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Associations of serum microRNA-20a, -27a, and -103a with cognitive function in a Japanese population: The Yakumo study

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ABSTRACT

Objectives: MicroRNAs (miRNAs) dysregulate gene expression by binding to target messenger RNAs, and play an important role in the pathogenesis of various diseases, including cancers, cardiovascular diseases and diabetes. Circulating miRNAs have increasingly been recognized as biomarkers for detecting and diagnosing those diseases. Few studies have investigated the association of circulating miRNA with the early stages of cognitive impairment, such as mild cognitive impairment, in the general population. The purpose of this study was to examine the association between cognitive function and several serum miRNAs levels related to amyloid precursor protein (APP) proteolysis in a Japanese general population who had never been diagnosed with dementia.
Methods: We conducted a cross-sectional study of 337 Japanese subjects (144 men, 193 women) who attended a health examination. The short form of the Mini-Mental State Examination (SMMSE) was used to assess cognitive function. Serum levels of 6 miRNAs (let-7d, miR-17, miR-20a, miR-27a, miR-34a, miR-103a) were measured by quantitative real-time polymerase chain reaction.
Results: Multivariable-adjusted odds ratios (ORs) for lower SMMSE score (SMMSE score < 28) were significantly increased in the lowest tertile of serum miR-20a (OR, 2.08; 95% confidence interval (CI), 1.09–4.04) and miR-103a (OR, 1.91; 95%CI, 1.00–3.69) compared to the highest tertile. Moreover, serum levels of miR-20a, -27a, and -103a were linearly and positively associated with SMMSE scores after adjustment for confounding factors.
Conclusion: Low serum levels of miR-20a, -27a, and -103a are independently associated with cognitive impairment.

1. Introduction

The increasing number of dementia patients with the progression of aging populations represents a global issue. In 2015, around 46.8 million people were estimated to be suffering from dementia worldwide, and this number is expected to almost double every 20 years (74.7 million in 2030, 131.5 million in 2050) (Alzheimer's Disease International, 2015). The worldwide estimated cost of dementia is considered likely to rise dramatically with increases in the number of older individuals (Wimo et al., 2017). The increase in dementia patients has recently developed into not only a social issue, but also an economic problem.

Mild cognitive impairment (MCI) is an intermediate stage between normal and dementia. Some longitudinal studies have reported that the conversion rate from MCI to dementia is 10–15% per year, while the reversion rate from MCI to normal cognition is about 20% per year (Larrieu et al., 2002; Plassman et al., 2008; Ravaglia et al., 2008; Roberts & Knopman, 2013). Early detection and treatment of MCI is

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necessary for the prevention of dementia. However, blood biomarkers to identify dementia and MCI before onset have not yet been established, even though some studies have been carried out (Karim et al., 2014; Stanga, Lanni, Sinforiani, Mazzini, & Racchi, 2012; Uchida et al., 2015).

MicroRNAs (miRNAs) are small (18–22 nucleotides), single-stranded, non-coding RNAs. Humans are known to show over 2500 types of miRNAs in various bodily fluids: serum, plasma, urine, tears, saliva, and breast milk (Cortez & Calin, 2009; Hanke et al., 2010; Park et al., 2009; Weber et al., 2010; Zubakov et al., 2010). These miRNAs dysregulate gene expression by binding to the 3′-untranslated region (UTR) of complementary target messenger RNAs (mRNAs). Given their stability in the blood, circulating miRNAs have been widely reported as potential biomarkers of diseases such as cancers, cardiovascular diseases and diabetes (Mitchell et al., 2008). Many studies (Dong et al., 2015; Sheinerman, Tsivinsky, Abdullah, Crawford, & Umansky, 2013; Sorensen, Nygaard, & Christensen, 2016; Weinberg, Mufson, & Counts, 2015; Xie et al., 2015) have reported that several circulating miRNAs are associated with Alzheimer's disease (AD) and MCI. Dong et al. found that four serum miRNAs (miR-31, -93, -143 and -146a) levels were significantly lower in AD compared with controls (Dong et al., 2015). Xie et al. investigated serum miRNA levels of MCI patients and normal controls, and reported that serum miR-206 and -132 levels were positively associated with the Montreal Cognitive Assessment score in MCI (Xie et al., 2015). The studies conducted by Weinberg, R.B. (case = 20 and control = 12), Sorensen, S.S. (case = 10 and control = 10), and Sheinerman, K.S. (case = 50 and control = 50) have reported that several circulating miRNAs were associated with AD or MCI, but were case-control studies with small number of subjects. Most of previous studies have been case-control study design and few reports appear to have examined the association of circulating miRNA with early-stage cognitive impairment conditions before MCI onset among general population.

