

Differential miRNA expression in neural-enriched extracellular vesicles as potential biomarker for frontotemporal dementia and bipolar disorder

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ABSTRACT

Behavioral variant of Frontotemporal Dementia (bvFTD) and Bipolar Disorder (BD) share overlapping symptoms, complicating diagnosis. BvFTD, especially linked to C9orf72 expansions, often mimics BD, highlighting the need for reliable biomarkers. This study aimed to differentiate bvFTD from BD using miRNA profiles in neural-enriched extracellular vesicles (NEVs). A cohort of 100 subjects was analyzed: 40 bvFTD (20 sporadic, 20 C9orf72 carriers), 40 BD, and 20 healthy controls. NEVs were isolated from plasma and profiled using real-time PCR. Among 754 miRNAs, 11 were significantly deregulated in bvFTD and BD. MiR-152-5p was downregulated in sporadic bvFTD, while let-7b, let-7e, miR-18b, and miR-142-5p were altered in C9orf72 carriers. BD patients showed distinct patterns in miR-331-5p, miR-335, and miR-345 compared to bvFTD. Bioinformatics analyses revealed that let-7e, let-7b, miR-18b, and miR-142-5p share common long non-coding RNA (lncRNA) targets, including XIST, NEAT1, and OIP5-AS1, suggesting their involvement in molecular networks relevant to C9orf72-related bvFTD. These miRNA signatures can differentiate bvFTD from BD, especially in C9orf72-related cases, and offer insights into disease pathways. Further research is needed to validate these findings and explore their clinical application.

1. Introduction

BvFTD and BD are two distinct neuropsychiatric conditions that can present with overlapping behavioral and cognitive symptoms, complicating the differential diagnosis. BvFTD and BD share also common pathological mechanisms, including elevated levels of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), which contribute to neuroinflammation and synaptic dysfunction. Genetic variations in HLA genes suggest a shared inflammatory component in both disorders. Additionally, oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress have been implicated in the neurodegenerative processes underlying both FTD and BD, further supporting common molecular pathways (Nascimento et al., 2019; Vismara et al., 2020).

BvFTD is characterized by progressive degeneration of the frontal and temporal lobes, leading to symptoms such as changes in personality, behavior, and language impairments (Maia da Silva, 2021). A significant subset of bvFTD cases is associated with expansions in the C9orf72 gene, which is the most common genetic cause of bvFTD and Amyotrophic Lateral Sclerosis (ALS) (DeJesus-Hernandez et al., 2011). Individuals with C9orf72 expansions often exhibit psychiatric symptoms, including those that overlap with BD, further complicating the diagnostic process (Galimberti et al., 2013). BD, on the other hand, is a mood disorder marked by episodes of mania and depression, which can also include significant changes in behavior and cognitive function (Roman Meller et al., 2021). The overlapping symptoms between these two conditions, such as mood swings, impulsivity, and cognitive decline, often pose

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diagnostic challenges (Ceylan et al., 2020; Özmen and Göverti, 2023). Therefore, biomarkers to facilitate differential diagnosis would be needed. Among potential candidates, there are micro(mi)RNA (miRNA) contained in extracellular vesicles (EVs). These include exosomes and microvesicles, are nano-sized particles released by cells that carry proteins, lipids, and nucleic acids, including microRNA. These vesicles are abundant in body fluids and can cross the blood-brain barrier, reflecting the pathological state of their cells of origin. EVs play a crucial role in intercellular communication, transferring miRNA and other molecules to recipient cells, thereby modulating various physiological and pathological processes including neurodegenerative diseases (D’Anca et al., 2019). NEVs are a specific subset of EVs enriching from neurons (Nogueras-Ortiz et al., 2024). NEVs have garnered attention for their potential as biomarkers in neurological disorders due to their ability to carry neuron-specific proteins and nucleic acids (Fiandaca et al., 2015; Goetzl et al., 2015; Kapogiannis et al., 2015). The biogenesis of NEVs involves the formation of multivesicular bodies (MVBs) that fuse with the plasma membrane, releasing their contents into the extracellular space as mature vesicles. These vesicles can be taken up by recipient cells, enabling horizontal transfer of their cargos, which include miRNAs (Serpente et al., 2020; Visconte et al., 2023a).

Recent studies have shown that miRNA profiles in EVs can distinguish between various neurodegenerative diseases. For instance, miRNA derived from EVs can discriminate between Alzheimer’s disease, Parkinson’s disease, ALS and bvFTD, suggesting their potential utility for differentiating bvFTD from BD as well (Chen et al., 2023b; Gámez-Valero et al., 2019). Next-generation sequencing (NGS), high-throughput real-time PCR system and bioinformatics analysis have facilitated the identification of miRNA signatures specific to each condition (Rajkumar et al., 2021). Patients with bvFTD associated with mutations in the *C9orf72* gene exhibit distinct miRNA expression profiles. Analyzing miRNA in these subgroups of patients provides deeper insights into the pathogenesis of bvFTD and helps in developing specific biomarkers for this condition (Fenoglio et al., 2022; Visconte et al., 2023a)..

Identifying miRNA specific to bvFTD and BD may have significant clinical implications since this could not only improve diagnostic accuracy but also pave the way for new targeted therapeutic strategies. .

In this study, we analyzed a cohort of 100 subjects, including 40 with bvFTD, 40 with BD and 20 healthy controls. We isolated NEVs from the plasma of these individuals and examined the miRNA content using real-time PCR. This approach aims to identify specific miRNA signatures that can differentiate between bvFTD and BD, ultimately contributing to improved diagnostic precision and understanding of the molecular underpinnings of these disorders.

2. Methods

2.1. Study design and sample selection

For the present study, 40 bvFTD (20 sporadic and 20 *C9orf72* expansion carriers), 40 BD patients and 20 controls (CTRLs) were enrolled. Patients were recruited at Neurodegenerative Disease and Psychiatric Units of Fondazione Ca’ Granda, IRCCS Ospedale Maggiore Policlinico. The diagnostic workup at the former Unit includes a standard battery of examinations, in particular: medical history, physical and neurological examination, screening laboratory tests, neuro-cognitive evaluation, and imaging. The Clinical Dementia Rating (CDR), the Mini-Mental State Examination (MMSE), the Frontal Assessment Battery (FAB), the Wisconsin Card Sorting Test (WCST), and the Tower of London test assessed the degree of cognitive impairment. The presence of significant vascular brain damage was excluded (Hachinski Ischemic Score < 4). BvFTD patients were diagnosed according to current consensus criteria (Rascovsky et al., 2011). Sporadic cases were screened for the three main genes associated with FTD (*C9orf72*, *GRN*, and *MAPT*) and tested negative for pathogenic mutations. BD patients

were diagnosed according to the criteria outlined in the DSM-5; in particular, the clinical work-up is composed by a comprehensive patient history to identify episodes of mood swings, manic or hypomanic episodes, depressive episodes, and any mixed features. Family history of mood disorders and substance use history was also explored. A Mental Status Examination (MSE) was conducted to evaluate the patient’s appearance, behavior, mood, thought processes, cognition, insight, and judgment. The CTRLs group consisted of volunteers matched for ethnic background and age, and without memory and psycho-behavioral dysfunctions (MMSE ≥28). Informed consent to participate in this study was given by all subjects or their caregivers. The study was approved by the local ethical committee (ID 1935–number 1935_SA_18.01.2024_P bis by Comitato Etico Territoriale (CET) Lombardia 3).

For each subject enrolled, an EDTA-blood was withdrawn and was allowed to sit at room temperature for a minimum of 30 min and a max of 2 h. Plasma was isolated by centrifugation at 2500 ×g for 15 min at room temperature, divided in 500-µl aliquots and stored at –80 °C until use. Details of the clinical, and biological variables of patients and CTRL are reported in Table 1.

2.2. C9orf72 genetic screening

Samples were genotyped with AmplideXPCR/CE C9ORF72 Kit (Asuragen, Inc), an optimized C9orf72 PCR amplification reagent as previously described (Serpente et al., 2021). The PCR products were converted to the number of G4C2 repeats using size and mobility conversion factor with AmplideX PCR-CE Reporter Core (v.3.03, Asuragen, Inc). Subjects carrying pathogenic G4C2 expansions exhibit >145 repeats and the classic sawtooth pattern.

2.3. Isolation of neural enriched extracellular vesicles

Aliquots of 500 µl of plasma were centrifuged at 2000 and 10,000 ×g for 20 min to remove cells and debris. The supernatant was transferred to a sterile tube and 0.5 volumes of PBS 1× (filtered with 0,1 µm) and 0.2 volumes Exosome Precipitation Reagent (from plasma, ThermoFisher Scientific) were added. Samples were incubated for 10 min at room temperature and centrifuged at 10000 ×g for 20 min to obtained pellets contained total EVs. These pellets were then re-suspended in 400 µl of 1× PBS (0.1 µm filtered) and stored at –80 °C until use.

To enrich for neural EVs, anti-human CD171 (L1 Cell Adhesion Molecule-L1CAM) biotinylated antibody (clone 5G3, e Bioscience, San Diego) was used; specifically, total EVs were incubated with 4 µg of anti CD171 antibody in 50 µl of 3 % BSA for 4 h at 4 °C with continuous gentle mixing on a rotational mixer. Next, suspensions received 15 µl of streptavidin-agarose Ultralink resin (Thermo Scientific, Rockford, IL, USA) and 25 µl of 3 % BSA and were further incubated for 60 min at 4 °C on a rotational mixer with continuous gentle mixing. Pellets, re-suspended in 200 µl of 0.1 M glycine-CTRL solutions (pH 3.0), were vigorously mixed for 10 s and centrifuged at 4500 ×g for 5 min at 4 °C to detach L1CAM+ EVs from the bead-antibody complex. Supernatants containing L1CAM+ EVs were transferred to clean tubes containing 15 µl of 1 M TRIS-CTRL pH 8.0 (to neutralize the pH) and mixed. Final

Table 1
Details of the clinical, and biological variables of patients and CTRL.

	bvFTD(n = 40)	BD (n = 40)	CTRLs (n = 20)
<i>C9orf72</i> expansion Carriers (n)	20	0	0
Gender distribution (F:M)	17:23	19:21	11:9
Age at sampling, mean years (SD)	68(9)	58 (12)*	65(15)
Age at onset, mean years (SD)	64(9)	38 (17)**	–
Positive history for psychiatric disorders (%)	22(22.7 %)	27(27.8 %)	–

Age at sampling *p < 0.001; age at onset** p < 0.001; bvFTD: behavioral variant Frontotemporal Dementia; BD: Bipolar Disorder; CTRLs: Healthy Controls.

suspensions of NDVs were stored at -80°C until immediately prior to assays (Nogueras-Ortiz et al., 2020; Théry et al., 2018).

2.4. Neural enriched extracellular vesicles characterization

The size distribution and concentration of the particles in the total EVs were measured with NanoSight (NS300) (Malvern Panalytical Ltd., Malvern, UK) equipped with nanoparticle tracking analysis (NTA) software (NanoSight NS300; Malvern Panalytical Ltd., Malvern, UK) (Visconte et al., 2023b). To characterize NEVs by immunoblotting, each tube received 1 volume of M-PER mammalian protein extraction reagent (Thermo Scientific, Inc., Waltham, MA, USA) containing protease and phosphatase inhibitors. Aliquots of EV lysates were separated using SDS-PAGE on 4–15 % acrylamide gels, and proteins were transferred to PVDF membranes. After blocking for 2 h with 5 % BSA in TBS, the membranes were incubated overnight at 4°C with the desired primary antibodies diluted in TBS. In the present study, the following antibodies and dilutions were used: anti-L1CAM at 1:200 from Abcam ab24345, anti-ALIX from Covalab (00013156) and anti-CD81 from Fine Test (FNab01501), both used at 1:500. Membranes were washed and incubated with an appropriate peroxidase-conjugated secondary antibody (1:10,000 dilution), and proteins were visualized with a chemiluminescence reaction. All the experiments reported in this study were repeated at least three times, and comparable results were obtained. The blots reported in the figures are representative images. Images of reactive bands were acquired using an Odyssey Fc imager (LI COR).

Specimens for transmission electron microscopy (TEM) were prepared using the conventional negative stain protocol. Briefly, a small drop of a sample was adsorbed into a formvar-coated copper grid, washed with two drops of deionized water, and stained with a drop of 2 % uranyl acetate in bidistilled water. Samples were observed using a Talos L120C transmission electron microscope (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a 4 K Ceta CMOS digital camera (Thermo Fisher Scientific). Twenty microscopic fields were observed, and representative images were selected.

2.5. RNA extraction and quality control

Total RNA, including small RNAs, was purified from NEVs using the miRNeasy Micro Kit (Qiagen, Hilden, Germany) with QIAzol Lysis Reagent/Chloroform extraction. After adding 1.5 volumes of 100 % ethanol, samples were passed through RNeasy MinElute spin columns to immobilize the RNA. The column was washed, and RNA was eluted with 15 μl of RNase-free water per the manufacturer's instructions. RNA was then analyzed using an Agilent 2100 Bioanalyzer and RNA 6000 Pico kit (Agilent Technologies, Santa Clara, CA, USA) to assess concentration, purity, and integrity, with a peak spanning 25–200 nucleotides.

