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miR-146a and miR-181a are involved in the progression of mild cognitive impairment to Alzheimer's disease

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ABSTRACT

The identification of mechanisms associated with Alzheimer's disease (AD) development in mild cognitive impairment (MCI) would be of great usefulness to clarify AD pathogenesis and to develop preventive and therapeutic strategies. In this study, blood levels of the candidate microRNAs (small noncoding RNAs that play a pivotal role in gene expression) miR-146a, miR-181a, miR-181b, miR-24-3p, miR-186a, miR-101, miR-339, miR-590, and miR-22 have been investigated for association to AD conversion within 2 years in a group of 45 patients with MCI. Baseline miR-146a ($p = 0.036$) and miR-181a ($p = 0.026$) showed a significant upregulation in patients with MCI who later converted to AD. These alterations were related to AD hallmarks: a significant negative correlation was found with amyloid beta cerebrospinal fluid concentration for miR-146a ($p = 0.006$) and miR-181a ($p = 0.001$). Moreover, higher levels of miR-146a were associated to apolipoprotein E ε4 allele presence, smaller volume of the hippocampus ($p = 0.045$) and of the CA1 ($p = 0.013$) and the subiculum ($p = 0.027$) subfields. Increased levels of miR-146a ($p = 0.031$) and miR-181a ($p = 0.002$) were also linked with diffusivity alterations in the cingulum. These data support a role for miR-146a and miR-181a in the mechanisms of AD progression.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative pathology characterized by memory loss, multiple cognitive abnormalities, and intellectual impairments that represents the most common cause of dementia (Apostolova, 2016).

Concerning the pathophysiology, AD is characterized by synaptic dysfunctions, neuronal loss, intracellular neurofibrillary tangles, extracellular neuritic plaques, and mitochondrial structural and functional abnormalities (Reddy et al., 2010). Defects in amyloid beta (Aβ) processing play a crucial role in AD because Aβ deposits are neurotoxic and initiate a series of events involving oxidative stress, inflammation, and dysregulation of lipid metabolism, leading to synaptic loss and cell death (Walsh and Selkoe, 2007). Moreover, the increased release of Tau protein, a microtubule-binding protein, is involved in AD neurodegeneration process. Indeed, its phosphorylated and truncated forms aggregate in neurofibrillary tangles inside nerve cell bodies, contributing to the breakage of microtubules and eventually collapsing the neuronal transport and leading to degeneration. Higher levels of total Tau

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(t-Tau) and phosphorylated Tau (p-Tau) have been reported in the cerebrospinal fluid (CSF) of patients with AD (Zetterberg, 2017). Furthermore, a genetic susceptibility factor for AD development is the presence of the ε4 allele in the apolipoprotein E (ApoE) gene, coding for a protein involved in brain lipid metabolism (Liu et al., 2013).

Mild cognitive impairment (MCI) is a clinical condition characterized by a greater cognitive decline than expected according to individuals' age and education level, but not remarkably affecting daily activities (Bora and Yener, 2017). Subjects clinically defined as MCI are heterogeneous and about 10%–15% per year develop AD (Petersen et al., 2009). The identification of the biological pathways underlying the differences between subjects who progress to AD (progressor MCI [pMCI]) and subjects which remain cognitively stable over time (stable MCI [sMCI]) would be of great usefulness in the clinical practice for the setup of pharmacological and non-pharmacological preventive treatment strategies.

Neuroimaging studies indicated that in MCI patients, the progression of neurodegeneration, the worsening of cognitive symptoms, and the functional impairment status are accompanied by cortical hypometabolism measured by fluorodeoxyglucose positron emission tomography and by medial temporal atrophy on structural magnetic resonance imaging (MRI), with the hippocampal area CA1 showing the greatest involvement (Frisoni et al., 2008; Kehoe et al., 2014; Marcus et al., 2014). Studies on CSF biomarkers for the prediction of MCI conversion showed a predictive potential for different combinations of Aβ isoforms and for an increased activity of beta-secretase 1 (BACE1), a rate-limiting protein cleaving enzyme in the production of Aβ (Zetterberg et al., 2008).

