Original Article
Relationship between vitamin D receptor gene polymorphism and mild cognitive impairment in elderly Uygur people

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Abstract: To explore the relationship between vitamin D receptor gene (ApaI, BsmI) genotypes and allele frequency and mild cognitive impairment in Xinjiang Uygur population. The polymorphisms of the VDR genotypes (ApaI and BsmI) were analyzed by Snapshot method in 124 MCI patients and 124 controls. A allele of ApaI gene increased the risk of MCI [*OR* = 1.62, 95% CI (1.13-2.31)]; AA genotype increased the risk of MCI [*OR* = 3.49, 95% CI (1.57-7.74)]. T allele of BsmI gene increased the risk of MCI [*OR* = 1.94, 95% CI (1.24-3.05)]. The risk of MCI increased accompanied with higher TG and SBP level. VDR (ApaI) AA genotype, a allele and VDR (BsmI) T allele probably associated with elderly MCI patients in Uygur ethnicity, higher level of TG and SBP were risk factors to elderly people with MCI among Uygurs.

Keywords: BsmI, apai, vitamin D receptor, mild cognitive impairment, elderly

Introduction

The mild cognitive impairment (hereinafter referred to as MCI) has caught many attention, the prevalence of findings across the country on elderly MCI is inconsistent, while the overall prevalence of MCI is high. The MCI prevalence survey Xinjiang Uyghur and Han ethnic with age over 60 showed [1]: Uygur, Han two national standardized prevalence rate of MCI is 10.58%; the prevalence rate of Uyghur elderly is 9.61%. Studies have shown that there is a high risk of MCI to AD conversion [2]. The increase of the prevalence of dementia, significantly increase the economic burden and affect the quality of life on society for such a being developing country, therefore domestic and foreign scholars began to focus on the impact of MCI factors.

Currently, the research on MCI factors mainly focus on sociological factors, disease, and molecular genetics effect. The study on vitamin D receptor (VDR) gene polymorphisms become popular in recent years, most studies focused on VDR gene polymorphism and cognitive function relationships for patients with AD, only few for patients with MCI. Previous studies found that VDR gene locus associated with cognitive function may have ApaI, BsmI [3-6]; these two sites are both in start site of intron VIII of the 12th chromosome 3' transcriptional, involved in gene expression. This study is to have a better understanding on the correlation between VDR gene ApaI and BsmI gene polymorphism and elderly Uyghur MC.

Material and methods

Objects

Patients are selected from participants of Xinjiang Uygur epidemiological investigation (October 2010) using random, stratified cluster sampling method. The total number of patients is 3346, among which there are 124 MCI cases of Uyghur elderly patients with complete data. Of the 124 patients, there are 69 male cases, 55 female cases, with an average age (65.63 ± 7.46) years. There are another 124 patients selected as gender, age-matched control subjects, of which 70 male and 54 female patients, with an average age (64.44 ± 6.20) years. All participants in this study have agreed and signed the consent form; this experiment has

been examined and approved by Xinjiang Medical Ethics Committee.

MCI diagnostic criteria

MCI diagnosis is based on the diagnostic criteria for mild cognitive impairment from American Psychiatric Association and Statistical Manual of Mental Disorders Fourth Revision (DSM-IV). The criteria includes: A subjective symptoms of memory loss. There is MCI related evidence such as: MMSE score: illiteracy (18-21 minutes), primary schools (21-24 minutes), and secondary schools (over 24-27 minutes). There is a decline of daily life and social function. Symptoms continue for 3 months or more. Hachinski ischemic Index Scale (HIS) < 4 points (to exclude specific causes of cognitive decline). The diagnostic criteria for dementia is not meet (CDR scale = 0.5 points). In our survey, the final diagnosis is based on comprehensive analysis by expert, history, physical examination results and scores for the results.

Inclusion criteria for control group

Patients who do not comply with diagnostic criteria of MCI from Statistical Manual of Mental Disorders of the American Psychiatric Association revised fourth edition (DSM-IV), but have the same or similar life background, age, gender with selected MCI patients.

Exclusion criteria

Exclude patients with neurological diseases that can cause cognitive impairment: ischemic cerebrovascular disease, Parkinson's disease, brain tumors. Exclude patients with a family history of dementia and Down's syndrome. Exclude patients with depression and other schizophrenia. Exclude patients with serious endocrine dysfunction, severe heart and lung liver and kidney dysfunction, severe infectious diseases as well as patients with toxic encephalopathy. Exclude patients with history of head injury, and special drug use history. 6 Exclude patients who have biological relations based on blood samples tests.