2.6. Expression analysis of microRNA using TaqMan® OpenArray® MicroRNA panels

RNA from NEVs was reverse-transcribed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) with Megaplex RT Primers PoolA and PoolB. Due to low RNA amounts, cDNA was pre-amplified using TaqMan PreAmp Master Mix and Megaplex PreAmp Primers PoolA and PoolB. The diluted preamplified cDNA (1:20) was mixed with TaqMan OpenArray Real-Time Master Mix for real-time PCR on an OpenArray plate. A 5 μl sample of the PCR reaction was loaded into each well of a 384-well plate, then transferred to the OpenArray plate using the OpenArray AccuFill System. PCR was performed on the QuantStudio 12 K Flex Real-Time PCR System (Thermo Fisher Scientific).

2.7. Workflow of miRNA expression data analysis

After NEVs isolation and miRNA profiling, the data analysis followed

three main approaches:

1. **Preliminary analysis:** Comparison between bvFTD (sporadic cases and C9orf72 expansion carriers), BD, and CTRLs to identify potential miRNAs specific to bvFTD and/or those that differentiate bvFTD from BD.
2. **First subgroup analysis of:** The expression of miRNAs that showed statistically significant differences between bvFTD and CTRLs was stratified according to genetic status (sporadic vs C9orf72 expansion carriers vs. CTRLs) to verify further differences.
3. **Second subgroup analysis:** The expression of miRNAs that were statistically different between bvFTD and BD was re-analyzed by comparing sporadic, C9orf72 expansion carriers, and BD to identify common patterns between C9orf72 expansion carriers and BD and/or between BD and sporadic cases. (Fig. 1).

2.8. Statistical analysis

The statistical analysis was performed on the expression values of miRNAs that were amplified in at least 80 % of the entire dataset. Data were analyzed using the statistical spreadsheets Jamovi v2.5.6.0 (<http://www.jamovi.org>). MiRNA expression levels (expressed as replicate $2^{-\Delta\text{Ct}}$ values for each gene) and age at sampling, age at onset and disease duration were considered continuous variables and expressed as mean $\pm$ standard deviation (SD). Conversely, gender, biological group (CTRLs, bvFTD, C9orf72 expansion carriers and BD) and positive history for psychiatric disorders were all considered categorical variables. Significant statistical threshold was set at 0.05. Shapiro–Wilk's test of normality was performed for continuous variables, and intergroup comparisons were carried out using Kruskal–Wallis test. Post hoc analyses for multiple comparisons were performed with Dwass–Steel–Critchlow–Fligner pairwise comparisons method (“The jamovi project (2024). jamovi (Version 2.5) [Computer Software]. Retrieved from <http://www.jamovi.org>,” n.d.). The W value reported in tables and in the text corresponds to the test statistic for the Dwass–Steel–Critchlow–Fligner pairwise comparisons. This rank-based statistic assesses significant differences between pairs of groups. The correlations between miRNA expression levels and age at onset, age at sampling and disease duration were analyzed using Spearman's rank correlation coefficient. To assess gender differences in miRNA expression levels, we used the Mann–Whitney U test. Box plots were created in BioRender (www.biorender.com).

2.9. Target prediction and pathway enrichment analysis

To identify the potential targets of the miRNAs under investigation, we utilized FunRich, a comprehensive functional enrichment analysis (tool available at <http://www.funrich.org/>) (Fonseka et al., 2021; Pathan et al., 2017). FunRich allowed us to determine the functional roles and biological pathways associated with the miRNAs. Putative target genes of the selected miRNAs were retrieved also using TargetScan 8.0 (https://www.targetscan.org/vert_80) which predicts conserved and non-conserved miRNA binding sites in protein-coding genes. The miRnet platform (<https://www.mirnet.ca>), was employed to predict the lncRNA targets of the four miRNAs. MiRnet provided an integrated environment for identifying interactions between miRNAs and lncRNAs, leveraging a wide array of databases and advanced prediction algorithms (Chang et al., 2020). To visualize and analyze the clusters of target genes, we utilized STRING (Search Tool for the Retrieval of Interacting Genes/Proteins), accessible at <https://string-db.org/>. STRING provided a platform to construct and display PPI networks, facilitating the identification of functional clusters and potential biological significance of the target genes (Szklarczyk et al., 2019). The integration of data from these bioinformatics tools enabled a comprehensive analysis of the miRNA–lncRNA–gene regulatory network. The combined use of FunRich, miRnet and STRING allowed us to

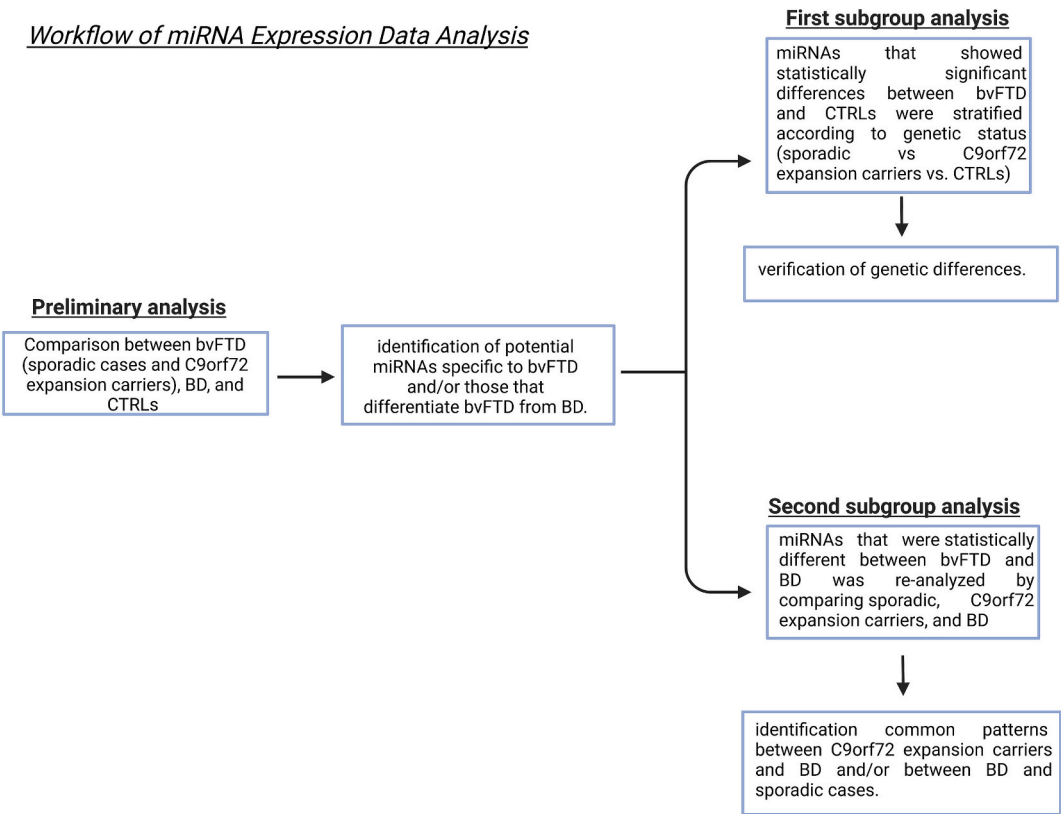


Fig. 1. Workflow of miRNA Expression Data Analysis. The analysis begins with a comparison between bvFTD (sporadic and C9orf72 expansion carriers), BD, and CTRLs to identify specific or differentiating miRNAs. Subgroup analyses stratify miRNAs based on genetic status (sporadic vs. C9orf72) and re-evaluate differences between bvFTD and BD to identify shared patterns.

systematically predict and visualize the complex interactions within the dataset. This multi-step approach ensured the robustness of our predictions and provided insights into the functional implications of the miRNA and lncRNA interactions in the biological context under study.

3. Results

3.1. Characterization of NEVs from plasma

First of all, we characterized the presence of specific surface markers, size, and morphology of NEVs isolated from frozen plasma samples, as suggested by the ISEV (Théry et al., 2018). Approximately $3.5 \times 10^9 \pm$

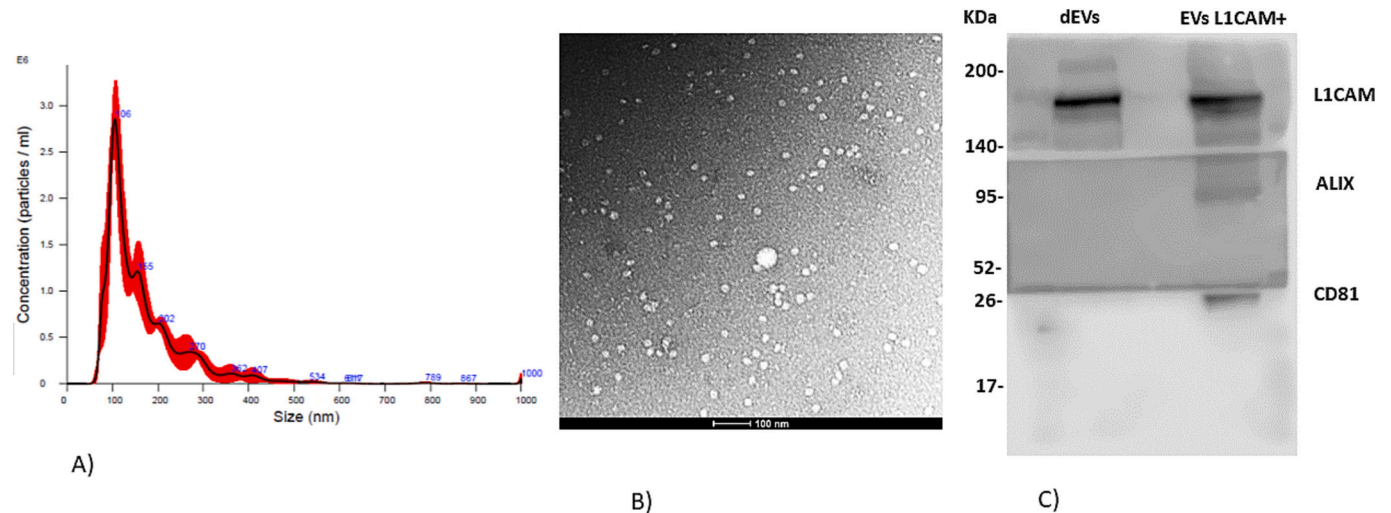


Fig. 2. Characterization of Extracellular Vesicles (EVs): (A) Nanoparticle Tracking Analysis (NTA) of isolated EVs. The concentration of particles (particles/ml) is plotted against their size (nm). Peaks indicate the most common particle sizes. (B) Transmission Electron Microscopy (TEM) image of isolated EVs. The scale bar represents 100 nm, showing the typical morphology and size distribution of EVs. (C) Representative western blot image of EV markers. EVs were probed for L1CAM, ALIX, and CD81. The lanes show dEVs (left) and L1CAM+ EVs (right), confirming the presence of these EV markers. Additional Figs. 1 and 2 provide the original, uncropped PVDF membrane images used for the Western blot analysis.

1.01×10^6 particles/ml could be isolated from 500 ml of plasma with a small size, ranging from 95 to 284 nm (mean 160 nm) (Fig. 2a). Additionally, the EV samples were subjected to a transmission electron microscope analysis (TEM), confirming the presence of heterogeneous vesicles with the expected size and morphology (Fig. 2b). Then, we further characterized, with Western blotting, our NEVs by staining for 3 EV markers: L1CAM (CD171 neuronal marker), tetraspanin CD81 and ALIX (Fig. 2c). Moreover, Depleted EV fraction (DEVs) and plasma sample were used as negative and positive controls.

3.2. MiRNAs expression profile in NEVs

RNA from NEVs was isolated from 40 bvFTD patients (sporadic and *C9orf72* expansion carriers), 40 BD patients, and 20 CTRLs, and the quality and integrity of the extracted RNA were verified using an Agilent 2100 Bioanalyzer. The amount of RNA detected in our samples was very low, with an approximate average $\pm$ SD of 500 ± 100 pg. Afterwards, miRNAs were profiled with RT-qPCR using the TaqMan Open Array miRNA panel, which enables the testing of 754 human miRNAs. RNU48, and U6snRNA were used as endogenous controls for data normalization. Out of the 754 miRNAs analyzed, 142 were successfully amplified, representing approximately 18.83 % of the total. For the preliminary analysis, we excluded miRNAs that were undetectable in more than five samples for each subgroup. After this filtering process, 109 miRNAs were expressed (76 % of all detectable miRNA) in at least 80 % of the dataset. To assess significant differences in the modulation of the expressed miRNAs, we then applied non-parametric statistical tests for multiple group comparisons. Of the 109 miRNAs analyzed, 11 were found to be significantly de-regulated in bvFTD (sporadic + *C9orf72* expansion carriers) and BD patients compared to CTRLs.

