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Validation study of microRNAs previously associated with antidepressant response in older adults treated for late-life depression with venlafaxine

Victoria S. Marshe[a,b], Farhana Islam[b,c], Malgorzata Maciukiewicz[b,l], Laura M. Fiori[d], Volodymyr Yerko[d], Jennie Yang[d], Gustavo Turecki[d], Jane A. Foster[e,f], Sidney H. Kennedy[a,e,g,h], Daniel M. Blumberger[a,b,g], Jordan F. Karp[i,j], James L. Kennedy[a,b,g], Benoit H. Mulsant[a,b,g], Charles F. Reynolds III[i], Eric J. Lenze[k], Daniel J. Müller[a,b,c,g,*]

[a] Institute of Medical Science, University of Toronto, Toronto, ON, Canada
[b] Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada
[c] Department of Pharmacology & Toxicology, University of Toronto, Toronto, ON, Canada
[d] McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Verdun, Quebec, Canada
[e] Department of Psychiatry, University Health Network, Toronto, ON, Canada
[f] Department of Psychiatry and Behavioral Neurosciences, McMaster University, Hamilton, ON, Canada
[g] Department of Psychiatry, University of Toronto, Toronto, ON, Canada
[h] Keenan Research Centre, St. Michael's Hospital, Toronto, ON, Canada
[i] Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA
[j] VA Pittsburgh Health System, Geriatric Research Education and Clinical Center, Pittsburgh, PA, USA
[k] Healthy Mind Lab, Department of Psychiatry, Washington University, St. Louis, MO, USA
[l] Center of Experimental Rheumatology, Department of Rheumatology, University Hospital Zurich, Switzerland

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ABSTRACT

Background: MicroRNAs (miRNAs) are small 22 nucleotides long, non-coding RNAs that are potential biomarkers for antidepressant treatment response. We aimed to replicate previous associations of miRNAs with antidepressant treatment response in a sample of older adults diagnosed with late-life depression.

Methods: Our sample included 184 older adults diagnosed with moderately severe depression that received open-label venlafaxine (up to 300 mg/day) for approximately 12 weeks. We quantified miRNA expression levels at baseline and week 12 for miRNAs miR-1202, miR-135a-5p, miR-16-5p, miR-146a-5p, miR-146b-5p, miR-425-3p, and miR-24-3p to explore their association with remission status, response trajectories, and time-to-remission.

Results: At T0 and T12, there were no differences in miRNA expression levels between remitters and non-remitters. However, remitters showed a trend toward higher baseline miR-135a-5p (*Median* = 11.3 [9.9, 15.7], $p = .083$). Prior to correction, baseline miR-135a-5p expression levels showed an association with remission status (*OR* = 1.8 [1.0, 3.3], $p = .037$). Individuals with higher baseline miR-135a-5p showed better response trajectories ($F = 4.5$, FDR-corrected $p = 4.4 \times 10^{-4}$), particularly at weeks 10 and 12 ($p < .05$). In addition, individuals with higher miR-135a-5p expression reached remission faster than those with lower expression (*HR* = 0.6 [0.4, 0.9], FDR-corrected $p = .055$).

Limitations: Although the sample size was relatively modest, our findings are consistent with the literature suggesting that higher miR-135a-5p levels may be associated with better antidepressant treatment response.

Conclusions: However, the miRNA signature of antidepressant response in older adults may be different as compared to younger adults.

1. Introduction

Affecting up to 16% of older adults, late-life onset depression (LLD) is depression diagnosed later in life (age ≥ 50–60 years) (McCusker et al., 2009; Naismith et al., 2012). While the first episode of depression may onset in late-life, depression may also be recurring from earlier in

* Corresponding author at: Pharmacogenetics Research Clinic, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, 250 College Street, Toronto, ON M5T1R8, Canada.
E-mail address: daniel.mueller@camh.ca (D.J. Müller).

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life. Notably, LLD is pathophysiologically distinct from depression in young- and middle-aged adults; patients present with greater medical comorbidities due to underlying neurodegenerative and cerebrovascular changes (Naismith et al., 2012). Given these particular characteristics associated with LLD, including others such as polypharmacy, > 50% of patients experience relapse or do not achieve remission. In turn, persisting LLD symptomatology is associated with progressive cognitive decline, as well as, an increased risk for dementia and stroke (Deng et al., 2018; Klap et al., 2003; Whyte et al., 2004). Therefore, it is increasingly important to identify biomarkers that can illuminate the underlying biological mechanism of depression and also guide medication selection and dosing that is better matched to the patient.

