertheless, the exact mechanism is complex and remains unclear.

The activity of GSK-3β may increase when insulin signaling function is weakened. The activation of GSK3β inhibits the secretory cleavage of the amyloid precursor protein (APP), increasing the production of the $A\beta_{42}$ peptide [3], and leads to memory impairment. Additionally, total GSK-3 protein increases in both AD and MCI without a compensatory decrease in activity [4]. It has been reported that GSK-3β can phosphorylate numerous proteins, such as tau, a

microtubule-associated protein which is mainly expressed in neurons and related to AD [5]. Aβ accumulation and tau hyperphosphorylation, the major pathological features of AD, have also been suggested to be involved in the pathogenesis of diabetic MCI [6, 7]. A light and electron microscopy study showed that GSK-3β expression elevates in intracellular locations and tangles in an old bigenic mouse with combined amyloid and tau pathology (BiAT) mice [8]. These data suggest that GSK-3β overexpression in specific brain areas may be associated with the pathology of diabetic MCI, and GSK-3β assays might be an available diagnostic marker.

In addition, it was reported that rs334558 polymorphism in GSK-3β gene was associated with an increased risk of AD [9-11]. GSK-3β (-50) TT/CT genotype is associated with an increased risk for AD, especially the late-onset AD. Genotypes with TT and T allele raises the risk of

developing AD compared to the control individuals [9, 11]. Previous studies revealed that very low expression has been found when the region between nt-171 and +29 was deleted. GSK-3β (-50) polymorphism is localized within the effective promoter region, indicating the possible functional role of GSK-3β rs334558 [12].

GSK-3β or GSK-3β rs334558 polymorphism and cognitive decline have been extensively investigated. However, the role of GSK-3β in diabetes-related cognitive deficits and the underlying genetic factor in this population are still not well understood. China is a country with high-incidence of type 2 diabetes mellitus (T2DM). This study was designed to investigate the association between the GSK-3β rs334558 polymorphism and MCI in Chinese diabetic patients. We also intended to seek the association between serum GSK-3β levels and cognition performance. Our findings may provide us more insights into the potential pathogenesis of T2DM with MCI and therapeutic targets for cognitive impairment.

Materials and methods

Clinical subjects and study design

This case-control study was conducted in the Endocrinology Division of Zhongda Hospital of Southeast University. All of the individuals provided written informed consent before they participated in the study, which was approved by the Research Ethics Committee of the Affiliated Zhongda Hospital of Southeast University. This trial was registered with ClinicalTrials.gov, number ChiCTR-OCC-15006060.

This study recruited a total of 88 subjects (36 women and 52 men) that all satisfied the diagnostic criteria of type 2 diabetes [13]. All of the participants were right-handed Han Chinese. 51 subjects (24 women and 27 men, mean age: 62.71 ± 9.19 years) satisfied the diagnostic criteria proposed by the MCI Working Group of the European Consortium on Alzheimer's Disease [14]: (1) cognitive complaints coming from patients themselves or their families; (2) reporting a decline in cognitive functioning relative to that of the past year by the patient or informant (CDR score of 0.5); (3) cognitive disorders as evidenced by clinical evaluation (impairment in memory or some other cognitive domains); (4) absence of major repercussions on daily life

(ADL score <26); and (5) absence of dementia (DSM-IV). And 37 T2DM patients with healthy cognition (12 women and 25 men, mean age: 59.86 ± 7.95 years) matched in terms of age, sex were also selected into our study.

Participants with diabetic ketoacidosis, hyperosmolar nonketotic diabetic coma, severe hypoglycemia, acute cardiovascular, cerebrovascular accident, a past history of known stroke, head injury, alcoholism, Parkinson's disease, epilepsy, major depression (excluded by SDS) or other mental or psychiatric illnesses (excluded by clinical assessment and case history), major medical illnesses (e.g., cancer, anemia, and thyroid dysfunction), and severe visual or hearing loss were excluded from this study.