3.2.1. Preliminary statistical analysis: comparative analysis of bvFTD, BD patients and CTRLs results

As mentioned above we found 11 miRNAs deregulated in our population. In particular, miRNA let-7b ($X^2(2) = 7.95$, $p = 0.025$), let-7e ($X^2(2) = 6.10$, $p = 0.047$), miR-142-5p ($X^2(2) = 8.47$, $p = 0.014$), miR-152-5p ($X^2(2) = 10.2$, $p = 0.006$), miR-18b ($X^2(2) = 17.2$, $p < 0.001$), miR-218 ($X^2(2) = 9.20$, $p = 0.010$), were deregulated in bvFTD as compared to CTRLs also after Dwass-Steel-Critchlow-Fligner pairwise comparisons. On the other hand, miR-331-5p ($X^2(2) = 10.8$, $p = 0.013$), miR-335 ($X^2(2) = 6.99$, $p = 0.009$), miR-345 ($X^2(2) = 6.31$, $p = 0.043$), miR-146a ($X^2(2) = 7.55$, $p = 0.029$) and miR-125a-5p ($X^2(2) = 5.93$, $p = 0.028$) resulted deregulated, after Dwass-Steel-Critchlow-Fligner pairwise comparisons, in bvFTD compared to BD patients. (Fig. 3). The details of the pairwise comparisons from preliminary analysis are listed, as well as the descriptive statistics (miRNA expression levels as $2^{-\text{DCT}}$ mean $\pm$ SD, 95 % CE split by biological group), in Table 2 and Table 3, respectively.

3.2.2. Results from first subgroup analysis: comparative analysis of sporadic bvFTD, *C9orf72* expansion carriers and CTRLs

The expression of miRNAs that showed statistically significant differences between bvFTD and CTRLs was re-analyzed and stratified according to genetic status (sporadic vs. *C9orf72* expansion carriers vs. CTRLs) to test whether the presence of the expansion is associated with a specific profile. Kruskal-Wallis analysis and subsequent pairwise comparisons showed that let-7b ($X^2(2) = 6.98$, $p = 0.031$; $W = -3.64$, $p = 0.027$), let-7e ($X^2(2) = 7.41$, $p = 0.025$; $W = 3.47$, $p = 0.037$), and miR-142-5p ($X^2(2) = 6.59$, $p = 0.037$; $W = -3.34$, $p = 0.048$) are down-regulated, while miR-18b ($X^2(2) = 12.70$, $p = 0.002$; $W = 5.06$, $p = 0.001$) were up-regulated in *C9orf72* expansion carriers compared to CTRLs. MiR-152-5p ($X^2(2) = 14.50$, $p < 0.01$; $W = -5.00$, $p = 0.001$) was down-regulated in sporadic bvFTD compared to CTRLs (Fig. 4). No difference was observed for miR-218. Descriptive statistics are detailed

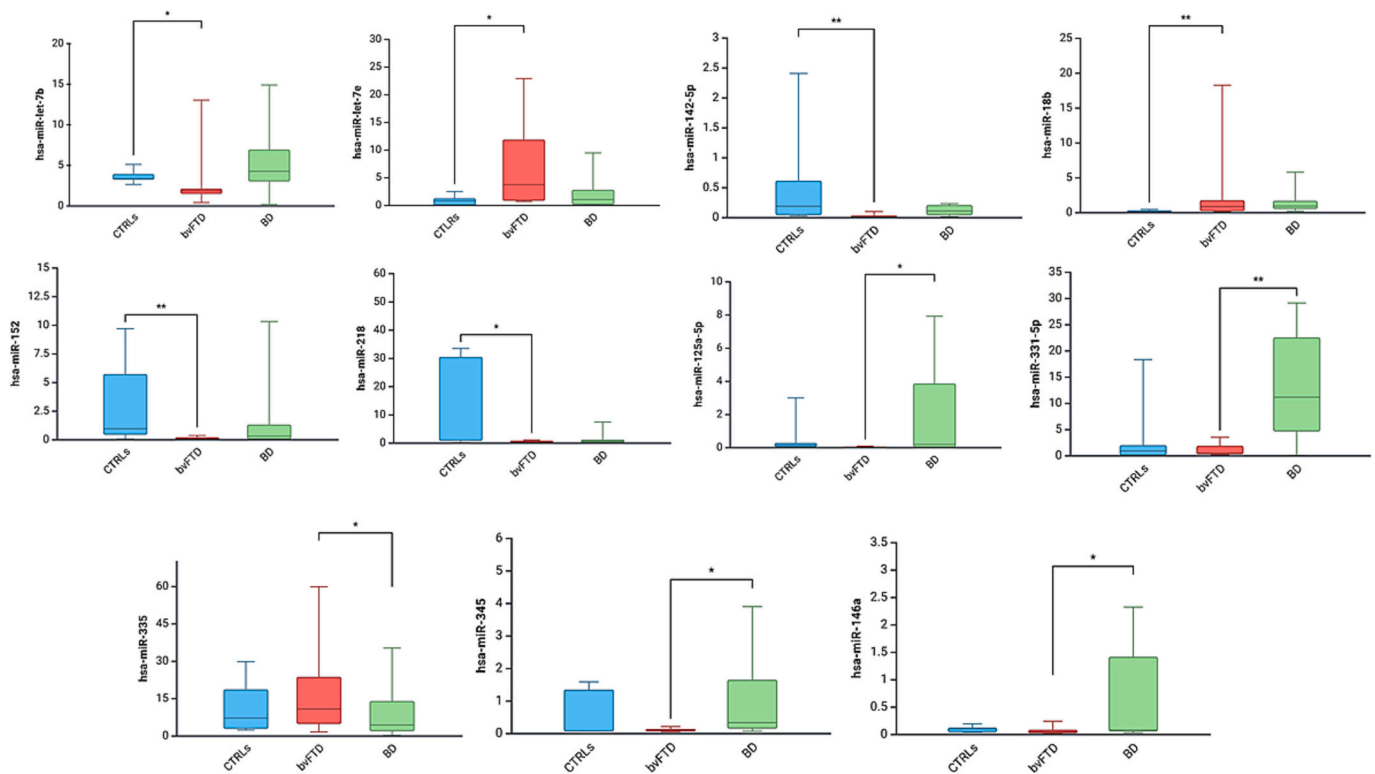


Fig. 3. Expression levels of deregulate miRNAs between bvFTD, BD compared to CTRLs after preliminary statistical analysis: Box plots illustrating the statistically significant expression levels (expressed $2^{-\text{DCT}}$ mean $\pm$ SD) of eleven different miRNAs (let-7b, let-7e, miR-142-5p, miR-18b, miR-152-5p, and miR-218 hsa-miR-125a-5p, hsa-miR-331-5p, hsa-miR-335, hsa-miR-345, and hsa-miR-146a).

Table 2
Details of the pairwise comparisons from preliminary analysis performing after Kruskal-Wallis test. Statistically significant comparisons are highlighted in bold.

Pairwise Comparisons	CTRLs- BD	CTRLs- bvFTD	BD- bvFTD
<i>hsa-let-7b</i>	$p = 0.0149$	$W = -3.35$; $p = 0.047$	$p = 0.075$
<i>hsa-let-7e</i>	$p = 0.727$	$W = 3.09$; $p = 0.042$	$p = 0.157$
<i>hsa-miR-142-5p</i>	$p = 0.986$	$W = -3.686$; $p = 0.025$	$p = 0.05$
<i>hsa-miR-152-5p</i>	$p = 0.210$	$W = -4.49$; $p = 0.004$	$p = 0.232$
<i>hsa-miR-18b</i>	$p = 0.186$	$W = 4.693$; $p = 0.003$	$p = 0.989$
<i>hsa-miR-218</i>	$p = 0.056$	$W = -3.919$; $p = 0.015$	$p = 0.62$
<i>hsa-miR-125a-5p</i>	$p = 0.542$	$p = 0.142$	$W = -3.42$; $p = 0.042$
<i>hsa-miR-331-5p</i>	$p = 0.05$	$p = 0.846$	$W = -3.783$; $p = 0.020$
<i>hsa-miR-335</i>	$p = 0.273$	$p = 0.475$	$W = 4.25$; $p = 0.008$
<i>hsa-miR-345</i>	$p = 0.660$	$p = 0.942$	$W = -3.727$; $p = 0.023$
<i>hsa-miR-146a</i>	$p = 0.288$	$p = 0.131$	$W = -3.02$; $p = 0.043$

bvFTD: behavioral variant Frontotemporal Dementia; BD: Bipolar Disorder; CTRLs: Healthy Controls; W: test statistic for the Dwass-Steel-Critchlow-Fligner pairwise comparisons. The comparisons are rank-based and conducted following a significant Kruskal-Wallis test.

in Table 4. Applying Spearman’s correlation, there were no significant correlations between miRNA levels and age at onset and age at sampling. There were no significant differences in the expression levels of let-7b ($p = 0.657$), let-7e ($p = 0.618$), miR-142-5p ($p = 0.385$), miR-152-5p ($p = 0.324$), and miR-18b ($p = 0.054$) between genders.

3.2.3. Results from second subgroup analysis: comparative analysis of sporadic bvFTD, C9orf72 expansion carriers and BD patients

The miRNAs that exhibited statistically significant differences in expression between bvFTD and BD in the preliminary analysis were re-analyzed by comparing sporadic, C9orf72 expansion carriers, and BD. Kruskal-Wallis analysis and subsequent pairwise comparisons revealed that miR-331-5p was up-regulated in BD compared to sporadic bvFTD ($X^2(2) = 8.43, p = 0.015$; $W = -3.5, p = 0.033$) whereas miR-335 and miR-345 were respectively down and up-regulated in BD as compared to C9orf72 expansion carriers ($X^2(2) = 6.91, p = 0.031$; $W = 3.69, p = 0.025$; $X^2(2) = 6.28, p = 0.043$; $W = -3.69, p = 0.029$) (Fig. 5). No differences were observed for miR-125a-5p and miR-146a. Descriptive statistics are detailed in Table 5. Applying Spearman’s correlation, there were no significant correlations between miRNA levels and age at onset and age at sampling. Based on the Mann-Whitney U test, there was a significant difference in the expression levels of miR-345 between genders ($p < 0.001$). The mean expression level of miR-345 was higher in females (1.26 ± 0.33) compared to males (0.14 ± 0.047), indicating a notable gender-based difference in miRNA expression. However, there were no significant differences in the expression levels of miR-331-5p ($p = 0.283$) and miR-335 ($p = 0.479$) between genders.

3.3. Bioinformatics-based analysis of deregulated NEVs miRNA and their gene networks

To further investigate the functional impact of the deregulated miRNAs identified in our study, we performed a series of bioinformatics analyses. Given the different comparisons in which these miRNAs were found to be altered, we structured our workflow into three main analyses. First, we examined let-7e, let-7b, miR-18b, and miR-142-5p, which were deregulated in C9orf72 expansion carriers compared to

Table 3
Descriptive statistics of preliminary analysis (miRNA expression levels as 2^{-DCt} mean $\pm$ SD, 95 % CE split by biological group).

		95 % Confidence Interval			
		Mean	Lower	Upper	SD
	Biological Group				
<i>hsa-let-7b</i>	CTRLs	3.5912	2.72085	4.4616	0.9411
	BD	6.7885	3.23895	10.3380	5.5866
	bvFTD	3.5300	-0.11919	7.1792	5.7434
<i>hsa-let-7e</i>	CTRLs	1.0046	0.40612	1.6031	0.9904
	BD	1.3694	0.54452	2.1943	1.2983
	bvFTD	5.5139	1.59981	9.4279	6.1603
<i>hsa-miR-142-5p</i>	CTRLs	0.5759	-0.18235	1.3342	0.9865
	BD	0.1412	0.06297	0.2195	0.0846
	bvFTD	0.0293	-0.00280	0.0615	0.0418
<i>hsa-miR-152-5p</i>	CTRLs	3.6940	0.85167	6.5363	4.4735
	BD	1.7727	-0.39304	3.9383	3.5838
	bvFTD	0.1249	0.03883	0.2110	0.1355
<i>hsa-miR-18b</i>	CTRLs	0.1646	0.06580	0.2633	0.1555
	BD	1.7320	0.73989	2.7242	1.9297
	bvFTD	7.8726	1.46386	14.2814	14.4545
<i>hsa-miR-218</i>	CTRLs	12.3209	3.02773	21.6141	14.6264
	BD	1.7263	-0.76072	4.2134	3.9143
	bvFTD	0.4415	0.17954	0.7034	0.3661
<i>hsa-miR-331-5p</i>	CTRLs	3.3093	-0.02242	6.6410	5.7704
	BD	13.5453	6.02921	21.0613	11.8294
	bvFTD	1.0497	0.36354	1.7358	1.1884
<i>hsa-miR-335</i>	CTRLs	11.2905	5.36631	17.2147	10.6977
	BD	6.3691	3.62935	9.1088	6.3356
	bvFTD	20.2201	12.39544	28.0448	21.7027
<i>hsa-miR-345</i>	CTRLs	0.7456	-0.05092	1.5421	0.7590
	BD	1.2843	0.18298	2.3856	1.5395
	bvFTD	0.2716	-0.09025	0.6333	0.6266
<i>hsa-miR-125a-5p</i>	CTRLs	0.7484	-0.86846	2.3652	1.5407
	BD	3.0609	1.09470	5.0271	3.5505
	bvFTD	0.0626	0.00905	0.1161	0.0696
<i>hsa-miR-146a</i>	CTRLs	0.0945	0.06256	0.1264	0.0553
	BD	4.1332	-3.75961	12.0260	9.4409
	bvFTD	0.1033	0.00681	0.1997	0.2061

bvFTD: behavioral variant Frontotemporal Dementia; BD: Bipolar Disorder; CTRLs: Healthy Controls

