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Micro RNA Predicts Cognitive Performance in Healthy Older Adults

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Abstract

The expression of micro-RNA (miRNA) is influenced by ongoing biological processes, including aging, and has begun to play a large role in the measurement of neurodegenerative processes in central nervous system. The purpose of this study was to utilize machine learning approaches to determine whether miRNA can be utilized as a blood-based biomarker of cognitive aging. A random forest regression combining miRNA with biological (brain volume), clinical (comorbid conditions), and demographic variables in 115 typically-aging older adults explained the greatest level of variance in cognitive performance compared to the other machine learning models explored. Three miRNA (miR-140-5p, miR-197-3p, and miR-501-3p) were top-ranked predictors of multiple cognitive outcomes (Fluid, Crystallized, and Overall Cognition) and past studies of

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these miRNA link them to cellular senescence, inflammatory signals for atherosclerotic formation, and potential development of neurodegenerative disorders (e.g. Alzheimer's disease). Several novel miRNA were also linked to age and multiple cognitive functions, findings which together warrant further exploration linking these miRNA to brain-derived metrics of neurodegeneration in typically-aging older adults.

Keywords

Machine Learning; miRNA; neurocognitive function; cognitive aging

1.1. INTRODUCTION

Age-related cognitive decline has been well-characterized in recent years (Alexander et al., 2012; Anton et al., 2015; Dotson et al., 2015; Febo and Foster, 2016; Nissim et al., 2017; O'Shea et al., 2016; Porges et al., 2017; Woods et al., 2013). Domains such as memory, executive functioning, and processing speed have been shown to decline at a differential or accelerated rate in normal aging (Craik, F. I. M. & Salthouse, 2008; Salthouse, 2009, 1998). Given that these declines have functional and health implications and are not consistent among all individuals nor within an individual, their characterization is often performed through the use of screening instruments and bundled neurocognitive batteries in clinical and research settings. The Montreal Cognitive Assessment (MoCA) is widely used both as a clinical assessment and in research as a screening measure (Nasreddine et al., 2005), while the NIH Toolbox has been more widely utilized to characterize a broad range of cognitive performance in several recent research studies (Akshoomoff et al., 2013; Bauer and Zelazo, 2013; Weintraub et al., 2013). Through the use of measures like these, we can begin to address the need for an easily obtained objective biomarker of cognitive function.

MicroRNAs (miRNAs) are broadly defined as a family of non-coding ribonucleic acids (RNAs) that account for nearly 1% of the human genome and act as modulators of gene expression at the post-transcriptional level (Piscopo et al., 2019). As an epigenetic approach, miRNAs have been found to be modified with experiences, and variability in the expression of miRNA is influenced by ongoing processes, including aging. Further, miRNAs have begun to play a large role in the measurement of neurodegenerative processes in the central nervous system, which provides evidence that peripheral levels of circulating miRNA can serve as markers of cognitive function. In this regard, specific miRNA have been found to express at certain times in development or in certain regions of the brain and have even been linked to neurogenesis and synaptic plasticity (Grasso et al., 2014). A number of recent studies have found miRNAs to predict cognitive function and/or impairment in both rodents (Shaltiel et al., 2013) and humans (Hansen et al., 2013). Specific to diseases of aging and neurodegeneration, one study found specific miRNAs to predict mild cognitive impairment (MCI) in asymptomatic humans prior to disease development over time (Sheinerman et al., 2013). Another study utilizing a rodent model of Alzheimer's disease found that overexpression of certain miRNA were associated with disease-related pathology in the brain, and direct injection of specific miRNAs into the hippocampus resulted in impaired learning processes *in vivo* (Banzhaf-Strathmann et al., 2014). However, few studies, if any,

have examined the ability of miRNA to provide accurate predictions of cognitive functioning in normally-aging older adults.

Our group has previously demonstrated the capability of miRNA to distinguish healthy elderly individuals with impairment on the MoCA (<23/30) from those with normal cognition (23) (Rani et al., 2017). The current study extends our previous work by expanding findings relating miRNA to a broad cognitive screening instrument to a more detailed and neuropsychologically-relevant cognitive functioning measure through the use of a number of powerful machine learning models.