MicroRNAs (miRNAs) are small noncoding RNAs playing a major role in regulating gene expression at the post-transcriptional level (Khoury and Tran, 2015). They are involved in many cellular processes including cell proliferation, development, and aging (Kawahara, 2014; Noren Hooten et al., 2013). Among all the known miRNAs, almost 70% are found to be expressed in the brain (Nowak and Michlewski, 2013) and their exosomal and microvesicular forms can cross the blood-brain barrier and be released in the CSF and in blood circulation (Turchinovich et al., 2013).

Recent evidence suggested that miRNA alterations could contribute to AD pathology (Tan et al., 2013) and many miRNAs can regulate crucial disease genes such as the amyloid beta precursor protein and the BACE1. Altered patterns of miRNA expression have been observed in animal models and in postmortem brains, CSF, and blood of AD and MCI patients, whereas miRNA signatures of presymptomatic AD and MCI due to AD are still missing (Kumar and Reddy, 2016; Maffioletti et al., 2014; Nagaraj et al., 2019; Piscopo et al., 2019).

A differential expression of miR-22 and miR-101 has been observed in the hippocampus and cerebral cortex of the senescence accelerated mouse-prone 8 mouse, an animal model of AD (Cheng et al., 2013). Moreover, a miR-101 sponge was shown to modulate the processing of RAN-binding protein 9 and amyloid beta precursor protein in hippocampal neurons, a key mechanism in AD development (Barbato et al., 2014). A study on exosome-enriched plasma samples evidenced low levels of miR-24-3p in patients with AD compared with nondemented controls (Lugli et al., 2015). Higher levels of miR-146a, a miRNA involved in brain aging, have been found in the brain cortex of AD animal models (Li et al., 2011b), as well as in the superior temporal lobe neocortex (Sethi and Lukiw, 2009) and in the CSF of patients with AD (Alexandrov et al., 2012). Moreover, studies in AD model mice reported an upregulation of miR-181 in the hippocampus and this increase was shown to reduce the expression of c-Fos and sirtuin 1 (SIRT1), 2 genes involved in synaptic plasticity and memory processing (Rodriguez-Ortiz et al., 2014). Furthermore, the downregulation of miR-186a in murine cerebral cortex directly regulates the expression of BACE1 (Kim et al., 2015); the concentration of miR-339, another miRNA able to modulate BACE1 levels, was found reduced in postmortem brains of patients with AD (Long et al., 2014). Finally, a decreased expression of miR-590 was observed in AD postmortem brains and this was correlated with expression levels of the heterogeneous nuclear ribonucleoprotein A1, a protein playing many roles in neuronal functions (Villa et al., 2011).

On these bases, the aim of this study was to evaluate whether 9 candidate miRNAs (miR-146a, miR-181a, miR-181b, miR-24-3p, miR-186a, miR-101, miR-339, miR-590, and miR-22) measured in the blood of patients with MCI at the baseline, that is before possible conversion to AD, were differentially expressed in patients who later converted to AD compared with patients who did not convert. We also intended to investigate if the identified alterations were correlated with AD-related risk factors and neuropathological markers, such as the presence of the ApoE ε4 allele, the levels of CSF biomarkers, hippocampal volumes, and brain connectivity measures, with the objective to clarify the role of these miRNAs in AD pathogenetic mechanisms.

2. Material and methodology

2.1. Study participants

Forty five patients with MCI were selected from a larger cohort enrolled in the context of The European Innovative Medicines Initiative PharmaCog project (European Alzheimer's Disease Neuroimaging Initiative) (Galluzzi et al., 2016). The inclusion criteria were age between 55 and 90 years; complaints of memory loss by the patient or a relative; Mini–Mental State Examination (Folstein et al., 1975) score of 24 or higher; overall clinical dementia rating (Morris, 1993) score of 0.5; logical memory test (Axelrod and Woodard, 2000) score lower than 1 standard deviation from the age-adjusted mean; and 15-item Geriatric Depression Scale (Brown et al., 2015) score of 5 or lower. The exclusion criteria were significant other neurologic, systemic, or psychiatric illness; use of antidepressant drugs with anticholinergic side effects, high dose of neuroleptics or chronic sedatives or hypnotics, antiparkinsonian medication, and use of narcotic analgesics. The MCI status could be single or multi-domain.