CTRLs. As an initial step, we used FunRich to construct a Venn diagram and determine the extent of overlap among the predicted protein-coding gene targets of these miRNAs. The analysis (Fig. 6a) revealed that no protein-coding target genes were shared among all four miRNAs. However, specific overlaps were observed, particularly between let-7e and let-7b, which shared 668 target genes, while 7 and 11 genes were unique to each, respectively. In contrast, miR-18b and miR-142-5p exhibited more distinct target profiles, with 171 and 80 unique genes, respectively. To validate this result, we performed an independent analysis using TargetScan, which confirmed that none of the four miRNAs shared a common protein-coding target. The full list of predicted targets for each miRNA is provided in Supplementary File 1. Given the absence of common protein-coding target genes, we extended our analysis to investigate long non-coding RNA (lncRNA) targets using miRNet. This analysis (Fig. 6b) identified several lncRNAs targeted by multiple miRNAs, with XIST, NEAT1, OIP5-AS1, and KCNQ1OT1 emerging as key shared targets (highlighted as yellow nodes). The presence of these shared lncRNAs suggests a potential regulatory role in C9orf72 expansion carriers, possibly influencing disease mechanisms through post-transcriptional regulation. In a separate analysis, we examined miR-152-5p, which was deregulated in sporadic bvFTD patients compared to CTRLs. To explore its potential biological functions, we used FunRich to identify its predicted targets and analyze their cellular localization. The results (Fig. 6a) indicated that a substantial

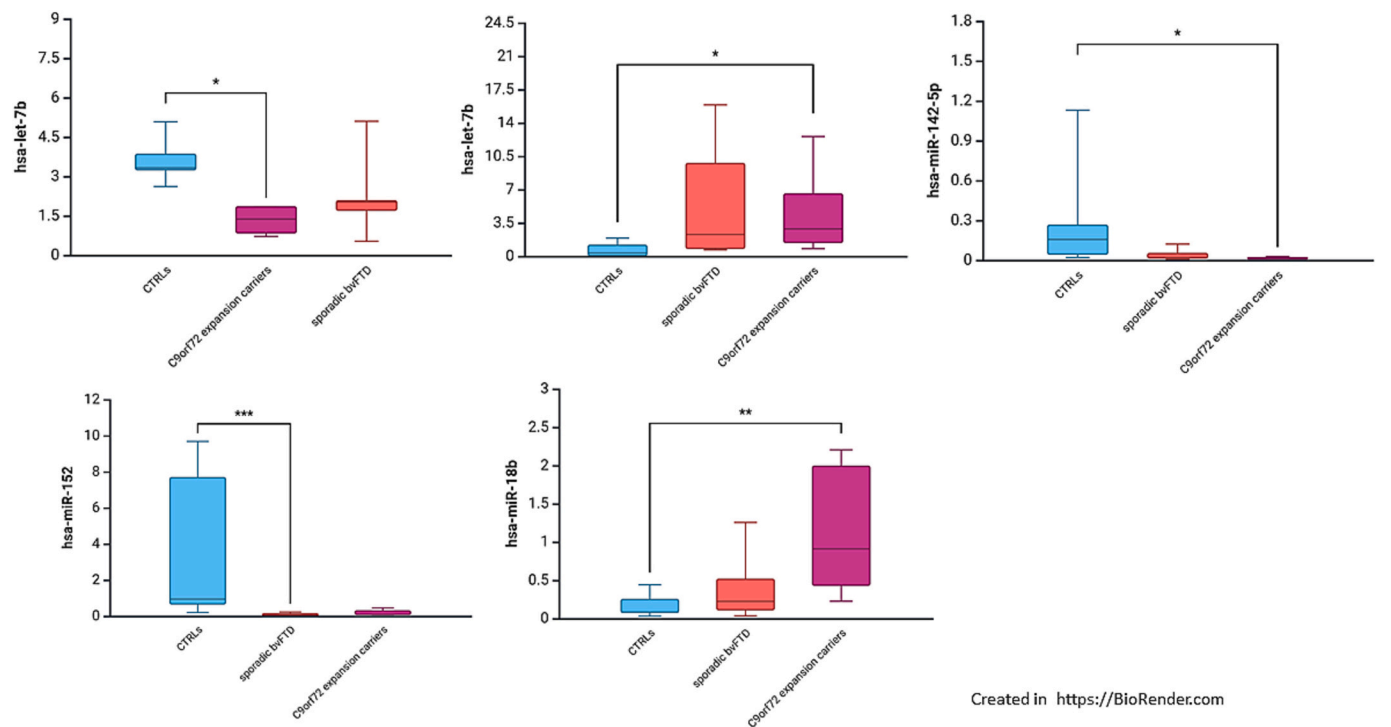


Fig. 4. Comparative analysis of sporadic bvFTD, C9orf72 expansion carriers and CTRLs: Box plots illustrating the statistically significant expression of let-7b, let-7e, miR-142-5p, miR-152-5p, and miR-18b. Statistically significant differences between groups are indicated with p-values. Let-7b ($p = 0.027$), let-7e ($p = 0.037$), miR-142-5p ($p = 0.048$), miR-18b ($p = 0.001$) are deregulated in C9orf72 expansion carriers compared to CTRLs. miR-152-5p ($p = 0.001$) is deregulated in sporadic bvFTD patients compared to CTRLs.

Table 4
Descriptive statistics of first subgroup analysis (miRNA expression levels as $2^{-\Delta\Delta CT}$ mean $\pm$ SD, 95 % CE split by biological group).

	Biological group	Mean	95 % Confidence Interval		
			Lower	Upper	SD
hsa-let-7b	CTRLs	3.6229	2.54533	4.7005	1.02685
	C9orf72 expansion carriers	1.3173	0.34274	2.2918	0.61245
	bvFTD	4.6364	-1.12423	10.3970	6.89051
hsa-let-7e	CTRLs	0.7372	0.19153	1.2829	0.81229
	C9orf72 expansion carriers	10.0901	-5.22741	25.4077	12.33631
	bvFTD	5.7148	0.21123	11.2183	6.58301
hsa-miR-142-5p	CTRLs	0.3234	-0.15845	0.8052	0.52100
	C9orf72 expansion carriers	0.0160	0.00495	0.0270	0.00886
	bvFTD	0.0461	-0.05310	0.1453	0.06234
hsa-miR-152-5p	CTRLs	3.4884	0.87595	6.1009	3.88875
	C9orf72 expansion carriers	0.2212	-0.34730	0.7896	0.22884
	bvFTD	0.0928	0.02623	0.1594	0.08660
hsa-miR-18b	CTRLs	0.1794	0.07596	0.2828	0.15392
	C9orf72 expansion carriers	10.3386	-0.77093	21.4482	17.48521
	bvFTD	4.9134	-2.09560	11.9225	9.79795

bvFTD: behavioral variant Frontotemporal Dementia; BD: Bipolar Disorder; CTRLs: Healthy Controls.

proportion of miR-152-5p target genes were associated with exosomes (26.2 %) and lysosomes (23.8 %), suggesting a role in vesicle-mediated transport and cellular degradation pathways. To gain further insights, we performed a STRING analysis (Fig. 6b) to examine the interactions between the predicted target genes. The network visualization revealed

several clusters of functionally related proteins. Notably, TFRC and VAMP3 were involved in vesicular transport, ALDH1A3 and FGF2 were associated with metabolic processes, and FOXE2 and ASXL2 were linked to transcriptional regulation. These findings suggest that miR-152-5p may play a role in modulating cellular transport, metabolism, and gene expression in sporadic bvFTD. (See Fig. 7.)

Finally, we investigated miR-331-5p, miR-335, and miR-345, which were deregulated in BD patients compared to sporadic bvFTD patients and/or C9orf72 expansion carriers. Initial analyses using miRNet (Fig. 6c) revealed that none of these three miRNAs shared a single common target gene. To further explore their functional impact, we selected genes that were shared between pairs of these miRNAs and analyzed their interactions using STRING. This approach (Fig. 6d) identified clusters of interacting proteins involved in cell cycle regulation, immune response, and transcriptional control. Among the most relevant targets, CDKN1A was identified as a crucial regulator of cell cycle progression, while E2F6 was involved in transcriptional repression during the cell cycle. Additionally, PPP1R1A emerged as a potential modulator of the immune response, and NFAT5 was implicated in osmoregulation and immune function. While no single target gene was shared among all three miRNAs, these shared functional clusters suggest that miR-331-5p, miR-335, and miR-345 may influence key biological processes related to cell cycle regulation, transcriptional control, and immune function.

4. Discussion

Herein, we characterized the presence, size, and morphology of NEVs isolated from plasma samples. Using RT-qPCR, we profiled the miRNA expression in NEVs from patients with bvFTD (sporadic and C9orf72 expansion carriers), BD patients, and CTRLs with the aim of identifying potential molecular signatures that could distinguish these group of patients. Out of the 109 miRNAs that passed the various quality control filters, 11 were found to be deregulated in our population after

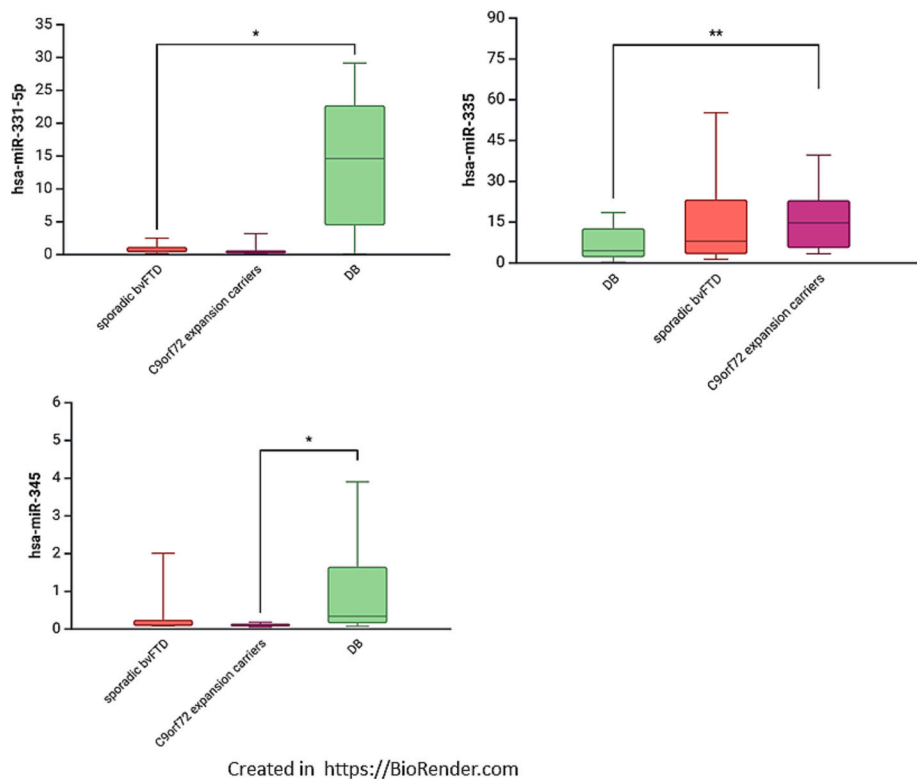


Fig. 5. Comparative analysis of BD patients, sporadic bvFTD cases and C9orf72 expansion carriers: Box plots illustrating the statistically significant expression of miR-331-5p, miR-335 and miR-345. Statistically significant differences between groups are indicated with p-values. miR-331-5p is up-regulated in BD compared to sporadic bvFTD patients ($p = 0.033$); miR-335 and miR-345 are deregulated in C9orf72 expansion carriers compared to BD patients ($p = 0.025$; $p = 0.029$ respectively).

Table 5
Descriptive statistics of second subgroup analysis (miRNA expression levels as 2^{DCt} mean $\pm$ SD, 95 % CE split by biological group).

	Biological group	95 % Confidence Interval			
		Mean	Lower	Upper	SD
hsa-miR-331-5p	BD	14.0029	7.0865	20.919	11.4454
	C9orf72 expansion carrier	0.8568	-0.7284	2.442	1.2767
	bvFTD	1.1568	0.2332	2.080	1.2015
hsa-miR-335	BD	6.7221	4.3248	9.119	6.1825
	C9orf72 expansion carrier	13.4073	8.5525	18.262	9.1108
	bvFTD	9.1543	4.8668	13.442	8.3391
hsa-miR-345	BD	1.0869	0.3668	1.807	1.3513
	C9orf72 expansion carrier	0.0996	0.0638	0.135	0.0466

bvFTD: behavioral variant Frontotemporal Dementia; BD: Bipolar Disorder.

preliminary statistical analysis. In the first subgroup analysis (sporadic bvFTD vs C9orf72 expansion carriers vs CTRLs), we observed significant differences in the expression of certain miRNAs among the groups. Specifically, C9orf72 expansion carriers showed downregulation of let-7b, let-7e, and miR-142-5p compared to CTRLs, while miR-18b was upregulated. In sporadic bvFTD cases, miR-152-5p was significantly downregulated compared to CTRLs. These findings suggest that the C9orf72 expansion might be associated with a specific miRNA expression profile that is distinct from sporadic cases, offering potential insights into the disease's biogenesis. In the second subgroup analysis (sporadic bvFTD vs C9orf72 expansion carriers vs BD patients), we compared miRNA expression between sporadic cases, C9orf72

expansion carriers, and BD patients to identify potential common or differential signatures. MiR-331-5p was found to be upregulated in BD patients compared to sporadic bvFTD cases, while miR-335 and miR-345 were downregulated and upregulated, respectively, in BD patients compared to C9orf72 carriers. These data suggest the presence of distinct molecular signatures among the different groups, with specific miRNAs that could serve as potential peripheral biomarkers. Additionally, miR-345 showed a significant difference in expression between genders, with higher levels in females than in males, indicating a possible gender-based modulation in this context.