AD is the most major form of dementia in the elderly and characterized by slow cognitive decline, particularly in the memory domain. AD progression is related to amyloid-beta peptide (Aβ) deposits in the brain and its cytotoxicity causes the death of nerve cells, resulting in decreasing cognitive function. In the pathological mechanisms of AD onset, Aβ is cleaved from amyloid precursor protein (APP) by β-secretase and γ-secretase, and aggregates (Allsop & Mayes, 2014). APP proteolysis is the starting point of Aβ production. Recent studies have reported that several miRNAs (let-7d, miR-17, -20a, -27a, -34a and -103a) regulate APP proteolysis (Hebert et al., 2009; Jian et al., 2017; Jiao et al., 2017; Luo et al., 2015; Sala Frigerio et al., 2013). The present study therefore examined the association between cognitive function and serum miRNAs levels related to APP proteolysis in a Japanese general population who had never been diagnosed with dementia.

2. Methods

2.1. Study subjects

Of 557 residents who attended a health examination in Yakumo, a town in Hokkaido, in August 2012, the 337 subjects (144 men, 193 women) who had undergone cognitive function testing were included in this study. These subjects had never been diagnosed with dementia. All participants provided written informed consent. The ethics review committee at Fujita Health University approved the study protocol (approval No. 164).

2.2. Cognitive function test

We conducted a short version of the Mini-Mental State Examination (SMMSE) using Nagoya University Cognitive Assessment Battery version 2 to screen cognitive function (Hatta, 2004). SMMSE is used widely around the world, and is reported to offer the same level of screening power as the original MMSE (Folstein, Folstein, & McHugh, 1975; Iwahara & Hatta, 2016). We used a cut-off SMMSE score of 27/28 to distinguish subjects with early cognitive impairment (Bartos & Raisova, 2016) and defined the subjects with SMMSE score < 28 as lower SMMSE scores.

2.3. Data and sample collection

Public health nurses administered a questionnaire assessing health and lifestyle, including drinking habit, smoking habit and history of major illnesses. Fasting serum samples were taken from all subjects during the health examination and sera were separated from blood samples within 1 h of collection by centrifugation, and stored at −80 °C until analysis. Biochemical analysis was conducted using an auto-analyzer (JCA-BM9130; Nihon Denshi, Tokyo, Japan) at Yakumo General Hospital.

2.4. Measurement of miRNA

Levels of 6 miRNAs in sera (let-7d, miR-17, -20a, -27a, -34a, and -103a) were measured by quantitative real-time polymerase chain reaction as previously described (Suzuki et al., 2016; Yamada et al., 2013). These miRNAs are reportedly associated with APP proteolysis involved in AD pathology (Hebert et al., 2009; Jian et al., 2017; Jiao et al., 2017; Luo et al., 2015; Sala Frigerio et al., 2013). Serum levels of miRNA expression were calibrated relative to those among subjects with normal SMMSE (≥ 28).

2.5. Statistical analysis

We conducted all statistical analyses using JMP version 12 (SAS Institute, Cary, NC). Because serum miRNA levels and SMMSE scores showed log-normal distributions, these values were log-transformed for statistical analysis. Normally distributed variables are expressed as mean (standard deviation). SMMSE scores are expressed as geometric mean and interquartile range. Serum levels of miRNAs are expressed as relative geometric mean and interquartile range based on subjects with normal cognition. Student's *t*-test was used to compare continuous parameters between subjects with lower SMMSE scores and normal subjects, and Pearson's chi-square test was used to compare categorical variables. Pearson's correlation analysis was used to show the relationship between serum miRNA levels and SMMSE scores. We calculated confounding factor-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of serum miRNA levels for lower SMMSE scores (SMMSE score < 28) using the highest tertile group of miRNAs as the reference by logistic regression. Moreover, multivariate regression analyses were performed to estimate the continuous association between serum miRNA levels and SMMSE scores. We used sex, age, educational level (6–9, 12, 14–16 years), body mass index (BMI), hemoglobin (Hb) A_{1c}, low-density lipoprotein-cholesterol (LDL-C), systolic blood pressure (SBP), smoking habit (current smoker, never smoker, or former smoker) and drinking habit (current drinker, never drinker, or former smoker) as confounding factors, because these factors are known to be associated with MCI (Niu et al., 2013; Xu et al., 2009; Zou et al., 2014) and cognitive decline (Sabia et al., 2012). All statistical tests were two-tailed, and values of $p < 0.05$ were considered significant.

