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FEATURED ARTICLE

Genome-wide profiling of circulatory microRNAs associated with cognition and dementia

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Abstract

Introduction: MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression. Their role in the pathophysiology of dementia and potential as biomarkers remains undetermined.

Methods: We conducted a single- (one-by-one) and multi-marker (joint) analysis to identify well-expressed circulating miRNAs in plasma (total = 591) associated with general cognition and incident dementia, for 1615 participants of the population-based Rotterdam Study.

Results: During single-marker analysis, 47 miRNAs were nominally ($P \leq .05$) associated with cognition and 18 miRNAs were nominally associated with incident dementia, after adjustment for potential confounders. Three miRNAs were common between cognition and dementia (miR-4539, miR-372-3p, and miR-566), with multi-marker analysis revealing another common miRNA (miR-7106-5p). In silico analysis of these four common miRNAs led to several putative target genes expressed in the brain, highlighting the mitogen-activated protein kinase signaling pathway.

Discussion: We provide population-based evidence on the relationship between circulatory miRNAs with cognition and dementia, including four common miRNAs that may elucidate downstream mechanisms.

KEYWORDS
biomarkers, cognition, dementia, microRNA, pathophysiology, population based

HIGHLIGHTS

- MicroRNAs (miRNAs) are involved in the (dys)function of the central nervous system.
- Four circulating miRNAs in plasma are associated with cognition and incident dementia.
- Several predicted target genes of these four miRNAs are expressed in the brain.
- These four miRNAs may be linked to pathways underlying dementia.
- Although miRNAs are promising biomarkers, experimental validation remains essential.

1 | INTRODUCTION

Dementia, with Alzheimer's disease (AD) as the most common subtype, is a multifactorial neurodegenerative syndrome characterized by progressive decline in cognitive function that interferes with activities of daily living.[1] Recent advances in -omics, particularly genome-wide association studies (GWAS), have facilitated the identification of important loci and biological pathways, such as endocytosis and immune response pathways, for dementia.[2] Importantly, the genetic variants in many identified loci map to genomic regions that do not code for proteins.[3,4] Most non-coding regions of the genome, however, are transcribed into non-coding RNAs (ncRNAs). The biological relevance of ncRNAs and their potential for clinical application as early biomarkers of dementia at the population level remains undetermined.

MicroRNAs (miRNAs) are the best characterized ncRNAs, generally comprising 21 to 23 nucleotides, with a fundamental role in post-transcriptional regulation of gene expression. As functional units involved in both physiological and pathological processes, miRNAs are capable of targeting hundreds, if not thousands, of genes, via complementary binding of their seed sequence to the target messenger (m)-RNA.[5] A vast array of biological processes contribute to release miRNAs from cells into bodily fluids,[6] such as cerebrospinal fluid or plasma, where miRNAs circulate encapsulated by microvesicles, exosomes, or apoptotic bodies.[7] Due to their stability in bodily fluids over time, these circulatory miRNAs may serve as biomarkers of many complex traits, including dementia.[8] Accumulating evidence suggests that miRNAs are involved in growth and differentiation, as well as dysfunction of the central nervous system.[9] Besides regulating key genes of AD, such as *APP* and *BACE1*, miRNAs have been associated with deficits in neuronal plasticity, amyloid beta (Aβ)-induced synaptic dysfunction, and hyperphosphorylation of tau proteins.[10] Hence, miRNAs may be involved in the transition from healthy cognitive functioning to dementia. Although various miRNAs have been suggested as biomarkers of dementia in previous case-control studies,[11–14] the temporality of the association remains elusive, as few miRNAs have been examined in longitudinal settings.

In this study, we aim to identify circulatory miRNAs in plasma associated with cognition and incident dementia, in a prospective population-based cohort. Subsequently, we aim to assess whether these miRNAs can serve as early biomarkers of dementia. By studying the putative target genes of identified miRNAs, we intend to further elucidate molecular mechanisms underlying cognitive decline, leading up to dementia.

2 | METHODS

2.1 | Study population

This study was conducted within the Rotterdam Study, a prospective population-based cohort in the Netherlands, details of which have been published earlier[15] (Methods S1 in supporting information).

The baseline of the current study was between 2002 and 2005, when participants visited the research center for blood sampling and extensive cognitive assessments. A random subset of 1000 participants from the fourth visit of the first cohort (RS-I-4) and 1000 participants from the second visit of the second cohort (RS-II-2) was included, for whom miRNA expression levels in plasma were determined ($n = 2000$). From this subset, we excluded 29 participants with prevalent dementia at baseline, 1 participant with miRNA data of insufficient quality, 7 participants with insufficient data on dementia status at baseline, and 350 participants for whom the G-factor could not be calculated, due to missing cognitive tests. Our baseline study population thus consisted of 1615 participants and all were followed for incident dementia until January 1, 2016.

2.2 | Informed consent and ethics approval

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC University Medical Center and the Dutch Ministry of Health, Welfare and Sport. All participants provided written informed consent.