The PharmaCog project has been designed as a typical clinical trial of antiamyloid drugs: MCI subjects were followed up for 2 years or until they converted to AD, according to the National Institute on Aging and the Alzheimer's Association criteria (McKhann et al., 2011). CSF was collected at the baseline for the evaluation of AD pathological biomarkers, whereas MRI was performed at the baseline and every 6 months to monitor the progression of the disease. The subgroups of patients with MCI enrolled for the present work included all those patients with MCI who converted to AD within 2 years (progressor, pMCI) and a group of patients with MCI who did not convert to AD (stable, sMCI). The pMCI and sMCI groups were matched for age and gender.

The study was approved by the Ethics Committee of the coordinating site and then by those of the respective countries. A written informed consent was obtained from all the participants. The demographic and clinical features of the patients at the baseline are reported in Table 1.

2.2. Discovery and confirmative stages

The study was performed in 2 stages; in the first one, the discovery stage, the expression of all the 9 candidate miRNAs was evaluated in a subcohort of 25 patients with MCI, including 14 pMCI and 11 sMCI. In the second one, the confirmative stage, the miRNAs

Table 1
Demographic and clinical features of the patients at the baseline

	pMCI	sMCI	*p*-value
Number of patients	19	26	
Age (y)	66.68 ± 5.8	66.23 ± 7.1	$p = 0.82$
Sex (females/males)	10/9	13/13	$p = 0.55$
Education	11.21 ± 4.9	10.11 ± 4.2	$p = 0.42$
ApoE ε4 carriers (%)	73.7	7.7	$p < 0.01$
Aβ (pg/mL)	562.84 ± 168.7	977.0 ± 233.6	$p < 0.01$
p-Tau (pg/mL)	96.15 ± 33.7	43.23 ± 14.3	$p < 0.01$
t-Tau (pg/mL)	686.23 ± 263.0	277.11 ± 150.7	$p < 0.01$
MMSE score, baseline	25.36 ± 1.3	27.42 ± 1.7	$p < 0.01$
MMSE score, 24 mo for sMCI/moment of conversion for pMCI	21.2 ± 4.4	27.0 ± 2.1	-

For continuous variables, the values are reported as mean ± standard deviation (SD).
Key: Aβ, amyloid beta; MMSE, Mini–Mental State Examination.

detected as differentially expressed between pMCI and sMCI in the discovery stage were evaluated in the whole group of 45 patients, which included the 25 patients used in the discovery stage. This whole group comprised 19 pMCI and 26 sMCI.

2.3. Blood collection and microRNA isolation from blood

Peripheral venous blood samples were obtained at the baseline by venipuncture using the PAXGene Tubes (Qiagen) in the morning, after an overnight fast. Total RNA was extracted from 2.5 mL of blood with the PAXGene Blood miRNA Kit (Qiagen), designed for the simultaneous isolation of small and large RNAs. RNA quantification and quality control were carried out using spectrophotometric analysis (Nanodrop 2000, Thermo Fisher Scientific). The purity of each sample was determined by evaluating the A260/280 and A260/230 ratios, with acceptable values ranging from 1.8 to 2.2.

2.4. Determination of the expression levels of the 9 candidate microRNAs by RT-qPCR

Reverse transcription was carried out using the TaqMan MicroRNA Assays (Thermo Fisher Scientific), preparing a primer pool to simultaneously get cDNA for miR-146a (probe ID: 000468), miR-181a (000480), miR-181b (001098), miR-24-3p (000402), miR-186a (002285), miR-101 (002253), miR-339 (002257), miR-590 (002677), miR-22 (002301), and 2 endogenous controls, RNU44 (001094) and RNU48 (001006). Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was conducted using the TaqMan MicroRNA Assays, following the manufacturer's instructions. The Ct values were normalized according to the deltaCt method on the geometric mean of RNU44 and RNU48. The stability values of these small nucleolar RNAs was calculated and evaluated through the software NormFinder (Andersen et al., 2004).