3. Results

The characteristics of study subjects are shown in Table 1. A total of 122 subjects (36.2%) showed lower SMMSE scores (SMMSE

Table 1
Characteristics of study subjects by cognitive status.

	Cognitive status		
	Normal (n = 215)	Lower SMMSE n scores (n = 122)	*p*-value
Women, n / %	138 / 64.2	55 / 45.1	< 0.001 [d]
Age, years [a]	60.9 (9.9)	67.6 (9.7)	< 0.001 [e]
Educational level			
6-9 years, n / %	45 / 41.7	63 / 58.3	< 0.001 [d]
12 years, n / %	104 / 71.7	41 / 28.3	
14-16 years, n / %	66 / 78.6	18 / 21.4	
BMI, kg/m^2 [a]	23.0 (3.4)	24.4 (2.8)	< 0.001 [e]
HbA_{1c}, % [a]	5.4 (0.5)	5.6 (0.8)	0.037 [e]
LDL-C, mg/dl [a]	124.5 (29.7)	123.7 (34.6)	0.832 [e]
SBP, mmHg [a]	132.4 (19.2)	140.4 (19.2)	< 0.001 [e]
DBP, mmHg [a]	75.1 (12.1)	79.4 (13.3)	< 0.001 [e]
Current smoker, n / %	29 / 13.5	24 / 19.7	0.325 [d]
Current drinker, n / %	90 / 41.9	46 / 37.7	0.744 [d]
SMMSE score, points [b]	29 (29-30)	25 (25-26)	< 0.001 [e]
let-7d [c]	1.00 (0.15-4.22)	0.89 (0.14-4.31)	0.557 [e]
miR-17 [c]	1.00 (0.41-1.88)	0.93 (0.37-2.53)	0.659 [e]
miR-20a [c]	1.00 (0.35-2.69)	0.70 (0.22-2.18)	0.035 [e]
miR-27a [c]	1.00 (0.28-3.16)	0.64 (0.22-1.86)	0.010 [e]
miR-34a [c]	1.00 (0.58-1.65)	1.19 (0.70-2.16)	0.164 [e]
miR-103a [c]	1.00 (0.16-6.55)	0.51 (0.09-4.10)	0.014 [e]

Normal: SMMSE ≥ 28, Lower SMMSE scores: SMMSE < 28, BMI, body mass index; HbA_{1c}, hemoglobin A_{1c}; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein-cholesterol.

[a] Data are expressed as mean value (standard deviation).

[b] Data are expressed as geometric mean value (interquartile range).

[c] Data are expressed as relative geometric means (interquartile range) based on normal subjects.

[d] Pearson's chi-square test.

[e] Student's *t*-test. (SMMSE scores and serum miRNA levels were log-transformed for analysis.).

score < 28). The proportion of women was significantly lower among subjects with lower SMMSE scores compared to normal subjects. Subjects with lower SMMSE scores were significantly older than normal subjects. Moreover, subjects with lower SMMSE scores displayed a significantly lower educational level and significantly higher BMI, HbA_{1c}, SBP, and diastolic blood pressure (DBP) compared to normal subjects. Serum levels of miR-20a, -27a and -103a in subjects with lower SMMSE scores were significantly lower than in those with normal cognition (Table 1, Fig. 1). Also, although these correlation coefficients were relatively low (miR-20a: r = 0.113, p = 0.043; miR-27a: r = 0.139, p = 0.014; miR-103a: r = 0.150, p = 0.007, respectively), serum miR-20a, -27a and -103a levels had significant positive correlation with SMMSE scores.