Novel advances in post-transcriptional regulation of gene expression and ribonucleic acid (RNA) silencing offer additional avenues of predictive biomarker discovery for antidepressant treatment response. MicroRNAs (miRNAs) are small 22 nucleotide-long non-coding RNAs which have been extensively investigated in psychiatry in recent years and have demonstrated their potential to be diagnostic biomarkers for depression (Lopez et al., 2018; Tavakolizadeh et al., 2018). MiRNAs disrupt or prevent the translation of target messenger RNA (mRNA) expression by binding to complementary miRNA responsive element sequences (MREs) of the 3′ UTR of their target mRNA transcripts, thereby regulating gene expression (Hansen and Obrietan, 2013). Of note, a single miRNA may target multiple genes, and multiple miRNAs may target a single gene. Collectively, miRNAs are regulators of > 60% of all the protein-coding genes, therefore their dysregulation can disrupt molecular signalling pathways and cellular homeostasis (Bocchio-Chiavetto et al., 2013; Hansen and Obrietan, 2013).

The role of miRNAs in the pathophysiology and treatment outcomes of MDD has been highlighted by several findings. Circulating miRNAs in blood and peripheral fluids seem to reflect physiological changes as a result of treatment effects (Lopez et al., 2017b). For instance, peripheral levels of miR-1202, which regulates metabotropic glutamate receptor-4 gene expression, has been associated with treatment response to citalopram, with remitters expressing increased miR-1202 levels after eight weeks of treatment, whereas no differences were observed in non-remitters and healthy controls (Lopez et al., 2014). Changes in circulating miR-1202 levels were also observed to be negatively correlated with brain activity changes in the right precuneus within the default-mode network following desvenlafaxine treatment (Lopez et al., 2017b). Likewise, circulating miRNAs 146a/b-5p, 425-3p and 24-3p have been associated with antidepressant response (Lopez et al., 2017a). Responders to duloxetine treatment demonstrated a downregulation of these miRNAs in blood samples, whereas non-responders showed no changes in the expression (Lopez et al., 2017a). These findings were replicated in a different cohort treated with escitalopram (Lopez et al., 2017a). Interestingly, miRNAs 146a/b-5p, 425-3p and 24-3p are regulators of expression of > 30 genes involved in mitogen-activated protein kinase (MAPK) and Wnt signalling pathways (Lopez et al., 2017a). Inhibition of these pathways is associated with depressive symptoms, while activation has been strongly associated with alleviation of depressive symptoms and improved antidepressant response (Lopez et al., 2017a). Lastly, levels of miR-16 in the cerebrospinal fluid have been associated with depressive symptoms, with decreased levels observed in MDD patients but not in healthy controls (Song et al., 2015).

The majority of biomarker studies have been conducted in early-to-middle life MDD patients. Association studies of miRNA expression levels have yet to be conducted in older adults treated with antidepressants. Therefore, the current investigation aimed to validate replicate previous associations of miR-1202, miR-135a-5p, miR-16-5p, miR-146a-5p, miR-146b-5p, miR-425-3p, and miR-24-3p in a cohort of older adults treated with venlafaxine (Lopez et al., 2017a).

2. Methods

2.1. Sample

For our analysis, we included a subset of older adults (≥60 years; n = 311) from Phase 1 from the NIH-funded clinical trial IRL-GREY (Incomplete Response in Late-Life Depression: Getting to Remission; NCT00892047; see Table 1). Individuals were diagnosed with MDD via the DSM-IV, were moderately depressed at baseline (Montgomery-Åsberg Depression Rating Scale, MADRS score ≥ 15), and were not cognitively impaired (Folstein Mini-Mental State Examination, MMSE < 24). In brief, individuals received open-label venlafaxine (37.5 mg/day, up to 300 mg/day) and were assessed at eight timepoints for approximately 12 weeks. Detailed study design, inclusion and exclusion criteria, as well as full subset analyses are described in-depth in earlier publications (Lenze et al., 2015; Marshe et al., 2017). Within this subset, the remission rate was 51.1%.