2.3 | MiRNA expression profiling

Expression levels of 2083 miRNAs in plasma were analyzed using the HTG EdgeSeq miRNA Whole Transcriptome Assay (WTA, HTG Molecular Diagnostics) and Illumina NextSeq 500 sequencer (Illumina). Out

RESEARCH IN CONTEXT

1. **Systematic Review**: The authors searched PubMed for literature on microRNAs (miRNAs) associated with cognition and dementia. While several miRNAs have been proposed as biomarkers of dementia in previous small case-control studies, few have been examined in prospective population-based cohorts. In addition, the relevance of miRNAs in the pathophysiology of dementia remains elusive.
2. **Interpretation**: Our findings provide population-based evidence on the relationship between circulatory miRNAs in plasma and cognition, including the potential downstream mechanisms leading up to dementia. Four plasma-based circulatory miRNAs (miR-4539, miR-372-3p, miR-566, and miR-372-3p) were associated with cognition and incident dementia. Their putative target genes are expressed in the brain, possibly enacting through the mitogen-activated protein kinase signaling pathway, with implications for cellular signal transduction and synapse function.
3. **Future Directions**: Although the identified circulatory miRNAs may be appealing biomarkers from a clinical perspective, experimental validation is warranted. Circulatory miRNAs can improve our understanding of mechanisms underlying dementia.

of 2083 miRNAs in total, 591 miRNAs were well expressed and used for subsequent analyses. For a full description of miRNA expression profiling methods, we refer to Methods S1.

2.4 | Assessment of cognition, surveillance of dementia, and covariates

Between 2002 and 2005, during the same time as blood sample collection, the protocol of the Rotterdam Study was expanded with an extensive neuropsychological test battery for cognitive assessments.[16] General cognition was summarized into a "G-factor," using the first component (PC1) of the principal component analysis of the Stroop interference test,[17] Letter-Digit-Substitution test,[18] Word Fluency Test,[19] the Purdue Pegboard Test, and a 15-word learning test.[20] The variance in cognitive test scores explained by the G-factor was 50.3%. Subsequently, as described in our previous publications, all participants were followed-up for dementia.[21] Participants were screened in person for dementia at baseline and at subsequent center visits with the Mini-Mental State Examination (MMSE) and the Geriatric Mental State (GMS) Schedule organic level. Those with an MMSE score <26 or a GMS score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly. Information from in-person screening was supplemented by data from the electronic linkage of the study database with medical records from all general practitioners and the regional institute for outpatient mental health care. A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (Diagnostic and Statistical Manual of Mental Disorders 3rd edition revised) and Alzheimer's disease (National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer's Disease and Related Disorders Association). Follow-up was complete until January 1, 2016, and participants were censored within this follow-up period at date of dementia diagnosis, date of death, date of loss to follow-up, or January 1, 2016, whichever came first.

Information on covariates was obtained during center visits and home interviews. Extended details on cognitive assessments, surveillance of dementia, and covariates are noted in Methods S2 in supporting information.

2.5 | Statistical analysis

To identify circulatory miRNAs associated with cognition (G-factor) and incident dementia, from in total 591 well-expressed miRNAs, we conducted a single-marker (one-by-one) and a multi-marker analysis (joint identification).

2.5.1 | Part I: Single-marker analysis

During single-marker analysis, we tested the association of each miRNA with cognition and dementia one by one. We used linear regression models to study the cross-sectional association between each circulatory miRNA and G-factor, from which we obtained mean differences and corresponding 95% confidence intervals (95% CI). Subsequently, we used Cox proportional hazards models to determine the association of each circulatory miRNA with incident dementia, from which we obtained hazard ratios (HR) and corresponding 95% CIs. All models were adjusted for age, sex, and cohort (model a); additionally adjusted for smoking, apolipoprotein E ε4 carriership, and education (model b); and additionally adjusted for cardiovascular risk factors, such as systolic blood pressure, diastolic blood pressure, use of blood pressure lowering medication, type II diabetes, total cholesterol, high-density lipoprotein cholesterol, and use of lipid-lowering medication (model c). Multiple testing correction was applied using the false discovery rate (FDR) of Benjamini-Hochberg. We then identified common miRNAs between cognition and dementia.

2.5.2 | Part II: Multi-marker analysis

Given that the underlying correlation structure between miRNAs is not accounted for in single-marker analysis, we also performed a multi-marker analysis. MiRNA data are high-dimensional including many multicollinear variables (especially when miRNAs are co-expressed); therefore, joint analysis of miRNAs using conventional regression techniques may yield inflated effect sizes and standard errors.[22] Hence,

we used elastic net regularization models for multi-marker analysis that reduce the dimensionality of all 591 well-expressed miRNAs *collectively*, penalizing highly collinear miRNAs that explain little to no variance for cognition or dementia. The algorithm of the elastic net is an extension of the ordinary least squares (OLS) model, that facilitates regularization and variable selection by combining penalties from ridge regression (L1) and lasso regression (L2).[23–25] While the loss function is solved, both multicollinearity and the grouping structure of data are accounted for, and a subset of potentially explanatory variables is generated. This dimensionality reduction property of the elastic net has been successfully applied to various types of omics data (e.g., proteomics, RNA-sequencing data, and gene-expression analysis).[26–28] For extended details on the elastic net algorithm, including its mathematical foundation, we refer to Methods S3 in supporting information.

MiRNAs that survived the elastic net penalty (with non-zero regression coefficients) were identified for cognition and dementia, separately. To test their robustness, common miRNAs between both phenotypes were then subjected to Cox proportional hazards regression for incident dementia, adjusting for the same potential confounders as described earlier.