Table 2 shows confounding factor-adjusted ORs and 95%CIs for lower SMMSE scores according to serum miRNA levels. Sex, age, and educational level-adjusted ORs and 95%CIs (Model 1) for lower SMMSE scores were significantly higher in subjects with low serum levels of miR-20a, -27a, and -103a, compared to those with high levels. Even with further adjustment for BMI, HbA_{1c}, LDL-C, SBP, smoking habits, and drinking habits (Model 2), significantly higher OR and 95%CI (miR-20a: OR, 2.08; 95%CI, 1.09–4.04, miR-103a: OR; 1.91; 95%CI, 1.00–3.69) for lower SMMSE scores were obtained in subjects with low serum levels of miR-20a and miR-103a. Moreover, multivariate regression analysis was performed to estimate the association between serum miRNA levels and SMMSE scores (Table 3). Serum levels of miR-20a, -27a, and -103a were linearly associated with SMMSE scores after adjustment for confounding factors.

4. Discussion

This study identified that low serum miR-20a, -27a, and miR-103a levels were significantly associated with cognitive impairment in Japanese individuals who had never been diagnosed with dementia. Although serum levels of let-7d, miR-17 and miR-34a reportedly correlate with APP proteolysis (Hebert et al., 2009; Jian et al., 2017; Luo et al., 2015), no significant associations between these miRNAs and SMMSE scores were observed after adjusting for sex, age, educational level, BMI, HbA_{1c}, LDL-C, SBP, smoking habit and drinking habit. These results suggest that serum levels of miR-20a, -27a, and 103a, that were reportedly associated with APP proteolysis involved in AD pathology, may already decrease at the early stage of cognitive impairment.

Intracellular miR-20a has been reported to regulate APP expression (Delay, Calon, Mathews, & Hebert, 2011; Fan et al., 2010). Fan et al. showed a negative correlation between intracellular miR-20a and APP protein level in ovarian cancer cells (Fan et al., 2010). They found that miR-20a could negatively regulate APP expression by directly binding to the 3′-UTR of APP mRNA in ovarian cancer cells (Fan et al., 2010). We infer that serum miR-20a levels might decrease as a result of intracellular miR-20a being altered due to changes in physiological condition.

Leidinger et al. identified downregulation of miR-103a-3p in the peripheral blood of AD patients compared to healthy controls (Leidinger et al., 2013). Interestingly, miR-103a binds to a disintegrin and metalloproteinase 10 (ADAM10), which shows α-secretase activity and is involved in APP proteolysis (Manzine et al., 2015). Overexpression of miR-103a inhibited ADAM10 protein expression in vitro using a model mouse of abdominal aortic aneurysm (Jiao et al., 2017). Postina et al. found that a moderate neuronal overexpression of ADAM10 reduced the formation of Aβ peptides and alleviates cognitive defects with an AD mouse model (Postina et al., 2004). However, ADAM10 levels were reportedly decreased in platelets (Colciaghi et al., 2004) and central nervous system tissue (Marcinkiewicz & Seidah, 2000) from AD patients, but increased in AD brain compared with those of control subjects (Gatta, Albertini, Ravid, & Finazzi, 2002). The epigenetic effect of miR-103a against ADAM10 might be associated with onset of AD, although the action of ADAM10 in AD patients and the biological mechanisms of miR-103a in human have not yet been fully elucidated.

A previous study showed that expression of hsa-miR-27a-3p was reduced in cerebrospinal fluid, and that this miRNA can interact in vitro with the 3′-UTR of AD-relevant transcripts (BACE1, GSK3β, MAPT, and PSEN1) (Sala Frigerio et al., 2013). Especially, BACE1 play the important role in amyloidogenic processing of APP. MiR-34a also regulated BACE1 protein expression reportedly as well as miR-27a (Jian et al., 2017), in addition, let-7d and miR-17 are reported to directly target APP related to AD pathology as well as miR-20a (Hebert et al., 2009; Luo et al., 2015). Let-7d, miR-17 and miR-34a could be expected to contribute to cognitive impairment, as they are reportedly associated with AD pathology. However, our results did not confirm the association between serum levels of these miRNAs and SMMSE scores.

