



Type II deiodinase polymorphisms and serum thyroid hormone levels in patients with mild cognitive impairment

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ABSTRACT. We investigated type II deiodinase (DIO₂) polymorphisms and serum thyroid hormone levels in subjects with mild cognitive impairment (MCI) in a Uygur population. We studied the DIO₂ Thr92Ala (rs225014) and ORFa-Gly3Asp (rs12885300) polymorphisms of 129 unrelated MCI cases and 131 matched controls. All subjects were genotyped using SNaPshot SNP genotyping assays. Serum thyroid hormone levels were measured by radioimmunoassay. Levels of serum triiodothyronine and thyroxine in the MCI group were significantly lower than those in the control group. Genotype and allele frequencies in the DIO₂ gene between the MCI and control groups were not significantly different. There was no association in genotype and allele frequencies of Thr92Ala between genders in both groups. ORFa-Gly3Asp genotype and allele frequencies were significantly different in patients and controls by gender. The Asp allele was less frequent among male MCI patients compared to controls (odds ratio = 0.471, 95% confidence interval = 0.261-0.848). However, female Asp carriers were more frequent among MCI patients than among controls (odds ratio = 2.842, 95% confidence interval = 1.326-6.09). Serum levels of triiodothyronine and thyroxine

were lower in individuals of the Ala/Ala genotype than in those with the Thr/Thr or Thr/Ala genotype. Serum levels of triiodothyronine were lower in male Gly/Gly carriers than in Gly/Asp or Asp/Asp carriers. Decreased serum levels of triiodothyronine and thyroxine may influence the incidence of MCI in the Uygur population. DIO₂ gene polymorphisms may play a role in the incidence of MCI in male patients.

Key words: Gene; Mild cognitive impairment; Polymorphism; Uygur; Thyroid hormone; Type 2 deiodinase

INTRODUCTION

Mild cognitive impairment (MCI) is an intermediate state between normal ageing and dementia, defined by the impairment of memory and other cognitive domains that are not sufficiently severe to be diagnosed as Alzheimer's disease (AD). We previously found that in addition to recognized independent risk factors for MCI such as hypertension, sugar metabolism disorders, and hyperlipidemia (Zhou et al., 2012), low triiodothyronine (T₃) level may be an independent risk factor for MCI. Previous studies have found that T₃ may promote the formation of central nervous system stem cells and glial cell differentiation and is closely related to cognitive function (Lazarus, 2012). Deiodinase is a key enzyme of thyroid hormone (TH) metabolism; in particular, type 2 deiodinase (DIO₂) is a key enzyme that regulates T₃ in brain tissues.

We hypothesized that genetic variations in the DIO₂ gene alter the bioactivity of THs associated with MCI. Thus, we performed a case-control study of a Uygur population of Hetian in Xinjiang based on previous epidemiological survey research.

MATERIAL AND METHODS

Subjects

Our sample was collected according to a cluster sampling design and geographical distribution. The study was carried out between August and October 2010. Using a random number table, 3346 Uygur participants were enrolled, aged 55 years or older, in Hetian. To evaluate MCI, the diagnostic criteria proposed by the Diagnostic and Statistical Manual of Mental Disorders 4th edition was used based on data from epidemiological surveys. Exclusion criteria included history of mental illness or mental retardation or suffering from severe heart or lung or kidney dysfunction, severe endocrine disease, severe infectious diseases, and toxic encephalopathy. Subjects with brain dysfunction such as stroke, Parkinson's disease, brain tumors, depression, a history of head trauma, or a history of psychotropic drug use, alcohol, or drug addiction in the past 6 months were also excluded. Those with primary thyroid disease and taking thyroid medication and iodine within 2 weeks were excluded. A total of 324 subjects diagnosed with MCI were recruited, including 156 male and 168 female Uygur cases. A total of 129 patients with MCI [74 males and 55 females with a mean \pm standard deviation (SD) age of 64.3 \pm 6.42 years] and 131 psychiatrically healthy control subjects (75 males and 56 females with a mean age of 64.4 \pm 6.45 years) were recruited for this case-control study. All subjects were unrelated native Chinese Uygur people. Control subjects matched by distribution, education, past history, and illness were included. All subjects participated in this study

voluntarily. Written informed consent was obtained from all subjects following a complete description of the study. The institutional Ethics Committees approved this study.