2.6 | Post hoc analysis of identified miRNAs and target genes

Only common miRNAs between cognition and dementia (either in part I or II) that survived adjustment for potential confounders were used for post hoc analyses. A four-step in silico analysis was conducted to see whether these miRNAs are potentially involved in underlying disease pathways. First, we used three miRNA target prediction databases (miRTarBase, miRDB, and TargetScan) to extract predicted target genes for identified miRNAs, but only if there was an overlap in at least two out three databases. Second, we examined whether single nucleotide polymorphisms (SNPs) located in these target genes were associated with cognition or dementia, using summary statistics data available from previous GWAS on general cognitive function[29] and dementia.[30,31] Only SNPs passing the genome-wide significance threshold ($P < 5 \times 10^{-8}$) and their annotated genes were retrieved. Host genes were used as proxy if a miRNA was located in the intronic region, as they are commonly co-expressed. Third, we investigated the expression of identified miRNAs and their predicted target genes or host genes in the brain, using the Human Protein Atlas (http://www.proteinatlas.org). As the final step, we assessed whether a specific pathway was highlighted for these putative target genes using g:Profiler (version: e105_eg52_p16_e84549f), and visualized them in a network using the Human Reference Interactome (HuRI) map (http://interactome-atlas.org).

2.7 | Exploratory analyses of miRNAs

Based on expression levels of common miRNAs, we stratified the population into a low- and high-risk group for incident dementia, using the maximally selected rank statistic as the cut-off point.[32] The Kaplan–Meier curves and the log-rank test are provided for descriptive purposes. Finally, we performed an additional exploratory analysis, in which we calculated a prognostic risk score for incident dementia using a linear combination of the miRNA expression levels weighted by the multivariable Cox regression coefficient (β): $\Sigma(\beta\text{-}_{\text{miRNA}} \times$ expression level of miRNA in log2 CPM).

3 | RESULTS

3.1 | Study population

Baseline characteristics of the study population and cognitive test scores are listed in Table 1. The median age of the study population ($n = 1615$) was 70.3 years (interquartile range [IQR], 65.6–76.5 years), and 58.1% was female. In this population, the G-factor was normally distributed (mean = 0.02, standard deviation [SD] ± 0.98). Data of the complete study population was used for the single-marker analysis. For the multi-marker analysis (elastic net), a random set of 1212 participants (75%) was allocated to the training set and the remaining 403 participants (25%) were part of the test set. Clinical characteristics and cognitive test scores for both groups were comparable, with the exception of a small difference in diastolic blood pressure (Table S1 in supporting information). Performance metrics of statistical models are mentioned in Methods S3.

3.2 | Part I: Single-marker analysis

Forty-seven miRNAs were nominally ($P \leq .05$) associated with cognition (G-factor) in fully adjusted linear regression models (Figure 1A), but none passed the FDR threshold. During a median follow-up of 10.7 years (IQR, 7.7–11.4 years), 180 participants developed dementia, of whom 150 (83.3%) had AD. Eighteen miRNAs were nominally associated with incident dementia in fully adjusted Cox proportional hazards models (Figure 1B), yet none passed the FDR threshold. From all nominally associated miRNAs (Table S2 in supporting information), three were common between cognition and dementia: miR-4539, miR-372-3p, and miR-566.

3.3 | Part II: Multi-marker analysis

Thirty-six miRNAs were identified by the elastic net, explaining 10% of the variation (R^2) in baseline cognition (residual mean squared error 0.92) and their penalized coefficients are displayed in Figure 2A. Forty-three miRNAs were identified for incident dementia using the elastic net (concordance index 0.59) and their penalized coefficients are displayed in Figure 2B. Seven miRNAs were common between cognition and dementia, from which three remained nominally associated with incident dementia after adjustment for potential confounders in Cox regression models, namely: miR-4539, miR-372-3p, and miR-7106-5p (Table S3 in supporting information).

TABLE 1 Baseline characteristics of the study population

Characteristic	Total ($n = 1615$)
Age, median [IQR], y	70.3 [65.6–76.5]
Female, no. (%)	938 (58.1)
Educational attainment, no. (%)	
Primary	144 (9.1)
Further	1201 (75.5)
Higher	246 (15.5)
Body mass index, mean (SD), kg/m^2	27.6 (4.1)
Smoking, no. (%)	
Never	474 (29.8)
Former	936 (58.9)
Current	180 (11.3)
Systolic blood pressure, mean (SD), mmHg	148.0 (20.8)
Diastolic blood pressure, mean (SD), mmHg	79.6 (10.8)
Blood pressure lowering medication, no. (%)	680 (42.4)
APOE ε4 carrier, no. (%)	404 (26.5)
Total cholesterol, mean (SD), mmol/L	5.7 (1.0)
HDL cholesterol, mean (SD), mmol/L	1.5 (0.4)
Lipid lowering medication, no. (%)	362 (22.6)
Type 2 diabetes, no. (%)	208 (13.3)
Cognitive test scores, mean (SD)	
Stroop 1 test, time to complete, s.	18.3 (4.1)
Stroop 2 test, time to complete, s.	24.4 (5.3)
Stroop 3 test, time to complete, s.	58.2 (27.0)
Letter digit substitution test, no. of correct answers	27.6 (7.0)
15-word learning test, immediate, no. of words	6.9 (2.0)
15-word learning test, delayed, no. of words	6.7 (2.7)
15-word learning test, recognition, no. of words	13.0 (2.2)
Verbal fluency test, number of correct answers	21.0 (5.2)
Purdue Pegboard Test, left hand, points per task	11.8 (1.9)
Purdue Pegboard Test, right hand, points per task	12.2 (2.0)
Purdue Pegboard Test, both hands, points per task	9.7 (1.8)
Mini-Mental State Examination	27.8 (2.0)
Cognition (G-factor)	0.02 (0.98)