Clinical measurements

Clinical data collection: Demographic characteristics, including age, gender, educational levels, contact information and ethnicity, were collected. Their medical history (including hypertension and coronary heart disease), and physical measurements (including blood pressure, weight, height) were also collected using standardized methods. Body mass index (BMI) was defined as the individual's body weight divided by the square of his or her height and was calculated as body weight (kg)/body height (m²). Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg. Blood samples were obtained to determine fasting blood glucose, HbA1c, triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C). The internal and external quality control procedures were performed by the Laboratory Center of Zhongda Hospital of Southeast University directed by the Chinese Laboratory Quality Control.

Neuropsychological test information: To evaluate each individual's cognitive function, including the function of semantic memory, attention, psychomotor speed, executive function, and visuospatial skills, a battery of neuropsychological tests were administrated to all subjects. Montreal Cognitive Assessment (MoCA), Digit Span Test (DST), Verbal Fluency Test (VFT), Clock Drawing Test (CDT), Word Similarity Test (ST), Trail Making Test-A and B (TMT-A and TMT-B) were performed. Other

Table 1. Demographic, clinical and cognitive characteristics

Characteristic	MCI (N=51)	Non-MCI (N=37)	p-value
Age (years)	62.71 ± 9.19	59.86 ± 7.95	0.134*
Female, n (%)	24 (47.06)	12 (32.43)	0.168‡
Education Levels (years)	9.00 (5.00-12.00)	11.50 (9.00-14.50)	0.003†
Smoking, n (%)	19 (37.25)	16 (43.24)	0.576‡
Alcohol, n (%)	16 (31.37)	9 (24.32)	0.455‡
BMI (kg/m^2)	25.19 ± 3.71	24.87 ± 2.86	0.749*
Hypertension, n (%)	35 (68.63)	21 (56.76)	0.439‡
CHD, n (%)	9 (17.65)	3 (8.11)	0.177‡
Diabetes duration (years)	11.12 ± 5.61	8.81 ± 4.43	0.041*
HbA1c (%)	9.16 ± 2.17	8.93 ± 1.94	0.609*
Triglyceride (mmol/L)	1.83 ± 1.42	2.08 ± 1.35	0.399*
Total cholesterol (mmol/L)	5.09 ± 1.46	4.94 ±1.09	0.612*
LDL-cholesterol (mmol/L)	3.05 ± 0.86	2.95 ± 0.68	0.549*
HDL-cholesterol (mmol/L)	1.20 ± 0.26	1.21 ± 0.19	0.819*
Cognition test levels			
MoCA	22.00 (16.75-23.25)	26.00 (27.00-28.00)	<0.001†
TMT-A	85.00 (64.00-107.00)	64.00 (51.50-70.00)	0.002†
TMT-B	195.00 (140.25-261.25)	155.00 (127-180.00)	0.011†
CDT	4.00 (2.00-4.00)	4.00 (4.00-4.00)	<0.001†
DST	11.00 (8.50-12.00)	12.00 (11.00-14.00)	<0.001†
VFT	16.00 (13.00-19.00)	18.00 (16.00-22.00)	0.003†
ST	7.00 (4.50-9.00)	10.00 (8.00-12.00)	<0.001†

Data are presented as n (%), mean ± SD, or median (interquartile range) as appropriate. *Student's t test. †Mann-Whitney U test. ‡Chi-square test. Abbreviations: MCI, mild cognitive impairment; Non-MCI, participations without MCI; BMI, body mass index; CHD, coronary heart disease; LDL, low density lipoprotein; HDL, high density lipoprotein; MoCA, Montreal Cognitive Assessment; TMT-A, Trail Making Test-A; TMT-B, Trail Making Test-B; CDT, Clock Drawing Test; DST, Digit Span Test; VFT, Verbal Fluency Test; ST, Similarities Test.

tests included Hamilton Depression Scale (HAMD), and self-rating depression scale (SDS). These tests were performed by an experienced neuropsychiatrist who was blinded to the study design. None of the participants displayed audiovisual or motor coordination deficits that could affect the process.