Bioinformatics analyses were performed to investigate the functional impact of all deregulated miRNAs. As a first step, we analyzed let-7e, let-7b, miR-18b, and miR-142-5p, which were deregulated in C9orf72 expansion carriers compared to CTRLs, identifying common lncRNA targets such as XIST, NEAT1, OIP5-AS1 and KCNQ1OT1. These lncRNAs are known to play key roles in neurodegenerative processes. Recent evidence suggests a potential link between the C9orf72 repeat expansion and the lncRNA NEAT1. The RNA foci formed by the hexanucleotide repeat expansion share characteristics with paraspeckles, nuclear structures in which NEAT1 plays a key role, suggesting that NEAT1 may be involved in the molecular pathology of C9orf72-related FTD by influencing RNA metabolism and nuclear dynamics (Anilkumar et al., 2024; Bajc Česnik et al., 2019). Additionally, a recent study found that NEAT1 is upregulated in symptomatic C9orf72 expansion carriers, further supporting the relevance of lncRNAs in the pathogenesis of FTD and their potential as biomarkers for disease progression (Serpente et al., 2024). Similarly, XIST has been linked to epigenetic regulation and immune responses, with increased expression reported in the serum of Parkinson's disease patients (Liang Zhang et al., 2024). OIP5-AS1, on the other hand, is associated with the regulation of autophagy and apoptosis, processes that are critical in neurodegeneration (Zhou et al., 2021). The identification of deregulated miRNAs and their shared

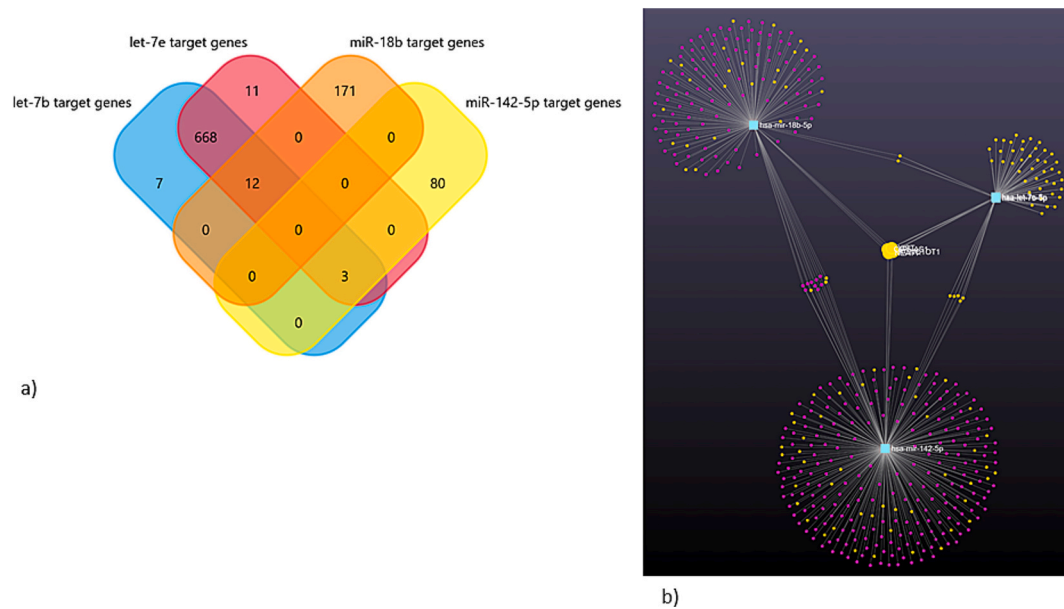


Fig. 6. *FunRich* and *miRnet* and *String* bioinformatic tools for miRNAs deregulated in *C9orf72* expansion carriers (a) Venn diagram showing the overlap of target genes among let-7b, let-7e, miR-18b, and miR-142-5p. (b) miRnet network analysis visualizing the interaction between target genes and miRNAs. Nodes represent miRNAs and their target genes, with edges indicating regulatory relationships.

lncRNA targets in *C9orf72* expansion carriers raises the intriguing possibility of a competing endogenous RNA (ceRNA) regulatory mechanism. In such a model, lncRNAs such as NEAT1, XIST, and OIP5-AS1 could act as miRNA sponges, modulating the availability of specific miRNAs to regulate their mRNA targets. While this hypothesis remains speculative, it suggests a complex post-transcriptional regulatory network that may contribute to frontotemporal dementia pathogenesis. Further experimental studies are required to validate this potential ceRNA interaction and its impact on disease-related gene expression.

In FTD, previous studies have reported alterations in miRNA expression in brain tissues and biofluids, suggesting their potential as biomarkers for the disease (Fenoglio et al., 2022; Grasso et al., 2019; Piscopo et al., 2018; Serpente et al., 2022). Specifically, miRNAs in EVs have been shown to be involved in the pathogenesis of bvFTD by regulating key signaling pathways and neuronal functions (Manzini et al., 2025; Pounders et al., 2022). For instance, miR-142-5p and miR-152-5p, deregulated in our cohort, have been implicated in neuro-inflammatory processes and neuronal survival. MiR-142-5p plays a significant role in neuroinflammation by modulating the expression of inflammatory cytokines and interacting with pathways that control immune cell activation and migration. Studies have shown that miR-142-5p can influence the production of pro-inflammatory cytokines such as IL-6 and TNF- α , which are crucial in the neuroinflammatory response (Zhu et al., 2022). Our findings contrast with previous studies, such as Talebi et al. (2017), where miR-142-5p was shown to regulate inflammation and T-cell differentiation in a murine model of multiple sclerosis. The observed decrease of miR-142-5p in EVs from FTD patients compared to controls may suggest a disrupted inflammatory regulation, highlighting a potential role in the neurodegenerative processes underlying FTD (Talebi et al., 2017). Interestingly, Saeedi et al. (2021) identified miRNA changes in extracellular vesicles in response to antidepressant treatment, using a methodologically similar approach (Saeedi et al., 2021). Their findings emphasize the relevance of EV-derived miRNAs as potential biomarkers of neuropsychiatric conditions, further supporting our observation of miR-142-5p downregulation and its possible involvement in the inflammatory response in bvFTD.

Similarly, miR-152-5p is involved in neuronal survival through its regulation of apoptosis-related genes and pathways. Indeed, research indicates that miR-152-5p can target genes involved in apoptotic

processes, such as BCL2 and CASP3, thereby modulating cell survival and death mechanisms in neurons (Chen et al., 2023a). Moreover, miR-152-5p has been associated with protective effects against oxidative stress, a common feature in neurodegenerative conditions, by regulating the expression of antioxidant genes (Zhang et al., 2019). Similarly, in BD, miRNAs have been found to be dysregulated in patients' blood and brain tissues, reflecting the complex molecular mechanisms underlying the disorder (Lim et al., 2016). MiRNAs in EVs have been proposed as biomarkers for BD due to their stability in biofluids and their ability to cross the blood-brain barrier (Lee et al., 2020). For example, miR-331-5p and miR-335 have been associated with mood regulation and synaptic plasticity (Winkler et al., 2022). MiR-331-5p is involved in the modulation of neurotransmitter systems and synaptic signaling pathways, with studies demonstrating that miR-331-5p can regulate the expression of genes involved in serotonin signaling, a key neurotransmitter system implicated in mood regulation (Gorinski et al., 2019; Melas et al., 2021). Importantly, recent findings from another study highlight the role of miRNA cargo in circulating vesicles from neurons in other psychiatric disorders, such as schizophrenia (Barnett et al., 2023). These results underscore the relevance of miRNA dysregulation in neuronal EVs, further supporting our observations in BD and bvFTD patient. Therefore, since the dysregulation of serotonin signaling is a well-known factor in mood disorders, alterations in miR-331-5p expression could contribute to the pathophysiology of these conditions. MiR-335, on the other hand, regulates synaptic plasticity, crucial for learning, memory, and emotional responses, by modulating the expression of synaptic proteins and glutamatergic receptors essential for excitatory neurotransmission (Franco et al., 2024). Additionally, miR-335 has been shown to influence the levels of brain-derived neurotrophic factor (BDNF), a key molecule involved in synaptic plasticity and neuronal survival (Duc Nguyen, 2023). Reduced BDNF levels have been associated with mood disorders, and the regulation of BDNF by miR-335 highlights its potential role in these conditions (Castrén and Monteggia, 2021). Furthermore, both miR-331-5p and miR-335 are involved in regulating genes associated with stress response and neuroinflammation, which are critical factors in the development and progression of mood disorders (Li et al., 2021). Their ability to modulate these pathways suggests that they could be potential therapeutic targets for mood stabilization and the treatment of BD and other related conditions. Moreover, *C9orf72* expansion, a genetic

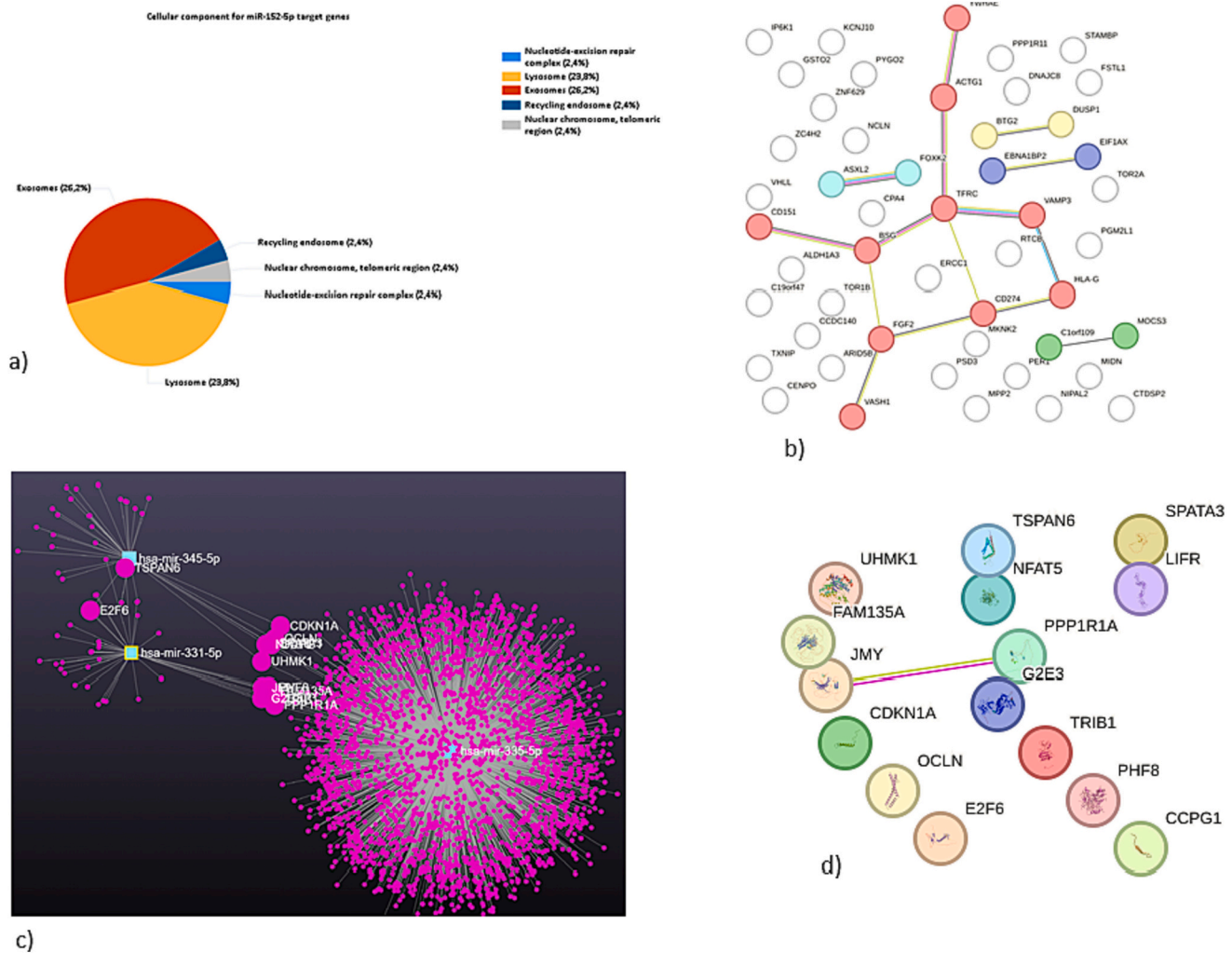


Fig. 7. Cellular components and interaction networks of miR-152-5p target genes and Interaction networks of miR-345-5p, miR-331-5p, and miR-335 target genes. a) Pie chart depicting the distribution of cellular components for miR-152-5p target genes. b) Protein-protein interaction (PPI) network of miR-152-5p target genes. Nodes represent target genes, with edges indicating interactions between them. Key nodes with significant interactions are highlighted, showing a complex network of interactions. (c) miRNet network analysis showing the interaction between miRNAs (hsa-miR-345-5p, hsa-miR-331-5p, and hsa-miR-335) and their target genes. The nodes represent miRNAs and their target genes, while the edges indicate regulatory relationships. Key genes such as TSPAN6, E2F6, CDKN1A, and PPP1R1A are highlighted, illustrating their interactions with multiple miRNAs. (d) String Protein-protein interaction (PPI) network of selected target genes of hsa-miR-345-5p, hsa-miR-331-5p, and hsa-miR-335. Nodes represent target genes, with edges indicating interactions between them. The network highlights central nodes such as G2E3, CDKN1A, and PPP1R1A.