2.5. Measurement of CSF biomarkers and ApoE genotyping

The measurement of CSF biomarkers and the genotyping of ApoE were performed at the project central site as previously described (Galluzzi et al., 2016). Briefly, Aβ, p-Tau and t-Tau were quantified in the CSF using dedicated ELISA kits (Innogenetics), according to the manufacturer's instructions. Blood DNA was used for ApoE genotyping through RT-PCR using dedicated TaqMan probes (Thermo Fisher Scientific).

2.6. MRI processing

All the MRI scans were performed on 3.0 Tesla machines. MRI protocols were harmonized, and pipelines were optimized and described in detail elsewhere (Jovicich et al., 2014, 2013; Marizzoni et al., 2015). Averaged T1 structural images were processed using FreeSurfer v6.0 (Dale et al., 1999; Fischl et al., 2004, 2002; Iglesias et al., 2015) on the neuGRID platform (https://neugrid4you.eu/). Diffusion tensor imaging analysis was performed using FMRIB's Software Library to extract diffusion maps and an atlas (JHU-ICBM atlas)-based method to define the white matter regions of interest (Jovicich et al., 2014). The present study was focused on markers which are of interest in AD: volume of the hippocampus and its subfields and diffusivity indexes, that is, fractional anisotropy (FA), axial diffusivity, radial diffusivity, and mean diffusivity in the cingulum of the hippocampus. These diffusivity metrics are indicative of fiber damage if decreased (FA, axial diffusivity, and mean diffusivity) or increased (radial diffusivity). Where possible, left and right hemisphere measures were averaged for each subject.

2.7. Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences, Version 17.0 (SPSS Inc). Normality of data distribution was evaluated by using the Shapiro-Wilk and the Kolmogorov-Smirnov tests. Possible differences between pMCI and sMCI patients in demographic and clinical features were evaluated through *t*-test for continuous and χ^2 test for categorical variables. Because data indicating miRNA levels were not normally distributed, a nonparametric test (Mann-Whitney test) was used to compare them between the groups. The outliers were identified using the SPSS boxplot method and removed from the analysis. A logistic regression model was used to assess the predictive potential of miRNA levels and Aβ and Aβ/p-Tau for AD conversion; goodness of fit model index was expressed as Nagelkercke R^2.

For the analyses including the volume of the hippocampus and its subfields, variations in individual head size were corrected by normalization using total intracranial volume measurement. Pearson's correlation analyses were performed to assess correlations between miRNA levels and CSF biomarkers/neuroimaging data.

3. Results

In the discovery stage, we observed a significant upregulation of miR-146a ($p = 0.006$, fold change [FC] = 2.96) and miR-181a ($p = 0.044$, FC = 2.74) in pMCI compared with sMCI patients (Supplementary Figure S1). We also identified a trend of increase for miR-24-3p ($p = 0.058$, FC = 2.73).

In the confirmative stage, the levels of miR-146a, miR-181a, and miR-24-3p were analyzed in the whole sample including 45 patients. Outliers (1 for miR-146a, 4 for miR-181a, and 2 for miR-24-3p) were removed from the analysis. The final analyses for miR-146a, miR-181a, and miR-24-3p were conducted on 44 (19 pMCI and 25 sMCI), 41 (17 pMCI and 24 sMCI), and 43 (19 pMCI and 24 sMCI) subjects, respectively.

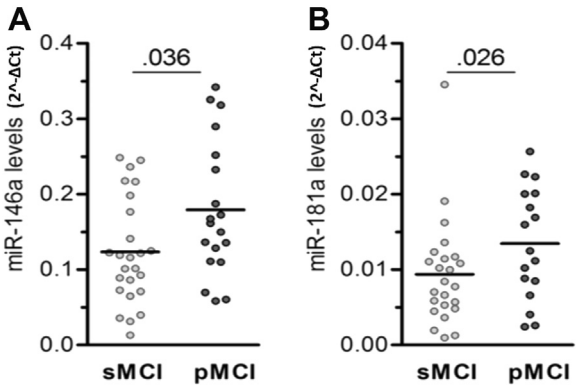

Fig. 1. Comparison of blood miRNA expression levels between sMCI and pMCI at the baseline in the confirmative cohort: significant upregulation of miR-146a (A) and miR-181a (B) in pMCI compared with sMCI subjects. Abbreviations: MCI, mild cognitive impairment; sMCI, stable MCI; pMCI, progressor MCI; miRNAs, microRNAs.