Genotyping of GSK-3β rs334558 polymorphism

Genomic DNA was extracted from EDTA-treated venous blood using the DNA purification kit (Puregene, GentraSystem, Minneapolis, MN). Polymerase chain reaction (PCR) restriction fragment length polymorphism-based genotyping was performed to genotype the variants of the GSK-3β gene (rs334558). The following sense and antisense primers were respectively used: 5'-CTCGCTTCCTTCCTTCCTTT-3' and 5'-CCGTCCTCAACTCTTCAAGC-3'. PCR was conducted in 30 µL reaction mixtures containing 20.8 µL H$_2$O, 3 µL 10×PCR buffer, 2 µL DNA, 1 µL primer forward (10 pmol), 1 µL primer reverse (10 pmol), 0.2 µL Ex Taq and 2 µL dNTP. The amplification condition was initiated at 96°C for 5 mins, followed by 30 cycles consisting of denaturation at 96°C for 20 s, 62°C for 20 s, and 72°C for 60 s, with a final extension step at 72°C for 5 min. PCR amplification product was electrophoresed on a 1.5% agarose gel at 100 V for 20 min. The presence of 220 base pair (bp) and 124 bp bands indicated the existence of T allele, the presence of 344 bp bands indicated the existence of C allele, and the presence of 344 bp, 124 bp and 220 bp bands indicated the existence of AG heterozygote.

Measurement of serum level of GSK-3β

Blood samples (2 mL) of diabetic patients were collected in EDTA-containing tubes and centrifuged at 1000 *g* for 15 min. Serum was collected and kept frozen at -80°C until assayed. GSK-

Table 2. Distributions of GSK-3β genotype and allele frequencies between groups

Group	Genotype, N (%)				Allele, N (%)		
	CC	TC	TT	*p*-value*	C	T	*p*-value*
MCI group	16 (31.37)	30 (58.82)	5 (9.80)	0.112	62 (60.78)	40 (39.22)	0.859
Control group	15 (40.54)	14 (37.84)	8 (21.62)		44 (59.46)	30 (40.54)	

Data are presented as n (%). *Chi-square test. Abbreviations: MCI, mild cognitive impairment; GSK-3β, glycogen synthase kinase-3β.

Table 3. Serum level of GSK-3β (ng/ml) in MCI and normal control patients with T2DM

Group	Total	Genotype			*p*-value†
		CC	TC	TT	
Total	17.33 ± 16.33	14.50 ± 12.83	18.74 ± 18.19	19.37 ± 17.46	0.753
Non-MCI	18.63 ± 16.07	14.83 ± 13.29	19.54 ± 17.14	24.18 ± 19.04	0.461
MCI	16.40 ± 16.61	14.19 ± 12.82	18.37 ± 18.93	11.67 ± 12.63	0.665
p-value*	0.355	0.968	0.529	0.143	

Data are presented as mean ± SD. *Student's t test. †Analysis of variance (ANOVA). Abbreviations: GSK-3β, glycogen synthase kinase-3β; MCI, mild cognitive impairment; T2DM, type 2 diabetes mellitus.

3β concentration was assessed using the ELISA kits (Uscn Life Science Inc, Wuhan, Hubei, China) according to the manufacturer's instructions. Serum levels of GSK-3β of all subjects were measured on the same day to minimize the assay variance.

Statistical analysis

Statistical analyses were conducted using SPSS version 19.0 (SPSS Inc., Chicago, IL). All of the tests were two sided, and statistical significance was defined as $P<0.05$. Data were reported as mean ± standard deviation (SD), median (interquartile range) or percentage as appropriate. Student's *t* test and ANOVA were employed for normally distributed variables; nonparametric Mann-Whitney *U* and Kruskal-Wallis tests were employed for asymmetrically distributed variables. Chi-squared (χ^2) test was used for the Hardy-Weinberg equilibrium, which compares allelic and genotypic distributions (Santiago Rodriguez, Tom R. Gaunt, and Ian N. M. Day, Hardy-Weinberg Equilibrium Testing of Biological Ascertainment for Mendelian Randomization Studies). The reference group comprised the carriers of the genotype CC. The association between polymorphism and the risk of MCI was estimated using odds ratio (OR) and 95% confidence interval. Crude OR was computed using Mantel-Haenszel χ^2 test. The correlation between serum GSK-3β concentration and clinical parameters were examined by Pearson or Spearman rank correlation. Multiple

step-wise regression analysis was used to explore the relationship between the cognitive measures and demographic factors, serum GSK-3β levels.