2.2. MicroRNA quantification

From the initial subset of 311 individuals of European ancestry, we

Table 1
Sample demographics (n = 184).

	Total (n = 184)	Non-remitters (n = 91)	Remitters (n = 93)	P-value
Categorical	n (%)	n (%)	n (%)	χ^2
Sex				0.006
Female	118 (64.1)	49 (53.8)	69 (74.2)	
Site				
University of Pittsburgh	74 (40.2)	25 (27.5)	49 (52.7)	0.002
University of Toronto	58 (31.5)	33 (36.3)	25 (26.9)	
Washington University	52 (28.3)	33 (36.3)	19 (20.4)	
Continuous	Mean (SD)	Mean (SD)	Mean (SD)	Wilcoxon p-value
Age (years)	68.1 (6.9)	66.8 (5.9)	69.4 (7.6)	0.020
Age of onset (years)	41.1 (21.2)	38.3 (21.5)	43.8 (20.7)	0.058
Duration in treatment (weeks)	13.5 (2.8)	13.2 (2.7)	13.9 (2.8)	0.017
Duration of current MDE (years)	5.5 (12.2)	7.2 (14.2)	3.8 (9.6)	< 0.001
Baseline MADRS score	26.6 (5.8)	28.2 (5.8)	25.1 (5.4)	0.001
Final MADRS score	14.5 (13.1)	27.1 (5.4)	2.1 (1.5)	< 0.001
Absolute change in MADRS score	−12.1 (11.9)	−1.1 (4.1)	−23.0 (5.1)	< 0.001
Change in MADRS score (%)	−0.48 (0.46)	−0.03 (0.16)	−91.7 (0.05)	< 0.001
Time to remission (weeks)	–	–	6.7 (4.3)	–
Venlafaxine dose (mg/day)	233.6 (74.8)	279.0 (45.1)	189.1 (71.4)	< 0.001

Abbreviations. MADRS, Montgomery-Åsberg Depression Rating Scale; MDE, major depression episode; SD, standard deviation.

Table 2
Comparison of baseline (T0) and end-of-treatment (T12) miRNA expression levels, in the total sample (n = 184), non-remitters (n = 91) and remitters (n = 93).

	T0 expression levels (Median [Min, Max])			T12 expression levels (Median [Min, Max])		
	Whole sample	Non-remitters	Remitters	Whole sample	Non-remitters	Remitters
miR-1202	11.7 [10.4, 16.6]	11.6 [10.4, 14.2]†	11.8 [10.5, 16.6]	11.7 [10.5, 16.7]	11.7 [10.8, 16.7]	11.8 [10.5, 14.9]
miR-135a-5p	11.3 [9.9, 15.7]	11.1 [10.1, 14.0]†	11.3 [9.9, 15.7]	11.2 [9.9, 16.5]	11.2 [10.0, 16.5]	11.3 [9.9, 14.2]
miR-16-5p	21.5 [19.2, 23.9]	21.5 [19.2, 23.1]	21.6 [20.0, 23.9]	21.5 [20.5, 23.3]	21.5 [20.7, 23.3]	21.5 [20.5, 23.3]
miR-146a-5p	17.7 [15.5, 20.1]	17.6 [15.8, 18.9]†	17.7 [15.5, 20.0]	17.7 [15.5, 19.4]	17.7 [15.6, 19.4]	17.7 [15.5, 19.0]
miR-146b-5p	17.3 [15.4, 19.1]	17.3 [16.0, 18.6]	17.3 [15.4, 19.1]	17.3 [15.2, 18.9]	17.3 [15.2, 18.9]	17.2 [15.6, 18.4]
miR-425-3p	12.2 [10.7, 16.3]	12.1 [10.7, 14.2]	12.3 [10.9, 16.3]	12.2 [10.8, 16.4]	12.2 [10.8, 16.4]	12.2 [10.8, 14.4]
miR-24-3p	18.5 [16.1, 20.0]	18.4 [16.1, 19.3]	18.5 [17.2, 20.0]	18.4 [16.2, 20.3]	18.4 [16.2, 20.3]	18.4 [16.9, 19.7]

Significance. †$p < .1$, prior to FDR-correction in comparison to remitters (Wilcoxon test).

selected the top 100 remitters and bottom 100 non-remitters (based on ordered percentage change in MADRS scores) for miRNA quantification. We quantified expression levels of hsa-miR-1202, hsa-miR-135a-5p, hsa-miR-16-5p, hsa-miR-146a-5p, hsa-miR-146b-5p, hsa-miR-425-3p, hsa-miR-24-3p at baseline (T0) and end of treatment (week 12, T12).

