

# Plasma Exo-miRNAs Correlated with AD-Related Factors of Chinese Individuals Involved in A $\beta$ Accumulation and Cognition Decline

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## Research Article

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# Abstract

Numerous studies have investigated the risk factors of Alzheimer's disease (AD), however, AD- risk factors related miRNAs were rarely reported. In this study, AD- risk factor related miRNAs of 119 Chinese individuals (47 AD patients and 62 cognitively normal controls) were investigated. The results showed that education, tea consumption, leisure activity, and social connection were low Body Mass Index (BMI), depression, and smoking were potentially risk factors for AD patients. Moreover, we screened exosome microRNAs (miRNAs) for the aforementioned lifestyle-related factors. Canonical correlation analyses revealed that our clinical observations were correlated with the miRNA expression profile for education, depression, leisure activity, and social connection. Furthermore, we predicted the targets of differentially expressed miRNAs and found that many of them were reported to be involved in the generation and clearance of Amyloid-beta (A $\beta$ ), important molecules related to cognition, and disease-activated microglia response to AD. Our results indicated that certain education and depression factors can contribute to AD progression by modulating miRNA expression, implying that preventive interventions might alter AD progression in Chinese patients.

## Introduction

Alzheimer's disease (AD) is a neurodegenerative disease and causes most of patients with dementia over the world. After decades of research, there are still no effective therapy for AD. During the slow progression of AD, the steady cognitive and functional decline are modulated by genetic and environmental protective and risk factors, including vascular, lifestyle-related and psychological factors [1,2]. Observational studies have revealed that statistically a third of the AD cases worldwide can be attributed to the following seven commonly modifiable hazard factors: midlife hypertension, depression, diabetes mellitus, midlife obesity, low education, physical inactivity, and smoking [1,3-6]. Social integration and leisure activity have also been shown to protect against cognitive decline and dementia [7-10].

Exosomes with diameters 40 - 160 nm (average 100 nm) are extracellular vesicles (EVs) derived from the late endosome [11]. They contain many cellular components including proteins, lipids, and nucleic acids (DNA, mRNAs transcripts, and non-coding RNAs (ncRNAs)), mostly miRNAs [12], and have the ability to pass through the Brain Blood Barrier (BBB) [13], making them a promising source of biomarkers to reflect the pathological process in brain. In the central nervous system (CNS), 70% of the miRNAs released by human brain cells and possibly regulate the transcription of more than one-third of the genes [14,15]. Differential miRNA expression in AD patients has been observed in exosomes from the plasma [16], serum [17], and cerebrospinal fluid [18]. Recent studies have demonstrated that exosome miRNA (exo-miRNA) profile can be potentially used for an early diagnosis of AD [19]. In fact, miRNA expression level could be influenced by lifestyle factors. The expression of miRNAs such as miR-935 and miR-4772 in peripheral blood mononuclear cells have been reported to be downregulated in weight loss participants under energy-restricted treatment [20]. Studies have shown that diet as a lifestyle factor that can change the miRNA expression profile in hepatocellular carcinomas [21,22] and esophageal carcinoma [23].

Whether AD-related lifestyle factors can change the miRNA expression profile of plasma exosome remains unreported and should be investigated.

In this study, we evaluated the miRNA expression profile in the plasma exosome Chinese patients with AD and normal individuals using next-generation sequencing data and found the correlations with AD-related factors. We found that AD-related factors, such as education, leisure activity, and depression were correlated with changes in miRNA expression. More important, many targets of these differentially expressed miRNAs were reported to modulate A $\beta$  generation and clearance, and/or important molecules involved in cognition, and disease-activated microglia response to AD.

## Materials And Methods

### Sample information and ethics statement

This study design included 107 Chinese participants of Han descent (47 patients with AD and 62 individuals with normal cognition) aged 60 years and older and performed miRNA expression profile analysis related to lifestyle factors. The data for the study population on sociodemographic, lifestyle variables, and history of diseases were collected by the standardized questionnaire. According to the recommendation from the National Institute on Aging-Alzheimer's Association workgroups [24], the patients with AD were screened using Mini-mental State Examination (MMSE) and then diagnosed by neurologists using a battery of tests including Clinical Dementia Rating (CDR), Geriatric Depression Scale (GDS) and Hachinski Ischemic Scale (HIS), Computerized Tomography (CT) scans for brain. The participants with Normal cognition were determined by trained interviewers using the Montreal Cognitive Assessment based on published cut-offs [25], the MMSE scores of participants with normal cognition were converted based on Montreal Cognitive Assessment (MoCA) scores according to a previous study [26]. Exosome miRNA data extracted from the blood samples of 45 patients with AD and 60 cognitively normal participants were described previously (Sun et al., 2021).

### Assessment of baseline characteristics

The potential influencing factors of Alzheimer's disease were collected based on previous report [27], including education, obesity, smoking, hypertension, diabetes, depression, physical inactivity, and social contact. In addition, numerous epidemiological studies also demonstrated that drinking tea, alcohol consumption, and leisure activity was associated with risks of Alzheimer's disease and dementia [28-30]. Thus, the above demographic information, lifestyle factors, and history of disease were collected in this study. Body Mass Index (BMI) was calculated as the ratio of body weight to the square of body height ( $\text{kg}/\text{m}^2$ , <18.5, 18.5-23.9, 24.0-27.9,  $\geq 28.0$ ). GDS was used to assess the depression of AD patients [31]. Individuals scoring 10 or higher in GDS were considered as suffering from depression. The education levels were categorized as the no-educated (illiteracy) and educated (including elementary school, middle school, high school, and college). The tea, smoke and alcohol consumption of individuals were categorized as Yes or No. Having leisure activity was defined as participating in at least one of following

mental items: reading, using the internet, playing cards/mahjong, calligraphy, handicrafts, watching TV, and playing chess. The social connection questionnaire included the following questions based on previous study with some details modified [32]: 1) living arrangement; 2) frequency of direct and remote contact with children; 3) frequency of contact with neighbors; 4) marital status. The following details were recorded: 1) living status was denoted as 1 for individuals living with family, or a live-in nurse, or in a nursing home, whereas it was denoted as 0 for individuals living alone; 2) frequency of direct and remote contact with children was denoted as 1 for individuals living with their children, had a close relationship with them, or with a contact frequency that was measurable in weeks, whereas it was denoted as 0 when the individual had a general or indifferent relationship with their children or a contact frequency that was measurable in months or years; 3) frequency of contact with neighbors was denoted as 1 for high frequency and 0 for medium or low frequency; 4) marital status was denoted as 1 for individuals in marriage (including remarriage), and as 0 for those not in marriage (including single, divorced, or widowed).