A significant upregulation of miR-146a ($p = 0.036$, FC = 1.45) and miR-181a ($p = 0.026$, FC = 1.43) was observed in pMCI compared with sMCI patients also in the confirmative stage (Fig. 1A and B). The increase of miR-24-3p in pMCI was only a trend also in the enlarged sample ($p = 0.056$, FC = 1.48).

To clarify if blood expression alterations of miR-146a and miR-181a could be associated with AD predictive markers, we analyzed correlations of their levels with CSF Aβ, p-Tau, and t-Tau contents in the same patients. We found a significant negative correlation between blood levels of miR-146a ($p = 0.006$, $r = -0.405$) and miR-181a ($p = 0.001$, $r = -0.494$) with Aβ concentration in CSF (Fig. 2A and B), whereas no association was evidenced for p-Tau and t-Tau.

We subsequently evaluated the potential value of miR-146a and miR-181a as predictive biomarkers of progression to AD by a logistic regression model. The results indicated that both miR-146a and miR-181a were predictive of conversion, with miR-181a showing a better performance in the model (miR-146a: $p = 0.034$, $R^2 = 0.15$ and miR-181a: $p = 0.017$, $R^2 = 0.21$). The CSF biomarkers Aβ and Aβ/p-Tau ratio showed higher predictive values ($p = 0.001$, $R^2 = 0.64$ and $p = 0.001$, $R^2 = 0.82$, respectively); in multiple models in which miR-181a measures were added to Aβ or Aβ/p-Tau ratio, no additive value was observed for the prediction of MCI conversion to AD.

Regarding the neuroimaging features in the 2 groups, as expected, pMCI reported volume reduction in the hippocampus as a whole and in several of its subfields (CA1, molecular layer, hippocampal tail, subiculum, dentate gyrus, CA4), as well as altered connectivity of the cingulum of the hippocampus compared with sMCI subjects (Supplementary Figure S2).

miR-146a and miR-181a blood levels were correlated with some neuroimaging features in patients with MCI. miR-146a blood levels showed a significant negative correlation with volumes in the subiculum, CA1, whole hippocampus and with FA in the cingulum of the hippocampus (Fig. 3), whereas miR-181a showed a significant negative correlation with FA in the cingulum of the hippocampus (Fig. 4).

Finally, the levels of miR-146a and miR-181a were compared between ApoE ε4 carriers and noncarriers. Only miR-146a showed a significantly upregulation ($p = 0.045$, FC = 1.50) in ApoE ε4 carriers (Fig. 5).

4. Discussion

The study findings indicate that blood miR-146a and miR-181a levels are higher in pMCI patients before conversion to AD than sMCI; moreover, they correlate with illness hallmarks such as increased CSF Aβ concentration, hippocampal atrophy and disconnections in critical white matter brain regions. The data suggest that these miRNAs could be involved in the pathogenic mechanisms underlying the conversion from MCI to AD, even if they do not show a potential clinical usefulness as predictive biomarkers, compared to the currently available ones.

miR-146a is one of the most studied miRNAs in AD, is a key regulator of the immune response (Taganov et al., 2006), and has been implicated in multiple neuroinflammatory processes related to different neurological diseases, including AD (Lukiw and Alexandrov, 2012). This miRNA was also described as involved in aging processes in AD animal models (Deng et al., 2017); studies in murine models of AD and *ex vivo* indicated that miR-146a expression increases with disease progression and correlates with senile plaque density and synaptic pathology (Li et al., 2011a,b). Modifications in the expression of miR-146a were reported in patients with AD compared with nonaffected controls, both in postmortem brains (Müller et al., 2014) and in peripheral tissues such as CSF, serum, and plasma (Dong et al., 2015; Kiko et al., 2014; Müller et al.,