2.1. METHODS

2.2. Participants:

After obtaining Institutional Review Board approval at the University of Florida, we retrospectively reviewed records from a cohort of healthy community dwelling older individuals living in North Central Florida. These participants were recruited through previous studies (Nissim et al., 2017; O'Shea et al., 2016) and completed a thorough medical history questionnaire from which demographic characteristics such as age, gender, and race were derived, along with health status and comorbid conditions, and a comprehensive neurocognitive evaluation. Individuals with miRNA measurements (see below for details), MoCA, NIH Toolbox, and complete survey data including detailed demographic and clinical information were included in the study. This resulted in a total sample size of 115 participants (mean age = 71.8, SD = 10.2), with 65 females and 50 males (Table 1).

2.3. Neurocognitive Measures:

The neurocognitive variables of interest for the current study included the MoCA test total score and several indices derived from the NIH Toolbox Cognitive Battery, including the global cognitive index, the fluid cognitive function index, and the crystallized cognitive function index. The MoCA is a cognitive screening instrument used clinically for the identification of dementia and in research for exclusion of participants with possible cognitive dysfunction or mild cognitive impairment (MCI) (Nasreddine et al., 2005). In the present study, the MoCA total score was used as both a continuous variable and a binary variable using a commonly used cutoff score of 23 (Larner, 2012; Lee et al., 2008; Luis et al., 2009) to dichotomize participants who would have been otherwise excluded from research for possible cognitive dysfunction or mild cognitive impairment (MCI). Individuals were divided into two groups based on their MoCA total score: those with a MoCA higher than or equal to the selected cut-off point (≥ 23; 81%) and those with a MoCA total score lower than the selected cut-off point (<23; 19%).

The NIH Toolbox Cognitive Battery (NIHTB) was administered as a brief yet comprehensive assessment of participants' current neurocognitive functioning status (Akshoomoff et al., 2013; Bauer and Zelazo, 2013; Weintraub et al., 2013). The NIHTB subtests can be combined to produce index scores of global cognitive functioning, fluid cognitive functioning (abilities that tend to decline with age), and crystallized cognitive functioning (abilities that improve or remain stable with age). The fluid cognition composite

is composed of the following tasks: Dimensional Change Card Sort, Flanker, Picture Sequence Memory, List Sorting, and Pattern Comparison. The crystallized cognition composite is composed of the Picture Vocabulary Test and the Oral Reading Recognition Test. For the purposes of this study, age, education, and gender were applied as covariates in all analyses.

2.4. MR Volume Calculation:

All neuroimaging data were collected on a Philips Achieva 3T MRI at the University of Florida (Philips Electronics, Amsterdam, The Netherlands) at the McKnight Brain Institute (University of Florida, Gainesville, FL) with a 32-channel receive-only head coil. A high-resolution T1-weighted MPRAGE whole-brain T1 image was acquired in 170 interleaved sagittal 1mm slices (TR=7.0ms, TE=3.2ms, FOV=240² mm). Structural T1 data were examined for imaging artifacts, and subsequently processed through the FreeSurfer version 6.0.0 automated pipeline (Fischl et al., 2002). Freesurfer output was manually inspected by trained research staff. During this process, data were reviewed in a manner consistent with Freesurfer documentation and previously validated against histological measures (Cardinale et al., 2014). In this process control points are added when necessary to ensure accurate delineation of tissue types throughout the brain (Morey et al., 2009). The resulting segmentation data for grey and white matter volumes from this process were then utilized for further analysis in the present study (labeled “MR Volumes” hereafter) through the use of machine learning models predicting cognitive function.

2.5. Sample Collection, RNA Isolation, Sequencing, and RNA Library Preparation:

Briefly, plasma samples were collected and processed within 30 minutes of blood draw. The samples were centrifuged before being stored at -80°C until RNA isolation. Cellular materials were removed by filtration, and microvesicles were isolated and morphologically assessed using Transmission Electron Microscopy (TEM). MiRNA were then purified from the microvesicles. Characterization and concentration of both total RNA and miRNA were obtained using the Agilent Bioanalyzer RNA 6000 Pico Assay and small RNA kit. The total RNA samples were found to contain a range of 49–90% miRNA.