### **Differential miRNA expression analyses**

miRNA expression data from a previous study were used [19]. For protective factors such as education (Fig. 1A), differentially expressed miRNAs were identified based on fold change > 1.1 of the following ratios: NC-uneducated/NC-educated, AD-uneducated / AD-educated, and AD-uneducated / NC-uneducated; or NC-educated / NC-uneducated, AD-educated/ AD-uneducated, and NC-uneducated/ AD-uneducated. The same criteria were used to identify miRNAs for leisure activities (Fig. 1B), social connection (Fig. 1C).

For risk factors such as depression (Fig. 1D), miRNAs were also screened based on fold change > 1.1 of the following ratios: NC-no depression / NC-depression, AD-no depression / AD-depression, and NC-no depression / NC-depression; or NC- no depression / NC-depression, AD-no depression / AD-depression, NC-no depression / AD-no depression. All above figures were performed by the Pheatmap package in R program.

### **Statistical analyses**

In this population, 47 patients with AD and 62 participants with normal cognition were performed the plasma miRNA analysis, and their basic characteristics were presented in Table 1. Canonical correlation analyses were performed to determine the correlations between lifestyle factors and expressed miRNAs specified in Table 2. The SPSS 24.0 was used for statistical analyses of the data presented in Tables 1-2. Prism 5.0 was used to perform one-way ANOVA analysis to determine the significant differences in miRNA expression for lifestyle factors such as education (Fig. 2B, 2E, 4A), leisure activity (Fig. 5B), and depression (Fig. 2F, 2I, 3A, 4D, 4G, 4H, 5A).

### **miRNA target prediction and functional enrichment analyses**

We predicted the targets of the miRNA using the R package multiMiR (V1.10.0). Validated targets in database were selected. Predicted cut-off and limit were set to 20 and 300, respectively. Functional enrichment analyses of target genes were performed using The Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8 [33,34] with default settings. Ten of the most enriched gene sets were selected for visualization (Fig. 2C, G, J, 3B, 4B, 4E). Mature miRNA sequences and the 3' UTR of target mRNAs were downloaded from miRbase and USUC, respectively.

## Results

### Exo-miRNA expression profile for AD-related factors

In order to determine whether miRNA sequencing results can provide insights to identify lifestyle factors involved in preventing AD, we screened the specific miRNAs related to above factors and AD by 45 ADs and 60 NCs from previous sequencing data (2 ADs and 2 NCs were excluded for miRNA screening analysis because of low quality sequencing data). Of all screened miRNAs, 8, 4, 6, 6, 12, 10 and 16 miRNAs were possibly correlated with AD and education, leisure activity, social connection, regular tea consumption, BMI, habitual smoking and depression respectively (Fig. 1 and Supplemental Table 1-3). Except hsa-miR-451a that was reported to be related to depression [35,36], all specific miRNAs were novel AD-associated-factor-related miRNAs. In order to further investigate the relationship between above factors-related miRNA profiles and the clinical measurement of these factors, we performed canonical correlation analysis, which revealed that miRNA expression was correlated with clinical measurements of education ( $R = 0.477$ ,  $P = 0.01$ ), depression ( $R = 0.605$ ,  $P < 0.001$ ), and leisure activity ( $R = 0.411$ ,  $P = 0.001$ ) (Table 2). Therefore, observational data for education, leisure activity and depression were correlated with miRNA expression.

### hsa-miR-20a-5p, hsa-miR-185-5p, hsa-miR-181c-5p, and hsa-miR-502-3p were involved in A $\beta$ generation

A $\beta$  was generated by the cleavage of Amyloid Protein Precursor (APP) by  $\beta$ -secretase to form CTF- $\beta$  (C-terminal fragment-beta), which was further cleaved by  $\gamma$ -secretase and then underwent several processes to form A $\beta$ .  $\alpha$ -secretase prevents the formation of A $\beta$  by catalyzing APP to  $\alpha$ APP- $\alpha$  (Alpha-cleaved APP-alpha) (Fig. 2A) [37,38]. In order to analyze the function of these miRNAs, validated targets of significantly expressed miRNAs were predicted using DAVID. A decreasing pattern in AD was alleviated by education for hsa-miR-20a-5p (Fig. 2B) that targets several genes enriched in AD (Fig. 2C), including APP (Fig. 2D) [39], which is a well-known culprit of AD [40]. Decreased expression of hsa-miR-20a-5p increased APP expression, which further promoted A $\beta$  generation, a significant signal for AD, which is a well-known culprit of AD and an experientially validated target of miR-20a-5p [40]. Decreased expression of hsa-miR-20a-5p may increase APP expression, which further promoted A $\beta$  generation, a significant signal for AD.

Presenilin 1 (PSEN1), a catalytic component of the  $\gamma$ -secretase complex [41], plays a pivotal role in generating A $\beta$  and increases its expression in AD patients. hsa-miR-185-5p was a decreasing miRNA in AD patients and the decreasing pattern was alleviated by education (Fig. 2E) and aggravated by depression (Fig. 2F). Functional enrichment analysis showed that validated targets were enriched in AD

(Fig. 2G). One of these targets, PSEN1, contained the predicted binding site for hsa-miR-185-5p (Fig. 2H); the same was applied to GSK3B (Glycogen Synthase Kinase 3 Beta), another key player in AD pathology, which regulates APP and PSEN1 [42]. Decreased hsa-miR-185-5p in AD improves the expression of PSEN1 and GSK3B, which further increases A $\beta$  generation.

