Mechanisms of Ageing and Development 225 (2025) 112063

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

miR-132–3p is down-regulated in plasma and CD171^{+} extracellular vesicles isolated from patients with mild Alzheimer's disease

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ARTICLE INFO

Keywords:
Alzheimer's disease
MicroRNA
Extracellular vesicles
Biomarkers
Neurodegeneration

ABSTRACT

Alzheimer's disease (AD), the most prevalent neurodegenerative disorder in aging populations, demands minimally invasive biomarkers for early diagnosis and monitoring. Circulating microRNAs (miRNAs) show promise as such biomarkers. In this study, we examined the levels of five selected miRNAs, implicated in neurodegenerative processes, in plasma and neuron-derived extracellular vesicles (EVs) from cognitively healthy controls (n = 5), and patients with mild (n = 10) and moderate AD (n = 10), stratified by Mini-Mental State Examination (MMSE). miR-23a-3p, miR-223a-3p, and miR-132–3p were significantly downregulated in both plasma and EVs of AD patients, with miR-132–3p emerging as the strongest biomarker candidate for mild AD. Plasma miRNA levels strongly correlated with EV cargo, supporting plasma-based assessments. To validate these findings, miR-132–3p levels were analyzed in expanded cohorts, including cognitively healthy subjects (n = 36), mild AD (n = 37), and moderate AD (n = 40), as well as a cohort of subjects with mild cognitive impairment (MCI, n = 31) and an additional external cohort of cognitively healthy subjects (CTR external, n = 37). Results confirmed miR-132–3p downregulation in AD patients and revealed a significant elevation in MCI individuals, suggesting a potential neuroprotective role in AD early stages. These findings highlight miR-132–3p as a promising, minimally invasive biomarker for early AD diagnosis and disease progression monitoring.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia among the elderly, with cases expected to quadruple in the next 25 years, making it a public health priority (Monteiro et al., 2023). As a chronic neurodegenerative disorder that gradually worsens, it impairs daily activities and leads to disability within years of diagnosis (Prince et al., 2016). Despite its significant social and health impact, diagnostic tools and effective therapies for AD are limited.

AD progression involves widespread neurodegeneration driven by

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https://doi.org/10.1016/j.mad.2025.112063
Received 19 December 2024; Received in revised form 28 March 2025; Accepted 16 April 2025
Available online 17 April 2025
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abnormal accumulation of extracellular amyloid plaques and intraneuronal neurofibrillary tangles, largely composed of amyloid-β (Aβ) peptides and tau proteins (Cai et al., 2022; El Fatimy et al., 2018; Femminella et al., 2015). These structures cause synaptic damage and neuronal dysfunction, resulting in memory loss and cognitive decline (Bloom, 2014). In advanced AD stages, brain structure changes reduce treatment efficacy, limiting options primarily to symptom management. Current diagnostics, based on cerebrospinal fluid (CSF) biomarkers and neuroimaging, are costly and invasive, restricting their use to specialized centers and making large-scale screening challenging (Jack et al., 2018). Consequently, identifying non-invasive blood-based biomarkers could enable easier, large-scale screening and early identification of at-risk individuals (Hampel et al., 2018).

The absence of definitive therapies for Alzheimer's disease (AD) highlights the need for early diagnosis. Mild cognitive impairment (MCI), which often precedes AD, represents a critical period for intervention before dementia develops. Recognized as an intermediate stage between normal aging and dementia, MCI is a heterogeneous condition, making accurate and timely diagnosis challenging. The American Academy of Neurology emphasizes the importance of identifying and monitoring MCI patients as a distinct clinical population, as early intervention may help slow AD progression. Assessing individuals for potential dementia, whether in community, primary, or secondary care, typically involves brief cognitive tests, informant questionnaires, or both (Arevalo-Rodriguez et al., 2015; Moyer and Force, 2014). Among these, the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) is the most widely used and recognized tool to screen for cognitive impairment in various settings (Arevalo-Rodriguez et al., 2015).

Circulating microRNAs (miRNAs) offer potential as prognostic indicators for monitoring AD progression. Dysregulated miRNAs contribute to pathological pathways linked to various diseases (Silvestro et al., 2019; Varesi et al., 2022). Approximately 70 % of known miRNAs are brain-expressed, where altered functioning affects neurogenesis, dendritic growth, and synaptic plasticity, likely contributing to pro-inflammatory pathways in AD (Eacker et al., 2009). We selected miRNAs previously identified as associated with AD, such as miR-23a-3p, miR-223a-3p, miR-100–5p, (Jiang et al., 2022; Mancuso et al., 2019; Serpente et al., 2020; Wei et al., 2018) miR-132–3p and miR-212, which are brain-expressed and extensively studied in AD pathology (Cha et al., 2019; Walgrave et al., 2021). An altered pattern of these miRNAs could be detected not only in brain tissue but also in blood. Circulating miRNA-based diagnostic tools could help overcome current limitations, allowing AD diagnosis with plasma or serum samples (Lee et al., 2021). However, their short half-life in biofluids and potential RNA contaminants challenge standardization. Alternatively, circulating exosomal miRNAs—due to their stability and role in cellular communication— offer a promising diagnostic tool for earlier and more accessible AD diagnosis. Exosomes, secreted by neurons and astrocytes, contain functional molecules that reflect the donor cell's condition and may serve as reliable, non-invasive AD markers (Manna et al., 2020; Su et al., 2022).

