Parkinsonism and Related Disorders 93 (2021) 77–84

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

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

# Exosomal miRNA as peripheral biomarkers in Parkinson's disease and progressive supranuclear palsy: A pilot study

Ida Manna [a,1], Andrea Quattrone [b,1], Selene De Benedittis [b], Basilio Vescio [c], Enrico Iaccino [d,*], Aldo Quattrone [a,e]

[a] Institute of Molecular Bioimaging and Physiology (IBFM), National Research Council (CNR), Section of Germaneto, 88100, Catanzaro, Italy
[b] Institute of Neurology, Department of Medical and Surgical Sciences, University "Magna Graecia", Germaneto, 88100, Catanzaro, Italy
[c] Biotecnomed, S.C.aR.L, 88100, Catanzaro, Italy
[d] Department of Experimental and Clinical Medicine, University "Magna Graecia" of Catanzaro, 88100, Catanzaro, Italy
[e] Neuroscience Research Center, University Magna Graecia, 88100, Catanzaro, Italy

ARTICLE INFO

*Keywords:*
microRNAs (miRNAs)
Biomarkers
Parkinson's disease (PD)

ABSTRACT

*Introduction:* Parkinson's disease (PD), a progressive neurodegenerative disease, can be misdiagnosed with atypical conditions such as Progressive Supranuclear Paralysis (PSP) due to overlapping clinical features. MicroRNAs (miRNAs) are small non-coding RNAs with a key role in post-transcriptional gene regulation. The aim was to identify a set of differential exosomal miRNAs biomarkers, which may aid in diagnosis.

*Methods:* We analyzed the serum level of 188 miRNAs in a discovery set, by using RTqPCR based TaqMan assay, in a small cohort of healthy controls, PD and PSP patients. Subsequently, the differentially expressed miRNAs, between PSP and PD patients, were further tested in a larger and independent cohort of 33 healthy controls, 40 PD and 20 PSP patients. The most accurate diagnostic exosomal miRNAs classifiers were identified in a logistic regression model.

*Results:* A statistically significant set of three exosomal miRNAs: miR-21-3p, miR-22-3p and miR-223-5p, discriminated PD from HC (area under the curve of 0.75), and a set of three exosomal miRNAs, miR-425-5p, miR-21-3p, and miR-199a-5p, discriminated PSP from PD with good diagnostic accuracy (area under the curve of 0.86). Finally, the classifier that best discriminated PSP from PD consisted of six exosomal miRNAs (area under the curve = 0.91), with diagnostic sensitivity and specificity of 0.89 and 0.90, respectively.

*Conclusions:* Based on our analysis, these data showed that exosomal miRNAs could act as biomarkers to differentiate between PSP and PD.

## 1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease characterized by bradykinesia associated with rigidity and or rest tremor, and multiple non-motor symptoms [1]. Dopamine imaging (PET or SPECT) serves to support clinical diagnosis but do not allow the differentiation between PD and atypical parkinsonisms. Progressive Supranuclear Palsy (PSP) is one of the most common atypical parkinsonism, and is characterized by vertical gaze palsy, dysarthria/dysphagia, and frontal dysexecutive syndrome [2]. The differential diagnosis between PD and PSP is often challenging, especially in the early stage of the disease, but is important because these diseases are characterized by different prognosis and response to dopaminergic therapy. The complex process of molecular pathogenesis of PD indicates that the disease, probably derives from several genetic and environmental factors. Considerable efforts have been made to identify the neuropathological, biochemical and genetic biomarkers of the disease so that a diagnosis can be established in the early stages. However, no biochemical marker is currently able to predict the course of the disease, the individual response to therapy or contribute to a clear differentiation between idiopathic PD and atypical parkinsonisms, such as PSP. It is known that at the base of neurodegenerative disorders, an important level of pathophysiological

* Corresponding author. Department of Experimental and Clinical Medicine, University "Magna Graecia" of Catanzaro, Catanzaro, 88100, Italy.
*E-mail addresses:* ida.manna@cnr.it (I. Manna), an.quattrone@hotmail.it (A. Quattrone), selene.db90@gmail.com (S. De Benedittis), basilio.vescio@biotecnomed.it (B. Vescio), iaccino@unicz.it (E. Iaccino), quattrone@unicz.it (A. Quattrone).
[1] These authors equally contributed.

https://doi.org/10.1016/j.parkreldis.2021.11.020
Received 21 June 2021; Received in revised form 16 November 2021; Accepted 18 November 2021
Available online 24 November 2021

complexity derives from the gene regulation of microRNAs (miRNAs) [3]. miRNAs have recently emerged as an important class of small RNAs (about 22 nucleotides) that act as post-transcriptional regulators of gene expression by base-pairing with their target mRNAs [4]. Several studies have shown a different expression of various miRNAs in the human brain, some of which regulate the expression of genes known to be associated with neurodegeneration [5]. Studies have linked several miRNAs to sporadic PD, like miR-133b was found to be specifically enriched in midbrain dopaminergic neurons of normal individuals and reduced in PD patients [6]; miR-433 binds to a polymorphism in the promoter region of the fibroblast growth factor 20 gene (FGF20) which is associated with PD [7]; miR-7 and miR-153 were shown to be predominantly expressed in the brain and to regulate α-synuclein expression levels [8]. On the contrary, few studies have investigated the role of miRNAs in PSP patients. *Tatura* et al. showed miR-147a and miR-158e dysregulation in the forebrain of a PSP cohort [9]. Numerous investigations have shown how these small molecules, previously identified in cells and tissues, are also present in extracellular biological fluids (plasma, serum, urine, saliva and cerebrospinal fluid), and suggested that miRNAs in the peripheral circulation are important biomarkers for the evaluation of diseases [10,11]. miRNAs were significantly enriched and more stable in exosomes when compared to cell-free biofluid samples [12]. Exosomes, extracellular vesicles with a diameter of 30–150 nm, are produced by a variety of cells and contain proteins, lipids, mRNA and miRNA, that are delivered to neighboring cells and/or are transported to distant sites. The exosome lipid bilayers protect miRNAs from nucleases degradation. Exosomes are also stable in blood and can be reliably detected at low concentrations using analytical methods. In this context, the study of exosomal miRNAs in body fluid is a good sample for noninvasive early diagnosis and prognostic evaluation for diseases. Even if the potential diagnostic value of cerebrospinal-derived exosomes has been demonstrated for PD [13], little is known about the exosomes released in the serum. In this pilot study, for the first time, we tested the hypothesis that a specific set of exosomal miRNAs could differentiate PD from PSP and thus represent a potential biomarker to be applied in clinical setting.