General information and blood samples collection methods

Using the combination of field surveys and household surveys, we initially completed the first questionnaire, measured the Mini-Mental State Examination (MMSE), and did further clinical examination including the overall decline scale (GDS), Hamilton Depression Scale (HAMD), Hachinski ischemic index scale (HIS), clinical dementia rating scale (CDR) and other testis to patients whose were mentioned recent memory impairment, and with MMSE scale significantly lower than the cutoff value. The patient's basic information was also collected including: height, weight, age, education level, medical history and other general information. The 5 ml cubital vein blood was collected from all participants, placed in -80°C research freezer. There were 16 physicians from different specialty (geriatrics, neurology, mental and psychology) joined in the survey work, including 8 Uyghur MD. All physicians were systematically trained before the survey.

DNA extraction and genotyping

Extraction of genomic DNA: The peripheral EDTA anticoagulant blood 5 ml was collected from all patients, and DNA was extracted based on peripheral blood genomic DNA extraction kit instructions. SNaPshot method was used to detect two sites.

Statistical methods

SPSS17.0 statistical software was used in data analysis, t test was used for measurement data, x^2 test was used for count data inspection, rank sum test was used for rating data, and multivariate logistic regression test was used to detect all relevant factors affecting the prevalence of MCI. Due to the difference of 6430 kb between the two sites, haplotype analysis is not meaningful; the linkage disequilibrium analysis was not applied.

Results

Peak graphs of multiplex PCR gel electrophoresis

The marks "BsmI (rs1544410)", "FIG ApaI (rs7975232)" indicate three genotypes of the two sites; of the, red, blue, green, black, represent the extension products of adding ddTTP, ddGTP, ddATP, ddCTP, respectively. The alleles were marked on the top of the peak (if the application of the extension primer is positive, then the join is consistent with the base extension, if the exten-
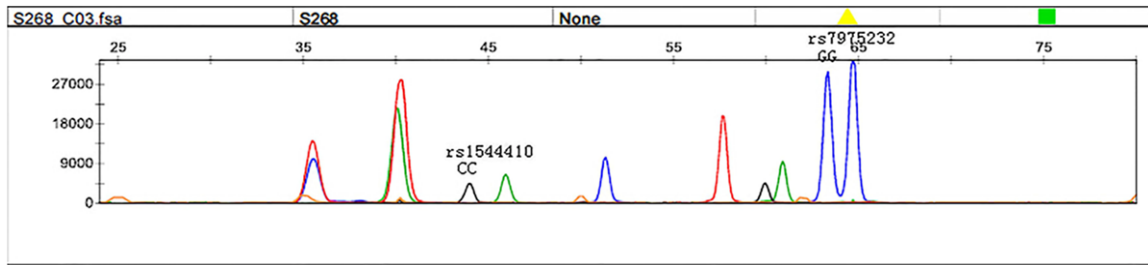
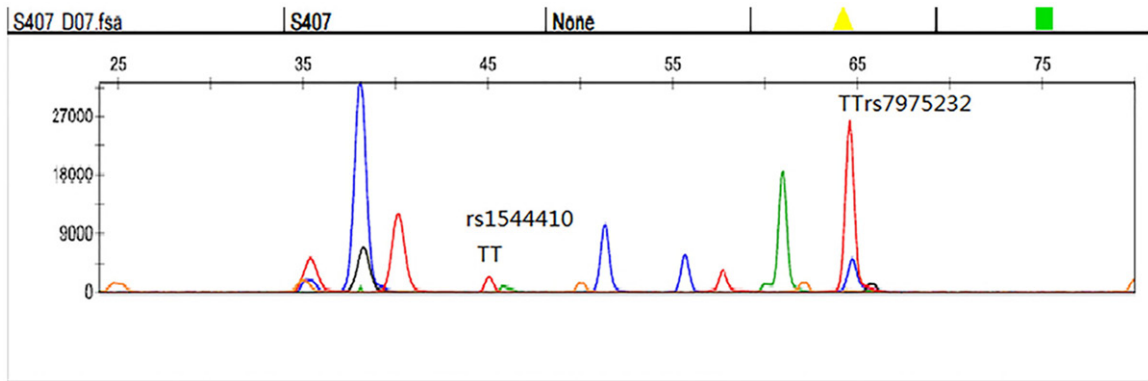**Figure 1.** No mutation on ApaI gene and BsmI gene locus.