Sequencing libraries were constructed using ~2 ng of total exosomal RNA and barcoded to allow multiplexed sequencing analysis. In addition, their concentration was assay quantified while their size distribution and molar concentration were determined by High Sensitivity D1000 Screen Tape kit (5067–5584) on 2200 TapeStation system (Agilent Technologies, Cat# G2964A) and Qubit dsDNA HS Assay (Thermo Fisher, Cat# 4475936) according to manufacturer’s protocol respectively.

Templates were prepared and sequenced with 25 µl of the pooled libraries at a final concentration of 50 pM. The resulting FASTQ files were then uploaded to Partek Flow servers for bioinformatics analysis. On average, each sample contained 13.8 million reads of 32 base pair (bp) length. Reads were trimmed based on size such that only reads between 15–32bp were considered. Following trimming, reads were aligned with the human genome reference (hg38) and checked for alignment quality. Gene annotation was completed with the miRBase mature miRNAs model and normalization by log transformation was

performed on total counts. The same procedure was repeated using hg38-Ensembl Transcripts in order to consider genes not included in the miRBase. For a more detailed description of the RNA sample collection and isolation process, please see our previously published work (Rani et al., 2017).

2.6. Statistical Analyses:

Descriptive analysis was conducted to assess demographic characteristics and cognitive performance across the sample, including chi-square tests and independent samples t-tests to compare between subgroups (Table 1). These analyses suggested the male portion of the sample was slightly more educated ($p < .05$) but otherwise males and females were equally matched across demographic and cognitive variables. When comparing those who scored in the impaired range on the MoCA test ($< 23/30$) to those above the cutoff for impairment, individuals in the impaired group were more likely to be less educated ($p < .05$) and African American ($p < .01$).

Logistic regression with greedy stepwise selection was then conducted using demographic characteristics, clinical variables, cognitive performance, and miRNA expression levels. This type of model uses training data to build the model and test it on the testing data sets. Several machine learning techniques were trained on the full variable set in addition to stepwise logistic regression. Specifically, these models included (i) a decision tree; (ii) a LogitBoost algorithm; and (iii) a random forest regression (optimizing number of trees up to 1,000). When treating MoCA as continuous outcome, 4 models were developed including (a) a boosted decision tree; (b) a random forest; (c) a machine learning based linear regression; and (d) a stepwise selection based on AUC value. We investigated the performances of these models with all available variables, and with miRNA expression levels alone. In addition to MoCA, we also examined how well these models predict other cognitive function measurements utilized as continuous variables, including NIHTB overall cognition, fluid cognition, and crystallized cognition.

The performance of all models was assessed in a 10-fold cross-validation framework. Precision, recall, F1 score, and AUC were used for binary MoCA outcome, and R-squared was used for continuous MoCA and other cognition function measurement. All statistical analyses were conducted using R statistics RWeka, pROC, caret, and MLmetrics packages (R Development Core Team, 2016). Predictors that were recorded in less than 50% of individuals were removed, and then missing values were imputed with population mean. A total of 345 predictors were included, they are 5 demographic variables (age, sex, race, ethnicity, years of education), 65 clinical variable/comorbidities, the MR Volumes described above, and 275 miRNA measurements.

3.1. RESULTS

When examining all miRNA for the highest level of expression achieved in the sample, replication of the analysis from our prior work (Rani et al., 2017) revealed similar results (Table 2). Results indicate the highest level of expression was found for miR-223-3p, similar to other investigations (Hunter et al., 2008; Mooney et al., 2015), and relatively high (> 1000 counts) expression for miR-451a, miR-191-5p, and miR-126-3p. The results presented here

differ from our previous analysis likely due to an increased sample size, such that three miRNA which previously fell at the lower end of the cutoff (>300) were no longer expressed at a quantity above 300 counts, and are thus not displayed (Table 2). For a full list of the 258 miRNA analyzed and their relationship to age and MoCA score, please see Supplemental Table 1.

Results also indicate that miRNA with the highest level of expression are not necessarily those which account for the highest level of variance in age or cognitive function on the MoCA test. It should be noted that only two miRNA overall (miR-486-3p, miR-1180-3p), regardless of count, achieved a Pearson correlation with MoCA score that exceeded $r = \pm 0.263$ ($p < 0.01$; $r = -0.274$ and $r = -0.266$, respectively) (Supplemental Table 1). Several miRNA expressed at >300 counts achieved this level of significance in relationship to age (Table 2), with 22 other miRNAs achieving this level of significance with an average expression count <300 (Supplemental Table 1).