Another decreased miRNA in AD patients was hsa-miR-181c-5p, whose decreasing pattern was aggravated by depression (Fig. 2I) and its targets were enriched in AD (Fig. 2J). Several targets of hsa-miR-181c-5p had binding sites in their 3' UTRs (Fig. 2K). Breast Cancer 1 (BRCA1), which has increased expression in AD patients, was predicted to be targeted by hsa-miR-181c-5p and reported to affect the turnover of PSEN1 (Fig. 2L) [43]. Methyl-CpG Binding Protein 2 (MeCP2), which was targeted by hsa-miR-181c-5p (Fig. 2K) and upregulated in AD patients, repressed  $\alpha$ -secretase, ADAM Metallopeptidase Domain 10 (ADAM10), to improve A $\beta$  generation (Fig. 2L) [44]. Arrestin Beta 2 (ARRB2), upregulated in AD patients, as another target of hsa-miR-181c-5p, was reported to regulate  $\gamma$ -secretase [45]. IL1A, was expressed in AD patients and directly related to A $\beta$  generation [46], with a predicted binding site in 3' UTR of hsa-miR-181c-5p (Fig. 2K).

Therefore, decreased expression of hsa-miR-20a-5p in AD patients increased APP expression and decreased hsa-miR-185-5p and hsa-miR-181c-5p, thereby increasing the expression of its targets, which further upregulates  $\beta$ -secretase and  $\gamma$ -secretase and downregulates  $\alpha$ -secretase to increase A $\beta$  generation and plaque formation. Education was the protective factor that alleviated A $\beta$  generation, whereas depression exacerbated A $\beta$  generation (Fig. 2L).

### **hsa-miR-20a-5p, hsa-miR-181c-5p, and hsa-miR-664a-3p, were involved in cognitive decline in Alzheimer's disease**

Brain-derived neurotrophic factor (BDNF) is well known as a beneficial marker for cognition; its deficit contributes to cognitive disorders and results in APP fragmentation [47]. A $\beta$  reduces BDNF mainly by lowering phosphorylated cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) [48]. hsa-miR-664a-3p has an increased expression in AD patients, and depression intensified its upregulation (Fig. 3A). CREB1 expression was inhibited by hsa-miR-664a-3p (Fig. 3B & C). RORA, a transcription factor that downregulates BDNF [49], was targeted by hsa-miR-181c-5p and hsa-miR-20a-5p that were correlated with depression and education, respectively (Fig. 2D & 3D). MeCP2 can also selectively repress the expression of BDNF [44]. A decrease in the expression pattern of these two miRNAs led to an increase in the expression of RORA, which in-turn inhibited BDNF expression in AD patients. Therefore, hsa-miR-664a-3p was upregulated in AD patients, which downregulated CREB1 and BDNF expression levels, thereby leading to a cognitive decline in AD patients. hsa-miR-181c-5p and hsa-miR-20a-5p were downregulated in AD patients and targeted both the transcription factors RORA that negatively regulated BDNF as well as MeCP2 whose upregulation further reduces the level of BDNF, thereby aggravating cognitive decline in AD patients (Fig. 3E).

### **hsa-miR-451a, hsa-miR-27a-3p, hsa-miR-497-5p, hsa-miR-181c-5p, and hsa-miR-320a were involved in A $\beta$ clearance in Alzheimer's disease**

We found that lifestyle- and AD-related miRNAs were also involved in A $\beta$  clearance. Kiyota et al. (2011) injected fibroblast growth factor 2 (FGF2) into APP/PS1 mice to reveal that it significantly improved spatial learning and A $\beta$  phagocytosis [50]. The expression of hsa-miR-497-5p was upregulated in AD patients and the upregulation pattern was inhibited by education (Fig. 4A). More than 20 targets of hsa-miR-497-5p were enriched in AD (Fig. 4B), and FGF2 level was regulated directly by hsa-miR-497-5p (Fig. 4C). Upregulated hsa-miR-497-5p in AD patients downregulated the expression of FGF2, thereby causing a decline in A $\beta$  phagocytosis (Fig. 4I).

Forkhead box O1 (FOXO1), which promotes the clearance of A $\beta$ , was reported to reduce the A $\beta$  level in the cortices of AD mice [51]. The expression level of hsa-miR-27a-3p was increased in AD patients and depression exacerbated such an increase (Fig. 4D). More than 10 targets of hsa-miR-27a-3p were enriched in AD (Fig. 4E). Of the 10 targets, FOXO1 was directly bonded by hsa-miR-27a-3p (Fig. 4F). Upregulated hsa-miR-27a-3p in AD patients downregulated the expression of FGF2, which caused a decline in A $\beta$  clearance (Fig. 4I).

Sterol O-acyltransferase 1 (SOAT1) inhibition has been reported to stimulate autophagy-mediated lysosomal proteolysis and increase A $\beta$  clearance [52]. miRNA hsa-miR-181c-5p was downregulated in AD patients, and their downregulation pattern was aggregated by depression (Fig. 4I). Although there was no binding site in their 3' UTRs, the two miRNAs were predicted to inhibit SOAT1 [53]. Downregulated hsa-miR-181c-5p caused an increase in SOAT1 expression, thereby inhibiting autophagy-mediated lysosomal proteolysis and A $\beta$  clearance (Fig. 4I).

Aquaporin 4 (AQP4) is involved in A $\beta$  clearance through the glymphatic pathway. AD led to a loss of polarity of AQP4 at the astrocytic endfeet, thereby causing more A $\beta$  deposition because of a dysfunction in the glymphatic system [54]. AQP4 was predicted to be inhibited by hsa-miR-320a, which is increased by depression in AD patients (Fig. 4H). Reduced AQP4 possibly increased A $\beta$  deposition owing to a dysfunction of the glymphatic system (Fig. 4I).

Metalloproteins, MMP2 and MMP9, have been reported to cleave soluble and fibrillar forms of A $\beta$  [55]. MMP2 and MMP9 [56] were targeted by hsa-miR-451a, which was decreased by depression in AD patients (Fig. 4I). It is possible that an increase in MMP2 and MMP9 facilitates the cleavage of A $\beta$ ; however, it cannot keep up with the speed of A $\beta$  accumulation in AD patients.