Methods

Blood sample measurement

We collected 5 mL fasting blood samples from participants at baseline between 8:30 a.m. and 10:00 a.m. Plasma and serum were separated from blood cells within 1 h of collection and stored at -80°C until assayed. Blood samples for blood lipids, blood glucose, and homocysteine concentration were collected in empty tubes and analyzed by a biochemical laboratory (Beckman, Brea, CA, USA). Serum T_3 , thyroxine (T_4), and thyroid stimulating hormone were measured using an [^{125}I] iodine triiodothyronine, [^{125}I] iodine thyroxine, and human thyrotropin radioimmunoassay kit from the North Biology Institute of Beijing.

Genotyping

Genomic DNA was isolated from ethylenediaminetetraacetic acid blood samples using the Genomic DNA Mini Kit (TIANGEN, Beijing, China). The primers for Thr92Ala and ORFa-Gly3Asp were designed using the Primer3 software (<http://frodo.wi.mit.edu/>). The primer sequences for analyzing DNA samples are listed in Table 1. Oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Multiplex polymerase chain reaction amplification included (20 μL): 1X GC Buffer I, 3.0 mM Mg^{2+} , 0.3 mM dNTPs, 1 U HotStarTaq polymerase (Qiagen, Hilden, Germany), 1 μL DNA, and 2 μL multiplex amplification primers. The cycling for amplification consisted of denaturation at 95°C for 2 min, 11 cycles of denaturation at 94°C for 20 s, annealing at 65°C , increasing by $0.5^{\circ}\text{C}/\text{cycle}$ for 40 s, and extension at 72°C for 90 s, followed by 24 cycles of denaturation at 94°C for 20 s, annealing at 59°C for 30 s, and extension at 72°C for 90 s, and final extension at 72°C for 2 min. According to the ABI PRISM SNaPshot Multiplex Kit Protocol, the amplified products were pooled for each sample and purified by digestion with 2 U exonuclease I and 5 U shrimp alkaline phosphatase at 37°C for 1 h to remove unutilized primers and dNTPs. The enzymes were heat-inactivated by incubation at 75°C for 15 min.

Table 1. Oligonucleotides used for cDNA polymerase chain reaction.

Primer	Sequence (5'-3')	Fragment (bp)
rs225014 (Thr92Ala)	F: CTGGCTCGTGAAAGGAGGTCAA	173
rs225014 (Thr92Ala)	R: CCAATTCCAGTGTGGTGCATGT	
rs12885300 (Gly3Asp)	F: GGCGTACTCGTCCCTAATCCAGT	170
rs12885300 (Gly3Asp)	R: TGGCAATTCAAGAAAGAAACAGGCTAC	

Genotyping was performed using the SNaPshot Multiplex kit (Applied Biosystems, Foster City, CA, USA). Briefly, approximately 2 μL purified amplicon was added to 5 μL SNaPshot Ready Reaction Mix and 1 μL of each SNaPshot oligonucleotide primer (Table 2). The reactions were carried out for 28 cycles at 96°C for 10 s, 52°C for 5 s, and 60°C for 30 s in a polymerase chain reaction Verity 96-well instrument (Applied Biosystems) and digested with shrimp alkaline phosphatase to dephosphorylate unincorporated fluorescent ddNTPs. The SNaPshot reaction products were analyzed in 3130xl genetic analyzer (Applied Biosystems)

by mixing 0.5 μ L SNaPshot product, and 0.5 μ L GS120 Liz Size Standard (Applied Biosystems) and 9 μ L Hi-Di Formamide (Applied Biosystems) for each sample and denaturing the injection mix at 95°C for 5 min. Subsequent automated genotype analysis was performed using GeneMapper 4.1 (Applied Biosystems).

Table 2. Oligonucleotides used for SNaPshot.