When utilizing machine learning models to predict binary MoCA score using a commonly used clinical and research cutoff of 23, results indicate that greedy stepwise selection produced the highest level of overall sensitivity and specificity (Youden $J = 0.096$). In general, these models produced consistently high sensitivity and relatively low specificity, with moderate area under the curve (AUC) values close to the chance level. To test whether these AUC values were significantly greater than chance, follow-up random binomial distributions were generated and used as predictors for the binary MoCA score which yielded similar results to the various machine learning models. Inclusion of all predictors, such as miRNA, MR volumes, demographic data, clinical diagnoses and comorbidities, and questionnaire data resulted in lower Youden J index scores compared to using miRNA values alone to predict the binary outcome, with the exception of decision tree, which performed moderately better with all predictors in the model (Table 3).

Machine learning models were used to predict continuous scores on the various cognitive performance variables, with the first iteration including all miRNA expression levels, demographic, clinical and diagnostic, MR volume, and questionnaire data to predict the outcomes. Results indicate that utilizing all predictors in a random forest model accounted for the highest level of variance across cognitive outcomes, with the exception of MoCA score, which was best predicted by a boosted decision tree model using miRNA only (Table 4).

An examination of the specific miRNA that contribute to cognitive performance was performed, and results demonstrate the top 10 most important microRNA for each predictor (Table 5). It is noted that within-variable rank importance is calculated, as is overall importance across all cognitive measures investigated. These rankings were calculated by reversing scoring the rankings for each miRNA such that a miRNA ranking first for a measure would have a score of 20 for that measure, one ranking second would have a score of 19, and so forth. MiRNAs that did not rank in the top 20 for a measure were automatically given a score of zero. Within-variable average rank indicates miR-139-5p to be the most important miRNA for predicting MoCA score at a cut-off of 23 for impairment detection, miR-335-5p predicting full MoCA score, miR-140-5p predicting Total Cognition on the NIH

Toolbox, miR-2110 predicting Fluid Cognition on the NIH Toolbox, and miR-181c-3p predicting Crystallized Cognition on the NIH Toolbox. When collapsing across cognitive variables, the two most important miRNA for predicting cognitive performance in terms of average rank were miR-335-5p and miR-2110. Further, a number of miRNA were highly ranked predictors across two cognitive measures, including miR-181c-3p, miR-497-5p, miR-425-3p and miR-221-3p. Lastly, several miRNA were highly ranked predictors across three cognitive measures, including miR-140-5p, miR-197-3p, and miR-501-3p (Figure 1).

The top ranked miRNA for predicting cognitive performance, beginning with those which predicted multiple cognitive variables, are listed in Table 6 along with their average level of expression in the present study and a summary of research findings related to each particular miRNA. Further, a number of these top-ranked miRNA were significantly associated with age (Table 6).

4.1. DISCUSSION

The present study provides further evidence that miRNA derived from extracellular microvesicle enriched blood plasma is highly predictive of cognitive functioning in typically-aging elderly individuals. We describe the most highly expressed miRNA in an aging sample of older adults, and the relationship of these highly expressed markers with age and cognitive function. Further, we utilize machine learning approaches to explore the ability of miRNA to predict impairment on a common cognitive screening measure (MoCA with a cutoff of 23/30) as well as to predict general level of cognitive performance on the MoCA and the NIH Toolbox. Results indicate that several miRNA independently predicted multiple cognitive measures, and many of these miRNA are associated with age in the present study and have been associated with cerebrovascular disease as well as neurodegenerative disease in prior studies. The present study provides useful information about the ability of miRNA to serve as predictive biomarkers for cognitive functioning in a sample of 115 typically-aging older adults.