For each participant, IRL-GREY buffy coat samples were collected using 6 mL K2-EDTA tubes. After blood collection, the tubes were spun at 1000*g* for 10 min at room temperature. Subsequently, the buffy coat layer was transferred to 1.5 mL tubes and stored at −80 °C until further RNA extraction. Total RNA was extracted from the buffy coat using the Zymo Research Direct-zol RNA miniprep kit (R2052) using the Tri-reagent method. RNA concentrations were then measured using a Nanodrop spectrophotometer and adjusted to 8 pg/uL for the Firefly assay.

Our custom Firefly assay was designed to test, in multiplex, seven miRNAs of interest (hsa-miR-1202, hsa-miR-135a-5p, hsa-miR-16-5p, hsa-miR-146a-5p, hsa-miR-146b-5p, hsa-miR-425-3p, hsa-miR-24-3p) and four miRNA as endogenous controls (hsa-miR-29b-3p, hsa-miR-19b-3p, hsa-let-7i-5p, hsa-let-7b-5p). We normalized miRNA levels using the Firefly Analysis Workbench software supplied by Abcam which has a built-in geNorm algorithm. This algorithm identified the endogenous miRNA probes (i.e., hsa-miR-29b-3p, hsa-miR-19b-3p, hsa-let-7i-5p, hsa-let-7b-5p) as the most stable in the set of experimental probes. The Firefly assay was conducted according to the manufacturer's instructions with FirePlex miRNA Assay Core Reagent Kit V2 – purified RNA (Abcam, Cat #ab218365). The fluorescence of Firefly particles was collected in Accuri C6 flow cytometer for 4000–8000 particle counts per sample using Abcam template.

To decode fluorescence data in correspondence to the assayed miRNA, we used Fireplex Analysis Workbench software. The Fireplex Analysis Workbench has a built-in normalization of Mean Fluorescence Intensity of miRNA of the sample to endogenous control miRNAs. Our samples were normalized to the geometric mean of four endogenous control miRNAs (hsa-mir-29b-3p, hsa-mir-19b-3p, hsa-let-7i-5p, hsa-let-7b-5p) using Mean Fluorescence Intensity. Lastly, we used four assay plates which had six calibrator samples whose means were used to normalize expression data between plates for each miRNA. After miRNA quality control, 188 out of the 200 samples were eligible for further analysis.

2.3. Statistical analysis

Within the samples that passed miRNA quality control (n = 188), our outcomes of interest included (a) remission status defined as a MADRS ≤10 at the end of treatment, (b) change in depressive severity (i.e., MADRS) from T0 to T12, (c) response trajectory of remitter and non-remitter groups across eight time-points, and (d) time-to-remission.

After $\log_2$ normalization of raw levels, we calculated fold change (FC) in miRNA expression levels from T0 to T12 (*R* package *gtools* v. 3.8.1). We excluded 4 significant multivariate outliers ($p < .05$) with extreme FC values based on Wilks' Mahalanobis distance test (Wilks, 1963) due to potential issues with RNA quality either at T0 or T12, resulting in a sample size of 184 individuals. To assess the significance of the change of miRNA expression levels from T0 to T12, we conducted paired Wilcoxon tests in remitters and non-remitters separately, followed by a two-sample Wilcoxon test.

Subsequently, to investigate whether miRNA expression levels at baseline were associated with remission status at the end of treatment or absolute change in depressive symptomatology, we conducted multivariate logistic and linear regressions, respectively. Regression models were adjusted for variables known to be associated with venlafaxine remission including sex, baseline age, duration of treatment, recruitment site, duration of the current major depressive episode, and baseline depressive severity (i.e., MADRS score) (Marshe et al., 2017). We use a likelihood ratio test to assess if the addition of miRNA expression levels significantly improved model fit.

In addition, we conducted analyses for MADRS score trajectories over assessment time-points using linear mixed effects models adjusted for the subject random effects and recruitment sites, as well as the fixed effects of other covariates, including baseline MADRS score within our subset of remitters and non-remitters. Lastly, we explored the effects of baseline miRNA expression on time-to remission using a Cox proportional hazards model. For each analysis, we corrected for multiple testing using an FDR-correction for seven miRNAs. All of the analyses were conducted in R.