mutation associated with both bvFTD and ALS, has been linked to miRNA dysregulation (Fenoglio et al., 2022). Studies have shown that miRNAs in EVs from *C9orf72* expansion carriers can modulate autophagy and stress response pathways, contributing to disease pathology (Bellini et al., 2022). Our bioinformatics analyses revealed that the deregulated miRNAs in our study are involved in critical cellular functions. For instance, the STRING analysis for miR-331-5p and miR-345 highlighted clusters related to cell cycle regulation (CDKN1A, E2F6), immune response (PPP1R1A, NFAT5), and transcription regulation (G2E3, PHF8). These findings suggest that miR-331-5p and miR-345 may influence cell proliferation, immune modulation, and gene expression. The analysis of miR-335 identified extensive interaction networks involved in chromatin remodeling, signal transduction, and DNA repair. This indicates that miR-335 may have broad regulatory roles across various cellular processes, potentially contributing to the pathophysiology of BD and bvFTD. The significant up-regulation of miR-345 in female gender could indicate its involvement in sex-specific pathways or responses. For instance, this miRNA might be implicated in hormone regulation, reproductive biology, or gender-specific

susceptibilities to certain diseases (Klinge, 2012). Previous studies have shown that miRNAs can be influenced by hormonal levels, which vary significantly between males and females (Sharma and Eghbali, 2014). This differential expression might also reflect underlying genetic or epigenetic mechanisms that are sex-specific. It is worth noting that while we refer to these vesicles as neural-enriched extracellular vesicles, there is an ongoing debate regarding the specificity of L1CAM as a marker for neuron-derived EVs. Although recent evidence supports the enrichment of neuronal content in L1CAM-positive EVs, the term 'neural-enriched' is considered more appropriate in light of current limitations and discussions in the field (Nogueras-Ortiz et al., 2024; Norman et al., 2021). This highlights the need for further refinement of isolation methods to ensure greater specificity for truly neuron-derived EVs.

Despite the promising findings, this study has several limitations. First, the sample size was relatively small, which may limit the generalizability of the results, despite the cohort being well-characterized from a diagnostic standpoint. Second, the low amount of RNA extracted from NEVs posed challenges in miRNA profiling, potentially affecting the sensitivity and accuracy of the detection. Third, while

bioinformatics analyses provided insights into the potential functions of the deregulated miRNAs, experimental validation by RT-PCR is necessary to confirm these findings and elucidate the underlying molecular mechanisms. Future studies should aim to validate the identified miRNA targets and their functional roles using in vitro and in vivo models. Additionally, longitudinal studies with larger cohorts are needed to assess the potential of these miRNAs as biomarkers for disease progression and treatment response. Integrating multi-omics approaches, such as proteomics and metabolomics, could provide a more comprehensive understanding of the molecular pathways involved in bvFTD and BD. Our study highlights the significant roles of deregulated miRNAs in NEVs in the pathogenesis of bvFTD, BD, and *C9orf72* expansion carriers. The identified miRNAs and their target genes may serve as potential biomarkers and therapeutic targets for these neurodegenerative and psychiatric disorders.

5. Conclusion

Our study provides novel insights into the role of deregulated miRNAs in NEVs isolated from plasma in bvFTD, BD, and *C9orf72* expansion carriers. We identified specific miRNA signatures that distinguish these patient groups, highlighting their potential as peripheral biomarkers for differential diagnosis and disease monitoring. Notably, miRNAs such as let-7b, let-7e, miR-18b, and miR-152-5p were associated with distinct molecular profiles in *C9orf72* expansion carriers and sporadic bvFTD cases, while miR-331-5p, miR-335, and miR-345 revealed unique roles in BD. Bioinformatics analyses further uncovered key cellular pathways, including neuroinflammation, vesicular transport, and transcription regulation, that are influenced by these miRNAs. Despite limitations such as small sample size and low RNA yield, our findings underscore the relevance of miRNA dysregulation in neurodegeneration and psychiatric disorders. Future studies should validate these biomarkers and investigate their functional roles to pave the way for targeted therapies and improved diagnostic tools.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.nbd.2025.106867>.

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CRedit authorship contribution statement

Maria Serpente: Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Giuseppe Delvecchio:** Investigation, Funding acquisition, Data curation, Conceptualization. **Chiara Fenoglio:** Methodology, Data curation. **Lorena Di Consoli:** Methodology. **Giulia Giudici:** Methodology. **Vittoria Borracchi:** Resources. **Emanuela Rotondo:** Resources. **Marina Arcaro:** Resources. **Luca Sacchi:** Resources. **Manuela Pintus:** Resources. **Laura Ghezzi:** Resources. **Adele Ferro:** Resources. **Cecilia Prunas:** Resources. **Antonio Callari:** Resources. **Elisa Scola:** Funding acquisition, Conceptualization. **Fabio M. Triulzi:** Writing – review & editing. **Andrea Arighi:** Writing – review & editing. **Paolo Brambilla:** Writing – review & editing, Supervision. **Daniela Galimberti:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors have no conflict of interest to report.

Data availability

Anonymized data may be shared upon request from a qualified academic investigator for the purpose of replication of procedures and results detailed in this article. All requests must be in agreement with EU legislation on general data protection and must be in line with the decisions from the Italian Ethical Review Board. Data sharing should be regulated in a material transfer agreement and/or data processing agreement as appropriate.

References

- Anilkumar, A.K., Vij, P., Lopez, S., Leslie, S.M., Duxtater, K., Khan, M.M., Yallapu, M.M., Chauhan, S.C., Maestre, G.E., Tripathi, M.K., 2024. Long non-coding RNAs: new insights in neurodegenerative diseases. *Int. J. Mol. Sci.* 25, 2268. <https://doi.org/10.3390/ijms25042268>.
- Bajc Česnik, A., Darovic, S., Prpar Mihevc, S., Štalekar, M., Malnar, M., Motaln, H., Lee, Y.-B., Mazej, J., Pohleven, J., Grosch, M., Modic, M., Fonović, M., Turk, B., Drukker, M., Shaw, C.E., Rogelj, B., 2019. Nuclear RNA foci from C9ORF72 expansion mutation form paraspeckle-like bodies. *J. Cell Sci.* 132, jcs224303. <https://doi.org/10.1242/jcs.224303>.
- Barnett, M.M., Reay, W.R., Geaghan, M.P., Kiltsewskij, D.J., Green, M.J., Weidenhofer, J., Glatt, S.J., Cairns, M.J., 2023. miRNA cargo in circulating vesicles from neurons is altered in individuals with schizophrenia and associated with severe disease. *Sci. Adv.* 9, eadi4386. <https://doi.org/10.1126/sciadv.adi4386>.
- Bellini, S., Saraceno, C., Benussi, L., Squitti, R., Cimini, S., Ricci, M., Canafoglia, L., Coppola, C., Puoti, G., Ferrari, C., Longobardi, A., Nicsanu, R., Lombardi, M., D'Arrigo, G., Verderio, C., Binetti, G., Rossi, G., Ghidoni, R., 2022. Plasma small extracellular vesicles with complement alterations in GRN/C9orf72 and sporadic frontotemporal lobar degeneration. *Cells* 11, 488. <https://doi.org/10.3390/cells11030488>.
- Castrén, E., Monteggia, L.M., 2021. Brain-derived neurotrophic factor signaling in depression and antidepressant action. *Biol. Psychiatry* 90, 128–136. <https://doi.org/10.1016/j.biopsych.2021.05.008>.
- Ceylan, D., Tufekci, K.U., Keskinoglu, P., Genc, S., Özerdem, A., 2020. Circulating exosomal microRNAs in bipolar disorder. *J. Affect. Disord.* 262, 99–107. <https://doi.org/10.1016/j.jad.2019.10.038>.
- Chang, L., Zhou, G., Soufan, O., Xia, J., 2020. miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. *Nucleic Acids Res.* 48, W244–W251. <https://doi.org/10.1093/nar/gkaa467>.
- Chen, S., Huang, Y., Liu, R., Lin, Z., Huang, B., Ai, W., He, J., Gao, Y., Xie, P., 2023a. Exosomal miR-152-5p/ARHGAP6/ROCK axis regulates apoptosis and fibrosis in cardiomyocytes. *Exp. Ther. Med.* 25, 165. <https://doi.org/10.3892/etm.2023.11864>.
- Chen, R., Shi, J., Yang, H., Zhang, M., Chen, Q., He, Q., 2023b. Dysregulation of MicroRNAs derived from plasma extracellular vesicles in schizoaffective disorder. *Mol. Neurobiol.* 60, 6373–6382. <https://doi.org/10.1007/s12035-023-03482-w>.
- D'Anca, M., Fenoglio, C., Serpente, M., Arosio, B., Cesari, M., Scarpini, E.A., Galimberti, D., 2019. Exosome determinants of physiological aging and age-related neurodegenerative diseases. *Front. Aging Neurosci.* 11, 232. <https://doi.org/10.3389/fnagi.2019.00232>.
- DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.C.A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.Y.R., Karydas, A., Seelye, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knopman, D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R., Rademakers, R., 2011. Expanded GGGGCC Hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72, 245–256. <https://doi.org/10.1016/j.neuron.2011.09.011>.
- Duc Nguyen, H., 2023. Neurotherapeutic effects of quercetin and its metabolite compounds on cognitive impairment and Parkinson's disease: an in silico study. *Eur. J. Drug Metab. Pharmacokinet.* 48, 151–169. <https://doi.org/10.1007/s13318-023-00816-w>.
- Fenoglio, C., Serpente, M., Visconte, C., Arcaro, M., Sorrentino, F., D'Anca, M., Arighi, A., Rotondo, E., Vimercati, R., Rossi, G., Scarpini, E., Galimberti, D., 2022. Circulating non-coding RNA levels are altered in autosomal dominant frontotemporal dementia. *Int. J. Mol. Sci.* 23, 14723. <https://doi.org/10.3390/ijms232314723>.
- Fiandaca, M.S., Kapogiannis, D., Mapstone, M., Boxer, A., Eitan, E., Schwartz, J.B., Abner, E.L., Petersen, R.C., Federoff, H.J., Miller, B.L., Goetzl, E.J., 2015. Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study. *Alzheimers Dement.* 11. <https://doi.org/10.1016/j.jalz.2014.06.008>, 600–7.e1.
- Fonseka, P., Pathan, M., Chitti, S.V., Kang, T., Mathivanan, S., 2021. FunRich enables enrichment analysis of OMICS datasets. *J. Molecular Biol. Comp. Res. Mol. Biol.* 433, 166747. <https://doi.org/10.1016/j.jmb.2020.166747>.