## 2. Materials and methods

### 2.1. Subjects, samples, and study design

A total of 108 subjects, 45 patients with PD, 24 patients with PSP and 39 healthy controls (HC) were enrolled in the current study. The patients were recruited consecutively between 2018 and 2020 among those referred to the Institute of Neurology at the University of Catanzaro, Italy. Clinical diagnosis of PSP and PD were established according to international diagnostic criteria by a neurologist with >10 years of experience in movement disorders. PSP patients were diagnosed according to the recent diagnostic criteria [2] as probable PSP-Richardson's syndrome (PSP-RS, n = 20) or PSP-Parkinsonism (PSP–P, n = 5). Clinical diagnosis PD was established according to recent international criteria [1]. For each patient, a clinical assessment was performed, including the Unified Parkinson's Disease Rating Scale – pars III (UPDRS-III) [14], the Hoen and Yahr rating scale (H–Y) and the Mini-Mental State Examination (MMSE), and the PSP rating scale (PSPRS) [15] in PSP patients. Exclusion criteria for patients were: history of neuroleptic use within the previous six months, clinical features suggestive of other diseases, and MRI abnormalities such as vascular lesions in the basal ganglia. HC were Caucasian unrelated subjects, who voluntarily agreed to donate serum samples to our study. None of HC had a history of neurological, psychiatric, or other major medical illnesses. Demographic and clinical characteristics of patients and healthy controls are summarized in Table 1. All participants gave written informed consent, and all study procedures and ethical aspects were approved by the institutional review board (Magna Graecia University review board, Catanzaro, Italy), according to the Helsinki Declaration.

**Table 1**
Demographic and clinical data of patients with Parkinson's disease, progressive supranuclear palsy and control subjects.

| Clinical parameters | PD patients | PSP patients | HC | p value |
|---|---|---|---|---|
| No of subjects | 45 | 24 | 39 | N/A |
| M/F | 26/19 | 15/9 | 15/24 | 0.11[d] |
| Age at sampling (years), mean (SD) | 66.4 ± 8.6 | 71.5 ± 5.4 | 63.7 ± 7.5 | 0.003[a] |
| Age of onset (years), mean (SD) | 62.8 ± 7.8 | 67.2 ± 3.4 | N/A | <0.001[b] |
| Disease duration (years), mean (SD) | 5.57 ± 4.1 | 4.43 ± 2.4 | N/A | 0.541[c] |
| MDS-UPDRS-III score, median (range) | 24 (19–66) | 47 (17–84) | N/A | <0.001[c] |
| H–Y score, median (range) | 2 (1–4) | 4 (2–5) | N/A | <0.001[c] |
| PSPRS score, median (range) | N/A | 46 (16–69) | N/A | N/A |
| LEDD (mg), mean (SD) | 498 ± 351 | 383 ± 284 | N/A | 0.213[c] |

Note - Abbreviations: PD = Parkinson's disease; PSP = progressive supranuclear palsy; MDS-UPDRS-III = Movement Disorder Society - Unified Parkinson's Disease Rating Scale pars III (motor examination); H–Y = Hoen-Yahr rating scale; PSPRS = progressive supranuclear palsy rating scale; LEDD = Levodopa equivalent daily dose.

[a] ANOVA test, followed by pairwise *t*-test: PD vs PSP, p = 0.03; PD vs HC, p = 0.3; HC vs PSP, p = 0.0004; p values adjusted according to Bonferroni.

[b] Two-sample *t*-test.

[c] Wilcoxon rank sum test.

[d] Fisher's exact test.

The study involves two phases: a first step of miRNA profiling analysis and a second step of validation of the results obtained on candidate miRNAs. For the first phase of global miRNA analysis, serum samples were collected from 15 subjects: 5 patients with PD (Age, mean ± SD: 71 ± 5.41), 5 patients with PSP (Age, mean ± SD: 72 ± 3.91) and 5 HC (Age, mean ± SD: 69 ± 4.38) using the TaqMan Advanced miRNA Human Serum/Plasma 96-well Plates, fast (Thermo Fisher Scientific, Waltham, MA, USA). This product contains a set of 2 plates for profiling of up to 188 unique miRNAs in serum samples, and includes endogenous and exogenous miRNA controls for normalization of data results. To approach the second phase of the study, on miRNAs selected and identified from the initial screening, a single assay analysis was performed on a new cohort of 92 subjects: 40 PD (Age, mean ± SD: 66 ± 9), 19 PSP (Age, mean ± SD: 70 ± 6) and 34 HC (Age, mean ± SD: 64 ± 8), not including the screening cohort. We investigated the potential diagnostic value of candidate miRNAs using receiver operator characteristic (ROC) curves. We also performed an *in silico* analysis in order to investigate whether the miRNAs differentially expressed in the study groups correlate with pathophysiologic mechanisms.

### 2.2. Serum processing and extraction of exosomes

Serum samples was collected from all subjects enrolled in the study. Before analysis, serum was processed within 2 h of collection by centrifugation at 3000*g* for 10 min at 4 °C. Exosomes from serum samples of all subjects were extracted and purified using ExoQuick Exosome Precipitation Solution kit (System Biosciences, Palo Alto, CA, USA), according to the manufacturer's protocol.