Results

Demographic, clinical and neuropsychological characteristics

The demographic, clinical, and neuropsychological tests of the participants were summarized in **Table 1**. The MCI and healthy-cognition diabetic patients were well matched in terms of age, sex, smoking, alcohol use, BMI, hypertension and coronary heart diseases (CHD) prevalence (all $P>0.05$). Education level of the MCI patients was lower and their diabetes duration was longer than that of non-MCI patients (all $P<0.05$). No significant differences were found in HbA1c and lipid levels between two groups (all $P>0.05$). The scores in the neuropsychological tests of T2DM patients with MCI were significantly lower than those with healthy cognition (all $P<0.01$).

Distributions of GSK-3β genotype and allele frequencies between groups

The GSK-3β genotypes and allele frequencies of MCI patients and control subjects were given in **Table 2**. The distributions of GSK-3β genotypes were consistent with Hardy-Weinberg equilibrium both in the non-MCI (χ^2=1.71, df=1,

Table 4. Comparison of cognitive test levels according to GSK-3β genotypic subgroups

Cognitive performances	MCI group				Non-MCI group			
	CC	TC	TT	*p*-value*	CC	TC	TT	*p*-value*
MoCA	22.00 (15.25-23.75)	22.00 (17.50-24.00)	22.00 (20.00-23.00)	0.869	27.00 (26.00-28.00)	27.00 (26.00-28.00)	27.00 (26.00-27.75)	0.720
TMT-A	87.00 (85.00-107.00)	77.00 (56.00-110.00)	71.00 (39.00-88.50)	0.186	65.00 (52.00-70.00)	65.00 (53.25-70.00)	55.00 (46.75-90.50)	0.861
TMT-B	198.00 (150.00-285.00)	180.50 (139.00-240.25)	135.00 (122.50-202.50)	0.220	136.00 (120.00-180.00)	157.50 (127.50-209.50)	158.50 (128.25-177.75)	0.586
CDT	4.00 (2.00-4.00)	3.00 (2.00-4.00)	4.00 (2.50-4.00)	0.840	4.00 (4.00-4.00)	4.00 (4.00-4.00)	4.00 (4.00-4.00)	0.490
DST	11.00 (8.00-11.75)	10.5. (8.25-12.00)	11.00 (10.00-13.50)	0.471	13.00 (11.00-14.00)	12.00 (11.00-13.00)	12.50 (9.75-14.50)	0.760
VFT	13.50 (12.00-18.75)	17.00 (13.25-19.00)	16.00 (13.50-20.00)	0.396	18.00 (17.00-22.00)	18.00 (16.75-22.25)	16.50 (13.25-24.75)	0.672
ST	7.50 (4.00-9.00)	8.00 (5.00-9.75)	6.00 (4.50-8.00)	0.605	10.00 (8.00-12.00)	9.00 (6.75-11.25)	9.00 (8.00-11.50)	0.526

Data are presented as median (interquartile range). *Kruskal-wallis test. Abbreviations: MCI: mild cognitive impairment; MoCA, Montreal Cognitive Assessment; TMT-A, Trail Making Test A; TMT-B, Trail Making Test B; CDT, Clock drawing test; DST, Digit Span Test; VFT, Verbal Fluency Test; ST, Similarities Test.

P>0.05) and MCI group (χ^2=2.79, df=1, P>0.05) [15]. No significant differences were found in GSK-3β genotype and allele distributions between the cases and the controls (χ^2=4.377, df=2, P=0.112 and χ^2=0.031, df=1, P=0.859, respectively).

Comparison of serum GSK-3β concentration between case-control groups and different GSK-3β genotypic subgroups

The serum GSK-3β concentration of the MCI and the control groups, stratified by GSK-3β genotypes were shown in **Table 3**. There was no significant difference in the serum GSK-3β concentration between MCI and healthy-cognition groups (16.40 ± 16.61 ng/ml *vs.* 18.63 ± 16.07 ng/ml, P>0.05). Nor difference was found between these two groups in terms of various GSK-3β genotypes (CC: 14.19 ± 12.82 *vs.* 14.83 ± 13.29, TC: 18.37 ± 18.93 *vs.* 19.54 ± 17.14 and TT: 11.67 ± 12.63 *vs.* 24.18 ± 19.04, ng/ml, all P>0.05). Among the three genotypic subgroups of the MCI group, there was no statistically significant difference in the serum level of GSK-3β.