Circulating miRNA levels are implicated in some physiological and pathological conditions, but those levels do not necessarily correlate with intracellular levels of miRNAs. Circulating miRNAs are protected from degradation by inclusion in protein or lipid vesicles. The role of circulating miRNA are not yet completely clear. Several lines of evidence support the hypothesis that miRNAs can be actively and selectively secreted from cells. Cells have been reported to selectively release some miRNAs that mediate cell-to-cell signaling via paracrine or endocrine routes, and circulating miRNAs exhibit hormone-like effects (Bayraktar, Van Roosbroeck, & Calin, 2017).

Several limitations must be kept in mind when interpreting the current results. First, this study was cross-sectional in design, and a

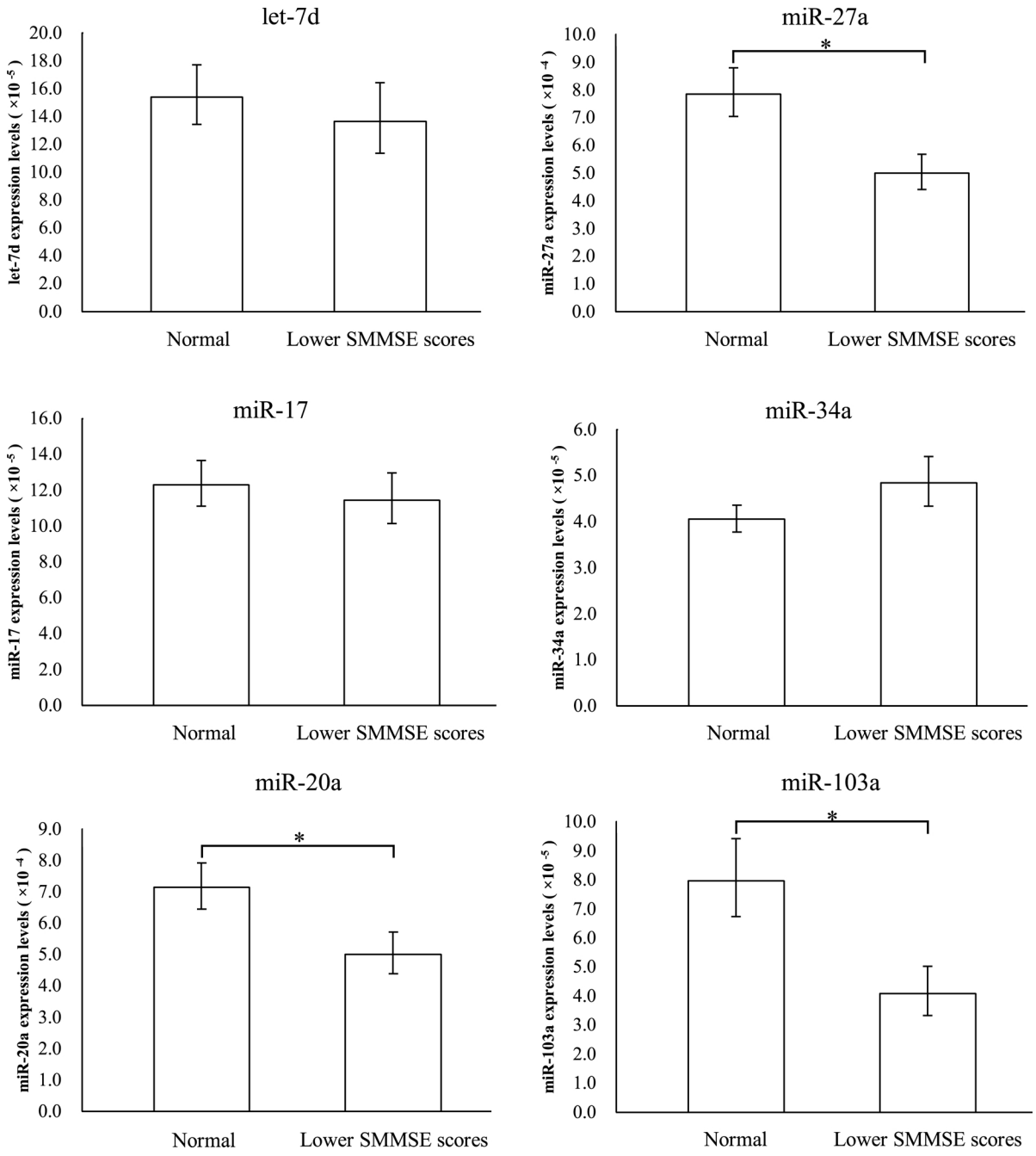

Fig. 1. Comparison of serum miRNAs (let-7d, miR-17, miR-20a, miR-27a, miR-34a, and miR-103a) levels between subjects with normal cognition and those with lower SMMSE scores.
MiRNAs expression levels are expressed as geometric mean values. Error bars represent standard errors.
* : $p < 0.05$.