Note: Unless specified otherwise, mean values and SD are displayed for continuous measures and frequencies (%) are presented for categorical values. Data presented in baseline table are not imputed.
Abbreviations: *APOE*, apolipoprotein E; HDL, high-desnsity lipoprotein; IQR, interquartile range; n, number of participants; no., number; SD, standard deviation.

Expression levels of all four common miRNAs between cognition and dementia, either identified during single- or multi-marker analysis (miR-4539, miR-372-3p, miR-566, and miR-7106-5p), are displayed in Figure 3 for various levels of cognition (G-factor) and cognitive test scores. Figure 4 illustrates the expression levels of these four miRNAs per dementia status and includes Kaplan Meier curves, in which the population was stratified into a low- and high-risk group for incident dementia based on the maximally selected rank statistic. Figure S1 in supporting information displays the correlation between these four miRNAs.

3.4 | Post hoc analysis of identified miRNAs

Predicted target genes of miR-4539, miR-372-3p, miR-566, and miR-7106-5p were extracted from miRTarBase (v8.0), miRDB (v6.0), and TargetScan (7.1) web tools. This resulted in the following amount of putative target genes: 349 for miR-4539, 620 for miR-372-3p, 36 for miR-566, and 928 for miR-7106-5p. We retrieved SNPs in these target genes and examined their associations with cognition and dementia using GWAS data. In a recent GWAS on general cognitive function from the CHARGE consortium,[33] 146 SNPs in 6 target genes of miR-4539, 356 SNPs in 22 target genes of miR-372-3p, 4 SNPs in 1 target gene of miR-566, and 1426 SNPs in 22 target genes miR-7106-5p were identified, passing the genome-wide significance threshold ($P < 5 \times 10^{-8}$). In two recent GWAS on dementia,[30,31] 34 SNPs in two target genes of miR-372-3p (*AGFG2* and *RABEP1*) and 115 SNPs in one target gene of miR-7106-5p (*SPI1*), reached the genome-wide significance threshold (Table S4 in supporting information). None of the miRNAs were previously reported to be expressed in the brain. However, miR-7106-5p is located in the intronic region of *DDX5*, and this host gene is expressed in brain tissue. Moreover, out of in total 51 predicted target genes of the four miRNAs, five were highly expressed (normalized expression > 50) in the cerebral cortex (*CAMK2N1*, *MAPT*, *PPP3R1*, *MEF2C*, *CADM2*), and two were highly expressed in the hippocampus (*CAMK2N1*, *PPP3R1*), according to the Human Protein Atlas (HPA) v20.1[34] (Figure S2 in supporting information). For the five highly expressed predicted target genes in the brain, the mitogen-activated protein kinase (MAPK) signaling pathway ($P = 2.26 \times 10^{-3}$) was identified in pathway enrichment analyses of KEGG (Kyoto Encyclopedia of Genes and Genomes). According to Gene Ontology (GO) cellular function databases, these genes are involved in synapse function ($P = 1.71 \times 10^{-4}$), postsynapse functions ($P = 6.71 \times 10^{-4}$), and cell junction ($P = 1.74 \times 10^{-3}$), possibly affecting cellular signal transduction. The provided *P*-values are corrected for multiple testing. In addition, the *MAPT*, *MEF2C*, and *PPP3R1* genes were part of an interactome (Figure S3 in supporting information).

3.5 | Exploratory analyses

The multivariable Cox regression coefficients of the three common miRNAs in multi-marker analysis (miR-4539, miR-7106-5p, and miR-372-3p) yielded the following formula for the prognostic risk of dementia: (0.3482 * miR-4539) + (0.3274 * miR-7106-5p) + (−0.2345 * miR-372-3p). Based on a maximally selected cut-off of 3.25, the cumulative incidence of dementia was 12.6% (95% CI: 10.5%–14.7%) in the high-risk group after 10.7 years, versus 6.8% (95% CI: 4.8%–8.8%) in the low-risk group (Figure S4 in supporting information).