### **Other miRNAs related to AD-related factors**

There were two more miRNAs that were correlated with AD and lifestyle factors, even though no validated targets were identified so far. hsa-miR-23b-3p was upregulated in AD patients, and the upregulating pattern was enhanced by depression (Fig. 5A). hsa-miR-502-3p was also upregulated in AD patients; however, this expression pattern was alleviated by leisure activity (Fig. 5B). Loss of TREM2 (Triggering Receptor Expressed On Myeloid Cells 2) function compromises the microglial response (the disease-activated microglia pattern) to the accumulation of A $\beta$  [57]. Skipping the transcription of exon 3 of TREM2 causes nonsense-mediated mRNA decay, thereby causing a reduction in functional TREM2 in AD



patients. CELF2 (CUGBP Elav-Like Family Member 2) has been linked to the exon3 transcription skipping, and its overexpression enhances TREM2 exon3 skipping [58]. hsa-miR-185-5p was downregulated in AD patients (Fig. 2E, F), which promoted CELF2 expression (Fig. 5C). Therefore, a decrease in the expression level of hsa-miR-185-5p in AD patients was enhanced by depression and reduced by education, thereby promoting its target CELF2, which reduced the TREM2 function in the microglia and further increased A $\beta$  accumulation (Fig. 5D).

## Discussion

Our study found that several AD-related factors, including education, leisure-time mental activity, and depression were correlated to miRNA expression. Compared to normal cognition group, a decreased expression pattern of hsa-miR-185-5p in AD group was enhanced by depression and alleviated by education significantly, this miRNA was involved in A $\beta$  generation (Fig. 2L) and accumulation (Fig. 5D). hsa-miR-181c-5p, which is a depression and AD-related miRNA (Fig. 2I), was involved in A $\beta$  generation (Fig. 2L), cognitive decline (Fig. 3E), and A $\beta$  clearance (Fig. 4I). hsa-miR-20a-5p, which was decreased in AD patients, was alleviated in people educated and involved in A $\beta$  generation (Fig. 2L) and cognitive decline (Fig. 3E).

To our knowledge, this is the first report to correlate clinical observations with miRNAs expression profiles in AD and AD - related factors. In this study, our results provide new insight on how lifestyle- and AD-related miRNAs promote AD progression through validated targets. APP and PSEN1 are critical molecules for process of A $\beta$  accumulation, the mainstream hypothesis for AD [37,38]. APP was one target of hsa-miR-20a-5p, reduced expression pattern of hsa-miR-20a-5p in AD patients improves APP expression, the subtract of A $\beta$ . The decreasing expression pattern of hsa-miR-20a-5p in AD was alleviated in educated people. Presenilin 1 (PSEN1) is a catalytic component of the  $\gamma$ -secretase complex [41], and plays a pivotal role in generating A $\beta$  and increases its expression in AD patients. PSEN1 is a target of hsa-miR-185-5p, a decreasing miRNA in AD patients. And its decreasing expression pattern was alleviated by education (Fig. 2E) and aggravated by depression (Fig. 2F). Besides, BDNF is a beneficial molecule for cognition and its expression level decreases in AD patient because of A $\beta$  accumulation [48]. Several molecules influence BDNF expression directly including CREB1, MeCP2, and RORA, which were targeted by hsa-miR-664a-3p, hsa-miR-181c-5p, and hsa-miR-20a-5p (Fig. 3E). The expression pattern of above three miRNAs in AD patients caused BDNF deficiency through validated targets, and the miRNA expression trends were alleviated by education and aggregated by depression.

Interestingly, the targets of our miRNAs are also enriched in various types of cancers. In fact, miRNAs participate in conducting and regulating many cellular processes such as proliferation, apoptosis, differentiation, metabolism, and immunity [59-64]. Abnormal miRNA expression is usually related to diseases such as cancers [65,66], AD [67], and the coronavirus pandemic [68,69]. Based on the miRNA expression panels, there is a hypothesis that AD and cancer (hematologic malignancies, colorectal, and lung cancer) are characterized by a shift in the expression of the same genes but in the opposite direction, which is supported by several reports [70-74]. Stánitz et al. [75,76] reported lifestyle-related

miRNA profile in gastric cancer and esophageal cancer samples. In gastric cancer samples, upregulated expression of miR-21 was related to low social status and habitual smoking, and the downregulation of miR-143 was related to habitual smoking. In esophageal cancer samples, increased expression of miR-205 was related to smoking, and reduced expression of miR-143 and miR-203 were related to low social status and smoking. Therefore, it is interesting to study the lifestyle factors involved in AD and cancer in future studies.

## Limitations

There were some limitations for the samples of miRNA expression used in this study, although our sample size (n=105) did provide insights into AD-related factors. For example, only one person was educated and suffered from AD, while no NCs suffered from depression (Table 1), these limitations indicate a scope for further study in this area. Moreover, although the validated targets of specific miRNA were predicted in this study, transcriptome or proteome would be used for validation and more information in our further research. Nevertheless, another validated group should be used for further validation for the specifically expressed miRNA and verifying the potential biomarkers for the AD -related lifestyle factors in our future study.

## Conclusion

In this study, we first reported AD and education /depression-related miRNAs. Specific expressed miRNAs were reported to be correlated with AD related factors, such as hsa-miR-185-5p, hsa-miR-20a-5p, hsa-miR-181c-5p and hsa-miR-497-5p. Besides, the predicted targets of these miRNAs were indicated to take part in A $\beta$  generation and clearance, cognitive decline, and progression of AD. These findings provided clues of AD preventive interventions in Chinese people.

## Declarations

### *Ethics approval and consent to participate*

All participants were approved by the BGI IRB (BGI-IRB 20149) and the Medical Ethics Committee of Medical College, Wuhan University of Science and Technology (20190049), and each of them provided informed consent before sample collection, and all samples were anonymized before further analysis.

### *Consent for publication*

Not applicable.

### *Availability of data and materials*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### *Competing Interests*

The authors declare no competing financial interests.