Primer	Sequence (5'-3')
rs225014SF	TTTTTCCACTGTTGTACCTCCTTCTG
rs12885300SR	TTTCGTTTAAAGAGCATAGAGACAATGAAAG

Statistical analyses

All continuous variables are reported as means \pm SD. Differences in continuous variables between MCI patients and control subjects were analyzed using the 2-tailed paired Student *t*-test and one-way analysis of variance. Chi-squared tests were used to determine whether samples were in Hardy-Weinberg equilibrium. Differences in the distributions of genotypes and alleles between MCI patients and control subjects were analyzed using the Fisher exact test. Risk factors for MCI, based on odds ratios (OR) and 95% confidence intervals (CI), were analyzed. $P < 0.05$ was considered to indicate statistical significance. Data were analyzed using SPSS V 17.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Clinical characteristics of study participants

Table 3 shows the clinical characteristics of the study participants. For total subjects, the following factors were significantly higher in the MCI patients compared to control subjects: systolic blood pressure, triglycerides, and low-density lipoprotein-cholesterol. The following factors showed no significant differences between MCI patients and control subjects: age, body mass index, diastolic blood pressure, glucose, total cholesterol, and high-density lipoprotein-cholesterol. MCI patients showed significantly lower T_3 and T_4 levels compared to control subjects ($P < 0.05$).

Table 3. Characteristics of study participants (means \pm SD).

	MCI patients	Control subjects	<i>t</i>	P value
Number of subjects (N)	129	131		
Age (years)	64.32 \pm 6.42	64.41 \pm 6.46	0.118	0.906
BMI (kg/m ²)	24.67 \pm 4.23	23.96 \pm 4.10	1.377	0.170
Systolic BP (mmHg)	140.72 \pm 24.07	133.49 \pm 24.21	2.416	0.016*
Diastolic BP (mmHg)	80.69 \pm 12.90	78.07 \pm 13.10	1.625	0.105
Fasting plasma glucose (mM)	6.03 \pm 2.96	5.47 \pm 1.81	1.829	0.069
Triglycerides (mM)	2.99 \pm 1.39	2.04 \pm 1.26	5.832	0.000*
Total cholesterol (mM)	4.70 \pm 0.87	4.50 \pm 1.02	1.751	0.081
HDL cholesterol (mM)	1.06 \pm 0.26	1.10 \pm 0.45	0.878	0.381
LDL cholesterol (mM)	2.77 \pm 0.73	2.40 \pm 0.61	4.355	0.000*
T_3 (ng/mL)	1.32 \pm 0.40	1.56 \pm 0.74	3.223	0.001*
T_4 (ng/mL)	84.98 \pm 17.91	93.50 \pm 23.21	3.318	0.001*
TSH (IU/mL)	6.08 \pm 2.63	5.68 \pm 2.13	1.369	0.172

* $P < 0.05$. BMI = body mass index; BP = blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; T_3 = triiodothyronine; T_4 = thyroxine; TSH = thyroid stimulating hormone.

SNaPshot method

The representative data are shown in Figure 1. For the rs12885300 polymorphisms, Gly/Gly genotype produced a blue peak, Asp/Asp genotype produced a green peak, and Gly/Asp genotype produced a blue and green peak. For the rs225014 polymorphisms, the Thr/Thr genotype produced a red peak, the Ala/Ala genotype produced a black peak, and the Thr/Ala genotype produced red and black peaks.