In this study, we selected five miRNAs — miR-23a-3p, miR-223a-3p, miR-100–5p, miR-132–3p, and miR-212 — based on an in-depth analysis of the most recent literature on this field, and analyzed their levels in neuron-derived EVs and plasma samples from cognitively healthy subjects and patients affected by AD. Finally, miRNAs with some potentiality as AD biomarkers were analyzed in a larger number of plasma samples, including cognitively healthy subjects from two different cohorts, subjects with mild cognitive impairment (MCI) who had not been diagnosed with AD, and patients with AD categorized into two groups based on their Mini-Mental State Examination (MMSE) scores: mild AD ($20 \leq$ MMSE ≤ 24) and moderate AD ($10 \leq$ MMSE ≤ 19). While the analysis of miRNAs contained in neuronal vesicles may provide more insight into disease mechanisms, the subsequent step of our study — validation in plasma samples — is justified for identifying new, minimally invasive biomarkers for the early diagnosis of neurodegenerative diseases.

2. Materials and methods

2.1. Study patients

Twenty patients with sporadic Alzheimer's disease (AD) and age- and gender-matched control subjects (CTR) were enrolled within the framework of the Italian National Cronos Project (CP), involving the Unit of Evaluation of AD (UVA) of IRCCS INRCA, Ancona (Santoro et al., 2010). AD was clinically diagnosed according to the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-R) and the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) guidelines (McKhann et al., 1984). Patients who had diabetes, severe cardiovascular conditions, chronic inflammatory disorders, or cancer were excluded. For validation of miR-132–3p, an external cohort of healthy, non-demented subjects (external CTR), and a cohort of subjects with MCI were included. Non-demented healthy subjects were selected from a larger group of ambulatory individuals visiting the IRCCS INRCA facilities for routine blood tests. Their health status was confirmed through questionnaires, laboratory tests, and physical examinations (Olivieri et al., 2009). Subjects with MCI were selected from the STRENGTH project, a prospective randomized controlled trial for the assessment of the effects of a multimodal intervention in subjects with MCI, recruited on the basis of the following inclusion criteria: (a) diagnosis of MCI according to the guidelines of the National Institute of Aging; (b) age 60 years or older, (c) availability during the intervention and testing phases, (d) presence of a caregiver; (e) capability to sign the informed consent (Giuli et al., 2020; Santillo et al., 2024). All the procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (IRCCS INRCA, Bioethics Advisory Committee, Ancona, Italy, approval code 18006) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The final protocol has been approved by the local ethics committee.

The cohorts of individuals considered for this study, and subsequent analyses are illustrated in Fig. 1.

2.2. RNA isolation and quantification by RT-qPCR

Total RNA was extracted from 100 μL of plasma-EDTA using the Total RNA Purification kit from Norgen Biotek Corporation (37500) according to the manufacturer's guidelines. microRNA expression was analyzed using TaqMan microassay through Real time PCR as previously described (Giuliani et al., 2021). Synthetic *cel*-miR-39–3p was spiked-in during RNA isolation for normalization in subsequent RT-qPCR.

2.3. CD171-positive extracellular vesicle isolation

CD171 (also known as L1 Cell Adhesion Molecule, L1CAM)-positive EVs were isolated from 0.75 ml of frozen human plasma containing EDTA. Samples were defibrinated with thrombin (System Biosciences, Inc., Mountainview, CA) for 30 minutes at room temperature. Defibrinated samples were combined with ExoQuick exosome solution (System Biosciences, Inc., Mountain View, CA) and incubated for 60 minutes to precipitate total extracellular vesicles. For exosome L1CAM enrichment, suspensions were incubated overnight with 4 μg of mouse anti-human CD171 biotinylated antibody (CD171, clone 5G3, eBioscience, San Diego, CA). Subsequently, streptavidin-agarose Ultralink resin (Thermo Scientific, Rockford, IL, USA) was added to 3 % BSA and incubated for 30 minutes at 4°C. After centrifugation and removal of the supernatant, the pellet was resuspended in IgG elution solution (Pierce ™ IgG Elution Buffer, Thermo Fisher Scientific), centrifuged to detach $L1CAM^{+}$ EVs from the bead-antibody complex and neutralized with Tris-HCl at pH 8. Final suspensions containing neuronal exosomes