### 2.3. miRNA isolation from serum and RT-qPCR analysis

Total RNA, including small RNAs was extracted from 400 μl of serum-derived exosomes using the *mir*Vana Paris extraction kit (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. During RNA isolation, cel-miR-39 was added to samples as a non-human spike-in control. We utilized TaqMan Advanced miRNA cDNA Synthesis Kit (A28007, Thermo Fisher Scientific) to prepare the complementary cDNA from 2 μl of total RNA. The qPCR amplification was performed according to the manufacturer's protocol on a

QuantStudio 7 Flex Real-Time PCR System (Thermo Fisher Scientific). For the screening phase, data generated was analyzed using Expression Suite Software Version 1.0.3 (Thermo Fisher Scientific), a data-analysis tool that utilises the comparative Ct (ΔΔCt) method to quantify relative gene expression across a large number of miRNAs and samples. To obtain an accurate miRNa profiling, we used the global median normalization methods: Ct values from each sample were normalized to the median Ct of the plates. Relative quantification or Fold Change (FC), was calculated from Ct values according to the equations: $\Delta Ct = Ct$ (miRNA) - Ct (global mean) and $FC = 2^{-(\Delta Ct \text{ target} - \Delta Ct \text{ controls pool})}$. Expression Suite performs an unpaired *t-test* for biological group comparisons, assuming that Ct values for both groups follow a normal distribution. In the validation phase only differentially expressed miRNAs were considered candidate biomarkers and investigated in an independent study cohort, by using single TaqMan Advanced miRNA Assays (Thermo Fisher Scientific). The miRNA level was expressed by FC and data were normalized with the mean of expression level of 2 endogenous miRNAs: hsa-miR-186-5p, and hsa-miR-16-5p. These miRNAs resulted among the most stable, ubiquitously expressed and without reported impact on diseases.

### 2.4. Statistical analysis

Differences in gender distribution was assessed by Fisher's exact test. The Shapiro-Wilk test was used to check for normality to decide whether parametric or non-parametric tests were appropriate for comparisons. Differences in age at examination was compared among groups using ANOVA test followed by pairwise *t*-test, with p-values adjusted according to Bonferroni. Age at disease onset was compared between PSP and PD using two sample *t*-test. Differences in disease duration, MDS-UPDRS-III score, H–Y score and Levodopa equivalent daily dose (LEDD) were assessed using the Wilcoxon rank sum test. The Mann–Whitney *U* test, followed by the Benjamini-Hochberg correction for false discovery rate (FDR), was performed to assess the differences in exosomal miRNA levels between PD *and* HC, between PSP *and* HC, and between PSP *and* PD: data are presented in boxplots as medians with the interquartile range. The diagnostic efficacy was evaluated on those miRNAs, matched for age and sex, that were identified to be significant, by using receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC), with 95% confidence intervals. The miRNAs with AUC value above 0.8 were considered having high sensitivity and specificity. Significant changes in miRNA expression were expressed in Fold Change and defined as $p$ (PD vs HC; PSP vs HC; PSP vs PD) $\leq$ 0.05. A Random Forest analysis was used to sort miRNAs according to their level of importance and logistic regression analysis was performed to evaluate the classification accuracy of miRNA combinations. All statistical analyses were performed using GNU R Statistical Software, version 3.5.2 (2018, The R Foundation for Statistical Computing).

### 2.5. miRNA target prediction and pathway analysis

In order to assess the biological functions of the gene targets of dysregulated miRNAs, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were employed using DIANA miRPath version 3.0 [16]. The lists of up-regulated miRNAs were uploaded as inputs to DIANA miRPath, and the Tarbase v7.0 database was selected to perform analysis of validated target [17]. The human KEGG and GO analyses were performed with *p-value* threshold at 0.01, and pathaway/categories union were selected to combine results. The output of the program provides an overview of the pathway modulated by selected miRNAs.

## 3. Results

### 3.1. Exosonal miRNAs discovery set analysis in PSP and PD patients

The characteristics of the participant included in this study are shown in Table 1. PSP patients showed higher disease severity than PD patients but similar disease duration and levodopa equivalent daily dose (LEDD). In the discovery phase, after a preliminar exosome purification and validation (Supplemental Fig. 1) to identify differentially expressed exosomal miRNAs in serum of PD and PSP patients, we profiled the expression of 188 miRNAs by using TaqMan miRNA assay. Gene Expression Suite Software was used to process miRNA expression data from the miRNA panel. The expression level of miRNAs was measured in serum exosomes of a small discovery cohort of patients, 5 PD and 5 PSP, and 5 HC. In this analysis, exosomal expression levels were compared between the groups. We identified 8 miRNAs with a FC of $\geq$1.4 and a *p*-value cutoff of $<$0.05 (Supplemental Table 1). We selected a low FC to ensure that we captured a set of differentially expressed miRNAs within the discovery phase of the study.

### 3.2. Validation set analysis of exosomal miRNAs in an independent cohort

In order to investigate these results, we tested the differentially expressed exosomal miRNAs in an independent validation cohort of 40 PD patients, 20 PSP patients, and 33 HC, employed a qRT-PCR based approach in three comparisons: PD vs HC, PSP vs HC, and PSP vs PD. Then, to evaluate the utility of serum miRNAs, and their diagnostic accuracy, ROC curves analysis was computed for differentially expressed miRNAs. In addition, the miRNAs highest importance evaluated by Random Forest analysis, was used to fit a logistic regression model, to establish the diagnostic profiles of miRNAs with the best performance achieved between disease groups and controls. In PSP and HC comparison we identified two miRNAs, miR-22-3p and miR-425-5p, significantly up-regulated in PSP patients (Fig. 1A), but these differences did not survive after controlling FDR (Supplemental Table 2A). However, ROC analysis showed that the combination of these miRNAs discriminated PSP patients from HC with AUC of 0.75, 95% CI 0.60 to 0.90, with sensitivity and specificity of 0.58 and 1, respectively (Fig. 1B). Between PSP and HC, the best miRNAs profile showed an AUC 0.90, 95% CI 0.80 to 0.99, with sensitivity and specificity of 0.89 and 0.94, respectively (Fig. 1 C). The comparison between PD patients and HC samples highlighted three significantly dysregulated miRNAs in PD patients: miR-22-3p, and miR-223-5p were up-regulated, and miR-21-3p down-regulated (Fig. 2A, and Supplemental Table 2B). Such combination discriminated PD patients from HC with AUC of 0.75, 95% CI 0.63 to 0.86, with sensitivity and specificity of 0.73 and 0.78, respectively (Fig. 2 B). The best miRNAs combination resulting from Random Forest analysis for PD *vs* HC showed an AUC 0.77, 95% CI 0.65 to 0.87, with sensitivity and specificity of 0.58 and 1, respectively (Fig. 2 C). Finally, the comparison PSP and PD revealed that miR-21-3p, miR-199a-5p, and miR-425-5p, were significantly up-regulated in PSP patients *vs* PD patients (Fig. 3A), and all these differences survived to FDR control (Supplemental Table 2C). This combination showed an AUC of 0.86, 95% CI 0.74 to 0.97, with sensitivity and specificity of 0.84 and 0.85, respectively (Fig. 3 B). The best miRNAs profile discriminating PSP from PD, was given by combination of miR-21-3p, miR-199a-5p, miR-425-5p, miR-483-5p, miR-22-3p, and miR-29a-3p with the highest AUC = 0.91, 95% CI 0.82 to 1, with diagnostic sensitivity and specificity of 0.89 and 0.90, respectively (Fig. 3 C).