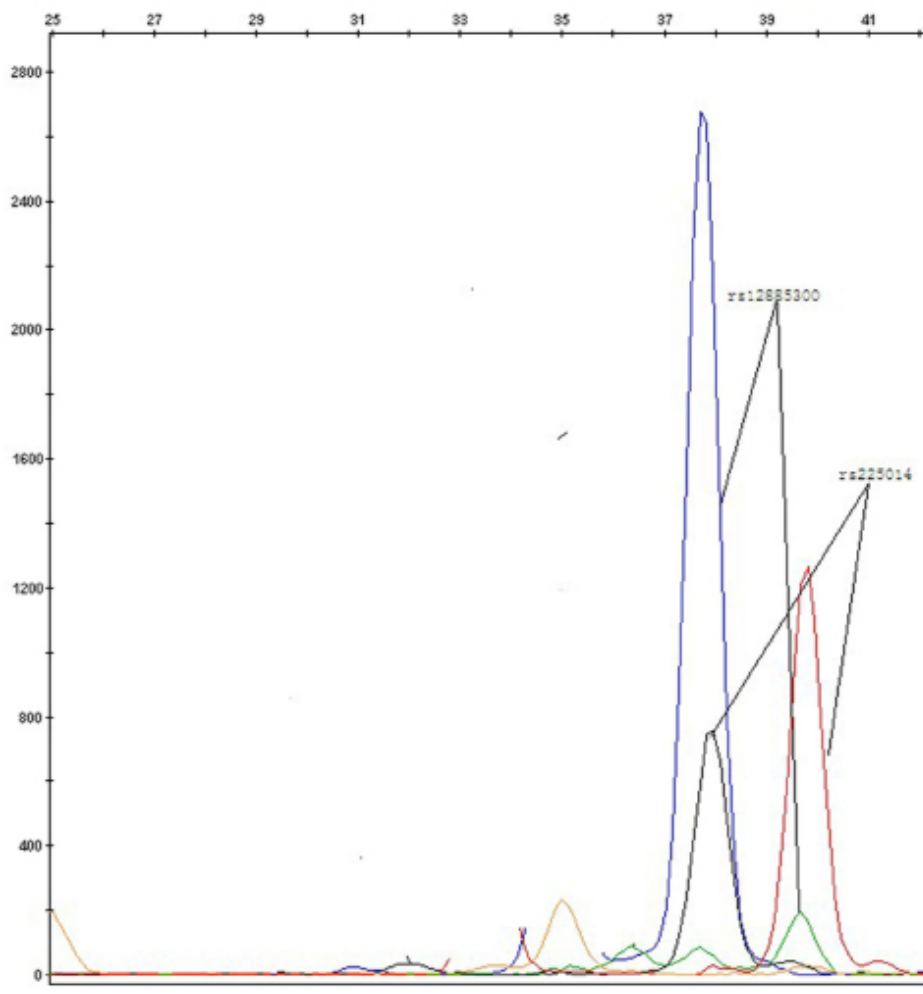


Figure 1. Blue = ddGTP; green = ddATP; red = ddTTP; black = ddCTP.

Genotype and allele frequencies of the DIO_2 gene

The distributions of genotypes of rs225014 and rs12885300 were both in Hardy-Weinberg equilibrium in the MCI and control groups. There was no association between geno-

type and allele frequencies in the DIO₂ gene common mutation and MCI group compared to control subjects ($P > 0.05$, Table 4).

Table 4. Genotype and allele frequencies for DIO₂ (N, %).

Locus	Type	MCI patients	Control subjects	χ^2	P value
rs225014	Thr/Thr	40 (31)	43 (32.8)	0.275	0.871
	Thr/Ala	57 (44.2)	59 (45)		
	Ala/Ala	32 (24.8)	29 (22.2)		
rs12885300	Thr	137 (53.1)	145 (55.3)	0.263	0.608
	Ala	121 (46.9)	117 (44.7)		
	Gly/Gly	88 (68.2)	83 (63.4)	3.625	0.163
	Gly/Asp	35 (27.1)	46 (35.1)		
	Asp/Asp	6 (4.6)	2 (1.5)	0.064	0.8
	Gly	211 (81.8)	212 (0.72)		
	Asp	47 (18.2)	50 (0.28)		

We observed no association in genotype frequencies and allele frequencies of rs225014 and gender in the MCI group or control group ($P > 0.05$). However, the Ala allele frequency for males in the MCI group was higher than that in the control group, although no significant difference was observed (48.6 vs 38.7%, $P = 0.08$). The genotype and allele frequencies of rs12885300 were significantly different between patients with MCI and controls according to gender ($P > 0.05$). Male carriers of the Gly/Gly and Asp/Asp genotypes and the Gly allele were more common among MCI patients than among controls ($P < 0.05$), but the Gly/Asp genotype and Asp allele were less frequently observed in MCI patients compared with controls (OR for male carriers of the Asp allele for MCI = 0.471, 95%CI = 0.261-0.848, $P = 0.011$). However, female carriers of the Asp allele were more frequent among MCI patients than among controls ($P < 0.05$). The OR for female carriers of the Asp allele for MCI was 2.842 (95%CI = 1.326-6.09, $P = 0.006$; Table 5).

Table 5. Genotype and allele frequencies for DIO₂ according to gender (N, %).