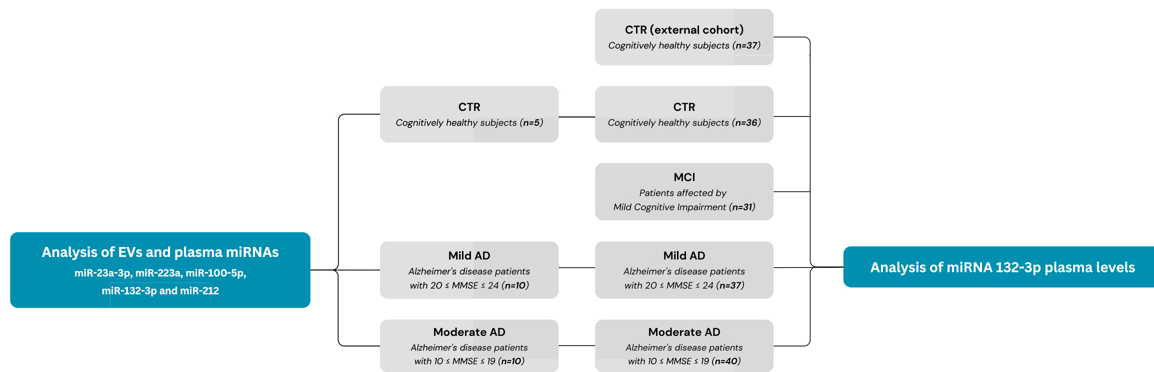

Fig. 1. Summary of study groups.

were stored at −80 °C.

2.4. Extracellular vesicle isolation and quantification

EV concentration and size were determined by NanoSight tracking analysis (NTA). The NTA measurements were carried out using NanoSight LM10 (NanoSight, Amesbury, UK). Three 30- or 60-second videos were recorded at room temperature, not exceeding 25°C, for each sample. NTA 3.1 software (NanoSight) was used for data acquisition and analysis. Results are presented as mean ± SD of three videos. Representative NTA plot is shown in Supplementary Figure 1A. Control beads of 100 and 400 nm, available from Malvern Instruments Ltd. (Malvern, UK) were used.

2.5. Cytofluorimetric analysis for CD171$^+$ EVs characterization

The EVs were resuspended in PBS + 0.1 % BSA and then labeled with ExoBrite, anti-CD9 FITC (BD Pharmingen, clone M-L13), anti-CD63 PerCP (BD Pharmingen, clone H5C6), anti-CD56 PE (eBioscience, CMSSB), and anti-L1CAM APC (eBioscience, clone 5G3). The cytometric analyses were performed by gating events smaller than 1 mm. Size beads (Ø 1–2 µm Polysciences Invitrogen, and Ø 5.2 µm DakoCyto-Count beads) were used to establish the proper gate for the analyses.

2.6. Western blotting analysis

10 µg of EV protein samples were mixed with Laemmli sample buffer 6x (1:6 ratio) and loaded onto each lane of 12 % SDS-PAGE gels. The proteins were then blotted onto a polyvinylidene difluoride (PVDF) membrane (Thermo). Primary antibodies were anti-CD63 (Thermo Fisher, clone TS63) and anti-CD81 (Cell Signaling Technology, clone D3N2D). The primary antibodies were incubated overnight at 4°C. After washing, specific HRP-conjugated secondary antibodies (Pierce) were applied. Immune complexes were visualized using Clarity WesternECL Substrate Luminol solution (Bio-Rad). Chemiluminescence was measured using a Bio-Rad ChemiDoc MP imaging system (Bio-Rad).

2.7. Statistical analysis

Continuous data distribution was analyzed using the Shapiro-Wilk normality test and presented as median and interquartile range (IQR). Non-parametric Mann–Whitney U-test was used for pair-wise comparisons between healthy controls and AD patients. Comparison among three groups was performed with the Kruskal–Wallis test. The diagnostic value of miR-132–3p was investigated by calculating the area (AUC) under the receiver operating characteristic (ROC) curve. Data analysis was carried out with the IBM SPSS Statistics 26.0 for Windows software (SPSS Incorporation, Chicago, IL, USA). Statistical significance was defined as a two-tailed p-value < 0.05.

3. Results

3.1. Isolation and characterization of L1CAM positive extracellular vesicles from CTR and AD plasma

We isolated EVs expressing L1CAM (also known as CD171) — a transmembrane neuronal adhesion protein, particularly abundant in cells of the nervous system — from the plasma of cognitively healthy subjects (CTR, n = 5) and subjects diagnosed with Alzheimer's disease (AD, n = 20). Demographic and clinical parameters of subjects are reported in Supplementary Table 1. After EV isolation using magnetic beads targeting surface expression of CD171, flow cytometric analysis of the tetraspanins CD9 and CD63, along with western blot analysis of CD81 and CD63, was performed to verify the quality of EV isolation (Supplementary Figure 1B and C). The presence of CD81 and CD63, both recognized exosome biomarkers, confirmed successful enrichment of exosomes. Flow cytometry analysis of CD171 and CD56 — proteins highly expressed in central nervous system cells — on the surface of EVs confirmed that we have successfully isolated EVs enriched with neuronal markers (Supplementary Figure 1D).