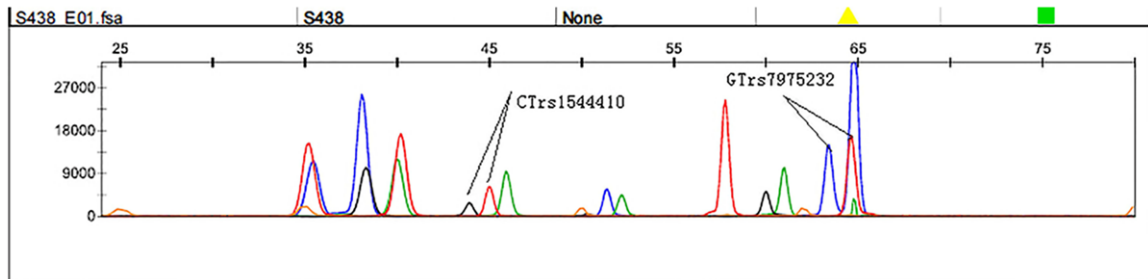**Figure 2.** Homozygous mutation on ApaI gene and BsmI gene locus.

Figure 3. Heterozygous mutation on ApaI gene and BsmI gene locus.

sion of the application of the reverse primer, then the join is complementary to nucleotide). SE, SR, respectively, refer to the extension of the forward or reverse primer. At the ApaI locus, the primer extension is rs7975232SR, it is a reverse primer, and the mark on the peak is GT, labeled as Pos. G:63.33; T:64.66. At the BsmI locus, the primer extension is rs1544410SF, it is a positive primer, and the mark on the peak is CT, labeled as Pos. C:43.31; T:45.11. **Figure 1** show there is no mutation on BsmI or ApaI locus. **Figure 2** shows there is homozygous mutation on BsmI, ApaI locus. **Figure 3** shows heterozygous mutation ApaI gene and BsmI gene

locus. (Corresponding to the peak marked genotype).

General information comparing between 2 MCI group and the control group

There is no significant difference between MCI group and control group in gender, diabetes, hypertension, and marital status, education level, age, weight, diastolic blood pressure, fasting glucose, total cholesterol, high density lipoprotein cholesterol, low-density lipoprotein cholesterol ($P > 0.05$). The Systolic difference between the two groups was statistically significant ($P < 0.05$) in blood pressure and triglycerides.

Table 1. Genotypes and allele distribution of VDR (ApaI) gene in MCI group and control group

| | N | Genotype | | | Allele | |
		CC (%)	CA (%)	AA (%)	C (%)	A (%)
MCI Group	124	32 (25.81)	63 (50.81)	29 (23.39)	127 (51.21)	121 (48.79)
Control Group	124	49 (39.52)	58 (46.77)	17 (13.71)	156 (62.90)	92 (37.10)
Total	248	81 (32.66)	121 (48.79)	46 (18.54)	283 (57.06)	213 (42.94)

Note: Comparison of genotypes: $x^2 = 6.904$, $P = 0.031$; Comparison of allele: $x^2 = 6.920$, $P = 0.008$.

Table 2. Genotypes and allele distribution of VDR (BsmI) gene in MCI group and control group

| | N | Genotype | | | Allele | |
		CC (%)	CT (%)	TT (%)	C (%)	T (%)
MCI Group	124	69 (55.65)	47 (37.90)	8 (6.45)	185 (74.60)	63 (25.40)
Control Group	124	89 (71.77)	33 (26.61)	2 (1.61)	211 (85.08)	37 (14.92)
Total	248	158 (63.71)	80 (32.26)	10 (4.03)	396 (79.84)	100 (20.16)

Note: Comparison of genotypes: $x^2 = 8.58$, $P = 0.014$; Comparison of allele: $x^2 = 8.46$, $P = 0.004$.

Table 3. MCI multivariate logistic regression analysis

| | B | Stb | Wald | P | OR | 95% CI for OR | |
						Lower	Upper
SBP	0.016	0.005	9.443	0.002	1.02	1.01	1.03
TG	0.288	0.086	11.217	0.001	1.33	1.13	1.58
ApaI			9.606	0.008			
ApaI AC vs. CC	0.568	0.306	3.442	0.064	1.77	0.97	3.21
ApaI AA vs. CC	1.249	0.407	9.398	0.002	3.49	1.57	7.74
Constant	-3.517	0.829	17.977	0.000			

Note: Stepwise regression is applied using SBP, TG, ApaI, and BsmI.