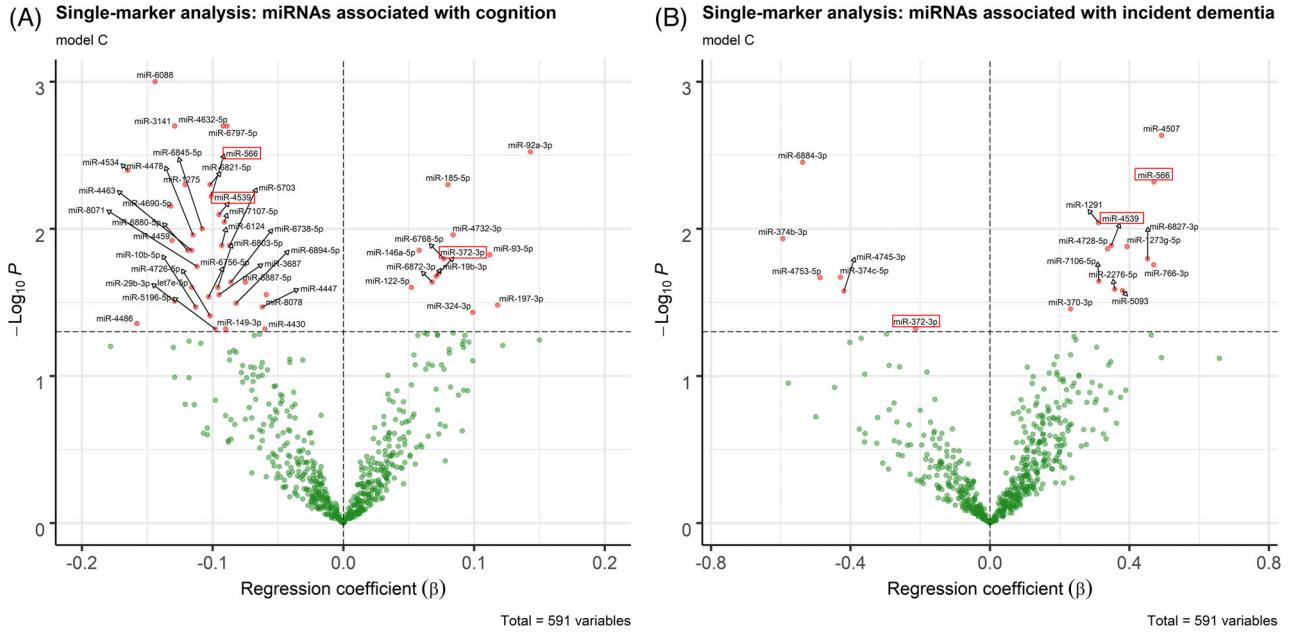

FIGURE 1 Single-marker analysis: microRNAs (miRNAs) associated with cognition and dementia. Volcano plots of miRNAs associated with cognition (A, linear regression) and incident dementia (B, Cox proportional hazard models), based on single-marker analysis. Model C is displayed, which is adjusted for age, sex, cohort, smoking, apolipoprotein E ε4 carriership, education, systolic blood pressure, diastolic blood pressure, use of blood pressure–lowering medication, type 2 diabetes, total cholesterol, high-density lipoprotein cholesterol, and use of lipid-lowering medication. Whereas 47 miRNAs were nominally associated with cognition ($p \leq .05$) and 18 miRNAs were nominally associated with dementia, none passed the false discovery rate threshold. Three miRNAs were common between both phenotypes: miR-4539, miR-372-3p, and miR-566, which are outlined (in red) on the graph. The effect estimates of displayed miRNAs including the 95% confidence intervals can be found in Table S2 in supporting information, which also displays the overlap with multi-marker analysis