Among the five selected microRNAs, four exhibited lower expression in neuronal-origin vesicles from individuals with Alzheimer's compared to cognitively healthy subjects. miR-212 did not show a significant difference between the two groups (Supplementary Figure 2A). Then, we grouped AD subjects with mild (20 ≤ MMSE ≤ 24, n = 10) or moderate (10 ≤ MMSE ≤ 19, n = 10) AD according to Mini-Mental State Examination (MMSE). None of the analyzed microRNAs showed statistically significant differences between mild and moderate AD groups; however, miR-132–3p expression is significantly lower in mild AD compared to cognitively healthy controls (CTR) (Fig. 2A). Moreover, miR-23a-3p and miR-223a were significantly down-regulated in moderate AD vs CTR.

To explore whether AD is associated with the release of neuronal EVs, we evaluated their number in CTR and AD patients. Our analysis revealed that AD patients release a lower amount of CD171$^+$ EVs compared to CTR (Fig. 2B). By grouping subjects with Alzheimer's according to the MMSE, we found that patients with either mild or moderate AD had a significantly lower number of CD171$^+$ EVs compared to CTR (Fig. 2C).

Then, to gain insight into the relative enrichment of miRNA in neuronal EVs, we normalized their expression with the number of CD171$^+$ EVs isolated from plasma. After normalizing for vesicle number, we confirmed that circulating levels of miR-23a-3p and miR-223a-3p were reduced in subjects with moderate AD. This finding suggests that the decrease may be partly due to reduced release of microRNAs via EVs from CD171$^+$ cells, including neurons. Interestingly, miR-132–3p retains the ability to discriminate between mild AD subjects and controls (Fig. 2D). In Supplementary Figure 2B we reported miRNA expression between CTR and AD (not stratified for MMSE).