This investigation was approved by the institutional review board and all individuals provided their informed consent.

3. Results

Our final sample (n = 184) included a larger proportion of individuals of the female sex (64.1%). On average, individuals were moderately depressed at baseline (MADRS, M = 26.6, SD = 5.8). The average time to remission was 9.9 weeks (SD = 4.9) at the end of treatment. For additional demographic details, see Table 1.

At T0 and T12, there were no significant differences in miRNA expression levels between remitters and non-remitters (see Table 2). However, remitters showed a trend toward higher baseline levels of miR-135a-5p (*Median* = 11.3 [9.9, 15.7], p = .083) and miR-146a-5p (*Median* = 17.7 [15.5, 20.1], p = .091). None of the miRNAs were significantly down- or up- regulated at the end of treatment in the whole sample or when stratifying by remission status. For all miRNAs, remitters showed decreased miRNA levels post-treatment compared to non-remitters (see Table 3). However, these differences were not significant after correction for multiple testing ($p > .1$). Compared to non-remitters, remitters showed a nominally significant decrease in miR-1202 (*Median* = −1.1 [−4.2, 6.3], *Median* = 1.1 [−10.6, 11.8], p = .052) and miR-425-3p levels (*Median* = −1.0 [−4.8, 5.2], *Median* = 1.1 [−2.6, 5.7], p = .05). In addition, remitters showed a nominally greater decrease in miR-24-3p levels (*Median* = −1.1 [−3.6, 2.5], *Median* = 1.1 [−2.4, 3.3], p = .088) than non-remitters.

Table 3
MiRNA expression levels from baseline to end-of treatment in remitters and non-remitters.

	Total (n = 184)			Non-remitters (n = 91)		Remitters (n = 93)	
miRNA	Median FC [Min, Max]	2-sample p-value	Paired p-value	Median FC [Min, Max]	Paired p-value	Median FC [Min, Max]	Paired p-value
miR-1202	−1.02 [−10.59, 11.76]	0.052	0.827	1.07 [−10.59, 11.76]	0.132	−1.12 [−4.19, 6.32]	0.26
miR-135a-5p	1.01 [−10.18, 9.04]	0.093	0.749	1.09 [−10.18, 9.04]	0.355	−1.09 [−4.34, 8.65]	0.139
miR-16-5p	−1.01 [−5.39, 5.80]	0.135	0.827	1.10 [−5.39, 5.80]	0.267	−1.04 [−2.24, 4.27]	0.336
miR-146a-5p	1.02 [−4.82, 4.09]	0.168	0.703	1.09 [−4.10, 4.09]	0.227	−1.07 [−4.82, 2.67]	0.507
miR-146b-5p	1.03 [−3.69, 3.76]	0.103	0.871	1.14 [−3.11, 3.76]	0.231	−1.01 [−3.69, 2.51]	0.306
miR-425-3p	1.01 [−4.78, 5.73]	0.05	1	1.08 [−2.61, 5.73]	0.165	−1.04 [−4.78, 5.23]	0.181
miR-24-3p	−1.07 [−3.55, 3.33]	0.088	0.127	−1.05 [−2.44, 3.33]	0.845	−1.11 [−3.55, 2.47]	0.022

Abbreviations. FC, fold-change.
Note. P-values are uncorrected and do not remain significant after FDR correction. One-sample, paired p-values assess differences from T0 to T12, whereas two-sample p-values assess the difference between remitters and non-remitters in terms of fold change.

Table 4
Association of baseline and fold-change miRNA expression levels with remission status, change in depressive severity, response trajectories and time-to remission.