Comparison of neuropsychological test scores according to GSK-3β genotypic subgroups

Neuropsychological test scores were not found significantly different between genotypic subgroups (CC, TC and TT) of GSK-3β rs334556 polymorphism in both the MCI group and control group (all P>0.05) (**Table 4**). These neuropsychological test scores included MoCA, TMT-A, TMT-B, CDT, DST, VFT and ST scores.

Discussion

As far as we know, for the first time we currently compared the distribution of genotype and allele frequencies of GSK-3β rs334558 (-50C/T) gene between T2DM patients with MCI and healthy control participants. Our results showed that diabetic patients with MCI had lower scores in the neuropsychological tests, but we failed to find a significant association between GSK-3β -50T and MCI in our diabetic patients. Furthermore, there were no statistically significant differences in serum GSK-3β level and neuropsychological test scores according to genotypic subgroups. Among the three genotypic (TT, CC, and TC) subgroups of above two groups, no statistically significant differences

were found in the serum GSK-3β concentration and neuropsychological test scores.

In contrast to our negative results, GSK-3β rs334558 polymorphism was demonstrated to be associated with AD, and -50T haplotype may significantly increase the risk of developing MCI and AD [9-11, 16]. The following factors may explain our negative results. The differences in the geographical distribution and demographic variables (e.g., ethnicity, level of education) of the participants may influence the susceptibility to the disease [17]. For GSK-3β gene, there is an age-specific association with AD reported in a past study, GSK-3β (-50) TT genotype is associated with an increased risk for late-onset (after the age of 72 years) AD [9], our negative finding might be attributable to age difference of participants between the studies. Several genes may contribute to the risk of MCI and AD, among which apolipoprotein E (APOE), amyloid precursor protein, presenilin 1, and presenilin 2 are considered the main risk factors for AD. Clusterin, complement receptor 1 and sortilin-related receptor have been identified novel genes that might be associated with AD, and GSK-3β gene may only have a small effect [18]; over five hundred SNPs are within GSK-3β gene which may confer the risk of AD, GSK-3β rs334558 could be in linkage disequilibrium with another nearby functional polymorphism [19]. Gene-environment interactions may also lead to this discrepancy. In addition to these interpretations, high blood glucose as a disorder of internal environment probably affects the role of GSK-3β gene in susceptibility to the disease.

Our data revealed that diabetic patients with MCI had significant lower scores in the neuropsychological tests than normal. These correspond with a number of studies and epidemiological data that diabetes is an important independent risk factor for mild cognitive impairment in elderly person [20-24], and diabetic patients experience multiple cognitive domains including memory, information-processing speed and executive functions [25, 26]. Although neuropsychological test scores were not found significantly different between the subgroups stratified by GSK-3β genotypes (CC, TC and TT) in the diabetic MCI group, the small sample size of the present study hindered the further stratification of the population excluding the gene

association between GSK-3β rs334558 and a broad range of cognitive domains.

Although several studies have previously investigated the association between GSK-3β protein levels, GSK-3β activity and cognitive performances in AD subjects, none have been performed in type 2 diabetic patients. Our results failed to reveal elevated GSK-3β levels among T2DM patients with MCI. Possible reasons may explain the results. First, GSK-3β is highly expressed in the brain, and also widely expressed in readily available tissue such as circulating lymphocytes and platelets [27-29]. Second, hyperactive GSK-3β has been reported to be associated with the formation of neurofibrillary tangles, which is important in the pathophysiology of AD [30-33]. Platelet GSK-3β activity, reflected by the proportion between phosphor-GSK-3β to total GSK-3β, was significantly increased in patients with mild cognitive impairment and AD. Serum GSK-3β level couldn't reflect the real level and activity in the brain, which is directly associated with cognition status. GSK-3β activity, other than GSK-3β level or not just in serum, should be further determined in the diabetes with MCI. In addition to this, another limitation is that our sample size was not large enough. A larger sample size and different ethnic populations can provide higher power to detect the associations between SNPs and disease.