Locus	Gender	Type	MCI patients	Control subjects	χ^2	P value
rs225014	Male	Thr/Thr	22 (29.7)	30 (40)	2.71	0.258
		Thr/Ala	32 (43.2)	32 (42.7)		
		Ala/Ala	20 (27)	13 (17.3)		
	Female	Thr	76 (51.4)	92 (61.3)	3.02	0.08
		Ala	72 (48.6)	58 (38.7)		
		Thr/Thr	18 (32.7)	13 (23.2)	1.446	0.485
		Thr/Ala	25 (45.5)	27 (48.2)		
		Ala/Ala	12 (21.8)	16 (28.6)		
		rs12885300	Male	Thr	61 (55.5)	53 (47.3)
Ala	49 (44.5)			59 (52.7)		
Gly/Gly	56 (76)			37 (48)	13.85	0
Gly/Asp	15 (20)			37 (49)		
Asp/Asp	3 (4)			1 (3)		
Female	Gly			127 (86)	111 (74)	7.81
	Asp		21 (14)	39 (26)		
	Gly/Gly		88 (68.2)	83 (63.4)	10.01	0.007
	Gly/Asp		35 (27.1)	46 (35.1)		
	Asp/Asp		6 (4.6)	2 (1.5)	10.17	0.001
	Gly		211 (81.8)	212 (0.72)		
Asp	47 (18.2)		50 (0.28)			

Relationship between TH level and SNP in DIO₂

The serum levels of T₃ and T₄ in subjects with the Ala/Ala genotype were lower than those in subjects with the Thr/Thr or Thr/Ala genotype (between groups, $P < 0.05$). Serum levels of T₃, T₄, and thyroid stimulating hormone in subjects with different genotypes of ORFa-Gly3Asp were not significant ($P > 0.05$; Table 6). Serum levels of T₃ in male Gly/Gly carriers were lower those in Gly/Asp or Asp/Asp carriers of different genders ($P < 0.05$). The serum levels of TH in female Gly/Gly carriers did not differ from those in Gly/Asp or Asp/Asp carriers ($P > 0.05$; Table 7).

Table 6. Relationship between TH level and DIO₂ SNP.

DIO ₂ gene locus		N	Frequency (%)	T ₃ (ng/mL)	T ₄ (ng/mL)	TSH (IU/mL)
rs225014	Thr/Thr	83	31.9	1.59 ± 0.65	91.78 ± 20.12	5.78 ± 1.98
	Ala/Thr	116	44.6	1.51 ± 0.60	90.85 ± 22.26	6.14 ± 2.85
	Ala/Ala	61	23.5	1.12 ± 0.42	82.86 ± 19.30	5.51 ± 1.91
rs12885300	Gly/Gly	171	65.8	1.41 ± 0.56	90.29 ± 20.50	6.06 ± 2.64
	Gly/Asp	81	31.2	1.49 ± 0.70	87.93 ± 22.84	5.46 ± 1.74
	Asp/Asp	8	3	1.44 ± 0.47	81.30 ± 16.44	6.23 ± 2.45

T₃ = triiodothyronine; T₄ = thyroxine; TSH = thyroid stimulating hormone. Levels of T₃ compared with Thr/Thr, Thr/Ala, and Ala/Ala carriers, $F = 12.862$, $P = 0.000$; levels of T₄ between groups, $F = 3.794$, $P = 0.024$; the levels of TSH between groups, $F = 1.469$, $P = 0.232$; levels of T₃ compared with 3 genotypes in rs12885300, $F = 0.441$, $P = 0.644$; levels of T₄ between groups, $F = 0.930$, $P = 0.396$, levels of TSH between groups, $F = 1.849$, $P = 0.16$.

Table 7. Thyroid hormone level and rs12885300 according to gender.

rs12885300		N	Frequency (%)	T ₃ (ng/mL)	T ₄ (ng/mL)	TSH (IU/mL)
Male	Gly/Gly	92	61.7	1.35 ± 0.51*	87.16 ± 21.62	6.15 ± 2.74
	Gly/Asp+Asp/Asp	57	38.3	1.60 ± 0.74	89.76 ± 22.01	5.59 ± 1.71
Female	Gly/Gly	79	71.2	1.31 ± 0.56	90.72 ± 19.05	5.99 ± 2.56
	Gly/Asp+Asp/Asp	32	28.8	1.40 ± 0.45	85.21 ± 18.47	5.41 ± 2.02