miRNA	Remission Status (OR [*95% C.I.*])		MADRS Change (β [95% C.I.])		Response Trajectory (*F-value*)	Time-to Remission (*HR* [*95% C.I.*])
	BL	FC	BL	FC		
miR-1202	1.64 [0.96, 2.8]†	0.88 [0.74, 1.05]	−1.73 [−3.92, 0.47]	0.44 [−0.31, 1.19]	1.75†	1.31 [0.86, 2.00]
miR-135a-5p	1.84 [1.04, 3.26]*	0.93 [0.78, 1.1]	−1.91 [−4.19, 0.37]	0.23 [−0.52, 0.97]	4.47***	1.76 [1.15, 2.68]**
miR-16-5p	1.5 [0.77, 2.93]	0.92 [0.73, 1.15]	−1.61 [−4.59, 1.37]	0.39 [−0.63, 1.41]	1.85†	1.30 [0.86, 1.99]
miR-146a-5p	1.52 [0.83, 2.79]	0.95 [0.77, 1.17]	−1.6 [−4.4, 1.2]	0.1 [−0.89, 1.08]	1.38	1.33 [0.87, 2.04]
miR-146b-5p	1.24 [0.64, 2.39]	0.97 [0.78, 1.2]	−0.61 [−3.8, 2.59]	0.04 [−1.02, 1.11]	0.78	0.96 [0.64, 1.52]
miR-425-3p	1.71 [0.97, 3.03]†	0.88 [0.72, 1.07]	−1.95 [−4.38, 0.48]	0.54 [−0.36, 1.43]	1.56	1.17 [0.76, 1.79]
miR-24-3p	1 [0.51, 1.97]	0.91 [0.71, 1.16]	0.54 [−2.74, 3.82]	0.43 [−0.74, 1.59]	0.57	0.92 [0.58, 1.44]

Significance. ***$p < .001$, **$p < .01$, *$p < .05$, †$p < .1$, prior to FDR-correction.
Abbreviations. BL, baseline; FC, fold-change; HR, hazard ratio; OR, odds ratio.

For all miRNAs, baseline expression was not significantly associated with remission status or change in depressive symptomatology after correction for multiple testing. However, baseline miR-135a-5p expression levels showed a trend association with remission status (OR = 1.8 [1.0, 3.3], p = .037, adjusted p = .167). Baseline miR-135a-5p levels were significantly associated with response trajectories ($F_{(176, 1241)}$ = 4.5, $p = 6.3 \times 10^{-5}$, FDR-corrected $p = 4.4 \times 10^{-4}$; see Table 4).

Specifically, individuals with low miR-135a-5p expression levels at baseline showed nominally higher mean MADRS scores at weeks 10 (M_{Low} = 15.2 ± 10.6, M_{High} = 12.1 ± 11.4; β = 3.6, p = .003), and 12 (M_{Low} = 15.2 ± 12.7, M_{High} = 13.7 ± 13.6; β = 2.4, p = .04). When dichotomizing individuals into low and high expressors using the median, miR-135a-5p showed only a nominal association with time-to remission (Mantel-Haenszel, χ^2 = 3.9, p = .05, FDR-corrected p = .34; see Table 4). Nonetheless, individuals with higher miR-135a-5p expression reached remission faster than those with lower expression (Cox regression, HR = 1.76 [1.15, 2.68], p = .009, FDR-corrected p = .063). (See Fig. 1).

Within our cohort, there was a substantial amount of comorbidity with anxiety-related disorders. Specifically, 44.1% of individuals (N = 105) were diagnosed with an anxiety-related disorder at baseline including generalized anxiety disorder (20.7%), specific phobias (12.8%), panic disorder (9.6%), social anxiety (9.6%), not-otherwise-specified anxiety disorders (6.4%), obsessive-compulsive disorder (3.7%), post-traumatic stress disorder (2.1%), and agoraphobia (2.1%). To further explore our observed association with miR-135a-5p, we looked at the interaction between the presence of anxiety symptoms and miR-135a-5p expression levels.

At baseline, high miR-135a-5p levels were nominally associated with higher chance of remission in individuals with anxiety (OR = 3.01 [1.29, 7.03]) as compared to those without anxiety (OR = 1.01 [0.62, 1.64], uncorrected p = .024). In those diagnosed with anxiety disorders, higher miR-135a-5p levels were associated with a greater change in MADRS scores at the end of treatment (β = −4.32 [−7.78, −0.84]) as compared to those without anxiety (β = 0.49 [−1.58, 2.57], uncorrected p = .017). However, there was no interaction between miR-135a-5p FC and anxiety symptoms. Longitudinally, the presence of anxiety appeared to moderate response trajectories ($F_{(177,1248)}$ = 1.86, uncorrected p = .073), but not time-to remission.