In summary, the current study showed that no evidence qualified the genetic association between the GSK-3β rs334558 polymorphism and MCI in Chinese Han T2DM patients. Further studies are needed to disclose the pathogenetic roles of GSK-3β in diabetic MCI.

Acknowledgements

Disclosure of conflict of interest

None.

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References

[1] Cheng G, Huang C, Deng H and Wang H. Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. Intern Med J 2012; 42: 484-491.

[2] Ohara T, Doi Y, Ninomiya T, Hirakawa Y, Hata J, Iwaki T, Kanba S and Kiyohara Y. Glucose tolerance status and risk of dementia in the community: the Hisayama study. Neurology 2011; 77: 1126-1134.

[3] Rockenstein E, Torrance M, Adame A, Mante M, Bar-On P, Rose JB, Crews L and Masliah E. Neuroprotective effects of regulators of the glycogen synthase kinase-3beta signaling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. J Neurosci 2007; 27: 1981-1991.

[4] Hye A, Kerr F, Archer N, Foy C, Poppe M, Brown R, Hamilton G, Powell J, Anderton B and Lovestone S. Glycogen synthase kinase-3 is increased in white cells early in Alzheimer's disease. Neurosci Lett 2004; 373: 1-4.

[5] Stoothoff WH and Johnson GV. Tau phosphorylation: physiological and pathological consequences. Biochimica Biophysica Acta 2005; 1739: 280-297.

[6] Sato N and Morishita R. Brain alterations and clinical symptoms of dementia in diabetes: aβ/tau-dependent and independent mechanisms. Front Endocrinol 2014; 5: 143.

[7] Liu Y, Liu F, Grundke-Iqbal I, Iqbal K and Gong CX. Deficient brain insulin signalling pathway in Alzheimer's disease and diabetes. J Pathol 2011; 225: 54-62.

[8] Gandy JC, Melendez-Ferro M, Bijur GN, Leuven F, Roche JK, Lechat B, Devijver H, Demedts D, Perez-Costas E and Roberts RC. Glycogen synthase kinase-3β (GSK3β) expression in a mouse model of Alzheimer's disease: a light and electron microscopy study. Synapse 2013; 67: 313-327.

[9] Mateo I, Infante J, Llorca J, Rodriguez E, Berciano J and Combarros O. Association between glycogen synthase kinase-3beta genetic polymorphism and late-onset Alzheimer's disease. Dement Geriatr Cogn Disord 2006; 21: 228-232.

[10] Kwok JB, Loy CT, Hamilton G, Lau E, Hallupp M, Williams J, Owen MJ, Broe GA, Tang N, Lam L, Powell JF, Lovestone S and Schofield PR. Glycogen synthase kinase-3beta and tau genes in-

teract in Alzheimer's disease. Ann Neurol 2008; 64: 446-454.

[11] Zhang N, Yu JT, Yang Y, Yang J, Zhang W and Tan L. Association analysis of GSK3B and MAPT polymorphisms with Alzheimer's disease in Han Chinese. Brain Res 2011; 1391: 147-153.

[12] Lau KF, Miller CC, Anderton BH and Shaw PC. Molecular cloning and characterization of the human glycogen synthase kinase-3beta promoter. Genomics 1999; 60: 121-128.

[13] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications, part 1: diagnosis and classification of diabetes mellitus: provisional report of a WHO consultation. Diabet Med Diab Med 1998; 15: 539-553.

[14] Portet F, Ousset PJ, Visser PJ, Frisoni GB, Nobili F, Scheltens P, Vellas B, Touchon J; MCI Working Group of the European Consortium on Alzheimer's Disease (EADC). Mild cognitive impairment (MCI) in medical practice: a critical review of the concept and new diagnostic procedure. Report of the MCI working group of the European consortium on Alzheimer's disease. J Neurol Neurosurg Psychiatry 2006; 77: 714-718.