T₃ = triiodothyronine; T₄ = thyroxine; TSH = thyroid stimulating hormone. Levels of T₃ compared with male Gly/Gly carriers and Gly/Asp+Asp/Asp carriers, $t = -2.502$, $P = 0.013$, levels of T₄ compared with Gly/Asp+Asp/Asp carriers, $t = -0.707$, $P = 0.480$, levels of TSH compared with Gly/Asp+Asp/Asp carriers, $t = 1.398$, $P = 0.164$; levels of T₃ compared with female Gly/Gly carriers and Gly/Asp+Asp/Asp carriers, $t = -0.718$, $P = 0.475$, levels of T₄ compared with Gly/Asp+Asp/Asp carriers, $t = 1.396$, $P = 0.166$, levels of TSH compared with Gly/Asp+Asp/Asp carriers, $t = 1.157$, $P = 0.250$. * $P < 0.05$.

DISCUSSION

Observational evidence from animal and human studies showed that TH may play an important role in the development and function of the central nervous system. Patients with hypothyroidism may suffer decreased cerebral blood flow, cerebral hypoxia, unresponsiveness, poor memory, comprehension decline, and dementia or other symptoms. Cognitive deficits resulting from dysfunction in thyroid function may promote the occurrence of AD. In addition, increasing evidence supports a strong interrelationship between TH and the cholinergic system. Specifically, there is extensive inter-reliance between TH and acetylcholine, nerve growth factor, and hippocampal function (Mafra and Fodale, 2008). TH plays a primary role in mnemonic processes by regulating insulin-like signaling and potentially modulating hip-

pocampal cognitive processes (Jahagirdar and McNay, 2012). Hypothyroidism also increased the expression of the amyloid- β protein precursor in animal experiments, which plays a key role in the pathogenesis of AD (O'Barr et al., 2006).

Additional data suggested that low TH is a risk factor for cognitive decline. Stern et al. (2004) found that higher T_4 levels within the normal range were associated with increased future cognitive function. Moreover, women without dementia with the lowest T_4 levels exhibited a higher risk for future cognitive decline. TH has a positive relationship with overall cognitive function in healthy, euthyroid older men (Prinz et al., 1999). Wang et al. (2011) also confirmed that AD and MCI patients show lower T_3 levels, and emphasized the relationship between lower T_3 and the development of MCI or AD. Bai et al. (2011) examined features of cognitive function impairment in elderly patients with subclinical hypothyroidism and found that the main cognitive impairment in elderly subclinical hypothyroidism was memory disorder. However, there was no difference in attention, executive function, visuospatial, and word abstract ability. However, a study by Quinlan et al. (2010) suggested that among those with MCI, T_3 levels were inversely associated with cognitive performance across all domains. After stratifying MCI cases according to T_3 levels, those with relatively high T_3 levels showed impairment in memory as well as in visuospatial and executive functions. Those with T_3 levels at or below the lower boundary of the normal range performed comparably to healthy controls. Thus, individuals with T_3 levels in the high normal range may suffer increased cognitive decline. Our control subjects were matched with cases for age, gender, education, hypertension, diabetes, and other medical history. We showed that T_3 and T_4 levels in MCI patients were still lower than that in the control group. Thus, TH level is one of the main factors affecting cognitive function. This result is consistent with those of Stern et al. (2004) and Wang et al. (2011), but not with those of Quinlan et al. (2010). Recently, an evidence-based analysis examining thyroid and aging reported that subclinical hyperthyroidism was associated with a higher incidence of cognitive impairment, but there was no evidence to support use of antithyroid drugs for dementia (Aggarwal and Ravzi, 2013).