### 3.3. In silico analysis of potential pathway and target

To explore the biological functions of dysregulated miRNAs in PSP serum when compared to PD, GO and KEGG pathways enrichment analyses were performed using DIANA miRPath v3.0 with *P* value

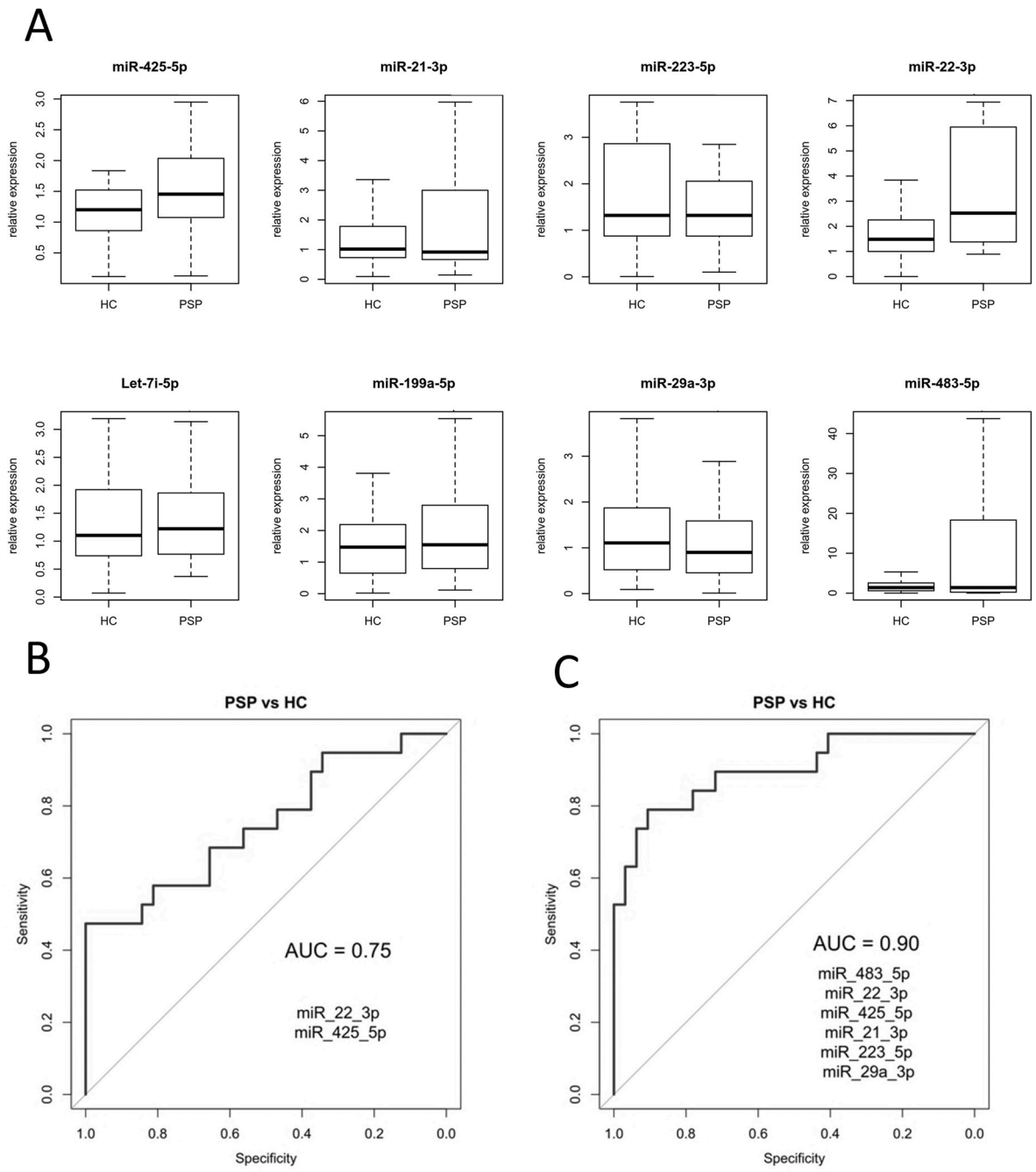


**Fig. 1. Differentially expressed miRNAs in PSP patients compared to HC.** (A) Box plot showing the relative expression levels of analyzed miRNAs in validation cohort, on the y-axis are reported FC values ($2^{-DDCt}$). Statistical significance was evaluated by Mann-Whitney *U* test (*p-value* < 0.05). (B) ROC curve of the subset of statically significant miRNAs. (C) ROC curve of the miRNAs based on logistic regression model created according to their level of importance established by Random Forest analysis.

threshold at 0.01 and TarBase v 7.0. GO enrichment analysis showed 72 biological processes associated with up-regulated miRNAs (Supplemental Table 2). KEGG pathway enrichment analysis showed that gene targets of up-regulated miRNAs were significantly involved ($P < 0.01$) in 9 pathways (Supplemental Table 3). Among them, *Fatty acid biosynthesis* (p-value <1e-325, 1 gene), *ECM-receptor interaction* (p-value <1e-325, 7 genes), *Fatty acid metabolism* (p-value 1.052277e-08, 5 genes), and *Hippo signaling pathway* (p-value 6.556339e-07, 20 genes) were the 4 most prominent pathways enriched. All biological pathways could be involved in development of PSP.