The implications of the present findings are numerous and include their potential for early identification of neurodegenerative diseases of senescence such as Alzheimer's disease as well as their ability to serve as biomarkers for common comorbid conditions known to affect cognitive functioning, such as cerebrovascular disease. Research has found that higher serum levels of miR-140-5p are associated with cellular senescence (aging)(Yoo et al., 2014) and higher miR-140-5p is associated with lower formation of atherosclerosis via inflammatory hypersensitive C-reactive protein (hs-CRP) signaling (Teng and Meng, 2019). Thus, miR-140-5p is upregulated in aging and protective for the development of atherosclerosis. In the present study, this miRNA subtype was found to be highly predictive of and positively associated with three cognitive performance measures. We also found that miR-497-5p, a miRNA linked to brain damage following stroke, was negatively associated with and a highly ranked predictor of two cognitive performance measures. Specifically, blocking the expression of -497 miRNA with antagomirs has a neuroprotective benefit, and results in decreased focal cerebral damage following ischemic or hemorrhagic stroke (Koutsis et al., 2013). Similarly, miR-221-3p, which was a significant predictor of cognitive function in the present study, is upregulated in stroke patients compared to controls

(Sørensen et al., 2014). In the present dataset, a post-hoc analysis indicates that higher levels of miR-497-5p and miR-221-3p were observed in individuals with diagnoses of hypertension, hyperlipidemia, and diabetes. Together, these findings provide preliminary evidence that higher peripheral blood levels of the -140, -497, and -221 miRNAs may be a prognostic marker of ongoing cerebrovascular disease commonly known to affect cognitive performance.

Our findings in a normal aging cohort are consistent with studies reporting serum levels of miR-140, miR-197, and miR-181c-3p, to easily distinguish MCI patients from normal controls (Kayano et al., 2016) and to be a significant predictor of Alzheimer's disease (Guo et al., 2017; Thounaojam et al., 2013). When utilizing a random forest regression, we found these miRNAs to be top-ranked predictors of age and cognitive performance, and also each one was highly predictive of multiple measures of cognitive performance derived from the NIH Toolbox, including Total Cognition, Fluid Cognition, and Crystallized Cognition (Table 5; Figure 1). Along these lines, miR-17-5p differentially predicted impairment on the MoCA (using a cutoff of 23 points) and was a top ranked predictor of fluid cognitive ability; a composite measure which declines with normal aging and at an accelerated rate with the progression of neurodegenerative diseases such as Alzheimer's disease. Post-hoc analysis revealed that higher serum levels of miR-17-5p were significantly associated with lower performance on the Fluid Cognition index. This is of particular interest given that miR-17-5p has been associated with the regulation and expression of cerebral amyloid precursor protein (APP)(Hébert et al., 2009), which is linked to the development of Alzheimer's disease. Similarly, a top-ranked predictor of age and multiple measures of cognitive function in the present study, miR-501-3p has been linked to cognitive functioning in Alzheimer's disease patients where its serum expression level is significantly downregulated ante-mortem compared to controls (Hara et al., 2017). Interestingly, Hara et al. found that in pathologically-confirmed AD patients, ante mortem serum expression levels of miR-501-3p were positively associated with MMSE performance whereas the present study found the opposite relationship with MoCA performance in typically-aging control participants. Regardless, findings suggest the importance of these microRNA as biomarkers of cognitive function, even in typically aging older adults without overt or diagnosed neurodegenerative pathology. Additional follow-up to these findings in relation to cognitive aging can include a systematic investigation of neuroimaging metrics of age-related change as they relate to these highly predictive miRNA. Further study in pathologically confirmed Alzheimer's disease patients pre-and post-mortem will be important to determine the ability of miRNA to be used as additional biomarkers for determining consensus diagnostic criteria *in vivo*.

It is important to note the caveat that these findings may be influenced by the co-occurrence of exosomes in the serum samples from the present analysis, as exosomes are known to cross the blood-brain barrier and may influence brain function (Alvarez-Erviti et al., 2011; Rani et al., 2017). Other limitations in the present study include the fact that the use of machine learning models for the prediction of binary MoCA score set at the common cutoff of 23 did not outperform a randomly generated binomial probability (chance prediction). This is potentially due to the arbitrary choice to use the common 23 point cutoff for MoCA rather than allowing the models to drive the selection of the optimal cutoff point. Future

investigations may benefit from such an investigation, though this would be of lesser clinical utility given that the score of 23 is most commonly used in research and practice. A final potential limitation may result from a number of miRNA which are not expressed to a significant degree for detection, but which may exert an influence on cognitive performance, and are therefore not investigated. However, we attempted to overcome this limitation with the use of machine learning regression models so as to provide the ability to input all potential detectable miRNA into the analyses.