Nervous system tissues contain a relatively high ratio of T_3/T_4 compared to circulation or non-nervous tissues (Nunez et al., 2008). DIO_2 and type 3 deiodinase are both expressed in the nervous systems and control TH activity. Eighty percent of T_3 in the brain is generated from T_4 by DIO_2 , which is expressed in glial cells. Thus, DIO_2 activity may affect the neurophysiology of neurons. DIO_2 is considered an important enzyme in the maintenance of intracellular T_3 levels in the central nervous system. The expression of DIO_2 in the brain and pituitary cell may increase during hypothyroidism to maintain T_3 level and cell function (Croteau et al., 1996). The DIO_2 gene is located on human chromosome 14q24.2-24.3 and includes 2 exons and 1 intron. The cDNA contains 918 nucleotides of the open reading frame that encodes a 30-kDa protein. Two commonly occurring polymorphisms have been described in the human DIO_2 gene and influence TH action. For example, the Thr92Ala polymorphism can reduce DIO_2 activity and T_3 concentration. In contrast, the ORFa-Gly3Asp variant can enhance the transcriptional activity of DIO_2 and T_3 concentration (Butler et al., 2010; Peltsverger et al., 2012).

The Thr92Ala polymorphism has also been linked to an increased risk of schizophrenia (Colak et al., 2013) and mental retardation (Guo et al., 2004). DIO_2 gene polymorphisms may be associated with intelligence development, but few studies have identified a relationship between DIO_2 gene polymorphisms and MCI or AD. Our study indicated that there were no statistical differences in genotype or allele frequencies in the DIO_2 gene (Thr92Ala or ORFa-Gly3Asp) and the MCI group compared with control subjects in a Uyghur population

($P > 0.05$). However, we observed that the Ala allele frequency in the MCI group for males (48.6%) was higher than that in the control group (38.7%), although the difference was not significant ($P = 0.08$). Male carriers of the Ala allele may have an increased incidence of MCI, but the difference is not significant. Further studies examining the correlation between different genotypes of Thr92Ala and TH found that the level of TH for Ala/Ala carriers was much lower than that of Thr/Thr and Thr/Ala carriers ($P < 0.05$), consistent with the results of Canani et al. (2005). Male carriers of the Ala allele accompanied by decreased TH showed an increased risk of MCI. No association was found between women carrying the Thr92Ala mutation and the incidence of MCI.

ORFa-Gly3Asp genotype and allele frequencies were significantly different between MCI patients and the control group according to gender ($P < 0.05$). Male carriers of the Asp allele were less common among MCI patients than among controls. The risk of MCI was decreased in male carriers of the Asp allele (OR = 0.471, $P < 0.05$). Examination of the relationship between different genotypes of ORFa-Gly3Asp and TH revealed that the serum level of T_3 in male carriers of the Asp allele was higher than that of Gly carriers, which was consistent with the results of Coppotelli et al. (2006). Thus, the Gly3Asp mutation in males may increase the serum level of T_3 , decrease the incidence of MCI, and act as a protective factor. In contrast, female carriers of the Asp allele were more frequent among MCI patients (OR = 2.842, $P < 0.05$). However, the serum levels of T_3 with Gly/Gly, Gly/Asp, and Asp/Asp genotypes showed no difference in women ($P > 0.05$). Thus, the effect of increased T_3 concentration on the ORFa-Gly3Asp mutation was limited. This may be because hormone levels can influence the cognitive function of postmenopausal women (Ryan et al., 2012), acting as confounding factors. The number of women enrolled in this study was low, and the frequency of the Gly3Asp mutation in the Hapmap database was very low in Asia. Being female is a risk factor for MCI. Therefore, a single gene mutation may not be the main factor altering TH level. However, the results are biased, and whether an association exists between the Gly3Asp mutation in females and MCI remains unknown. Thus, a common mutation in the DIO_2 gene may play a role in the incidence of male MCI.

In summary, we examined the relationship between common single-nucleotide polymorphisms in the DIO_2 and MCI through epigenetic analysis. The sample size was small and we did not conduct the analysis based on ethnicity. Additional studies examining differences in microRNA will increase the understanding of changes in RNA and protein levels and their regulation as well as the signaling pathways of nerve cells.