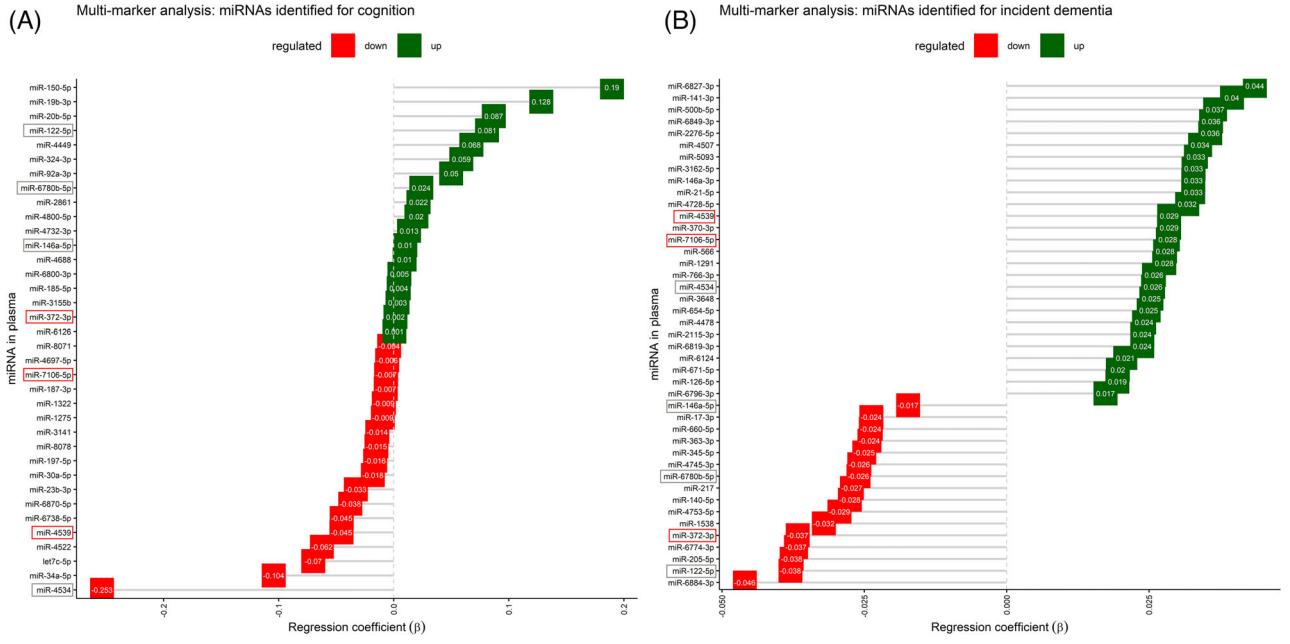

FIGURE 2 Multi-marker analysis: microRNAs (miRNAs) identified for cognition and dementia. Dotplots of miRNAs identified for cognition (A, G-factor) and (B) incident dementia, based on multi-marker analysis with elastic net regularization models. Displayed are 36 miRNAs selected by the elastic net for G-factor and 43 miRNAs selected by the elastic net for incident dementia. The common miRNAs between both phenotypes are outlined in gray. The miRNAs that survived adjustment for potential confounders (miR-4539, miR-372-3p, and miR-7106-5p) are outlined in red. Effect estimates of all common miRNAs based on multi-marker analysis, including their 95% confidence intervals, can be found in Table S3 in supporting information

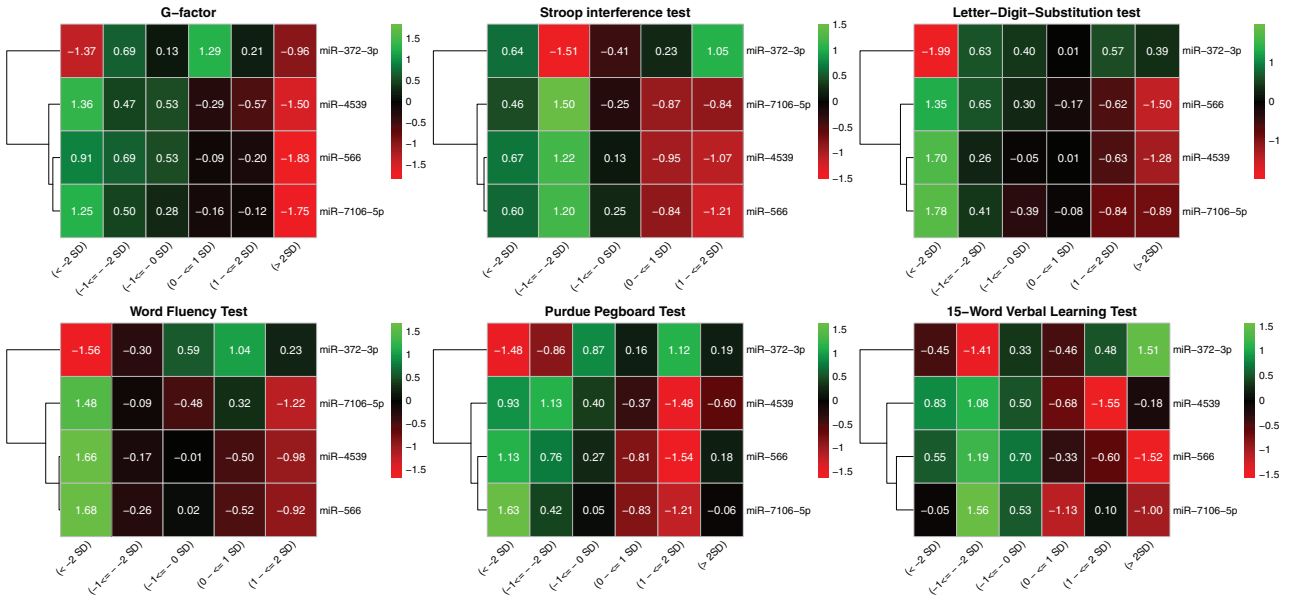

FIGURE 3 Hierarchical cluster plot of the four microRNAs (miRNAs) for cognitive function. Hierarchical cluster plots displaying relative mean expression levels of the four identified miRNAs that were common between cognition and dementia, after adjusting for potential confounders (miR-4539, miR-372-3p, miR-566, and miR-7106-5p). The G-factor and included cognitive tests are displayed with standardized test scores to facilitate comparison. The *x*-axis denotes the categories from severe cognitive dysfunction (far left, ← 2SD [standard deviation]) to cognitive function above average (far right, > 2SD). Upregulated miRNAs are displayed in green, downregulated miRNAs are displayed in red