## Discussion

There is a great interest in the field of diagnostic research in the identification of specific non-invasive biomarkers that can discriminate between PD and atypical parkinsonian syndromes such as PSP, because the symptoms are overlapping and heterogeneous, especially in the early stages of the disease, making clinical differentiation difficult. To date, the most robust biomarkers to distinguish between PSP and PD are based on MR imaging [18–20], and especially on morphometry of brainstem structures and third ventricle [21,22]. Another approach is the development of peripheral biomarkers utilizing biofluids, which reflect molecular changes in the brain and may be used alternatively or in

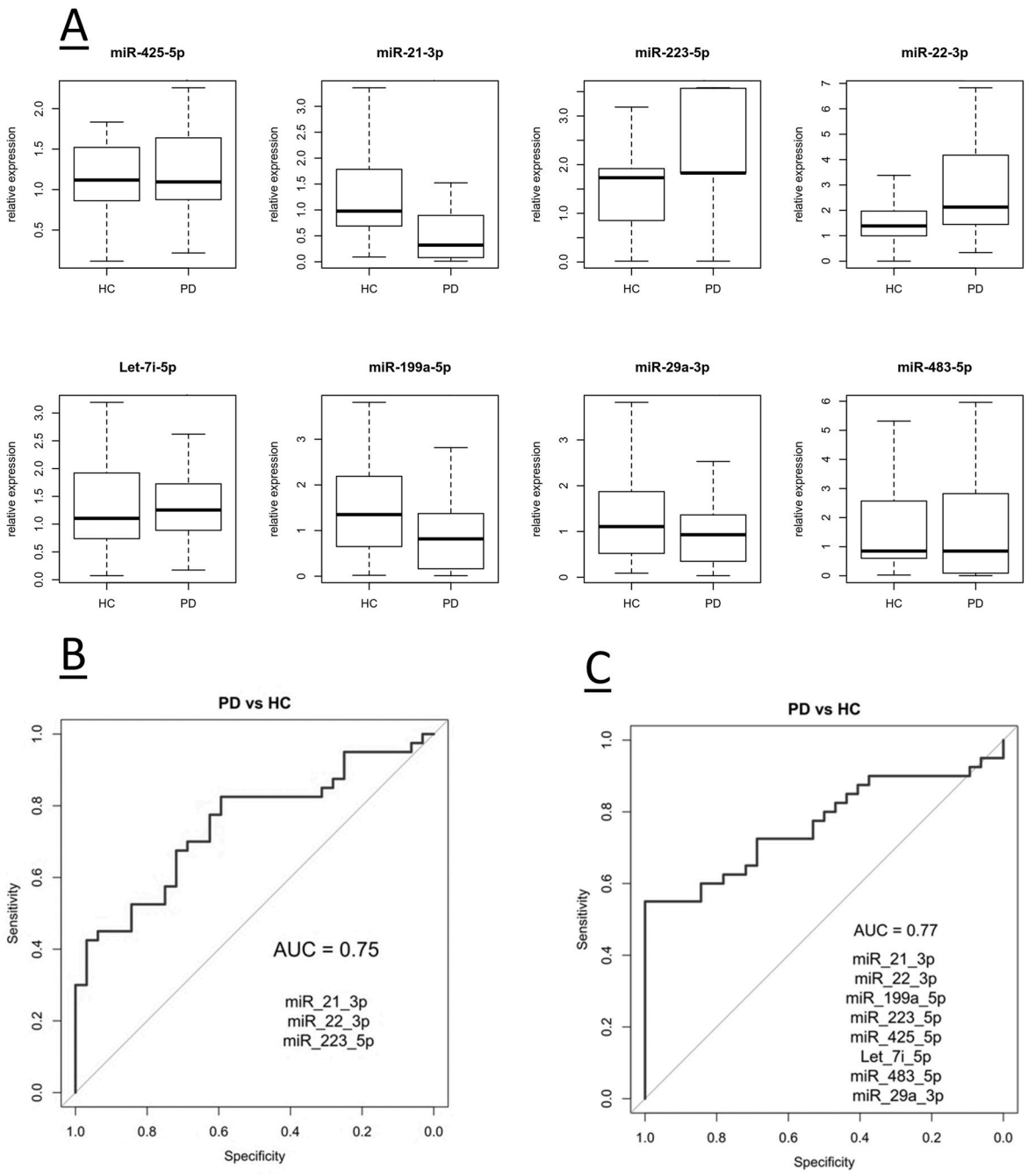


**Fig. 2. Differentially expressed miRNAs in PD patients compared to HC.** (A) Box plot showing the relative expression levels of analyzed miRNAs in validation cohort, on the y-axis are reported FC values ($2^{\wedge -DDCt}$). Statistical significance was evaluated by Mann-Whitney *U* test (*p-value* $< 0.05$). (B) ROC curve of the subset of statically significant miRNAs. (C) ROC curve of the miRNAs based on logistic regression model created according to their level of importance established by Random Forest analysis.

combination with imaging biomarkers. It is known that exosomes perform a cargo function, transporting various biomolecules, as proteins, functional mRNAs and miRNAs, thus mediating, also long-distance cell-to-cell communication processes [23]. In particular, exosomal miRNAs have emerged as candidate circulating biomarkers because they are protected from endogenous RNase and are present in a stable form, and are easily detectable even in small concentrations [24]. Actually, exosome-based biomarkers represent a non-invasive source for researching potential miRNA biomarkers for neurodegenerative disease [25], even if, a global view of miRNA roles in the involvement of neurodegenerative disease remains incomplete. Recently, numerous studies have reported the important functions of exosomal miRNAs in disease development and the potential clinical application as diagnostic biomarkers [26]. The use of exosomes as a source of miRNA biomarkers has led to standardized protocols for the isolation and analysis of exosomes from cell lines and biological fluids. Exosomes can be isolated from peripheral blood using several methods and, more recently, using exosomal isolation kits now available on the market. In this regard, a recent advance is the isolation of CNS-derived exosomes isolated from blood, which may represent a useful source of biomarkers for various neurological conditions. Enrichment of exosomes derived from specific types of brain cells, such as neurons, astrocytes and oligodendrocytes could provide a more specific and useful source of biomarkers. However, this application is not without methodological limitations [27].