CT, TT genotype is higher in the MCI group than the control group ($P < 0.05$). Allele T is higher in MCI group ($P < 0.05$). Further analysis shows that allele T increases MCI risk [OR = 1.94, 95% CI (1.24-3.05), $P < 0.05$] compared to C, genotype CT increased prevalence of MCI risk [OR = 1.84, 95% CI (1.07-3.17), $P < 0.05$] compared with the CC, TT genotype increases MCI risk [OR = 5.16, 95% CI (1.06-25.07), $P < 0.05$] compared with the CC.

Hardy-weinberg equilibrium test

MCI group and control group two loci three genotypes were in accordance with Hardy-weiberg genetic equilibrium. ApaI locus (MCI group = 0.030, $P = 0.982$; group = 0.000, $P = 0.999$), BsmI locus (MCI group = 0.000, $P = 1.00$; control group = 0.290, $P = 0.865$).

VDR gene polymorphism with apaI MCI

Table 1 shows the distribution of ApaI genotype and allele between the two groups. The A allele and AA genotype was higher in MCI group ($P < 0.05$). Based on further allele analysis at the ApaI locus, allele A increases risk of MCI [OR = 1.62, 95% CI (1.13-2.31), $P < 0.05$] compared to C, genotype AA increases risk of MCI [OR = 2.61, 95% CI (1.24-5.51), $P < 0.05$] compared to the CC.

VDR gene bsmI polymorphism with MCI

Table 2 shows the distribution of BsmI locus genotype and allele between the two groups.

Multivariate logistic regression analysis on factors of MCI

Based on the comparison of general information and the genotype chi-square test between MCI group and control group, the risk factors of MCI include genotype, SBP, TG. Therefore we included SBP, TG, ApaI, BsmI into multivariate logistic regression analysis. The results showed that ApaI locus, AA genotype may increase the risk of MCI [OR = 3.49, 95% CI (1.57-7.74), $P < 0.05$]. The increase of TG increases the risk of MCI [OR = 1.33, 95% CI (1.13-1.58), $P < 0.05$], the increase of SBP increases the risk of MCI [OR = 1.02, 95% CI (1.01-1.03), $P < 0.05$] (**Table 3**).

The distribution of combined gene of in case-control

Table 4 shows the distribution of the joint genetic differences between MCI group and control group was statistically significant ($x^2 = 16.910$, $P < 0.05$). The chi-square test was fur-

Table 4. Genotypes distribution of combined VDR BsmI and VDR ApaI genotypes in MCI group and control group

	MCI group	The control group	Total	x^2	P
CCAA (%)	7 (5.64)	10 (8.06)	17 (6.85)	0.57	0.451
CCCA (%)	30 (24.19)	31 (25.00)	61 (24.60)	0.022	0.883
CCCC (%)	32 (25.81)	48 (38.71)	80 (32.26)	4.724	0.030
CTAA (%)	14 (11.29)	7 (5.65)	21 (8.47)	2.549	0.110
CTCA (%)	33 (26.61)	26 (20.97)	59 (23.79)	1.090	0.297
TTAA (%)	8 (6.45)	0 (0.00)	8 (3.23)	0.004*	0.007
TTCA (%)	0 (0.00)	1 (0.81)	1 (0.40)	0.500*	1.000
TTCC (%)	0 (0.00)	1 (0.81)	1 (0.40)	0.500*	1.000
n	124	124	248		

Note: The overall comparison of combined gene x^2 = 16.910, P = 0.018; *denote probability based on Fisher's test.

ther applied to joint genome: Choose one of the joint genes, merge the remaining genes and apply multiple joint chi-square tests. The results show that the difference was statistically significant ($P < 0.05$) between MCI group with more BsmI (TT) genotype and ApaI (AA) genotype and the control group; the difference was statistically significant ($P < 0.05$) between MCI group with more BsmI (CC) genotype and ApaI (CC) genotype an the control group. The study did not found CTCC gene combinations.