- Franco, C., Giannella, A., Gasparotto, M., Zanatta, E., Ghirardello, A., Pettorossi, F., Rahmè, Z., Depascale, R., Ragno, D., Bevilacqua, G., Bellis, E., Iaccarino, L., Doria, A., Ceolotto, G., Gatto, M., 2024. Circulating extracellular vesicles and small non-coding RNAs cargo in idiopathic inflammatory myopathies reveal differences across myositis subsets. *J. Autoimmun.* 147, 103255. <https://doi.org/10.1016/j.jaut.2024.103255>.
- Galimberti, D., Fenoglio, C., Serpente, M., Villa, C., Bonisi, R., Arighi, A., Fumagalli, G.G., Del Bo, R., Bruni, A.C., Anfossi, M., Clodomiro, A., Cupidi, C., Nacmias, B., Sorbi, S., Piaceri, I., Bagnoli, S., Bessi, V., Marcone, A., Cerami, C., Cappa, S.F., Filippi, M., Agosta, F., Magnani, G., Comi, G., Franceschi, M., Rainero, I., Giordana, M.T., Rubino, E., Ferrero, P., Rogava, E., Xi, Z., Confaloni, A., Piscopo, P., Bruno, G., Talarico, G., Cagnin, A., Clerici, F., Dell'Osso, B., Comi, G.P., Altamura, A.C., Mariani, C., Scarpini, E., 2013. Autosomal dominant frontotemporal lobar degeneration due to the C9orf72 hexanucleotide repeat expansion: late-onset psychotic clinical presentation. *Biol. Psychiatry* 74, 384–391. <https://doi.org/10.1016/j.biopsych.2013.01.031>.
- Gámez-Valero, A., Campdelacreu, J., Vilas, D., Ispuerto, L., Reñé, R., Álvarez, R., Armengol, M.P., Borràs, F.E., Beyer, K., 2019. Exploratory study on microRNA profiles from plasma-derived extracellular vesicles in Alzheimer's disease and dementia with Lewy bodies. *Transl. Neurodegen.* 8, 31. <https://doi.org/10.1186/s40035-019-0169-5>.
- Goetzl, E.J., Boxer, A., Schwartz, J.B., Abner, E.L., Petersen, R.C., Miller, B.L., Kapogiannis, D., 2015. Altered lysosomal proteins in neural-derived plasma exosomes in preclinical Alzheimer disease. *Neurology* 85, 40–47. <https://doi.org/10.1212/WNL.0000000000001702>.
- Gorinski, N., Bijata, M., Prasad, S., Wirth, A., Abdel Galil, D., Zeug, A., Bazovkina, D., Kondaurou, E., Kulikova, E., Ilchibaeva, T., Zareba-Kozioł, M., Papaleo, F., Scheggia, D., Kochlamazashvili, G., Dityatev, A., Smyth, I., Krzystyniak, A., Włodarczyk, J., Richter, D.W., Strelakova, T., Sigrist, S., Bang, C., Hobu, L., Fiedler, J., Thum, T., Naumenko, V.S., Pandey, G., Ponimaskin, E., 2019. Attenuated palmitoylation of serotonin receptor 5-HT1A affects receptor function and contributes to depression-like behaviors. *Nat. Commun.* 10, 3924. <https://doi.org/10.1038/s41467-019-11876-5>.
- Grasso, M., Piscopo, P., Talarico, G., Ricci, L., Crestini, A., Tosto, G., Gasparini, M., Bruno, G., Denti, M.A., Confaloni, A., 2019. Plasma microRNA profiling distinguishes patients with frontotemporal dementia from healthy subjects. *Neurobiol. Aging* 84, 240.e1–240.e12. <https://doi.org/10.1016/j.neurobiolaging.2019.01.024>.
- Kapogiannis, D., Boxer, A., Schwartz, J.B., Abner, E.L., Biragyn, A., Masharani, U., Frassetto, L., Petersen, R.C., Miller, B.L., Goetzl, E.J., 2015. Dysfunctional phosphorylation of type 1 insulin receptor substrate in neural-derived blood exosomes of preclinical Alzheimer's disease. *FASEB J.* 29, 589–596. <https://doi.org/10.1096/fj.14-262048>.
- Klinge, C.M., 2012. miRNAs and estrogen action. *Trends Endocrinol. Metab.* 23, 223–233. <https://doi.org/10.1016/j.tem.2012.03.002>.
- Lee, S.-Y., Lu, R.-B., Wang, L.-J., Chang, C.-H., Lu, T., Wang, T.-Y., Tsai, K.-W., 2020. Serum miRNA as a possible biomarker in the diagnosis of bipolar II disorder. *Sci. Rep.* 10, 1131. <https://doi.org/10.1038/s41598-020-58195-0>.
- Li, L.-D., Naveed, M., Du, Z.-W., Ding, H., Gu, K., Wei, L.-L., Zhou, Y.-P., Meng, F., Wang, C., Han, F., Zhou, Q.-G., Zhang, J., 2021. Abnormal expression profile of plasma-derived exosomal microRNAs in patients with treatment-resistant depression. *Hum. Genomics* 15, 55. <https://doi.org/10.1186/s40246-021-00354-z>.
- Lim, C.H., Zainal, N.Z., Kanagasundram, S., Zain, S.M., Mohamed, Z., 2016. Preliminary examination of microRNA expression profiling in bipolar disorder I patients during antipsychotic treatment. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 171, 867–874. <https://doi.org/10.1002/ajmg.b.32457>.
- Maia da Silva, M.N., de Porto, F.H.G., Lopes, P.M.G., Sodré de Castro Prado, C., Frota, N. A.F., Alves, C.H.L., Alves, G.S., 2021. Frontotemporal dementia and late-onset bipolar disorder: the many directions of a busy road. *Front. Psychol.* 12, 768722. <https://doi.org/10.3389/fpsy.2021.768722>.
- Manzini, V., Cappelletti, P., Orefice, N.S., Brentari, I., Rigby, M.J., Lo Giudice, M., Feligioni, M., Rivabene, R., Crestini, A., Manfredi, F., Talarico, G., Bruno, G., Corbo, M., Pugliesi, L., Denti, M.A., Piscopo, P., 2025. miR-92a-3p and miR-320a are upregulated in plasma neuron-derived extracellular vesicles of patients with frontotemporal dementia. *Mol. Neurobiol.* 62, 2573–2586. <https://doi.org/10.1007/s12035-024-04386-z>.
- Melas, P.A., Scherma, M., Fratta, W., Cifani, C., Fadda, P., 2021. Cannabidiol as a potential treatment for anxiety and mood disorders: molecular targets and epigenetic insights from preclinical research. *Int. J. Mol. Sci.* 22, 1863. <https://doi.org/10.3390/ijms22041863>.
- Nascimento, C., Nunes, V.P., Diehl Rodriguez, R., Takada, L., Suemoto, C.K., Grinberg, L. T., Nitrini, R., Lafer, B., 2019. A review on shared clinical and molecular mechanisms between bipolar disorder and frontotemporal dementia. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 93, 269–283. <https://doi.org/10.1016/j.pnpbp.2019.04.008>.
- Nogueras-Ortiz, C.J., Mahairaki, V., Delgado-Peraza, F., Das, D., Avgerinos, K., Eren, E., Hentschel, M., Goetzl, E.J., Mattson, M.P., Kapogiannis, D., 2020. Astrocyte- and neuron-derived extracellular vesicles from Alzheimer's disease patients effect complement-mediated neurotoxicity. *Cells* 9, 1618. <https://doi.org/10.3390/cells9071618>.
- Nogueras-Ortiz, C.J., Eren, E., Yao, P., Calzada, E., Dunn, C., Volpert, O., Delgado-Peraza, F., Mustapic, M., Lyashkov, A., Rubio, F.J., Vreones, M., Cheng, L., You, Y., Hill, A.F., Ikezu, T., Eitan, E., Goetzl, E.J., Kapogiannis, D., 2024. Single-extracellular vesicle (EV) analyses validate the use of L1 cell adhesion molecule (L1CAM) as a reliable biomarker of neuron-derived EVs. *J. Extracell. Vesicles* 13, e12459. <https://doi.org/10.1002/jev2.12459>.
- Norman, M., Ter-Ovanesyan, D., Trieu, W., Lazarovits, R., Kowal, E.J.K., Lee, J.H., Chen-Plotkin, A.S., Regev, A., Church, G.M., Walt, D.R., 2021. L1CAM is not associated with extracellular vesicles in human cerebrospinal fluid or plasma. *Nat. Methods* 18, 631–634. <https://doi.org/10.1038/s41592-021-01174-8>.
- Özmen, G., Göverti, D., 2023. Psychiatric Symptoms & Misdiagnosis of Frontotemporal Dementia: A case report. *Eur. Psychiatry* 66, S917–S918. <https://doi.org/10.1192/j.eurpsy.2023.1941>.
- Pathan, M., Keerthikumar, S., Chisanga, D., Alessandro, R., Ang, C.-S., Askenase, P., Batagov, A.O., Benito-Martin, A., Camussi, G., Clayton, A., Collino, F., Di Vizio, D., Falcon-Perez, J.M., Fonseca, P., Fonseca, P., Fontana, S., Gho, Y.S., Hendrix, A., Hoen, E.N.T., Iraci, N., Kastaniegaard, K., Kislinger, T., Kowal, J., Kurochkin, I.V., Leonardi, T., Liang, Y., Llorente, A., Lunavat, T.R., Maji, S., Monteleone, F., Øverbye, A., Panaretakis, T., Patel, T., Peinado, H., Pluchino, S., Principe, S., Ronquist, G., Royo, F., Sahoo, S., Spinelli, C., Stensballe, A., Théry, C., van Herwijnen, M.J.C., Wauben, M., Welton, J.L., Zhao, K., Mathivanan, S., 2017. A novel community driven software for functional enrichment analysis of extracellular vesicles data. *J. Extracell. Vesicles* 6, 1321455. <https://doi.org/10.1080/20013078.2017.1321455>.
- Piscopo, P., Grasso, M., Puopolo, M., D'Acunto, E., Talarico, G., Crestini, A., Gasparini, M., Campopiano, R., Gambardella, S., Castellano, A.E., Bruno, G., Denti, M.A., Confaloni, A., 2018. Circulating miR-127-3p as a potential biomarker for differential diagnosis in frontotemporal dementia. *J. Alzheimers Dis.* 65, 455–464. <https://doi.org/10.3233/JAD-180364>.
- Pounders, J., Hill, E.J., Hooper, D., Zhang, X., Biesiada, J., Kuhnell, D., Greenland, H.L., Esfandiari, L., Timmerman, E., Foster, F., Wang, C., Walsh, K.B., Shatz, R., Woo, D., Medvedovic, M., Langevin, S., Sawyer, R.P., 2022. MicroRNA expression within neuronal-derived small extracellular vesicles in frontotemporal degeneration. *Medicine (United States)* 101, E30854. <https://doi.org/10.1097/MD.00000000000030854>.
- Rajkumar, A.P., Hye, A., Lange, J., Manesh, Y.R., Ballard, C., Fladby, T., Aarsland, D., 2021. Next-generation RNA-sequencing of serum small extracellular vesicles discovers potential diagnostic biomarkers for dementia with Lewy bodies. *Am. J. Geriatr. Psychiatry* 29, 573–584. <https://doi.org/10.1016/j.jagp.2020.10.012>.
- Rascovsky, K., Hodges, J.R., Knopman, D., Mendez, M.F., Kramer, J.H., Neuhaus, J., Van Swieten, J.C., Seelaar, H., Dopper, E.G.P., Onyike, C.U., Hillis, A.E., Josephs, K.A., Boeve, B.F., Kertesz, A., Seeley, W.W., Rankin, K.P., Johnson, J.K., Gorno-Tempini, M.L., Rosen, H., Prileau-Latham, C.E., Lee, A., Kipps, C.M., Lillo, P., Piguet, O., Rohrer, J.D., Rossor, M.N., Warren, J.D., Fox, N.C., Galasko, D., Salmon, D.P., Black, S.E., Mesulam, M., Weintraub, S., Dickerson, B.C., Diehl-Schmid, J., Pasquier, F., Derramecourt, V., Lebert, F., Pijnenburg, Y., Chow, T.W., Manes, F., Grafman, J., Cappa, S.F., Freedman, M., Grossman, M., Miller, B.L., 2011. No Title.
- Roman Meller, M., Patel, S., Duarte, D., Kapczynski, F., de Azevedo Cardoso, T., 2021. Bipolar disorder and frontotemporal dementia: A systematic review. *Acta Psychiatr. Scand.* 144, 433–447. <https://doi.org/10.1111/acps.13362>.
- Saeedi, S., Nagy, C., Ibrahim, P., Thérault, J.-F., Wakid, M., Fiori, L.M., Yang, J., Rotzinger, S., Foster, J.A., Mechawar, N., Kennedy, S.H., Turecki, G., 2021. Neuron-derived extracellular vesicles enriched from plasma show altered size and miRNA cargo as a function of antidepressant drug response. *Mol. Psychiatry* 26, 7417–7424. <https://doi.org/10.1038/s41380-021-01255-2>.
- Serpente, M., Fenoglio, C., D'Anca, M., Arcaro, M., Sorrentino, F., Visconte, C., Arighi, A., Fumagalli, G.G., Porretti, L., Cattaneo, A., Ciani, M., Zanardini, R., Benussi, L., Ghidoni, R., Scarpini, E., Galimberti, D., 2020. MiRNA profiling in plasma neuron-derived small extracellular vesicles from patients with Alzheimer's disease. *Cells* 9, 1443. <https://doi.org/10.3390/cells9061443>.
- Serpente, M., Fenoglio, C., Arighi, A., Fumagalli, G.G., Arcaro, M., Sorrentino, F., Visconte, C., Scarpini, E., Galimberti, D., 2021. Analysis of C9orf72 intermediate alleles in a retrospective cohort of neurological patients: risk factors for Alzheimer's disease? *J. Alzheimers Dis.* 81, 1445–1451. <https://doi.org/10.3233/JAD-210249>.
- Serpente, M., Ghezzi, L., Fenoglio, C., Buccellato, F.R., Fumagalli, G.G., Rotondo, E., Arcaro, M., Arighi, A., Galimberti, D., 2022. miRNA expression is increased in serum from patients with semantic variant primary progressive aphasia. *Int. J. Mol. Sci.* 23, 8487. <https://doi.org/10.3390/ijms23158487>.
- Serpente, M., Fenoglio, C., Arcaro, M., Carandini, T., Sacchi, L., Pintus, M., Rotondo, E., Borraacci, V., Ghezzi, L., Bouzigues, A., Russell, L.L., Foster, P.H., Ferry-Bolder, E., van Swieten, J.C., Jiskoot, L.C., Seelaar, H., Sánchez Valle, R., Laforce, R., Graff, C., Vandenbergh, R., de Mendonça, A., Tiraboschi, P., Santana, I., Gerhard, A., Levin, J., Sorbi, S., Otto, M., Pasquier, F., Ducharme, S., Butler, C.R., Le Ber, I., Finger, E., Tartaglia, M.C., Masellis, M., Rowe, J.B., Synofzik, M., Moreno, F., Borroni, B., Rohrer, J.D., Arighi, A., Galimberti, D., Genetic FTD Initiative GENFI, 2024. Long non-coding RNA profile in genetic symptomatic and Presymptomatic frontotemporal dementia: A GENFI study. *J. Alzheimers Dis.* 100, S187–S196. <https://doi.org/10.3233/JAD-240557>.
- Sharma, S., Eghbali, M., 2014. Influence of sex differences on microRNA gene regulation in disease. *Biol. Sex Differ.* 5, 3. <https://doi.org/10.1186/2042-6410-5-3>.
- Szklarczyk, D., Gable, A.L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N.T., Morris, J.H., Bork, P., Jensen, L.J., von Mering, C., 2019. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 47, D607–D613. <https://doi.org/10.1093/nar/gky1131>.
- Talebi, F., Ghorbani, S., Chan, W.F., Boghoozian, R., Masoumi, F., Ghasemi, S., Vojgani, M., Power, C., Noorbakhsh, F., 2017. MicroRNA-142 regulates inflammation and T cell differentiation in an animal model of multiple sclerosis. *J. Neuroinflammation* 14, 55. <https://doi.org/10.1186/s12974-017-0832-7>.
- The jamovi project, 2024. jamovi (Version 2.5) [Computer Software]. Retrieved from <https://www.jamovi.org>. n.d.