[15] Rodriguez S, Gaunt TR and Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 2009; 169: 505-514.

[16] Bai F, Shi Y, Yuan Y, Yue C, Zhuang L, Xu X, Liu X and Zhang Z. Association of a GSK-3beta polymorphism with brain resting-state function in amnestic-type mild cognitive impairment. J Alzheimers Dis 2012; 32: 387-396.

[17] Chen J, Zheng H, Bei JX, Sun L, Jia WH, Li T, Zhang F, Seielstad M, Zeng YX, Zhang X, Liu J. Genetic structure of the Han Chinese population revealed by genome-wide SNP variation. Am J Hum Genet 2009; 85: 775-785.

[18] Bagyinszky E, Youn YC, An SS and Kim S. The genetics of Alzheimer's disease. Clin Interv Aging 2014; 9: 535-551.

[19] Hohman TJ, Koran ME, Thornton-Wells TA; Alzheimer's Neuroimaging Initiative. Interactions between GSK3beta and amyloid genes explain variance in amyloid burden. Neurobiol Aging 2014; 35: 460-465.

[20] Roberts RO, Knopman DS, Geda YE, Cha RH, Pankratz VS, Baertlein L, Boeve BF, Tangalos EG, Ivnik RJ and Mielke MM. Association of diabetes with amnestic and nonamnestic mild cognitive impairment. Alzheimers Dement 2014; 10: 18-26.

[21] Gregg EW, Yaffe K, Cauley JA, Rolka DB, Blackwell TL, Narayan KM and Cummings SR. Is diabetes associated with cognitive impairment and cognitive decline among older women? Study of osteoporotic fractures research group. Arch Intern Med 2000; 160: 174-180.

[22] Logroscino G, Kang JH and Grodstein F. Prospective study of type 2 diabetes and cognitive decline in women aged 70-81 years. BMJ 2004; 328: 548.

[23] Knopman D. Cardiovascular risk factors and cognitive decline in middle-aged adults. Neurology 2001; 56: 42-48.

[24] Zemva J and Schubert M. Central insulin and insulin-like growth factor-1 signaling: implications for diabetes associated dementia. Curr Diabetes Rev 2011; 7: 356-366.

[25] Kalcza-Janosi K, Lukacs A, Barkai L and Szamoskozi I. Cognitive functions in type 1 and type 2 diabetes. Meta-analysis. Orv Hetil 2013; 154: 694-699.

[26] Qiu C, Sigurdsson S, Zhang Q, Jonsdottir MK, Kjartansson O, Eiriksdottir G, Garcia ME, Harris TB, van Buchem MA, Gudnason V and Launer LJ. Diabetes, markers of brain pathology and cognitive function: the age, gene/environment susceptibility-reykjavik study. Ann Neurol 2014; 75: 138-146.

[27] Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M and Gogos JA. Convergent evidence for impaired AKT1-GSK3[beta] signaling in schizophrenia. Nat Genet 2004; 36: 131-137.

[28] Nadri C, Kozlovsky N, Agam G and Bersudsky Y. GSK-3 parameters in lymphocytes of schizophrenic patients. Psychiatry Res 2002; 112: 51-57.

[29] Yang SD, Yu JS, Lee TT, Yang CC, Ni MH and Yang YY. Dysfunction of protein kinase Fa/Gsk-3 in lymphocytes of patients with schizophrenic disorder. J Cell Biochem 2004; 59: 108-116.

[30] Frame S and Cohen P. GSK3 takes centre stage more than 20 years after its discovery. Biochem J 2001; 359: 1-16.

[31] Lovestone S and McLoughlin DM. Protein aggregates and dementia: is there a common toxicity? J Neurol Neurosurg Psychiatry 2002; 72: 152-61.

[32] Kar S and Quirion R. Amyloid beta peptides and central cholinergic neurons: functional interrelationship and relevance to Alzheimer's disease pathology. Prog Brain Res 2004; 145: 261-274.

[33] Balaraman Y, Limaye AR, Levey AI and Srinivasan S. Glycogen synthase kinase 3beta and Alzheimer's disease: pathophysiological and therapeutic significance. Cell Mol Life Sci 2006; 63: 1226-1235.