Discussion

Mild cognitive impairment (Mild Cognitive Impairment, MCI) is between normal aging and dementia in an intermediate state, there is a high risk of progression to dementia, the risk was 10 times than normal [2]. Development of dementia is a slow process, further intervention in the final stages of development of the disease is able to slow the progression of dementia, but there is no apparent effect on the pathological damage that has formed, so the clinical effect is not obvious. Therefore, early detection of MCI, early intervention is the focus of current clinical work.

Current research studies of MCI mainly focus on the impact of factors, including: biological factors, social factors, vascular risk factors and the impact of molecular genetics. Previous studies on impact of vascular risk factors on MCI, the relationship between SBP and our study all agree that [7, 8]: SBP increases risk of MCI. The relationship between TG and MCI in this study and Zou Y et al [7] study are inconsistent, the latter result is based on the southern Chinese Han population, while our study fo-

cused on the Xinjiang Uygur people. There are many differences between the two groups in diet, lifestyle, location, genetic background, etc; it is possible that ethnicity affected results.

Current genetic studies of MCI include: SORL1 gene [9], VEGF gene [10], APOEε4 gene [11]. APOEε4 is now clearly proved to be an independent risk factor for AD [12, 13]. However, the relationship between VDR gene polymorphism and cognitive function in recent years has become a hot topic.

The effects of active vitamin D (1, 25 (OH) 2D3) on the nervous system include: Promote the synthesis of neurotropic factors. Decrease the expression of L-type calcium channels sensitivity and reduce the flow and excitotoxic effects of calcium ions. Vitamin D deficiency may cause chemical changes in the nervous system [14]. 1, 25 (OH) 2D3 plays a role in inhibition of Aβ by enhancing VDR gene transcription [15], however VDR gene polymorphism may change after the VDR gene transcription affect the role of Aβ clearance of, then affect cognitive function.

In this study, there are following tests results: AA genotype of ApaI locus gene is risk factor of MCI, A allele is related to MCI. There are few studies focusing on MCI and VDR gene polymorphism, studies on AD's, Gezen [4] and other studies have shown that in 2007 heterozygotes Aa was higher in the control group, and the difference was statistically meaningful, but the research data do not meet the Hardy-Weinberg genetic equilibrium. Donald et al [5] study shows ApaI T allele (i.e., A) is the prevalence of risk genes for AD. The results of this study along with Donald, etc. can be considered: ApaI T allele (i.e., A) is not only the risk of AD genes but also affecting cognitive function in the early stages of AD, namely related to MCI.

This study suggests: BsmI T allele may be associated with MCI. This is inconsistent with Kuningas, etc. [3] T inconsistency may be due to the combined effect of different ethnic, geographical, cultural background, genetic background and many other factors results. By comparing the gene Joint Genome visible TTAA joint between the two groups at higher MCI group,

we conclude: elderly Uighur individuals with Bs-mI (TT) genotype and ApaI (AA) genotype may have risk for MCI.

The biological effects of vitamin D may be changed as the intermediate passage is changed due to genetic polymorphism and VDR binding process [16]. Current research shows there are five kinds of VDR polymorphisms -FokI, BsmI, TaqI, ApaI and Tru9I. The FokI gene, outside of the 2nd VDR gene polymorphism in exon, changes the VDR protein structure (forming an additional 3 more elongated structure of amino acids). ApaI, BsmI, Tru9I gene polymorphism, in the 8th intron, not affect the protein structure of the VDR gene polymorphism in intron but 3'transcription start with a strong unbalanced chain and control VDR gene expression. ApaI restriction sites, located in intron 8 is located in the 3'VDR gene transcription initiation site, a ligand binding site of the gene encoding VDR [17], affects cognitive function. This is probably because we consider such a ligand binding site polymorphism affecting the regulation of gene expression by affecting chromatin conformation regulate gene transcription efficiency, thereby affecting cognitive function.

This article only conduct studies on relationship between VDR gene polymorphism and MCI for elderly patients from Uyghur population, results suggest that: VDR gene locus ApaI, BsmI are related to MCI for Uyghur elderly. Because this study focused on Uighur population, and the sample size is small, the test requires a large sample study to repeat this study, and it might be helpful to detect the correlation between VDR gene polymorphism and the MCI for the Han population using a large sample study. As there are few studies on relation between VDR gene polymorphism and MCI, and there is no found evidence of VDR gene existing in Chinese population, this research can provide ideas and information for research to further study the vitamin D receptor-associated signaling pathways regulation and vitamin D receptor gene expression.

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Disclosure of conflict of interest

None.

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