4. Discussion

In this sample of older depressed adults, prior to correction for multiple testing, we observed that individuals with higher miR-135a-5p expression levels at baseline showed significantly better response over time and reached remission approximately twice as quickly as individuals with low expression levels. Our findings are consistent with the literature suggesting that those with higher miR-135a-5p levels may respond better to antidepressant treatment. Previous studies have shown that not only do individuals with depression show lower central and peripheral miR-135a-5p levels compared to controls, but miR-135a-5p is also upregulated after antidepressant treatment (Issler et al., 2014). In addition, our findings suggest that there is an interaction between miR-135a-5p baseline levels and anxiety symptomatology in association with antidepressant treatment.

Showing a specific expression profile in serotonergic neurons, miR-135a-5p regulates transcription of the serotonin transporter (SERT) and 5-HT1A receptor (Issler et al., 2014), which are among the primary targets of antidepressants. Within acute and chronic murine stress models, stressed animals showed lower levels of miR-135a levels compared to controls (Mannironi et al., 2013; Zurawek et al., 2017). Similarly, in younger adults (n = 39 MDD, 36 controls), patients showed lower expression levels than controls and levels had a modest predictive capacity with a sensitivity of 94.4% and specificity of 41.0% (Gheysarzadeh et al., 2018). In a small cohort of 55 MDD patients (31 responders, 24 non-responders) treated with escitalopram or desvenlafaxine, there were no differences in miR-135a-5p levels (Fiori et al.,

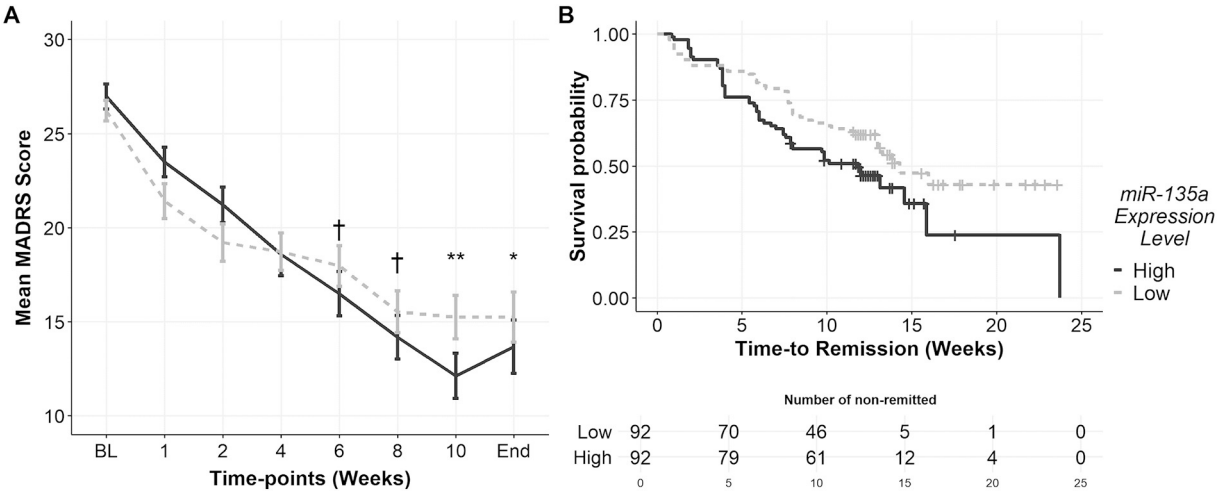

Fig. 1. (A) Response trajectories and (B) survival curves for low and high baseline miR-135a-5p expression levels.

2017). Within the second cohort of duloxetine-treated individuals (97 responders, 27 non-responders), compared to non-responders, responders showed lower miR-135a-5p levels were at baseline but higher levels after treatment (Fiori et al., 2017). While we did not observe any baseline differences between remitters and non-remitters at baseline, our findings show that in older adults, baseline miR-135a-5p levels are associated across various phenotype definitions (i.e., remission status, time-to remission, change in depressive symptoms, and response trajectories). However, changes in miR-135a levels across treatment were not associated remission.

An exploratory analysis revealed that there were baseline interaction effects between miR-135a-5p levels and the presence of anxiety-related diagnoses, suggesting that those with anxious symptomatology and high miR-135a-5p levels were more likely to be remitters. Although mechanistically this association is unclear, miR-135a-5p has been involved in stress response and may modulate the glutamatergic system which may be important for antidepressant effects (Mathews et al., 2012). Further studies are required to explore this association in larger samples with adequate power for subset analyses.