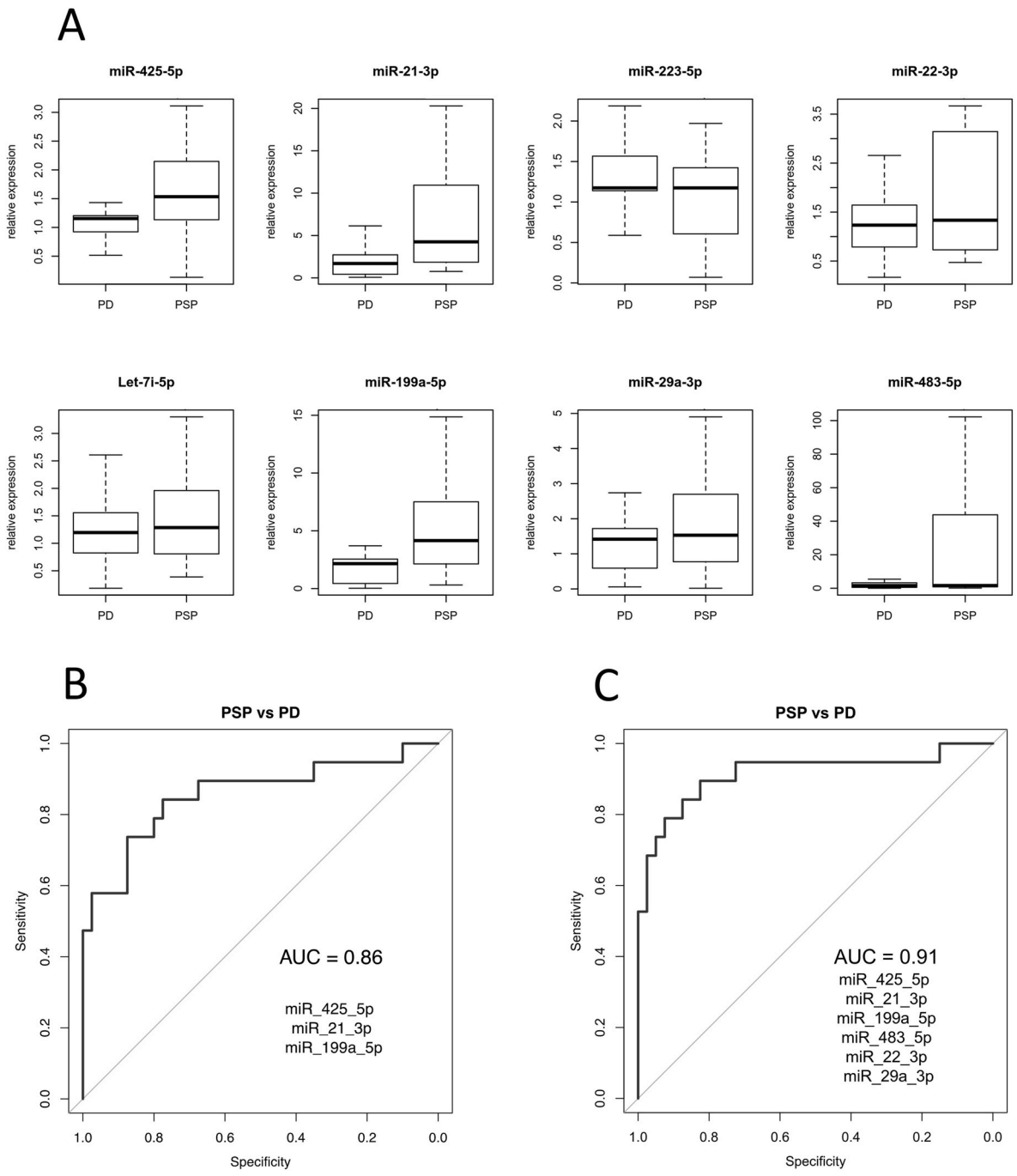


**Fig. 3. Differentially expressed miRNAs in PSP patients compared to PD.** (A) Box plot showing the relative expression levels of analyzed miRNAs in validation cohort, on the y-axis are reported FC values ($2^{-\Delta\Delta Ct}$). Statistical significance was evaluated by Mann-Whitney *U* test (*p-value* $< 0.05$). (B) ROC curve of the subset of statically significant miRNAs. (C) ROC curve of the miRNAs based on logistic regression model created according to their level of importance established by Random Forest analysis.

Searching for biomarkers in the blood exosome is a quite practical way to diagnose neurodegenerative diseases. To date, only a few studies have investigated the levels of miRNAs present in exosomes collected from peripheral biofluids and their potential as biomarkers of PD and/or parkinsonism but none of these concern patients with PSP [13]. Our study demonstrates that combinatory serum exosomal miRNAs signatures can differentiate between PD and PSP. The combination of a few miRNAs (hsa-miR-425-5p, hsa-miR-21-3p and hsa-miR-199a) in serum, showed good discrimination between these two diseases, with AUC of 0.86. In addition, a logistic regression analysis was performed to generate the best model for differentiating PSP from PD. The created model included a 6-miRNAs panel (miR-21-3p, miR-199a-5p, miR-425-5p, miR-483-5p, miR-22-3p, and miR-29a-3p), with the highest importance evaluated by Random Forest analysis. The AUC from the ROC analysis increased to 0.91. Hence, our preliminary results, from a pilot study, allow us to suggest exosomal miRNA panels provided good diagnostic discrimination. These results, if validated independent studies, could be used to support clinical differential diagnosis between PSP and PD. Finally, we performed a GO and KEGG pathway analysis to investigate the possible role of the identified dysregulated exosomal miRNAs in biological mechanisms involved in PSP. Results from GO analysis indicated that our dysregulated miRNAs regulate the expression

of key genes involved in several physiological process. Whereas, KEGG pathway analysis showed that these exosomal miRNAs are involved in different biological pathways that might be related to neurodegeneration. In PSP compared to PD, the most significant miRNAs targeted pathway are *Fatty acid biosynthesis*, *ECM-receptor interaction*, *Fatty acid metabolism*, and *Hippo signaling pathway*. The Hippo signaling pathway is known to be involved in cell proliferation and apoptosis and, to our knowledge, has not been reported to be associated with atypical parkinsonism, for which relevance can only be speculated. The extracellular matrix (ECM) is a dynamic structure that supports multiple physiological processes. It acts as an adhesion site for various cells and serves as a storage site for different signaling molecules, growth factors, and proteins in general, thus influencing development and migration of the cells. It has been shown that matrix metalloproteinases (MMPs) are involved in the degradation of ECM components [28], and the expression and activity of MMP-2 and -9, a subset of MMPs were found increased in PSP brain tissue and CSF samples [29]. Growing evidence indicates that alterations in metabolism and fatty acid levels lead to the onset and progression of neurodegenerative diseases, in particular, the metabolism of fatty acids is involved in neuroinflammation and neurodegeneration and at the same time is involved in neuronal repair mechanisms [30].