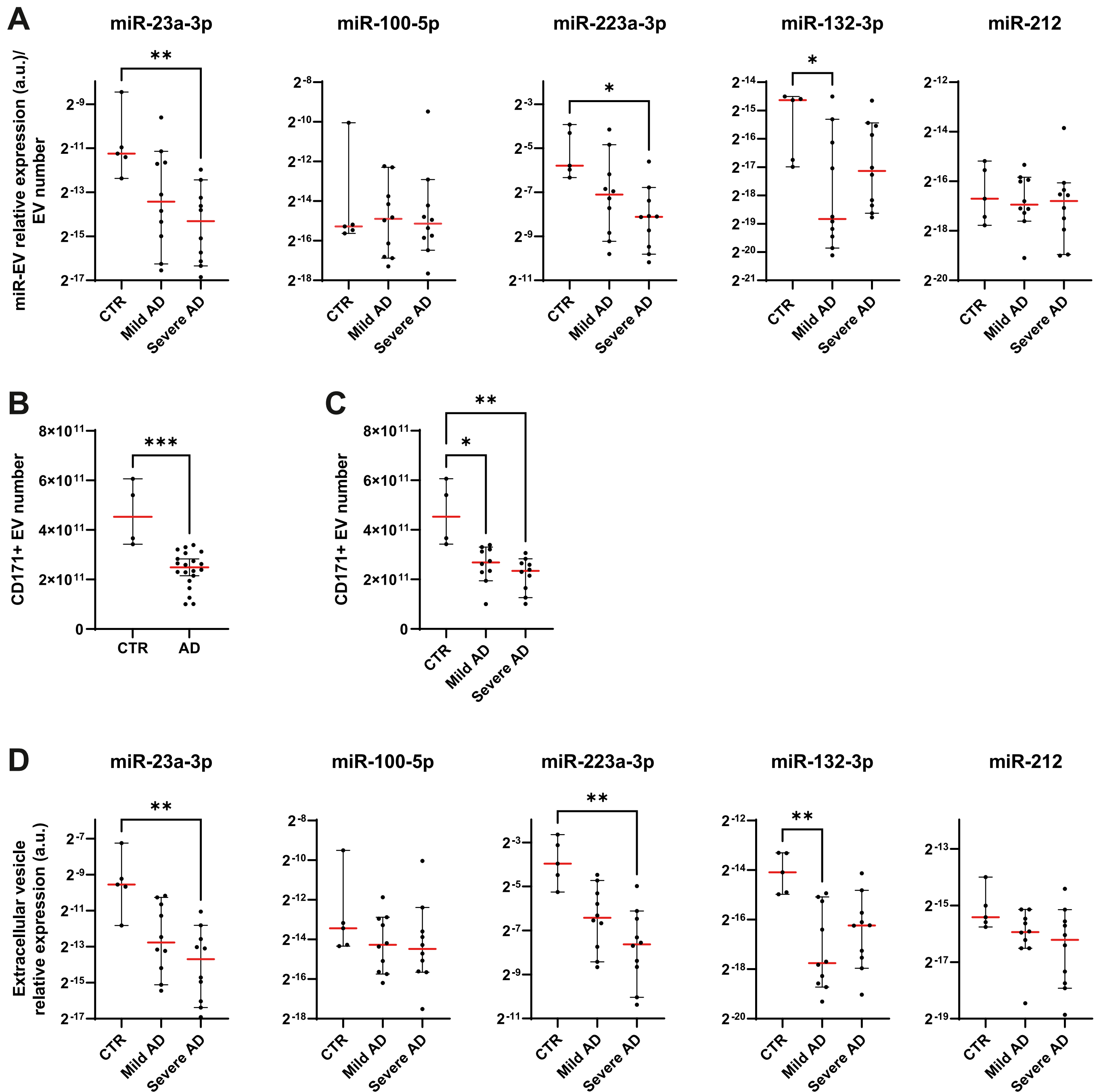

Fig. 2. miRNA expression levels in neuron-derived EVs isolated from peripheral plasma from subjects with AD (mild, n = 20; moderate, n = 20) compared with cognitive healthy controls (CTR, n = 5). **(A)** Relative expression of microRNAs evaluated through Real Time PCR. Data were normalized using *cel*-miR-39. **(B and C)** EV number evaluated with Nanoparticle Tracking analysis (NTA). **(D)** Relative expression of microRNAs evaluated through RT-qPCR. Data were normalized to g *cel*-miR-39 and the number of CD171$^+$ EVs. Data are median and 95 % CI. P-values for Dunn's post-hoc tests. * , $p < 0.05$; * *, $p < 0.01$; * ** , $p < 0.001$.

3.2. Circulating microRNAs in cognitively healthy controls and patients with Alzheimer's disease

To assess whether miRNA levels in whole plasma could replicate findings from EVs, we measured their expression in plasma samples from the same subjects used for EV isolation. Notably, we confirmed the down-regulation of the three miRNAs—miR-23a-3p, miR-223a-3p, and miR-132–3p—that showed reduced expression in CD171$^+$ EVs from subjects with AD. Furthermore, the levels of these miRNAs in plasma samples and in CD171$^+$ EVs demonstrated a strong reciprocal correlation, as shown by Spearman's rank test (miR-23a-3p, Spearman's rho=0.779, $p < 0.0001$; miR-223a-3p, Spearman's rho=0.731, $p < 0.0001$; miR-132–3p, Spearman's rho=0.499, $p = 0.0111$). Similarly, circulating miR-100–5p and miR-212 levels in plasma were unchanged (Supplementary Figure 2C). When AD subjects were grouped by MMSE score, three of the five miRNAs (miR-23a-3p, miR-132–3p, and miR-223a-3p) were less expressed in mild AD group compared to controls (Fig. 3). Additionally, miR-23a-3p and miR-223a-3p were significantly down-regulated in moderate AD compared to controls.

3.3. miR-132–3p levels as a potential biomarker of MCI and AD

Since miR-132–3p appeared to be the most promising miRNA as a biomarker of early disease and the same result was obtained by