longitudinal study is required to confirm the true direction of causality in the relationship between serum miRNA levels and cognitive function. It is necessary to verify the reproducibility by analysis using another population or cohort study. Second, some reports have indicated the need to establish appropriate cut-off points following educational level, because educational levels are a factor that may strongly affect MMSE scores (Kochhann, 2010; O'Bryant et al., 2008). However, we only adjusted educational level as a confounding factor for analysis in this study. Further study is needed for analysis using cut-off points established according to educational levels.

In summary, we found a positive association of serum miR-20a, -27a, and -103a levels with SMMSE scores in middle-aged and elderly individuals who had never been diagnosed with dementia, though the mechanism of lowering in these miRNAs is unclear. To the best of our knowledge, this is the first report to investigate the association between serum miRNA level and cognitive function in a general population. These findings imply that serum miR-20a, -27a and miR-103a may already decrease at the early stage of cognitive impairment.

Table 2
Multivariable-adjusted odds ratios and 95% confidence intervals for lower SMMSE scores according to serum miRNA levels.

Serum levels of miRNAs		Total n	Lower SMMSE n scores	Adjusted ORs (95%CIs)	
				Model 1	Model 2
let-7d	Low	102	38	0.71 (0.38-1.34)	0.75 (0.38-1.43)
	Middle	102	39	0.81 (0.43-1.49)	0.82 (0.43-1.56)
	High	102	40	1.00	1.00
miR-17	Low	106	43	1.04 (0.56-1.94)	1.00 (0.53-1.90)
	Middle	106	34	0.72 (0.39-1.34)	0.64 (0.33-1.22)
	High	106	41	1.00	1.00
miR-20a	Low	108	47	2.12 (1.14-4.00)	2.08 (1.09-4.04)
	Middle	108	39	1.51 (0.80-2.85)	1.43 (0.74-2.75)
	High	108	35	1.00	1.00
miR-27a	Low	105	43	2.02 (1.08-3.84)	1.88 (0.97-3.69)
	Middle	107	43	1.55 (0.83-2.94)	1.50 (0.79-2.89)
	High	105	33	1.00	1.00
miR-34a	Low	82	32	1.03 (0.50-2.10)	1.10 (0.53-2.30)
	Middle	83	32	1.11 (0.56-2.23)	1.16 (0.57-2.37)
	High	82	30	1.00	1.00
miR-103a	Low	106	48	1.99 (1.08-3.76)	1.91 (1.00-3.69)
	Middle	107	37	1.27 (0.68-2.40)	1.29 (0.68-2.48)
	High	106	33	1.00	1.00

Model 1 is adjusted for sex, age and educational level. Model 2 is adjusted for sex, age, educational level, BMI, HbA_{1c}, LDL-C, SBP, smoking habit and drinking habit. Lower SMMSE scores: SMMSE < 28.

Table 3
Linear regression analysis for serum miRNA levels with SMMSE scores.

miRNA	Model 1		Model 2	
	standardized β	*p*-value	standardized β	*p*-value
let-7d	0.007	0.896	0.008	0.887
miR-17	0.024	0.646	0.013	0.800
miR-20a	0.117	0.022	0.112	0.029
miR-27a	0.151	0.003	0.139	0.008
miR-34a	−0.028	0.637	−0.013	0.838
miR-103a	0.135	0.009	0.127	0.014

SMMSE scores and serum miRNA levels were log-transformed for analysis. Model 1 is adjusted for sex, age and educational level. Model 2 is adjusted for sex, age, educational level, BMI, HbA_{1c}, LDL-C, SBP, smoking habit and drinking habit.

Conflicts of interest

The authors declare they have no conflict of interest with respect to this research study and paper.

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