This study is not without limitations. First, our findings were obtained in patients with well-established disease, already under antiparkinsonian medications. However, no differences were found in disease duration and levodopa equivalent daily dose between PSP and PD patients. Second, this study was performed in cohort from a single center. Future studies to validate these findings in independent cohorts of PD and PSP patients, also at the early stage of the disease, are warranted to confirm the usefulness of exosomal miRNAs as biomarker for PD and PSP.

In summary, we performed a single-center pilot study on serum exosomal miRNAs profiles in PD and PSP patients, and we found that the exosome-derived miRNA subset is highly accurate for discriminating between PSP and PD. Profiles of exosomal miRNAs deserve more attention in order to establish their emerging and potential role in PSP.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Authors' roles

Ida Manna, PhD Study concept or design, Analysis or interpretation of data, Drafting/revising the manuscript for content.

Andrea Quattrone, MD Analysis or interpretation, Drafting/revising the manuscript for content.

Selene De Benedittis, PhD Analysis or interpretation of data, Drafting/revising the manuscript for content.

Basilio Vescio, PhD Statistical analysis, Analysis or interpretation of data.

Enrico Iaccino, PhD Study concept or design, Analysis or interpretation of data, Drafting/revising the manuscript for content.

Aldo Quattrone, MD Study concept or design, Analysis or interpretation of data, Drafting/revising the manuscript for content.

All authors have seen and approved the final version.

## Financial disclosures

I. Manna has nothing to disclose.
A. Quattrone has nothing to disclose.
S. De Benedittis has nothing to disclose.
B. Vescio has nothing to disclose.
E. Iaccino has nothing to disclose.
A. Quattrone has nothing to disclose.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.11.020.

## References

[1] R.B. Postuma, D. Berg, M. Stern, W. Poewe, C.W. Olanow, W. Oertel, J. Obeso, K. Marek, I. Litvan, A.E. Lang, G. Halliday, C.G. Goetz, T. Gasser, B. Dubois, P. Chan, B.R. Bloem, C.H. Adler, G. Deuschl, MDS clinical diagnostic criteria for Parkinson's disease, Mov. Disord. 30 (2015) 1591–1601.

[2] G.U. Höglinger, G. Respondek, M. Stamelou, C. Kurz, K.A. Josephs, A.E. Lang, B. Mollenhauer, U. Müller, C. Nilsson, J.L. Whitwell, T. Arzberger, E. Englund, E. Gelpi, A. Giese, D.J. Irwin, W.G. Meissner, A. Pantelyat, A. Rajput, J.C. van Swieten, C. Troakes, A. Antonini, K.P. Bhatia, Y. Bordelon, Y. Compta, J.C. Corvol, C. Colosimo, D.W. Dickson, R. Dodel, L. Ferguson, M. Grossman, J. Kassubek, F. Krismer, J. Levin, S. Lorenzl, H.R. Morris, P. Nestor, W.H. Oertel, W. Poewe, G. Rabinovici, J.B. Rowe, G.D. Schellenberg, K. Seppi, T. van Eimeren, G. K. Wenning, A.L. Boxer, L.I. Golbe, I. Litvan, Movement Disorder Society-endorsed PSP Study Group, Clinical diagnosis of progressive supranuclear palsy: the movement disorder society criteria, Mov. Disord. 32 (2017) 853–864.

[3] X.F. Jin, N. Wu, L. Wang, J. Li, Circulating microRNAs: a novel class of potential biomarkers for diagnosing and prognosing central nervous system diseases, Cell. Mol. Neurobiol. 33 (2013) 601–613.

[4] J.S. Satterlee, S. Barbee, P. Jin, A. Krichevsky, S. Salama, G. Schratt, D.Y. Wu, Noncoding RNAs in the brain, J. Neurosci. 27 (2007) 11856–11859.

[5] S.M. Eacker, T.M. Dawson, V.L. Dawson, Understanding microRNAs in neurodegeneration, Nat. Rev. Neurosci. 10 (2009) 837–841.

[6] K.C. Sonntag, MicroRNAs and deregulated gene expression networks in neurodegeneration, Brain Res. 1338 (2010) 48–57.

[7] J. Kim, K. Inoue, J. Ishii, W.B. Vanti, S.V. Voronov, E. Murchison, G. Hannon, A. Abeliovich, A MicroRNA feedback circuit in midbrain dopamine neurons, Science 317 (2007) 1220–1224.

[8] G. Wang, J.M. van der Walt, G. Mayhew, Y.J. Li, S. Züchner, W.K. Scott, E. R. Martin, J.M. Vance, Variation in the microRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of alpha-synuclein, Am. J. Hum. Genet. 82 (2008) 283–289.

[9] E. Doxakis, Post-transcriptional regulation of alpha-synuclein expression by mir-7 and mir-153, J. Biol. Chem. 285 (2010) 12726–12734.

[10] R. Tatura, M. Buchholz, D.W. Dickson, J. van Swieten, C. McLean, G. Hoglinger, U. Muller, microRNA profiling: increased expression of miR-147a and miR-518e in progressive supranuclear palsy (PSP), Neurogenetics 17 (2016) 165–171.

[11] S.K. Khoo, D. Petillo, U.J. Kang, J.H. Resau, B. Berryhill, J. Linder, L. Forsgren, L. A. Neuman, A.C. Tan, Plasma-based circulating MicroRNA biomarkers for Parkinson's disease, J. Parkinson Dis. 2 (2012) 321–331.

[12] K. Burgos, I. Malenica, R. Metpally, A. Courtright, B. Rakela, T. Beach, H. Shill, C. Adler, M. Sabbagh, S. Villa, W. Tembe, D. Craig, K. Van Keuren-Jensen, Profiles of extracellular miRNA in cerebrospinal fluid and serum from patients with Alzheimer's and Parkinson's diseases correlate with disease status and features of pathology, PLoS One 9 (2014), e94839.

[13] H. Dong, C. Wang, S. Lu, C. Yu, L. Huang, W. Feng, H. Xu, X. Chen, K. Zen, Q. Yan, W. Liu, C. Zhang, C.Y. Zhang, A panel of four decreased serum microRNAs as a novel biomarker for early Parkinson's disease, Biomarkers 21 (2016) 129–137.