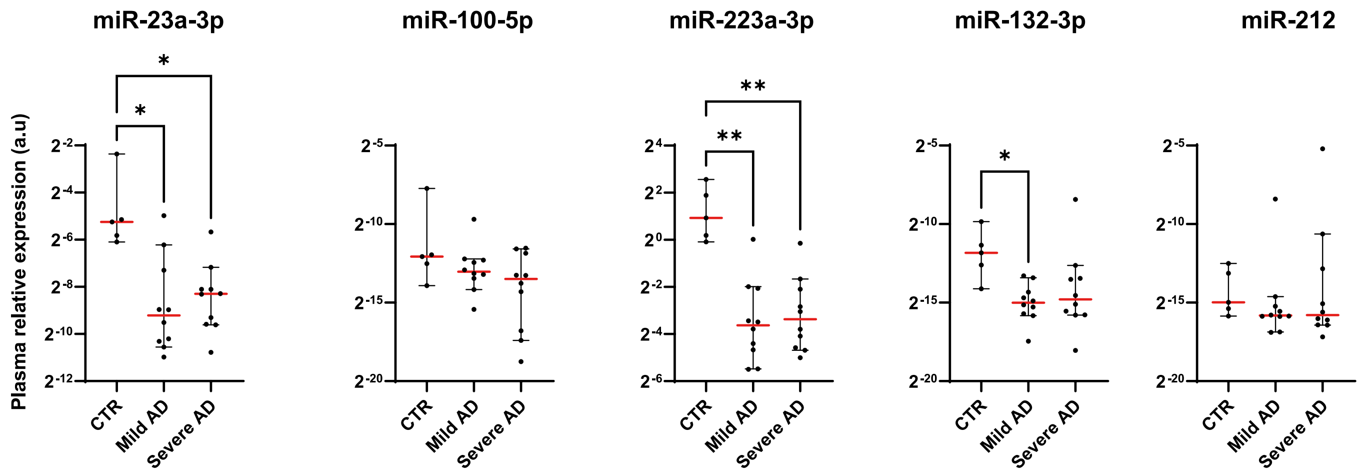

Fig. 3. miRNA expression levels in plasma of patients with AD (mild, n = 20; moderate, n = 20) and cognitively healthy controls (CTR, n = 5). MicroRNA expression was evaluated through RT-qPCR. Data were normalized using *cel*-miR-39. Data are median and 95 % CI. P-values for Dunn's post-hoc tests. * , p < 0.05; * *, p < 0.01; * ** , p < 0.001.

analyzing both plasma and EVs, we analyzed miR-132–3p levels in plasma samples from a larger number of subjects. These included cognitively healthy individuals from the original cohort (CTR, internal validation, n = 36), an additional cohort of older cognitively healthy subjects (CTR external validation, n = 37), a smaller cohort of subjects diagnosed with mild cognitive impairment (MCI, n = 31), a larger cohort of individuals with mild (n = 37) and moderate AD (n = 40) (Supplementary table 1).

To avoid batch effects in miRNA levels from different cohorts, samples were shuffled before analysis. Interestingly, miR-132–3p showed increased circulating levels in subjects with MCI, while was significantly down-regulated in mild AD compared to both cohorts of control subjects (Fig. 4A). We also observed a reduction of miR-132–3p in subjects with moderate AD compared to CTR subjects. The ROC curve illustrates the diagnostic performance of miR-132–3p in plasma for detecting mild AD. The test demonstrates that miR-132–3p can effectively differentiate between MCI and cognitively healthy CTR, with an AUC of 0.95 (p < 0.001), and between mild AD and controls, with an AUC of 0.89 (p < 0.001) (Fig. 4B).

Finally, given previous evidence from animal models indicating that miR-132 expression is modulated by sex and estrogen levels—with higher circulating miR-132 observed in females, potentially due to estrogen signaling (Hirsch et al., 2018)– we conducted an additional subanalysis stratifying our cohort by sex to investigate whether similar sex-related differences influenced the observed changes in miR-132–3p levels across cognitively healthy, MCI, and AD subjects. However, our subanalysis revealed no significant sex-related differences in circulating miR-132–3p levels, neither when considering all subjects together (non-parametric p-value = 0.709) nor when analyzing each cohort separately (non-parametric p-values; CTR internal validation, p = 0.159, CTR external validation, p = 0.745; MCI, p = 0.364; mild AD, p = 0.543; moderate AD, p = 0.111).

4. Discussion

Alzheimer's disease (AD), a progressive neurodegenerative disorder, highlights the need for non-invasive biomarkers to enhance diagnosis and monitoring. Circulating microRNAs, detectable in peripheral blood, have shown potential as indicators of disease progression, especially in at-risk populations. We analyzed the levels of five selected miRNAs in