### *Author Contributions*

L. W., C. N. and H. S. designed the research; L. W. analyzed bioinformatic data; L. W. analyzed the clinical observational data, H. Z., Y. S. and C. N. provided the sequencing data, S. R. and B. L. provided the clinical observational data and advices, Z. S., Z. L., and R. G. contributed unpublished reagents/analytic tools; Z. L., J. D., H. Y., X. X., and X. Z provided unpublished advices; L. W. wrote the paper. All the authors read and approved the final manuscript.

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## Tables

Table 1 Baseline characters of the study population

Characteristics	NC (n = 62)	AD (n = 47)	P	
Age, mean (SD), years	71.2 (5.6)	73.5 (7.2)	0.062	
Female, n (%)	43 (69.4)	41 (87.2)	0.028	
MMSE (SD)	28.28 (1.95)	12.87 (4.12)	<0.001	
BMI, kg/m <sup>2</sup>			<0.001	
Underweight (<18.5)	3 (5.1)	13 (27.7)		
Normal weight (18.5-23.9)	34 (57.6)	26 (55.3)		
Overweight (24.0-27.9)	19 (32.2)	8 (17.0)		
Obese (>=28)	3 (5.1)	0 (0.0)		
Education			<0.001	
No education	15 (24.6)	46 (97.9)		
Elementary school	15 (24.6)	1 (2.1)		
Middle school	12 (19.7)	0 (0.0)		
High school	6 (9.8)	0 (0.0)		
college	13 (21.3)	0 (0.0)		
Current smoking	4 (6.5)	12 (25.5)	0.012	
Alcohol consumption	9 (14.5)	7 (14.9)	0.928	
Tea drinking	18 (29.0)	2 (4.3)	0.002	
Hypertension	26 (42.6)	15 (31.9)	0.127	
Depression	0 (0.0)	24 (51.1)	<0.001	
Social connection			0.002	
	Poor	4 (6.5)	6 (12.8)	
	Moderate	23 (37.1)	31 (65.9)	
	Rich	35 (56.5)	10 (21.3)	
Leisure activity	49 (79.0)	2 (4.3)	<0.001	
Physical activity	60 (96.8)	43 (91.5)	0.476	
Social connection, mean (SD)	2.9 (1.1)	2.1 (1.2)	< 0.001	

Note: Data are presented as number (proportion %). Missing data: None.

Abbreviations: NC, normal cognition; AD, Alzheimer's disease; BMI, body mass index.

Chi-squared tests was used for statistical analysis of categoric variables by SPSS software.

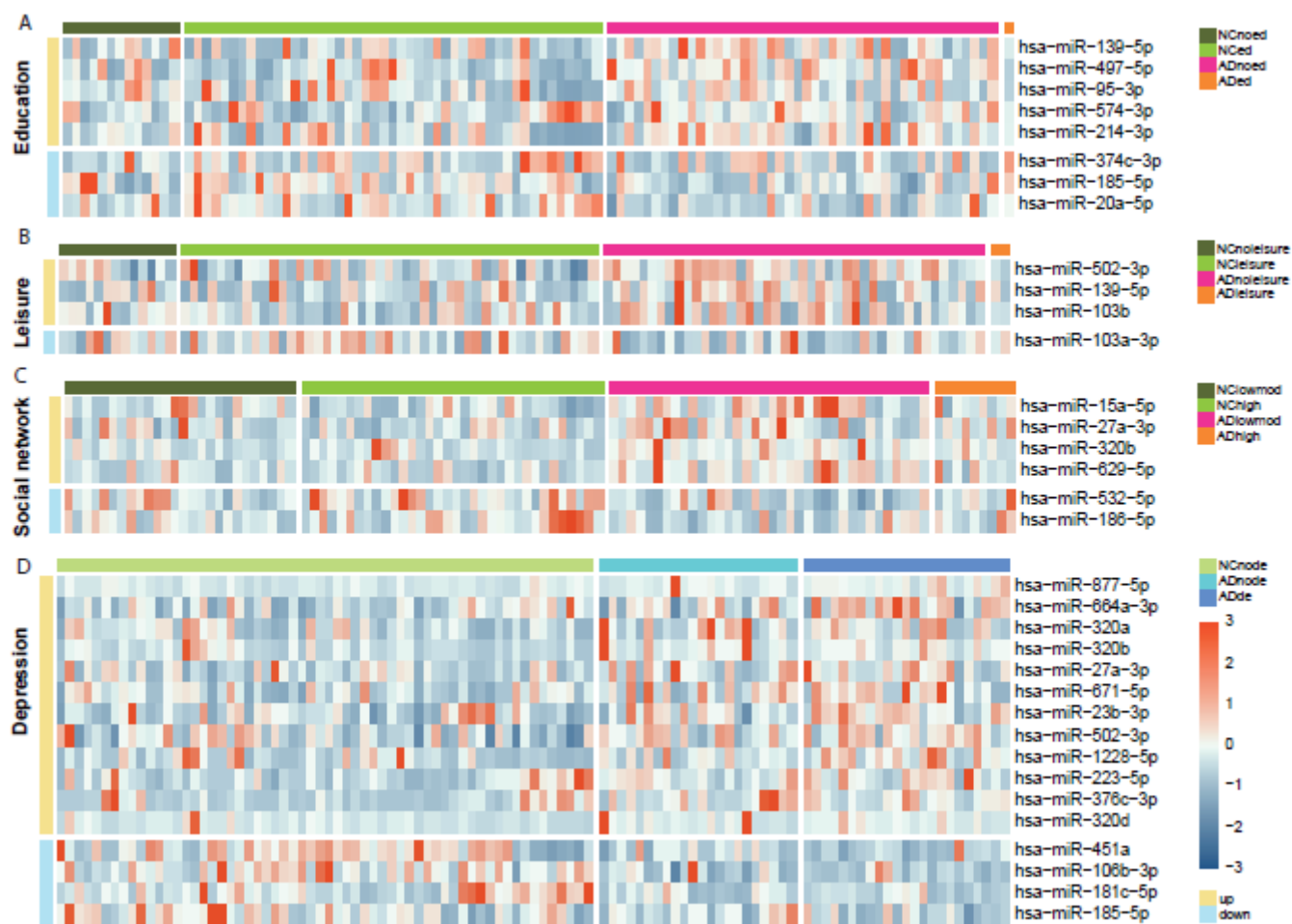
Table 2 correlation analysis of specific expressed miRNA and lifestyle factors

	Canonical correlation analysis	F value	P value
Education	0.477	3.498	0.001
Smoke	0.369	1.480	0.159
Tea	0.261	1.194	0.316
Depression	0.605	3.170	<0.001
BMI	0.221	0.713	0.661
Leisure activity	0.411	5.036	0.001
Social network	0.300	1.963	0.091

Abbreviations: BMI, body mass index.