5.1. CONCLUSION

In conclusion, a number of miRNA which have been previously linked to Alzheimer's disease, cerebrovascular disease, and cellular senescence appear to be significant predictors of cognitive functioning in typically aging individuals. Further, a number of novel miRNA which have not previously been examined in the extant literature were significantly related to age and identified as top predictors of cognitive performance, suggesting they may serve as biomarkers of cognitive aging. Together, these findings indicate that there may be epigenetic contributions to cognitive performance in typical aging and that highly expressed miRNA associated with fluid cognitive function may ultimately allow for the early identification of individuals at risk for neurodegenerative or cerebrovascular disease effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Machine learning can predict cognitive function using blood-based Micro-RNA (miRNA)
- Several miRNA linked to diseases of aging predict multiple cognitive measures
- Novel miRNA are also related to multiple cognitive functions, meriting further study

Top miRNA Predictors by Category

Figure 1.

This plot summarizes the most impactful miRNA for each cognitive measure. A score of 20 indicates the miRNA is the most predictive for that measure, whereas a score of 0 means the miRNA was not predictive.

Table 1.

Participant demographics

Variable	Total Mean (SD) (n = 115)	Male Mean (SD) (n = 50)	Female Mean (SD) (n = 65)	MoCA > 23 (SD) (n = 94)	MoCA < 23 (SD) (n = 21)
Age	71.88 (10.19)	72.60 (9.65)	71.32 (10.63)	71.78 (9.46)	72.29 (13.25)
Highest Education	16.35 (2.59)	17.08 (2.35) **	15.78 (2.64)	16.60 (2.50) *	15.24 (2.76)
Race					
Caucasian (%)	91.3	92	90.8	95.7 **	71.4
African-American (%)	6.1	4	7.7	1.1	28.6 **
LatinX (%)	0.9	2	1.5	1.1	0
Asian/Pacific Islander (%)	1.2	2	0	2.1	0
MoCA Total Score	25.48 (2.84)	24.98 (2.41)	25.86 (3.10)	26.52 (1.90) **	20.81 (1.08)
NIHTB Total Cognition	114.08 (11.94)	113.54 (12.03)	114.50 (11.95)	115.62 (12.10) **	107.18 (8.42)
NIHTB Crystallized Cognition	128.19 (13.87)	128.64 (17.67)	127.84 (10.17)	129.46 (14.32) *	122.53 (10.08)
NIHTB Fluid Cognition	96.05 (8.23)	96.00 (8.48)	96.08 (8.18)	96.93 (8.58) *	92.11 (5.32)

* p < .05 difference between groups;

** p < .01 difference between groups

Note: Adjusted Scaled Scores have a mean of 100 and standard deviation of 15; MoCA = Montreal Cognitive Assessment, education corrected total score; NIH Toolbox Crystallized Performance is comprised of the Picture Vocabulary Test and the Oral Reading Recognition Test; NIH Toolbox Fluid Performance is comprised of Dimensional Change Card Sort, Flanker, Picture Sequence Memory, List Sorting, and Pattern Comparison tasks.

Table 2.

Highly expressed miRNA relationships to age and a cognitive screener

miRNA	Average Expression	Pearson Correlation		Pearson Correlation for log-transformed	
		with age	with MoCA	with age	with MoCA
miR-223-3p	7604.2822	0.2058 *	-0.0562	0.2534 *	-0.0317
miR-451a	2481.8212	0.0725	-0.2015	0.0886	-0.2441
miR-191-5p	1403.5431	0.1792	-0.0894	0.2125	-0.0992
miR-126-3p	1006.2365	0.1918	-0.1075	0.2150 *	-0.1539
miR-126-5p	888.5534	0.2400 *	-0.1181	0.2814 **	-0.1373
miR-103a-3p	736.2916	0.1917	-0.0226	0.2561	-0.0471
miR-150-5p	735.5120	0.0553	-0.1834	0.1230 *	-0.1589
miR-23a-3p	719.1378	0.2469 **	-0.0466	0.3354 **	-0.0788
miR-484	607.3528	0.2742 *	-0.1237	0.3184 **	-0.1434
miR-19b-3p	605.6067	0.1938	-0.0229	0.2936 *	-0.0730
miR-26a-5p	596.2385	0.1653	-0.0236	0.1653	-0.0686
let-7a-5p	499.9165	0.1740	-0.1633	0.2188 *	-0.1500
miR-185-5p	399.4012	0.2580 *	-0.0276	0.3558 **	-0.0761
miR-320a	378.8306	0.2105	-0.0967	0.2515 *	-0.0813
let-7b-5p	343.4773	0.1456	-0.2156	0.2122 *	-0.2008