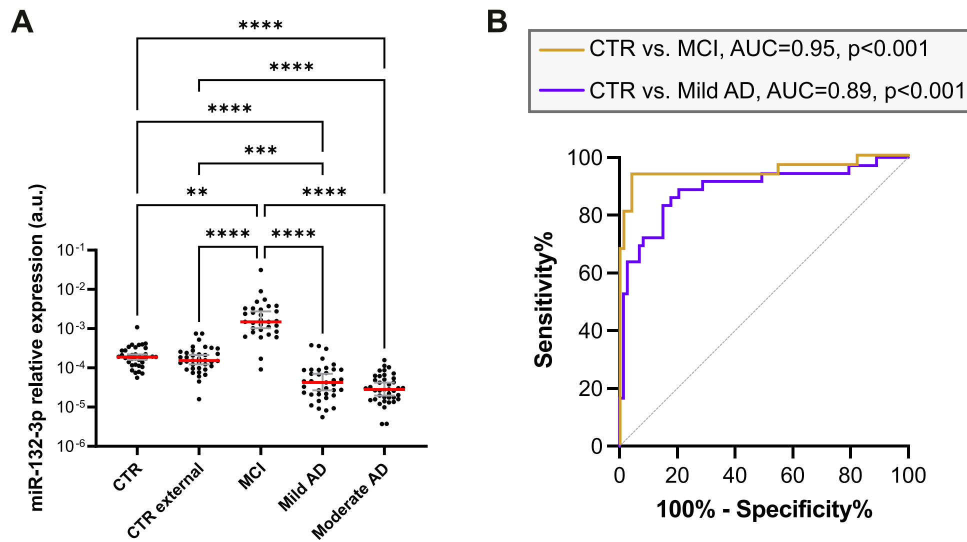

Fig. 4. **(A)** miR-132–3p expression levels in plasma of patients with AD (mild, n = 37; moderate, n = 40), MCI (n = 31), and cognitively healthy controls (CTR, n = 36; CTR external cohort, n = 37). Data were normalized using cel-miR-39. Data are median and 95 % CI. P-values for Dunn's post-hoc tests. **(B)** ROC curves for plasma miR-132–3p. * *, p < 0.01; * ** , p < 0.001; * ** *, p < 0.0001.

neuronally derived EVs and plasma samples from cognitively healthy controls and AD patients. Key findings include: i) CD171^{+} EVs were significantly reduced in AD patients; ii) miR-23a-3p, miR-223a-3p, and miR-132–3p levels were down-regulated in both plasma and CD171^{+} EVs from AD patients; and iii) miR-132–3p was reduced in individuals with mild AD ($20 \leq$ MMSE ≤ 24) compared to healthy controls in both CD171^{+} EVs and plasma.

We focused our attention on miR-132–3p for its ability, in our setting, to differentiate between mild cases of AD and controls subjects among the tested miRNAs. miR-132–3p is particularly abundant in nervous tissue, making it a subject of extensive research in neurodegenerative diseases (Klein et al., 2007). Indeed, it is consistently down-regulated in different brain regions and displays a negative correlation with Braak stage, a method for assessing the extent of AD pathology (Hansen et al., 2016). Specifically, miR-132–3p has been shown to play a protective role in the nervous system by regulating neuroinflammatory responses, enhancing synaptic plasticity, and promoting neuronal survival while mitigating neuronal apoptosis (Qu et al., 2021; Scott et al., 2012; Zeng et al., 2022). In this context, the downregulation of miR-132–3p in AD has significant implications due to its regulatory influence on key genes involved in neuronal function and survival (Hansen et al., 2013). Among its targets, synapsin 1 (SYN1), is essential for synaptic vesicle trafficking and neurotransmitter release, directly impacting synaptic plasticity. Another critical target, E1A binding protein p300 (EP300), functions as a histone acetyltransferase involved in chromatin remodeling and transcriptional regulation. EP300 influences genes associated with neuroinflammation and neuronal survival (Shimizu and Kawasaki, 2021). Additionally, PTEN (phosphatase and tensin homolog) is a validated target of miR-132 that counteracts pro-survival signals in cortical and hippocampal neurons by negatively regulating the PI3K/AKT signaling pathway. Furthermore, the transcription factor FOXO3a, a critical regulator of tau phosphorylation and aggregation, was identified as a direct target of miR-132, linking its downregulation to tau pathology (Wong et al., 2013). Recent studies have explored therapeutic strategies to modulate miR-132–3p levels. Nguyen et al. identified small molecules, such as cardiac glycosides, that can upregulate miR-132–3p expression in human induced pluripotent stem cell-derived neurons. These compounds were shown to downregulate known miR-132–3p targets, including Tau protein, and confer neuroprotection against various toxic insults (Nguyen et al., 2023).

Given that miR-132–3p appears to play a role in the pathogenesis of AD (Walgrave et al., 2023), it is reasonable to propose that this microRNA could act as a valuable biomarker for diagnosing and monitoring the progression of AD.

Most studies consistently report the down-regulation of miR-132–3p in AD, particularly in the frontal and temporal cortices and hippocampus (Martinez and Peplow, 2022; Wingo et al., 2022). However, limited research has explored its circulating levels (Sheinerman et al., 2012) and only few studies have examined miR-132–3p in individuals with MCI. Xie et al. found increased serum miR-132–3p levels in MCI patients, which contrasts with trends observed in AD studies (Xie et al., 2015). Additionally, plasma miR-132–3p levels were higher in T2D patients with MCI compared to T2D patients without MCI (Salama et al., 2020). However, serum miR-132–3p levels did not predict the progression from amnestic MCI to probable AD (Xie et al., 2017). In our study, we observed a U-shaped trend for plasma miR-132–3p, with higher levels in individuals with MCI who did not meet AD criteria and lower levels in patients with confirmed AD. This aligns with prior findings and suggests that elevated miR-132–3p release may play a neuroprotective role during the preclinical stage of the disease. Interestingly, although previous research suggested sex-specific modulation of miR-132 expression linked to estrogen signaling (Hirsch et al., 2018), our subanalysis revealed no significant sex-related differences in circulating miR-132–3p levels across the examined cohorts. Nonetheless, evidence regarding sex-related differences in miR-132 levels in AD remains conflicting, (Piscopo et al., 2021) even though differential expression of miRNAs, including miR-132, has been proposed to contribute to explaining the higher prevalence of AD in females (Llera-Oyola et al., 2024). Thus, additional studies are needed to clarify this aspect. Investigation into factors affecting circulating miR-132–3p levels outside conditions directly related to the nervous system is currently limited. Available literature suggests no significant association between circulating miR-132–3p levels and age or smoking status (Weber et al., 2017), although serum miR-132 has been linked to non-alcoholic fatty liver disease risk (Zong et al., 2020), and heterogeneous associations have been reported with various types of cancer (Rafat et al., 2021). To mitigate these potential confounders, we carefully excluded patients with significant comorbidities (e.g., cancer, diabetes, severe cardiovascular conditions, chronic inflammatory disorders), thus minimizing the likelihood that factors unrelated to AD influenced our observed downregulation of miR-132–3p.