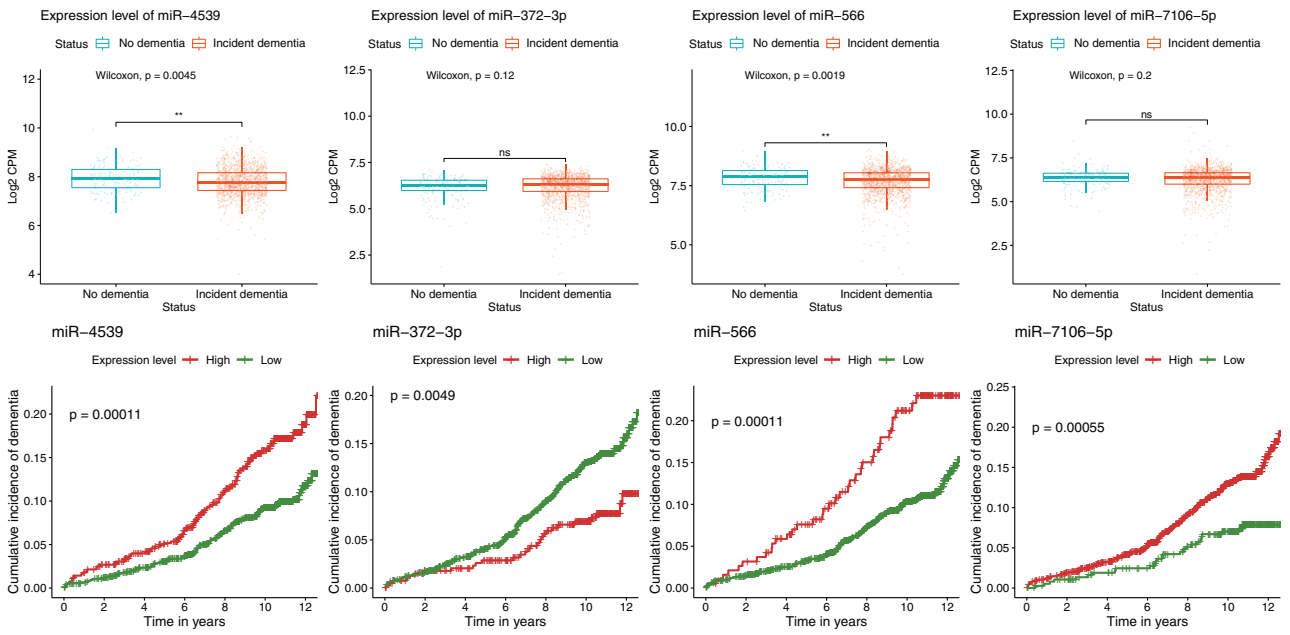

FIGURE 4 Expression levels of the four common microRNAs (miRNAs) between cognition and dementia. Median miRNA expression levels in plasma at baseline are displayed in log2, counts per million (upper graphs). Expression levels at baseline were compared using the Wilcoxon test. For Kaplan–Meier plots (lower graphs), the cut-offs in log2 counts per million based on the maximally selected rank statistic were 8.01 for miR-4539, 6.61 for miR-372-3p, 8.22 for miR-566, and 5.99 for miR-7106-5p. Abbreviations: ns, not significant; *P*, *P*-value

4 | DISCUSSION

In this study, we performed both a single- and multi-marker analysis to identify circulatory miRNAs for cognition (G-factor) and dementia. During single-marker analysis, 47 miRNAs were nominally associated with cognition and 18 miRNAs were nominally associated with incident dementia, after adjustment for potential confounders. Three miRNAs were common between cognition and dementia, namely, miR-4539,

miR-372-3p, and miR-566. While accounting for multicollinearity and grouping structure of data in multi-marker analysis, as well as adjustment for potential confounders, we identified one additional common miRNA (miR-7106-5p) between phenotypes. Several predicted target genes of these four miRNAs (e.g., *CAMK2N1*, *MAPT*, *PPP3R1*, *MEF2C*, *CADM2*) are highly expressed in the hippocampus, possibly involving the MAPK signaling pathway, with implications for cellular signal transduction and synapse function.

We identified many novel miRNAs for cognition, probably because the G-factor allows for a more comprehensive evaluation of cognitive function than cognitive screening tests, such as the Montreal Cognitive Assessment (MoCA) score. From all identified miRNAs for cognition, three (miR-150-5p, miR-19b-3p, and miR-185-5p) were previously correlated with age and the MoCA score in the plasma of 115 healthy community-dwelling older adults (mean age: 71.8 years).[35] In an ancillary study in which the authors corrected for age, miR-10b-5p and miR-23b-3p were also nominally associated with the MoCA score, which we similarly identified during single- and multi-marker analysis, respectively.[36] Upregulation of miR-10b-5p has been linked to impaired hippocampal neurogenesis in mice by downregulating the expression of brain-derived neurotropic factor (*BDNF*).[37] Upregulation of miRNA-23b-3p has been linked to oxidative stress and apoptosis of neuronal cells, via the *SIRT1/NRF2* signaling pathway.[38] One miRNA that was identified for cognition during multi-marker analysis only (miR-34a-5p) has previously been shown to affect both cognitive performance and AD by targeting amyloidogenic processing of *APP*.[39] This was demonstrated in animal models and by miR-34a knockout in transgenic *APP/PS1* mice.[40] Yet, miR-34a-5p was not identified for incident dementia in multi-marker analysis, probably due to a strong correlation with other miRNAs.

Among all miRNAs identified for dementia in our study, miR-146a is a well-recognized regulator of the neuroinflammatory immune response.[41,42] Upregulation of miR-146a increases susceptibility to neuronal damage via the nuclear factor kappa B pathway and its expression levels correlate with synaptic pathology as well as amyloid plaque density.[43] In addition, toll-like receptor 2 (*TLR2*) is a validated target gene of miR-146a, which encodes a primary receptor for Aβ.[44]

We distinguished four common miRNAs between cognition and dementia, including miR-4539 and miR-372-3p, that were identified in both single- and multi-marker analysis. MiR-566 was only associated with both phenotypes during single-marker analysis, probably due to a high correlation with miR-4539 (Pearson's correlation coefficient: 0.68, $P = 2.20 \times 10^{-16}$, Figure S1). MiR-7106-5p was nominally associated with incident dementia in single-marker analysis, and identified as a common miRNA in multi-marker analysis.