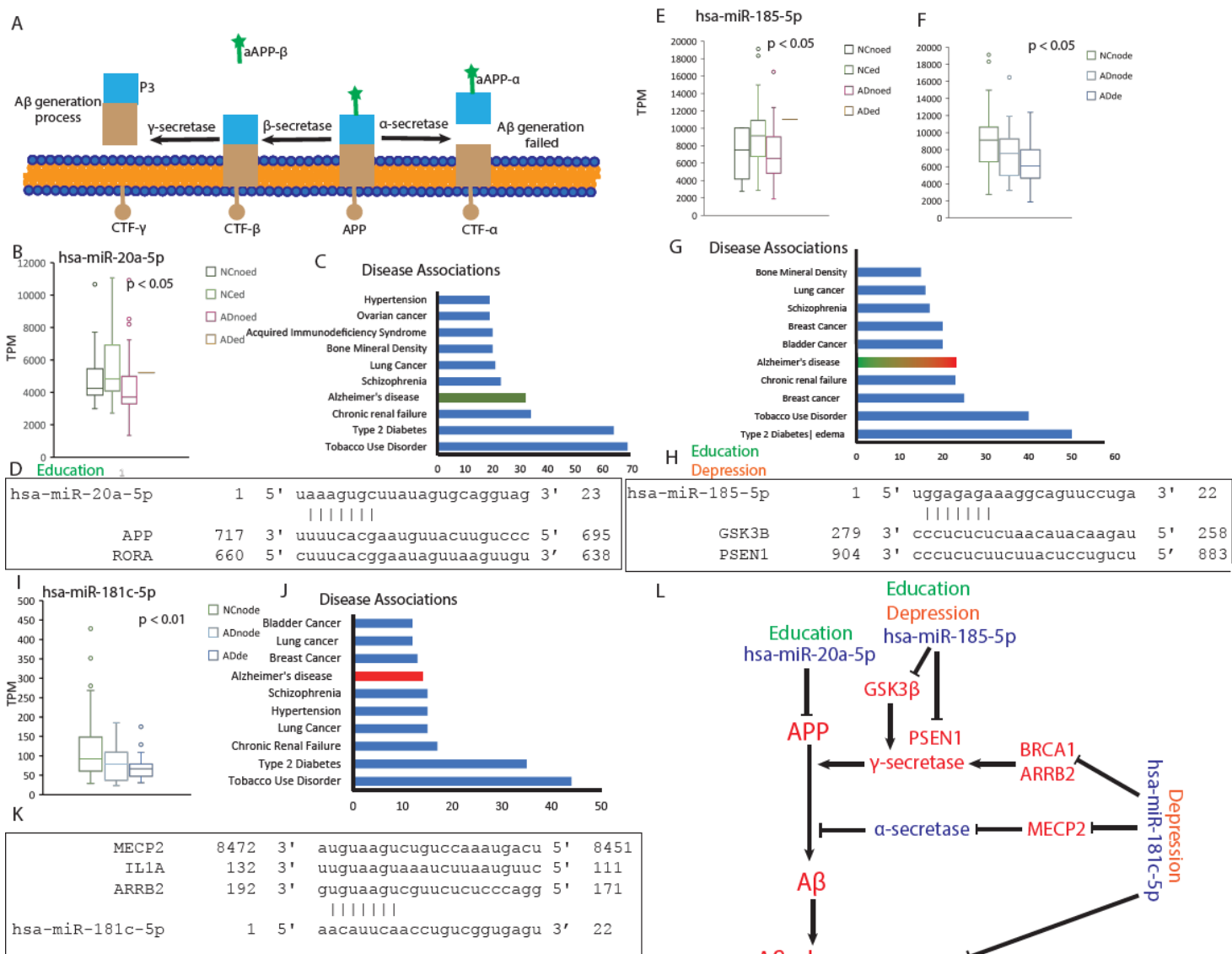
Canonical correlation analyses were performed to determine any correlations between lifestyle factors and expressed miRNAs specified.

## Figures



**Figure 1**

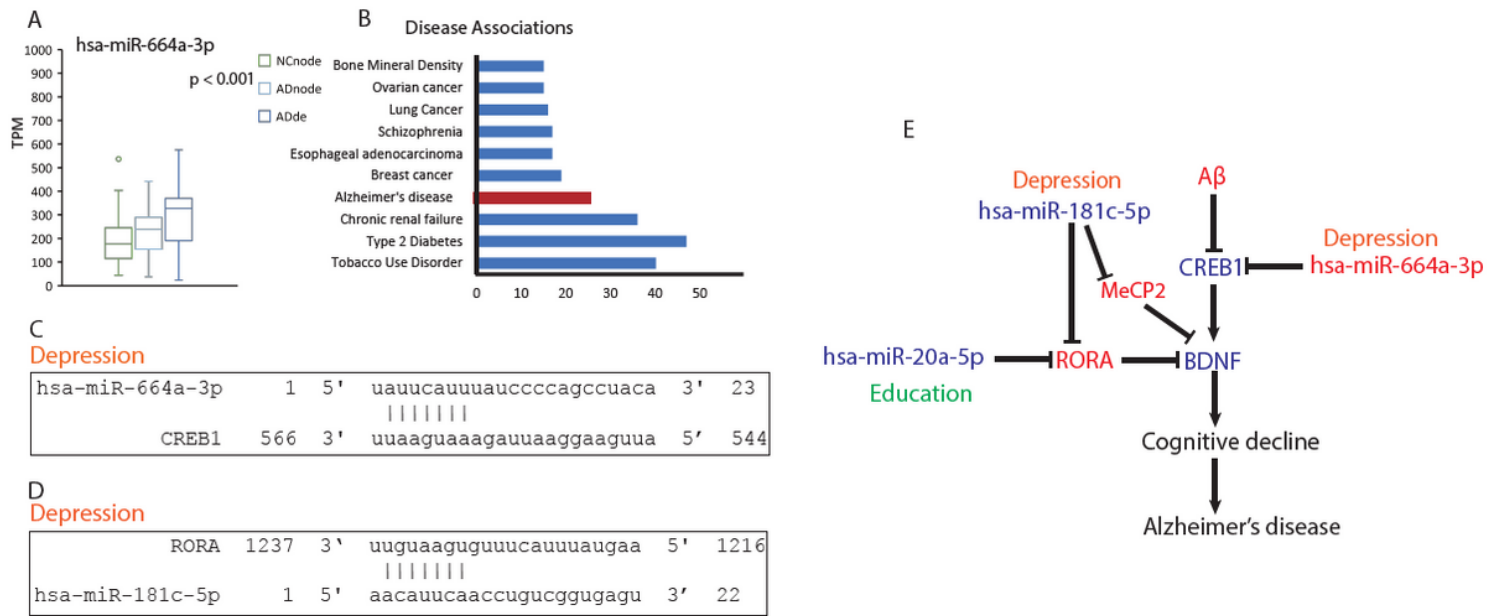
Heatmap of miRNAs for AD-related factors. A-D. Heatmap of miRNA expression level of all individuals with and without education (A), leisure activity (B), social connection (C), with and without depression (D).