There are several limitations to this analysis, particularly our modest sample size. Although we have used a well-characterized sample of older adults, future studies should include larger samples to increase power to detect significant associations. In comparing our cohort to previous cohorts were associations of miR-135a have been observed, our sample had a lower MADRS inclusion rate (MADRS score ≥ 15 versus MADRS score ≥ 26) (Fiori et al., 2017). Therefore, the lack of a significant association may be due to lower depressive symptomatology in our sample. In addition, there are significant differences in pharmacology between venlafaxine in our cohort and previously investigated cohorts treated with escitalopram, desvenlafaxine, or duloxetine (Fiori et al., 2017; Lopez et al., 2017a). Specifically, venlafaxine is a dual serotonin-norepinephrine reuptake-inhibitor (SNRI) while escitalopram is a selective-serotonin reuptake inhibitor. Although desvenlafaxine is an SNRI like venlafaxine, the unique exclusively-serotonergic action of venlafaxine at lower doses may result in different pharmacological mechanism regulated by miRNAs. Nonetheless, the similarity of our association with the duloxetine-treated cohort (Fiori et al., 2017; Lopez et al., 2017a) may reflect the fact that both venlafaxine and duloxetine are SNRIs.

Another limitation of our investigation was the mixed ancestry which forced us to split the sample into ancestry groups, and use the largest ancestry sample (i.e., Europeans) for our analysis. In addition, to avoid potential spurious findings due to differences across individuals of heterogeneous genetic ancestries, we conducted our analysis in the subgroup of individuals of confirmed, European genetic ancestry. In addition, conducting analyses in a small cohort would have resulted in a loss of statistical power.

Another limitation of our investigation is the lack of additional cohorts of older adults for investigation, given that previous studies have suggested that the effects of miR-135a-5p may vary across cohorts due to effects not assessed in the study (Fiori et al., 2017). As such, further replication studies are required to assess the presence of associations between miRNAs and remission in older adults, which may be pathophysiologically unique compared to younger adults (Naismith et al., 2012). In addition, it is unclear if the observed effects of miR-135a-5p are the results of antidepressant treatment, or maybe associated with general improvement which may be explored with a comparative placebo arm.

Furthermore, older adults are often subject to polypharmacy (i.e., the use of five or more concomitant medications) and may have comorbid neurodegenerative, as well as cerebrovascular comorbidities which may affect antidepressant treatment response (Naismith et al., 2012). Although it is not clear how polypharmacy would impact miRNA expression levels, it may be difficult to disentangle the effects of uncontrolled co-medications within the *IRLGREY* clinical trial. There is a substantial list of medications that may affect miRNA expression levels in relation to antidepressant treatment (Almenar-Pérez et al., 2019). As such, we may extrapolate that similar effects may be occurring in individuals receiving antidepressant treatment for depression and utilizing similar co-medications.

In sum, our sample shows nominal evidence for the association of miR-135a-5p with venlafaxine remission in older adults. To the best of our knowledge, this is the first investigation to examine the associations of miRNA levels with antidepressant remission for LLD. However, we observed a lack of associations between miR-1202, miR-16-5p, miR-146a-5p, miR-146b-5p, miR-425-3p, and miR-24-3p with remission. Therefore, the miRNA signature of response and remission after antidepressant treatment in older adults may be different as compared to younger adults. Further investigations are required to explore the miRNA contributions to antidepressant response in late-life.

Author Statment Contributors

- Victoria S. Marshe
- Farhana Islam
- Malgorzata Maciukiewicz
- Laura M. Fiori
- Volodymyr Yerko
- Jennie Yang
- Gustavo Turecki
- Jane A. Foster

- Sidney H. Kennedy
- Daniel M. Blumberger
- Jordan F. Karp
- James L. Kennedy
- Benoit H. Mulsant
- Charles F. Reynolds III
- Eric J. Lenze
- Daniel J. Müller

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Declaration of Competing Interest

- Dr. Lenze has received funding from Takeda, Lundbeck, Janssen, Alkermes, Aptynx, and PCORI, and is a consultant for Janssen and Jazz Pharmaceuticals.
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