In contrast to miR-4139, miR-566, and miR-7106-5p, for which upregulation is associated with impaired cognitive functioning (Figure 3), upregulation of miR-372-3p may promote expression of genes that enhance cognition, such as *TET2*,[45] which is a highly predicted target gene (Table S4). Among the four miRNAs, miR-7106-5p may be more specific to AD, compared to miR-4539, miR-372-3p, or miR-566. miR-7106 has the *MAPT* gene as one of the predicted target genes and, perhaps more importantly, is located in the intronic region of *DDX5*, which has previously been linked to hyperphosphorylation of tau.[46] miR-566 was previously identified as one of the top 10 differentially expressed miRNAs in patients with frontotemporal dementia and mutations in the progranulin gene (*PGRN*).[47] Although miR-4539 and miR-372-3p have not been associated with cognition or dementia before, each of them has been reported along a known risk factor for dementia including stroke[48] and traumatic brain injury.[49] Results from our in silico analysis suggest that the four miRNAs have putative target genes that are highly expressed in the cerebral cortex or hippocampus (e.g., *CAMK2N1*, *MAPT*, *PPP3R1*, *MEF2C*, *CADM2*), which may form a complex interactome, possibly enacting via the MAPK signaling pathway. This pathway is implicated in AD pathology, considering its involvement in synaptic plasticity and tau phosphorylation.[50] Future studies are warranted to validate the identified miRNAs in an experimental design and assess the clinical utility of the prognostic risk score developed in this study.

4.1 | Strengths and limitations

Some methodological advantages of this study include: (1) evaluation of cognitive performance, (2) measurement of miRNA expression, (3) the study design, and (4) statistical analysis techniques, which allowed for the identification of several novel miRNAs for cognition and dementia. While the MoCA or the MMSE predominantly serve as cognitive screening tools, the G-factor allows for a more comprehensive evaluation of cognitive performance. Also, expression levels of miRNAs may differ depending on the assays and/or the biological fluid (e.g., cerebrospinal fluid, serum, or plasma) used.[6,11] We had the opportunity to explore a large set of miRNAs in plasma ($n = 591$) using a highly reproducible assay. Furthermore, previous studies were predominantly cross-sectional in nature[11,12,36] and either used differential expression analysis with a *t*-test or classical regression methods to perform single-marker analysis of miRNAs for cognition and dementia. Such methods cannot handle multicollinearity, whereas multi-marker analysis techniques (such as the elastic net) can address this effectively. Other strengths of this study include use of next-generation sequencing technologies for measuring miRNA expression in plasma and a standardized assessment of cognitive function in a population of community-dwelling older adults, whom we followed over a decade for incident dementia. Furthermore, we selected miRNAs associated with cognition and dementia independently, reducing the risk of overestimating their true effect size.

Nevertheless, several limitations should be acknowledged. First, we were not able to validate the identified miRNAs nor their putative target genes in an experimental design, which hampers inferences on causality. Besides, epigenetic changes, such as histone modifications and DNA methylation, may alter the expression levels of miRNAs. Second, we lacked an external validation cohort for testing the prognostic risk score, hence it should be interpreted with caution. Third, we were limited in statistical power to evaluate miRNA expression among dementia subtypes. Last, the results from this predominantly White population may not be generalizable to other ethnicities.

5 | CONCLUSION

In conclusion, we identified several circulatory miRNAs in plasma associated with cognition and incident dementia, including four common well-expressed miRNAs (miR-4539, miR-372-3p, miR-566, and miR-7106-5p). Their link to both phenotypes and associated target genes suggests that these miRNAs could serve as potential biomarkers and may be involved in molecular pathways underlying dementia.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report. Author disclosures are available in the supporting information.

AUTHOR CONTRIBUTIONS

All listed authors made significant intellectual contribution to the study design (Mohsen Ghanbari, Mohammad Arfan Ikram), acquisition of data (Jaco M. Klap, Gerrit Jan Weverling, Paul Klatser, Just P.J. Brakenhoff, Mohsen Ghanbari, Mohammad Kamran Ikram, Michelle M.J. Mens, Amber Yaqub), analysis and interpretation of data (Amber Yaqub, Michelle M.J. Mens, Gennady V. Roshchupkin, Mohammad Kamran Ikram, Mohsen Ghanbari, Mohammad Arfan Ikram), drafting the manuscript (Amber Yaqub), or critically revising it for additional intellectual content (Amber Yaqub, Michelle M.J. Mens, Jaco M. Klap, Gerrit Jan Weverling, Paul Klatser, Just P.J. Brakenhoff, Gennady V. Roshchupkin, Mohammad Kamran Ikram, Mohsen Ghanbari, Mohammad Arfan Ikram). Amber Yaqub, Michelle M.J. Mens, Mohsen Ghanbari, and Mohammad Arfan Ikram had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All listed authors approved the decision to submit for publication.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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