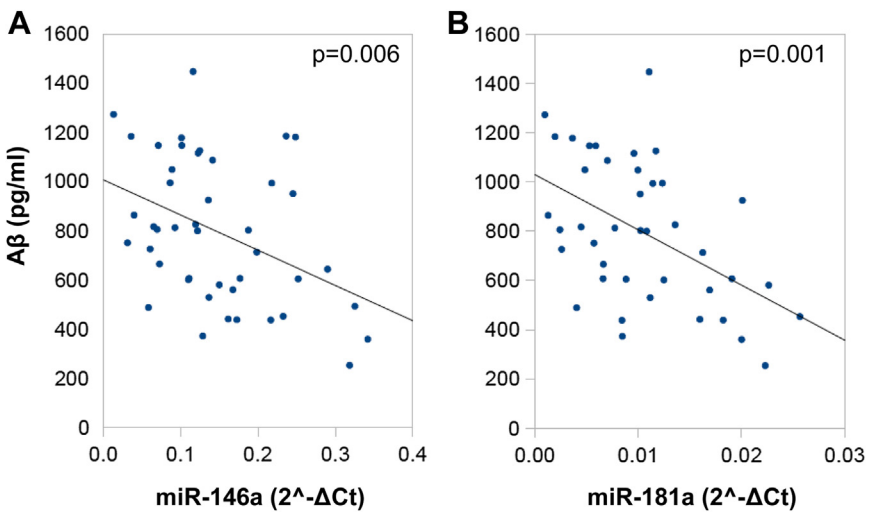

Fig. 2. Correlation between baseline miR-146a (A)/miR-181a (B) blood levels and Aβ concentration in the CSF of patients with MCI. Abbreviations: MCI, mild cognitive impairment; CSF, cerebrospinal fluid; Aβ, amyloid beta.