**Figure 2**

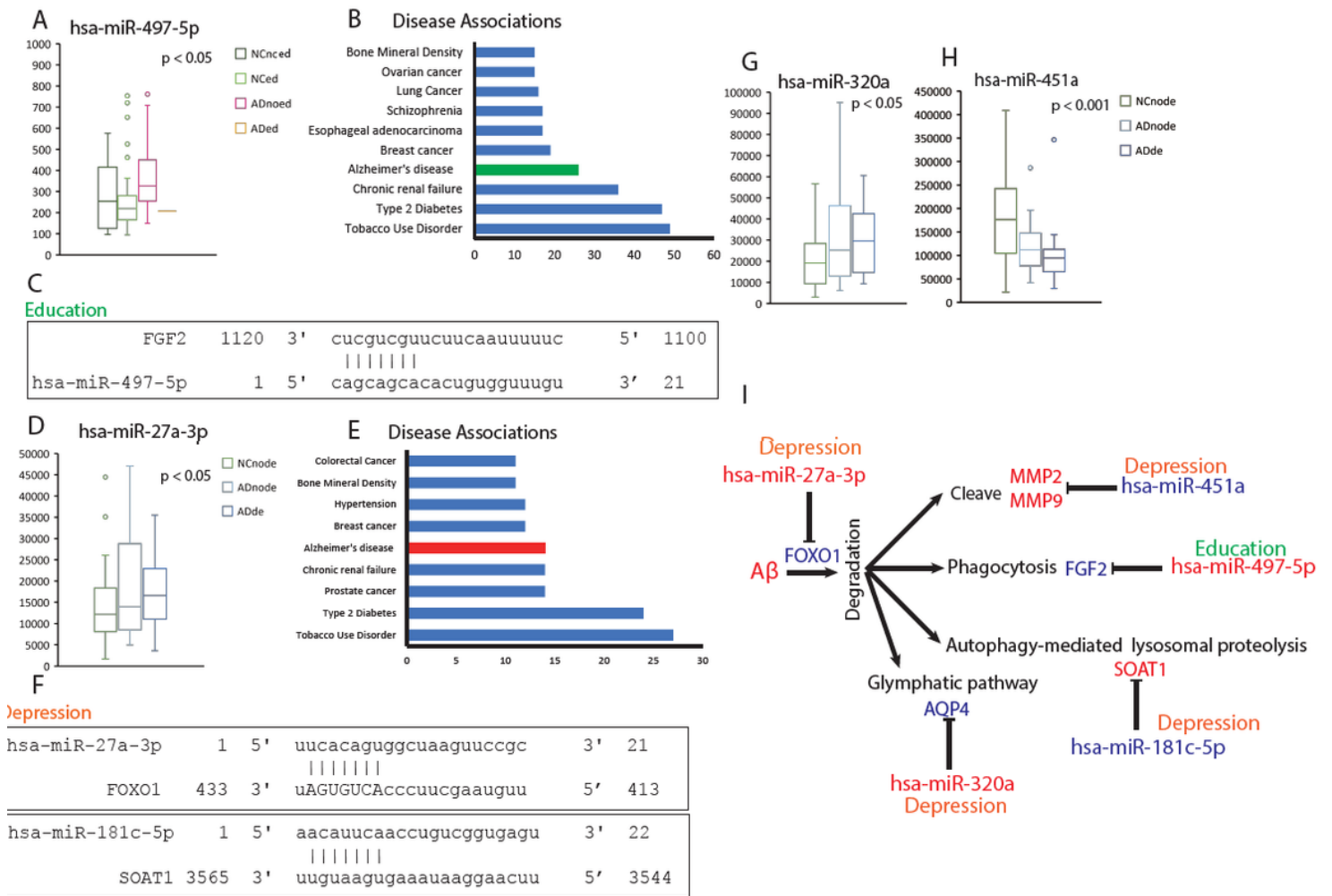
Education /depression and AD-related miRNAs were involved in A $\beta$  generation. A - A schematic of the catalytic enzyme in APP; B - The relative expression level of hsa-miR-20a-5p categorized for education. NCnoed = normal controls who had no education experience, NCed = normal controls who were educated, ADnoed = AD patients who had no education experience, ADed = AD patients who were educated, F (3, 103) = 3.676, P = 0.0146, one-way ANOVA followed by Tukey's Multiple Comparison Test; C - Target enrichment analysis for hsa-miR-20a-5p; D - Predicted binding site for hsa-miR-20a-5p and its targets; E - The relative expression level of hsa-miR-185-5p categorized for education, F (3, 103) = 3.064, P = 0.0314, one-way ANOVA followed by Tukey's Multiple Comparison Test; F - The relative expression level of hsa-miR-185-5p categorized for depression, F (2, 103) = 4.238, P = 0.0170, one-way ANOVA followed by Tukey's Multiple Comparison Test. NCnode = normal controls who did not suffer from depression, ADnode = AD patients who did not suffer from depression, ADde = AD patients who also suffered from depression; G - The relative expression level of hsa-miR-185-5p; H - Predicted binding site for hsa-miR-185-