* p<.05;

** p<.01

Note: The average expression and correlation coefficients for miRNA with >300 counts from this replication analysis are consistent with our previous work²², with slight differences in coefficients likely due to increased sample size.

Table 3.

Prediction models of MoCA screening cutoff score

Model	Predictors	Precision	Recall	F1 score	AUC	Sensitivity	Specificity	Youden
Random Forest	all	0.804	0.967	0.887	0.520	0.974	0.065	0.039
	miRNA	0.794	0.963	0.881	0.522	0.965	0.080	0.045
LogitBoost	all	0.799	0.867	0.853	0.478	0.841	0.116	-0.043
	miRNA	0.813	0.856	0.836	0.495	0.874	0.116	-0.01
Stepwise Selection	all	0.809	0.988	0.890	0.549	0.966	0.130	0.096
	miRNA	0.813	0.967	0.883	0.548	0.967	0.130	0.097
Decision Tree	all	0.785	0.867	0.869	0.541	0.907	0.174	0.081
	miRNA	0.784	0.859	0.828	0.477	0.837	0.116	-0.047

Note: Cut-point for binary MoCA is 23. All predictors include miRNA values, comorbid medical diagnoses, questionnaire data, calculated MR volumes, and demographic information. For all performance indices, a higher value indicates better performance for the prediction of the binary outcome. The optimal sensitivity and specificity were selected using the Youden Index (*J*) as indicated by bold numbers.

Table 4.

Resulting R-squared values for full prediction models of selected cognitive function measurements

Model	Predictors	MoCA ^a	Total Cognition ^b	Fluid Cognition ^b	Crystalized Cognition ^b
Random Forest	all	0.116	0.303	0.199	0.238
	miRNA	0.129	0.148	0.143	0.080
Boosted Tree	all	0.108	0.227	0.079	0.072
	miRNA	0.145	0.177	0.142	0.090
Linear	all	0.078	0.109	0.119	0.122
	miRNA	0.098	0.174	0.110	0.100
Stepwise Selection	all	0.081	0.157	0.071	0.131
	miRNA	0.122	0.038	0.090	0.090

^aTotal Score.^bDerived from the NIH Toolbox Cognitive Battery.

Note: All predictors include miRNA values, comorbid medical diagnoses, MR volumes, questionnaire data, and demographic information. For the R² measurement, a higher value indicates better performance for the prediction of the cognitive variable. Bold indicates the best model for predicting each outcome.

Table 5.

Top 10 most important miRNA for predicting cognitive performance based on Random Forest regression

Rank	Binary MoCA ^a	Continuous MoCA	Total Cognition ^b	Fluid Cognition ^b	Crystallized Cognition ^b	Overall (Mean) Rank
1	miR-139-5p	miR-335-5p	miR-140-5p	miR-2110	miR-181c-3p	miR-335-5p miR-2110
2	miR-584-5p	miR-425-3p	miR-197-3p	miR-140-5p	miR-221-3p	miR-493-5p miR-1287-5p
3	miR-30a-5p	miR-497-5p	miR-501-3p	miR-493-5p	miR-1287-5p	miR-143-3p miR-425-5p miR-652-5p miR-33b-5p
4	miR-1304-3p	miR-143-3p	miR-425-5p	miR-652-5p	miR-33b-5p	miR-140-5p *** miR-181c-3p **
5	miR-483-5p	miR-106b-5p	miR-532-3p	miR-589-3p	miR-339-3p	miR-106b-5p miR-532-3p miR-589-3p miR-339-3p
6	miR-30c-5p	miR-29b-3p	miR-378a-5p	miR-25-3p	miR-197-3p	miR-497-5p ** miR-29b-3p miR-378a-5p miR-25-3p
7	miR-6852-5p	miR-584-5p	miR-411-3p	miR-17-5p	miR-1180-3p	miR-197-3p *** miR-425-3p **
8	miR-17-5p	miR-33a-3p	miR-181c-3p	miR-501-3p	miR-22-5p	miR-501-3p *** miR-221-3p **
9	miR-320b	miR-652-5p	miR-497-5p	miR-106a-5p	miR-186-5p	miR-320a
10	miR-376c-3p	miR-6852-5p	miR-214-3p	miR-532-3p	miR-140-5p	miR-483-5p