Overall, there is evidence that the expression of miR-132–3p may vary with age, cognitive decline, and the deterioration of nervous tissue, suggesting a potential implication in neurodegeneration. Lower levels of miR-132–3p could indicate increased susceptibility to neurodegenerative diseases, underscoring its importance as a biomarker for cognitive health (Zhang and Bian, 2021). Thus, further research is necessary to resolve these differences and validate the findings.

Our results gain significance as we successfully isolated circulating neuronal vesicles and confirmed a decrease in miR-132–3p levels, also after normalization for the number of EVs. Our results align with another study in which miR-132–3p levels were lower in neuron-derived plasma exosomes of both early-stage AD and MCI patients relative to the healthy controls (Cha et al., 2019). Moreover, miR-132–3p exhibited high sensitivity and specificity in diagnosing AD, although the study did not clearly distinguish between AD and MCI patients (Cha et al., 2019). Furthermore, we observed that patients with AD release fewer CD171^{+} vesicles compared to age- and sex-matched healthy controls, corroborating findings from a previous study (Visconte et al., 2023). Overall, we can speculate that the degeneration of neural tissue in this condition may influence both the quantity and the mechanisms of EV release. This aligns with existing literature, which suggests that alterations in vesicle dynamics are often associated with neurodegenerative conditions (Goetzl et al., 2016; Xing et al., 2021). Analyzing EV cargo could be helpful for identifying early biomarkers of certain neurodegenerative diseases, though further validation and technical standardization are needed to clarify the relationship between neuronal deterioration and extracellular vesicle behavior (Thompson et al., 2016). Notably, in this study we demonstrated that measuring the levels of miR-132–3p and other AD-related miRNAs in plasma samples may serve as a reliable indicator of their levels in neuron-derived EVs.

Our study acknowledges several limitations. The retrospective design restricts our ability to establish temporal relationships and causal inferences, which could be better addressed by a prospective study. Additionally, the small cohort size limits the generalization of the results, suggesting that larger samples are needed for stronger conclusions. Conducted at a single center, our findings may lack external validity; thus, multicentric studies with diverse AD populations are crucial for confirming the applicability of our results. However, to corroborate our findings, we used an external cohort of control subjects. Moreover, while we isolated CD171^{+} EVs, we cannot completely rule out the presence of EVs originating from non-neuronal cells, such as glial cells or other types of immune cells. Nonetheless, the concomitant enrichment in CD56 supports the neuronal origin of the isolated EVs. Additionally, while our primary normalization strategy for miRNA quantification was based on the exogenous spike-in cel-miR-39, future studies might explore alternative endogenous references (e.g., U6 or miR-16–5p) to further enhance data consistency and reproducibility. Nevertheless, our key findings regarding miR-132–3p remained significant even after additional adjustment for the number of CD171^{+} EVs.

5. Conclusions

In conclusion, miR-132–3p holds potential as a diagnostic biomarker for Alzheimer's disease; however, further large-scale studies are required to yield clinically significant findings. Given its key role in the pathogenesis of Alzheimer's, even in the early-stages, it is plausible to suggest that miR-132–3p contained in neuron-derived EVs could also serve as a preclinical biomarker for neurodegeneration, especially in pre-symptomatic individuals and high-risk populations. Considering the current unmet need for effective diagnostic tools and therapeutic strategies in Alzheimer's disease, exploring the role of miRNAs like miR-132–3p offers a valuable avenue for advancing early intervention and personalized disease management. Moreover, the analysis of plasma miRNAs may provide a valuable surrogate to the analysis of EVs, which presents challenges in terms of analytical standardization, thus further supporting the potential utility of miR-132–3p in clinical applications.

Funding

This study was supported by the "Ricerca Finalizzata" grant from the Italian Ministry of Health (grant number GR-2016–02363041) to Cinzia Giuli and IRCCS INRCA, and "Ricerca corrente" funding from the Italian Ministry of Health to IRCCS INRCA.

CRediT authorship contribution statement

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mad.2025.112063.

Data Availability

Data will be made available on request.

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