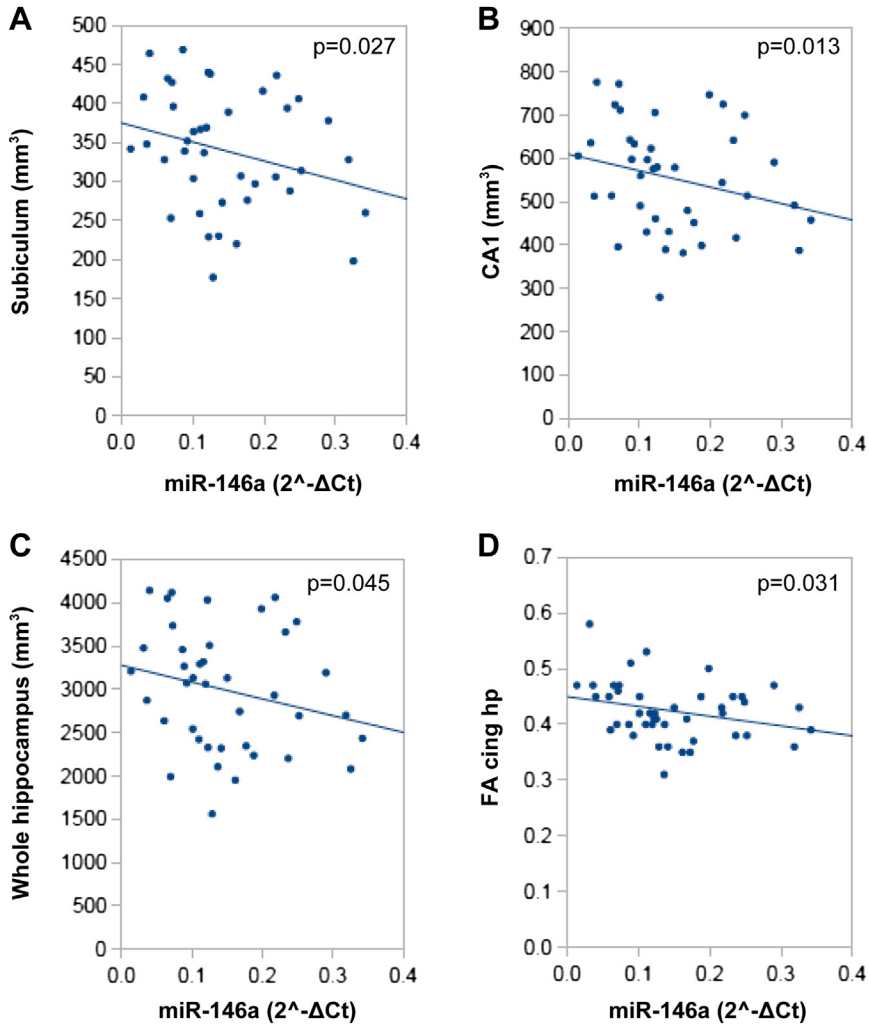

Fig. 3. Correlation between baseline blood miR-146a levels and hippocampus/subfields volumes/connectivity measures: (A) subiculum ($p = 0.027$); (B) CA1 ($p = 0.013$); (C) whole hippocampus ($p = 0.045$); (D) fractional anisotropy in the cingulum of the hippocampus (FA cing hp) ($p = 0.031$). Abbreviations: FA, fractional anisotropy.

2016). These data are in line with our results, which indicated that miR-146a blood expression levels are higher in patients with MCI who later converted to AD, suggesting that the upregulation of miR-146a could represent a molecular mechanism contributing to the onset of AD.

Importantly, one of the validated target genes of miR-146a is the Toll-like receptor 2 (TLR2) (Jurkin et al., 2010). It has been shown that TLR2 can recognize deposited Aβ and plays roles in its clearance, delaying the cognitive decline in an animal model of AD (Ravari et al., 2017). Increased miR-146a levels could downregulate the expression of TLR2, leading to a reduced clearance of deposited Aβ. Finally, miR-146a might stimulate inflammation in AD brains by downregulating other genes such as the complement factor H, interleukin-1 receptor, and tetraspanin-12 (Lukiw et al., 2008; Wang et al., 2012). Moreover, we observed an increase in miR-146a blood levels in ApoE ε4 carriers. At this regard, studies in transgenic mice indicated that ApoE expression is able to suppress neuroinflammation by enhancing miR-146a levels in monocytes and macrophages (Li et al., 2015) and ApoE ε4 allele is associated to decreased miR-146a levels both in the brain and plasma in transgenic mice (Teter et al., 2016). These data are partially conflicting with our findings and further studies in humans are needed to clarify the relation between ApoE and miR-146a.

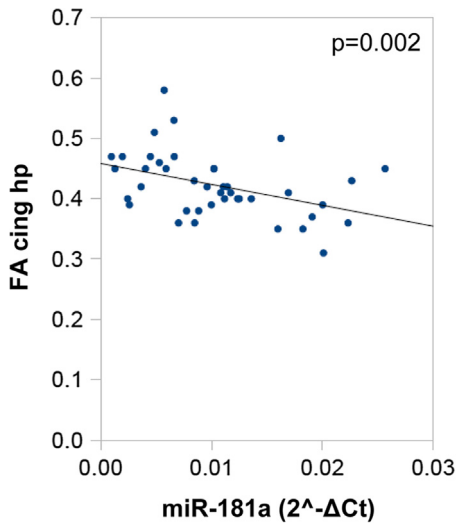

Fig. 4. Correlation between baseline blood miR-181a levels and fractional anisotropy in the cingulum of the hippocampus (FA cing hp) ($p = 0.002$). Abbreviations: FA, fractional anisotropy; miRNAs, microRNAs.

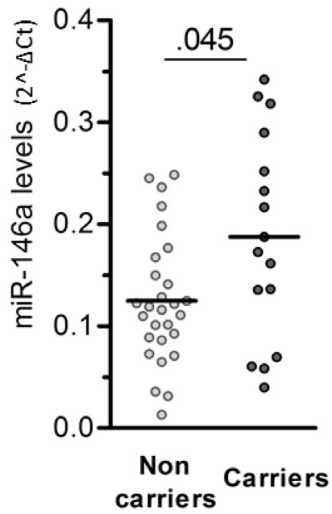

Fig. 5. Significant differential expression of baseline blood miR-146a between ApoE-ε4 carriers versus noncarrier patients with MCI. Abbreviations: MCI, mild cognitive impairment; ApoE, apolipoprotein E; CA, cornu ammonis; FA, fractional anisotropy; GC-ML-DG, granule cells in the molecular layer of the dentate gyrus; hp, hippocampus; RD, radial diffusivity (10^{-3} mm^2/s).