^aCut-point for impairment detection < 23;

^bDerived from the NIH Toolbox Cognitive Battery.

***Top ranked predictor of two cognitive variables (excluding binary MoCA).

***Top ranked predictor of three cognitive variables (excluding binary MoCA). Note: Higher rank denoted by a lower number indicates that specific miRNA better predicts the cognitive variable of interest.

Table 6. Expression level of top-ranked miRNA cognitive predictors, their association with age and prior associations in research

miRNA	Average Expression mean (SD)	Prior Associations
miR-140-5p ***	9.62 (7.77) [†]	• Upregulated in cellular senescence(Yoo et al., 2014) • Protective for the development of atherosclerosis(Teng and Meng, 2019) • Treatment with miR-140-5p attenuates Pulmonary Artery Hypertension(Rothman et al., 2016) • Enhances expression of A Disintegrin and Metalloproteinase 10, which has been linked with Alzheimer's disease, in the brain(Akhter et al., 2018) • Attenuates neuroinflammation and brain injury in rats(Wang et al., 2019)
miR-197-3p ***	111.17 (62.72) ^{††}	• No relevant previous research found for serum-based miRNA. • Significant differences between controls and Alzheimer's patients for 197- miRNA taken from cerebrospinal fluid (CSF)(Cogswell et al., 2008)
miR-501-3p ***	3.40 (2.33) [†]	• Downregulated in Alzheimer's disease(Hara et al., 2017) • Associated with the development of neuropathy in diabetics(Massaro et al., 2019) • Associated with blood brain barrier disruption and vascular cognitive decline(Toyama et al., 2018)
miR-181c-3p **	1.27 (0.80)	• Early biomarker of Alzheimer's disease(Guo et al., 2017) • Associated with multiple sclerosis(Regev et al., 2017) • Deregulation of expression is associated with deficits in cortical glucose metabolism and increased plasma levels amyloid-β(Manzano-Crespo et al., 2019)
miR-497-5p **	2.12 (1.77) [†]	• Linked to brain damage following stroke(Koutsis et al., 2013) • Higher levels observed in our participants with hypertension, hyperlipidemia and diabetes. • May be a biomarker of cerebrovascular disease
miR-425-3p **	13.38 (7.12)	• Upregulated in patients with major depressive disorder(Maffioletti et al., 2016)
miR-221-3p **	116.50 (118.66)	• Upregulated in stroke patients(Sørensen et al., 2014) • Inhibits Schwann cell myelination(Zhao et al., 2018)
miR-335-5p	20.91 (23.78) [†]	• Dysregulated in Parkinson's disease(Patil et al., 2019) • Overexpression is detrimental to spatial memory(Capitano et al., 2017)

miRNA	Average Expression mean (SD)	Prior Associations
miR-2110	1.22 (0.94)	No relevant previous research found
miR-493-5p	1.17 (2.43) [†]	No relevant previous research found
miR-1287-5p	1.01 (0.88)	No relevant previous research found
miR-143-3p	3.18 (3.92)	• Associated with gestational hypertension(Hromadnikova et al., 2019) • Associated with the development of retinopathy in diabetics(Massaro et al., 2019)
miR-425-5p	222.32 (110.48) ^{††}	• Downregulated in Traumatic Brain Injury(Di Pietro et al., 2017)
miR-652-5p	2.59 (1.99)	No relevant previous research found
miR-33b-5p	0.91 (1.39)	No relevant previous research found

[†] p<.05 correlation with age;

^{††} p<.01 correlation with age

^{**} Top ranked predictor of two cognitive variables (excluding binary MoCA).

^{***} Top ranked predictor of three cognitive variables (excluding binary MoCA).