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REFERENCES

- Aggarwal N and Razvi S (2013). Thyroid and aging or the aging thyroid? An evidence-based analysis of the literature. *J. Thyroid Res.* 2013: 481287.
- Bai J, Gao Y and Zhao GP (2011). Features of cognitive function impairment in old patients with subclinical hypothyroidism. *J. Clin. Neurol.* 24: 68-70.
- Butler PW, Smith SM, Linderman JD, Brychta RJ, et al. (2010). The Thr92Ala 5' type 2 deiodinase gene polymorphism is associated with a delayed triiodothyronine secretion in response to the thyrotropin-releasing hormone-stimulation

- test: a pharmacogenomic study. *Thyroid* 20: 1407-1412.
- Canani LH, Capp C, Dora JM, Meyer EL, et al. (2005). The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. *J. Clin. Endocrinol. Metab.* 90: 3472-3478.
- Colak A, Akan G, Oncu F Yanbay H, et al. (2013). A association study of the dio2 gene as a susceptibility candidate for schizophrenia in the turkish population: a case-control study. *European Psychiatry* 28: 1.
- Coppotelli G, Summers A, Chidakel A, Ross JM, et al. (2006). Functional characterization of the 258 A/G (D2-ORFa-Gly3Asp) human type-2 deiodinase polymorphism: a naturally occurring variant increases the enzymatic activity by removing a putative repressor site in the 5'UTR of the gene. *Thyroid* 16: 625-632.
- Croteau W, Davey JC, Galton VA and St Germain DL (1996). Cloning of the mammalian type II iodothyronine deiodinase. A selenoprotein differentially expressed and regulated in human and rat brain and other tissues. *J. Clin. Invest.* 98: 405-417.
- Guo TW, Zhang FC, Yang MS, Gao XS, et al. (2004). Positive association of the DIO2 (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. *J. Med. Genet.* 41: 585-590.
- Jahagirdar V and McNay EC (2012). Thyroid hormone's role in regulating brain glucose metabolism and potentially modulating hippocampal cognitive processes. *Metab. Brain Dis.* 27: 101-111.
- Lazarus JH (2012). Thyroid hormones and cognitive function. *Expert Rev. Endocrinol. Metab.* 7: 365-367.
- Mafrica F and Fodale V (2008). Thyroid function, Alzheimer's disease and postoperative cognitive dysfunction: a tale of dangerous liaisons? *J. Alzheimers Dis.* 14: 95-105.
- Nunez J, Celi FS, Ng L and Forrest D (2008). Multigenic control of thyroid hormone functions in the nervous system. *Mol. Cell. Endocrinol.* 287: 1-12.
- O'Barr SA, Oh JS, Ma C, Brent GA, et al. (2006). Thyroid hormone regulates endogenous amyloid-beta precursor protein gene expression and processing in both *in vitro* and *in vivo* models. *Thyroid* 16: 1207-1213.
- Peltsverger MY, Butler PW, Alberobello AT, Smith S, et al. (2012). The -258 A/G (SNP rs12885300) polymorphism of the human type-2 deiodinase gene is associated with a shift in the pattern of secretion of thyroid hormones following a TRH-induced acute rise in TSH. *Eur. J. Endocrinol.* 166: 839-845.
- Prinz PN, Scanlan JM, Vitaliano PP, Moe KE, et al. (1999). Thyroid hormones: Postive relationship with cognition in healthy, euthyroid older men. *J. Gerontol. A Biol. Sci. Med. Sci.* 54: 111-116.
- Quinlan P, Nordlund A, Lind K, Gustafson D, et al. (2010). Thyroid hormones are associated with poor cognition in mild cognitive impairment. *Dement. Geriatr. Cogn. Disord.* 30: 205-211.
- Ryan J, Stanczyk FZ, Dennerstein L, Mack WJ, et al. (2012). Hormone levels and cognitive function in postmenopausal midlife women. *Neurobiol. Aging* 33: 1138-1147.
- Stern RA, Davis JD, Rogers BL, Smith KE, et al. (2004). Preliminary study of the relationship between thyroid status and cognitive and neuropsychiatric functioning in euthyroid patients with Alzheimer dementia. *Cog. Behav. Neurol.* 17: 219-223.
- Wang BY, Yang H, Zhong Y, Miao Y, et al. (2011). Clinical study on levels of blood fat, Folic acid, vitamin B₁₂ and thyroid hormone in elderly patients with mild cognitive impairment. *Modern J. Integrated Trad. Chin. West Med.* 20: 4464-4466.
- Zhou XH, Liang J, Dong LM, Zhang ZY, et al. (2012). Study of thyroid hormone level in mild cognitive impairment dysfunction patients in Xinjiang Uygur and Han nationalities. *Chin. J. Lab. Med.* 35: 921-925.