In addition, miR-181a could regulate the expression of AD-associated genes as Fidgetin, B-cell lymphoma 2, and SIRT1 that are validated targets of this miRNA (Femminella et al., 2015; Miya Shaik et al., 2018). Fidgetin is a microtubule-severing protein that cuts or breaks microtubules playing an important role in axonal and dendritic growth, a process which is compromised in AD (Leo et al., 2015). B-cell lymphoma 2 proteins have been reported to be involved in learning and memory processes, which are severely affected in amnestic MCI and AD (Sultana et al., 2010), whereas SIRT1 showed a role in Aβ pathology, Tau concentration and cognitive performance in animal models of AD (Julien et al., 2009). SIRT1 has recently been shown to suppress g-secretase activity in different in vitro models, thereby reducing the production of Aβ (Qin et al., 2006) and its high expression has been suggested to protect aged individuals from dementia (Hadar et al., 2018). miR-181a is also involved in hippocampal contextual fear memory consolidation in adult mice, by indirectly regulating mTOR pathway; indeed, miR-181a can target the mTOR upstream inhibitors PRKAA1 and REDD, determining an increased mTOR activity and facilitating the consolidation of fear memory (Xu et al., 2018).

A common validated target of both miR-146a and miR-181a is RyanR3, which mediates the release of calcium from the endoplasmic reticulum. A dysregulation of this process has been described as linked to synaptic loss and impaired cognitive function in AD. The deletion of RyanR3 in an animal model of AD was reported to accelerate the pathology course in young mice and decelerate it in older mice (Liu et al., 2014).

However, we observed an upregulation of miR-146a and miR-181a in the blood of pMCI patients and it is currently unclear if peripheral miRNA alterations directly reflect brain modifications. In this regard, it should be considered that miRNAs may cross the blood-brain barrier, both in free and microvesicular form (Creemers et al., 2012; Skog et al., 2008). Compared with cell-free matrices as serum and plasma, whole blood contains both extracellular and intracellular miRNAs from total hematocytes; thus, the observed peripheral miRNA alterations may be a direct consequence of their dysregulation in the brain or be part of a systemic pathological mechanism, possibly determined also to the presence of genetic risk factors as suggested by the differences observed according to the ApoE genotype. The lack of data concerning the expression of miRNAs in cell-free fractions should be considered as a study limitation.

Moreover, it has to be noted that for both miRNAs, a fine expression modulation has been involved in hippocampal plasticity mechanisms (van Spronsen et al., 2013). In this regard, for the first time, we report in patients with MCI a negative correlation between miR-146a levels and volumes of hippocampal CA1, subiculum and presubiculum, among the brain regions affected by tangle pathology (Braak and Braak, 1991) and found as altered in AD (Carlesimo et al., 2015; Frisoni et al., 2008). Moreover, the study data indicate an association of both miRNA alterations with reduced connectivity in the cingulum of the hippocampus, a white matter tract connecting the hippocampus to other brain areas and involved in memory processes (Ezzati et al., 2016).

In conclusion, the study results indicate an increase in blood levels of miR-146a and miR-181a in patients with MCI with a progressive cognitive decline, as well as a correlation of these alterations with AD risk factors and markers of the pathology. All these data together support an involvement of these miRNAs in the neuropathological mechanisms that characterize the MCI/AD conversion. Further studies, investigating changes in miRNA levels during the MCI/AD progression will be useful to clarify the involvement of these small noncoding RNAs in the dementia pathogenesis and their potential usefulness as disease markers.

Disclosure

The authors report no conflicts of interest.

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The list of participants to the PharmaCog Consortium WP5 working group is reported in the supplementary materials S2.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.06.005.

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