- Théry, C., Witwer, K.W., Aikawa, E., Alcaraz, M.J., Anderson, J.D., Andriantsitohaina, R., Antoniou, A., Arab, T., Archer, F., Atkin-Smith, G.K., Ayre, D.C., Bach, J.-M.M., Bachurski, D., Baharvand, H., Balaj, L., Baldacchino, S., Bauer, N.N., Baxter, A.A., Bebak, M., Beckham, C., Bedina Zavec, A., Benmoussa, A., Berardi, A.C., Bergese, P., Bielska, E., Blenkiron, C., Bobis-Wozowicz, S., Boillard, E., Boireau, W., Bongiovanni, A., Borràs, F.E., Bosch, S., Boulanger, C.M., Breakefield, X., Breglio, A. M., Brennan, M.A., Brigstock, D.R., Brissan, A., Broekman, M.L.D.L., Bromberg, J.F., Bryl-Górecka, P., Buch, S., Buck, A.H., Burger, D., Busatto, S., Buschmann, D., Bussolati, B., Buzás, E.I., Byrd, J.B., Camussi, G., Carter, D.R.R.F., Caruso, S., Chamley, L.W., Chang, Y.-T.T., Chen, C., Chen, S., Cheng, L., Chin, A.R., Clayton, A., Clerici, S.P., Cocks, A., Cocucci, E., Coffey, R.J., Cordeiro-da-Silva, A., Couch, Y., Coumans, F.A.A.W., Coyle, B., Crescitelli, R., Criado, M.F., D'Souza-Schorey, C., Das, S., Datta Chaudhuri, A., de Candia, P., De Santana, E.F., De Wever, O., del Portillo, H.A., Demaret, T., Deville, S., Devitt, A., Dhondt, B., Di Vizio, D., Dieterich, L.C., Dolo, V., Domínguez Rubio, A.P., Dominici, M., Dourado, M.R., Driedonks, T.A.A.P., Duarte, F.V., Duncan, H.M., Eichenberger, R.M., Ekström, K., EL Andaloussi, S., Elie-Caille, C., Erdbrügger, U., Falcón-Pérez, J.M., Fatima, F., Fish, J.E., Flores-Bellver, M., Försonits, A., Frelet-Barrand, A., Fricke, F., Fuhrmann, G., Gabriellson, S., Gámez-Valero, A., Gardiner, C., Gärtner, K., Gaudin, R., Gho, Y.S., Giebel, B., Gilbert, C., Gimona, M., Giusti, I., Goberdhan, D.C.I.C., Görgens, A., Gorski, S.M., Greening, D.W., Gross, J.C., Gualerzi, A., Gupta, G.N., Gustafson, D., Handberg, A., Haraszti, R. A., Harrison, P., Hegyesi, H., Hendrix, A., Hill, A.F., Hochberg, F.H., Hoffmann, K.F., Holder, B., Holthofer, H., Hosseinkhani, B., Hu, G., Huang, Y., Huber, V., Hunt, S., Ibrahim, A.G.-E.E., Ikezu, T., Inal, J.M., Isin, M., Ivanova, A., Jackson, H.K., Jacobsen, S., Jay, S.M., Jayachandran, M., Jenster, G., Jiang, L., Johnson, S.M., Jones, J.C., Jong, A., Jovanovic-Talman, T., Jung, S., Kalluri, R., Kano, S. Ichi, Kaur, S., Kawamura, Y., Keller, E.T., Khamari, D., Khomyakova, E., Khvorova, A., Kierulf, P., Kim, K.P., Kislinger, T., Klingeborn, M., Klinke, D.J., Kornek, M., Kosanović, M.M., Kovács, Á.F., Krämer-Albers, E.-M.M., Krasemann, S., Krause, M., Kurochkin, I.V., Kusuma, G.D., Kuypers, S., Laitinen, S., Langevin, S.M., Languino, L.R., Lannigan, J., Lässer, C., Laurent, L.C., Lavieu, G., Lázaro-Ibáñez, E., Le Lay, S., Lee, M.-S.S., Lee, Y.X.F., Lemos, D.S., Lenassi, M., Leszczynska, A., Li, I.T.T.S., Liao, K., Libregts, S.F., Ligeti, E., Lim, R., Lim, S.K., Liné, A., Linnemannstons, K., Llorente, A., Lombard, C.A., Lorenovic, M.J., Lörincz, A. M., Lötvall, J., Lovett, J., Lowry, M.C., Loyer, X., Lu, Q., Lukomska, B., Lunavat, T.R., Maas, S.L.L.N., Malhi, H., Marcilla, A., Mariani, J., Mariscal, J., Martens-Uzunova, E. S., Martin-Jaular, L., Martinez, M.C., Martins, V.R., Mathieu, M., Mathivanan, S., Maugeri, M., McGinnis, L.K., McVey, M.J., Meckes, D.G., Meehan, K.L., Mertens, I., Minciacci, V.R., Möller, A., Möller Jørgensen, M., Morales-Kastresana, A., Morhayim, J., Mullier, F., Muraca, M., Musante, L., Mussack, V., Muth, D.C., Myburgh, K.H., Najrana, T., Nawaz, M., Nazarenko, I., Nejsun, P., Neri, C., Neri, T., Nieuwland, R., Nimrichter, L., Nolan, J.P., Nolte-t Hoen, E.N.N.M., Noren Hooten, N., O'Driscoll, L., O'Grady, T., O'Loughlin, A., Ochiya, T., Olivier, M., Ortiz, A., Ortiz, L.A., Osteikoetxea, X., Ostegaard, O., Ostrowski, M., Park, J., Pegtel, D.M., Peinado, H., Perut, F., Pfaffl, M.W., Phinney, D.G., Pieters, B.C.H.C., Pink, R.C., Pisetsky, D.S., Pogge von Strandmann, E., Polakovicova, I., Poon, I.K.H.K., Powell, B.H., Prada, I., Pulliam, L., Quesenberry, P., Radeghieri, A., Raffai, R.L., Raimondo, S., Rak, J., Ramirez, M.L., Raposo, G., Rayyan, M.S., Regev-Rudski, N., Ricklefs, F.L., Robbins, P. D., Roberts, D.D., Rodrigues, S.C., Rohde, E., Rome, S., Rouschop, K.M.A.M., Rugghetti, A., Russell, A.E., Saá, P., Sahoo, S., Salas-Huenuleo, E., Sánchez, C., Saugstad, J.A., Saul, M.J., Schiffelers, R.M., Schneider, R., Schöner, T.H., Scott, A., Shahaj, E., Sharma, S., Shatnyeva, O., Shekari, F., Shelke, G.V., Shetty, A.K., Shiba, K., Siljander, P.R.-M.R.M., Silva, A.M., Skowronek, A., Snyder, O.L., Soares, R.P., Sódar, B.W., Soekmadji, C., Sotillo, J., Stahl, P.D., Stoorvogel, W., Stott, S.L., Strasser, E.F., Swift, S., Tahara, H., Tewari, M., Timms, K., Tiwari, S., Tixeira, R., Tkach, M., Toh, W.S., Tomasini, R., Torrecillas, A.C., Tosar, J.P., Toxavidis, V., Urbanelli, L., Vader, P., van Balkom, B.W.M.W., van der Grein, S.G., Van Deun, J., van Herwijnen, M.J.C.J., Van Keuren-Jensen, K., van Niel, G., van Royen, M.E., van Wijnen, A.J., Vasconcelos, M.H., Vechetti, I.J., Veit, T.D., Vella, L.J., Velot, É., Verweij, F.J., Vestad, B., Viñas, J.L., Visnovitz, T., Vukman, K.V., Wahlgren, J., Watson, D.C., Wauben, M.H.M.H., Weaver, A., Webber, J.P., Weber, V., Wehman, A. M., Weiss, D.J., Welsh, J.A., Wendt, S., Wheelock, A.M., Wiener, Z., Witte, L., Wolfram, J., Xagorari, A., Xander, P., Xu, J., Yan, X., Yáñez-Mó, M., Yin, H., Yuana, Y., Zappulli, V., Zarubova, J., Zekas, V., Zhang, J., Ye, Zhao, Z., Zheng, L., Zheutlin, A.R., Zickler, A.M., Zimmermann, P., Zivkovic, A.M., Zocco, D., Zuba-Surma, E.K., 2018. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J. Extracell. Vesic.* 7, 1535750. doi:<https://doi.org/10.1080/20013078.2018.1535750>.
- Visconte, Caterina, Fenoglio, C., Serpente, M., Muti, P., Sacconi, A., Rigoni, M., Arighi, A., Borracchi, V., Arcaro, M., Arosio, B., Ferri, E., Golia, M.T., Scarpini, E., Galimberti, D., 2023a. Altered extracellular vesicle miRNA profile in prodromal Alzheimer's disease. *Int. J. Mol. Sci.* 24, 14749. <https://doi.org/10.3390/ijms241914749>.
- Visconte, C., Golia, M.T., Fenoglio, C., Serpente, M., Gabrielli, M., Arcaro, M., Sorrentino, F., Busnelli, M., Arighi, A., Fumagalli, G., Rotondo, E., Rossi, P., Arosio, B., Scarpini, E., Verderio, C., Galimberti, D., 2023b. Plasma microglial-derived extracellular vesicles are increased in frail patients with mild cognitive impairment and exert a neurotoxic effect. *Geroscience* 45, 1557–1571. <https://doi.org/10.1007/s11357-023-00746-0>.
- Vismara, M., Cernigliaro, G., Piccoli, E., Giorgetti, F., Molteni, L., Cremaschi, L., Fumagalli, G.G., D'addario, C., Dell'Osso, B., 2020. Crossing Borders between frontotemporal dementia and psychiatric disorders: an updated overview. *J. Alzheimers Dis.* 75, 661–673. <https://doi.org/10.3233/JAD-191333>.
- Winkler, I., Heisinger, T., Hammerl, M., Huber, E., Urbanek, M., Kiechl-Kohlendorfer, U., Griesmaier, E., Posod, A., 2022. MicroRNA expression profiles as diagnostic and prognostic biomarkers of perinatal asphyxia and hypoxic-ischaemic encephalopathy. *Neonatology* 119, 204–213. <https://doi.org/10.1159/000521356>.
- Zhang, A., Qian, Y., Qian, J., 2019. MicroRNA-152-3p protects neurons from oxygen-glucose-deprivation/reoxygenation-induced injury through upregulation of Nrf2/ARE antioxidant signaling by targeting PSD-93. *Biochem. Biophys. Res. Commun.* 517, 69–76. <https://doi.org/10.1016/j.bbrc.2019.07.012>.
- Zhang, Liang, Li, G., Lu, D., Dai, Q., 2024. High expression of lncRNA XIST assists in the diagnosis of Parkinson's disease. *Neurochem. J.* 18, 220–225. <https://doi.org/10.1134/S1819712424010276>.
- Zhou, S., Yu, X., Wang, M., Meng, Y., Song, D., Yang, H., Wang, D., Bi, J., Xu, S., 2021. Long non-coding RNAs in pathogenesis of neurodegenerative diseases. *Front. Cell Dev. Biol.* 9. <https://doi.org/10.3389/fcell.2021.719247>.
- Zhu, W., Huang, X., Qiu, S., Feng, L., Wu, Y., Shao, H., 2022. miR-142-5p encapsulated by serum-derived extracellular vesicles protects against acute lung injury in septic rats following remote ischemic preconditioning via the PTEN/PI3K/Akt Axis. *J. Innate Immun.* 14, 532–542. <https://doi.org/10.1159/000522231>.