5p and its target; I - The relative expression level of hsa-miR-181c-5p categorized for depression,  $F(2, 103) = 5.441, P = 0.0057$ , one-way ANOVA followed by Tukey's Multiple Comparison Test; J - Target enrichment analysis for hsa-miR-181c-5p; K - Predicted binding site for hsa-miR-181c-5p and its targets; L - A summary of miRNAs and their targets that are involved in A $\beta$  generation process. Decreased expression level of miRNA and genes were marked as diamond blue color, increased expression level of miRNA and genes were marked as red color. Factor education was using dark green color, factor depression was using orange color.



**Figure 3**

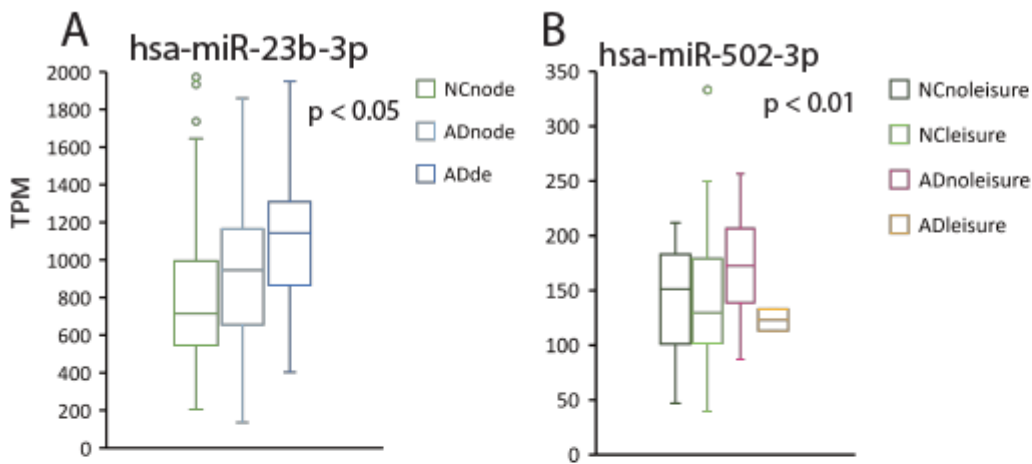
Education / depression- and AD-related miRNAs were involved in cognition decline. A - The relative expression level of hsa-miR-664a-3p categorized for depression,  $F(2, 103) = 8.439, P = 0.0004$ , one-way ANOVA followed by Tukey's Multiple Comparison Test; B - Target enrichment analysis for hsa-miR-664a-3p; C - Predicted binding site for hsa-miR-664a-3p and its target; D - Predicted binding site for hsa-miR-181c-5p and its target; E - A summary of miRNAs and their targets that are involved in cognitive decline in AD. Decreased expression level of miRNA and genes were marked as diamond blue color, increased expression level of miRNA and genes were marked as red color. Factor education was using dark green color, factor depression was using orange color.



**Figure 4**

Education / depression- and AD-related miRNAs were involved in A $\beta$  clearance. A - The relative expression level of hsa-miR-497-5p categorized for education,  $F(3, 103) = 3.500$ ,  $P = 0.0182$ , one-way ANOVA followed by Tukey's Multiple Comparison Test; B - Target enrichment analysis for hsa-miR-497-5p; C - Predicted binding site for hsa-miR-497-5p and its target; D - The relative expression level of hsa-miR-27a-3p categorized for depression,  $F(2, 103) = 3.338$ ,  $P = 0.0394$ , one-way ANOVA followed by Tukey's Multiple Comparison Test; E - Target enrichment analysis for hsa-miR-27a-3p; F - Predicted binding site for hsa-miR-27a-3p, or hsa-miR-181c-5p and their targets; G - The relative expression level of hsa-miR-320a categorized for depression,  $F(2, 103) = 4.605$ ,  $P = 0.0121$ , one-way ANOVA followed by Tukey's Multiple Comparison Test; H - The relative expression level of hsa-miR-451a categorized for depression,  $F(2, 103) = 9.595$ ,  $P = 0.0002$ , one-way ANOVA followed by Tukey's Multiple Comparison Test; I - A summary of miRNAs and their targets that are involved in cognitive decline in AD. Decreased expression level of miRNA and genes were marked as diamond blue color, increased expression level of miRNA and genes were marked as red color. Factor education was using dark green color, factor depression was using orange color.





**C**

### Depression

CEL22	4267	3'	uggagagaaaggcaguuccuga	5'	4289
hsa-miR-185-5p	1	5'	cccucucuuccccuuaauugga	3'	22

**D**



**Figure 5**

Other lifestyle- and AD-related miRNAs. A - The relative expression level of hsa-miR-23b-3p categorized for depression,  $F(2, 103) = 3.539$ ,  $P = 0.0326$ , one-way ANOVA followed by Tukey's Multiple Comparison Test; B - The relative expression level of hsa-miR-502-3p categorized for leisure activities,  $F(3, 101) = 2.979$ ,  $P = 0.0350$ , one-way ANOVA followed by Tukey's Multiple Comparison Test. NCnoleisure = normal controls who had no leisure activity, NCleisure = normal controls who had leisure activity, ADnoleisure = AD patients who had no leisure activity, ADleisure = AD patients who had leisure activity; C - Predicted binding site for hsa-miR-185-5p and its target; D - A summary of hsa-miR-185-5p and its targets that are involved in microglia response to A $\beta$  accumulation. Decreased expression level of miRNA and genes were marked as diamond blue color, increased expression level of miRNA and genes were marked as red color. Factor education was using dark green color, factor depression was using orange color.

## Supplementary Files

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