[14] L. Cheng, R.A. Sharples, B.J. Scicluna, A.F. Hill, Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood, J. Extracell. Vesicles 3 (2014).

[15] Y. Gui, H. Liu, L. Zhang, W. Lv, X. Hu, Altered microRNA profiles in cerebrospinal fluid exosome in Parkinson disease and Alzheimer disease, Oncotarget 6 (2015) 37043–37053.

[16] C.G. Goetz, B.C. Tilley, S.R. Shaftman, G.T. Stebbins, S. Fahn, P. Martinez-Martin, W. Poewe, C. Sampaio, M.B. Stern, R. Dodel, B. Dubois, R. Holloway, J. Jankovic, J. Kulisevsky, A.E. Lang, A. Lees, S. Leurgans, P.A. LeWitt, D. Nyenhuis, C. W. Olanow, O. Rascol, A. Schrag, J.A. Teresi, J.J. van Hilten, N. LaPelle, Movement disorder society UPDRS revision task force. Movement disorder society-sponsored revision of the unified Parkinson's disease rating scale (MDS-UPDRS): scale presentation and clinimetric testing results, Mov. Disord. 23 (2008) 2129–2170.

[17] L.I. Golbe, P.A. Ohman-Strickland, A clinical rating scale for progressive supranuclear palsy, Brain 130 (2007) 1552–1565.

[18] I.S. Vlachos, K. Zagganas, M.D. Paraskevopoulou, G. Georgakilas, D. Karagkouni, T. Vergoulis, T. Dalamagas, A.G. Hatzigeorgiou, DIANA-miRPath v3.0: deciphering microRNA function with experimental support, Nucleic Acids Res. 43 (2015) 460–466.

[19] S.D. Hsu, F.M. Lin, W.Y. Wu, C. Liang, W.C. Huang, W.L. Chan, W.T. Tsai, G. Z. Chen, C.J. Lee, C.M. Chiu, C.H. Chien, M.C. Wu, C.Y. Huang, A.P. Tsou, H. D. Huang, miRTarBase: a database curates experimentally validated microRNA-target interactions, Nucleic Acids Res. 39 (2011) D163–D169.

[20] J.L. Whitwell, G.U. Höglinger, A. Antonini, Y. Bordelon, A.L. Boxer, C. Colosimo, T. van Eimeren, L.I. Golbe, J. Kassubek, C. Kurz, I. Litvan, A. Pantelyat, G. Rabinovici, G. Respondek, A. Rominger, J.B. Rowe, M. Stamelou, K.A. Josephs, Movement Disorder Society-endorsed PSP Study Group. Radiological biomarkers for diagnosis in PSP: where are we and where do we need to be? Mov. Disord. 32 (2017) 955–971.

[21] A.L. Boxer, J.T. Yu, L.I. Golbe, I. Litvan, A.E. Lang, G.U. Höglinger, Advances in progressive supranuclear palsy: new diagnostic criteria, biomarkers, and therapeutic approaches, Lancet Neurol. 16 (2017) 552–563.
[22] G. Barbagallo, M. Morelli, A. Quattrone, C. Chiriaco, M.G. Vaccaro, D. Gullà, F. Rocca, M. Caracciolo, F. Novellino, A. Sarica, G. Arabia, U. Sabatini, A. Quattrone, In vivo evidence for decreased scyllo-inositol levels in the supplementary motor area of patients with Progressive Supranuclear Palsy: a proton MR spectroscopy study, Park. Relat. Disord. 62 (2019) 185–191.
[23] S. Nigro, A. Antonini, D.E. Vaillancourt, K. Seppi, R. Ceravolo, A.P. Strafella, A. Augimeri, A. Quattrone, M. Morelli, L. Weis, E. Fiorenzato, R. Biundo, R. G. Burciu, F. Krismer, N.R. McFarland, C. Mueller, E.R. Gizewski, M. Cosottini, E. Del Prete, S. Mazzucchi, A. Quattrone, Automated MRI classification in progressive supranuclear palsy: a large international cohort study, Mov. Disord. 35 (2020) 976–983.
[24] A. Quattrone, A. Antonini, D.E. Vaillancourt, K. Seppi, R. Ceravolo, A.P. Strafella, M. Morelli, S. Nigro, B. Vescio, M.G. Bianco, R. Vasta, P.P. Arcuri, L. Weis, E. Fiorenzato, R. Biundo, R.G. Burciu, F. Krismer, N.R. McFarland, C. Mueller, E. R. Gizewski, M. Cosottini, E. Del Prete, S. Mazzucchi, A. Quattrone, A new MRI measure to early differentiate progressive supranuclear palsy from de novo Parkinson's disease in clinical practice: an international study, Mov. Disord. 36 (2021) 681–689.
[25] H. Valadi, K. Ekstrom, A. Bossios, M. Sjostrand, J.J. Lee, J.O. Lotvall, Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (2007) 654–659.
[26] I. Manna, S. De Benedittis, A. Quattrone, D. Maisano, E. Iaccino, A. Quattrone, Exosomal miRNAs as potential diagnostic biomarkers in alzheimer's disease, Pharmaceuticals 13 (2020) 243.
[27] S. Rastogi, V. Sharma, P.S. Bharti, K. Rani, G.P. Modi, F. Nikolajeff, S. Kumar, The evolving landscape of exosomes in neurodegenerative diseases: exosomes characteristics and a promising role in early diagnosis, Int. J. Mol. Sci. 22 (2021) 440.
[28] I. Manna, A. Quattrone, S. De Benedittis, E. Iaccino, A. Quattrone, Roles of non-coding RNAs as novel diagnostic biomarkers in Parkinson's disease, J. Parkinsons 11 (2021) 1475–1489.
[29] S. Hornung, S. Dutta, G. Bitan, CNS-derived blood exosomes as a promising source of biomarkers: opportunities and challenges, Front. Mol. Neurosci. 19 (13) (2020) 38.
[30] X.Y. Cao, J.M. Lu, Z.Q. Zhao, M.C. Li, T. Lu, X.S. An, L.J. Xue, MicroRNA biomarkers of Parkinson's disease in serum exosome-like microvesicles, Neurosci. Lett. 644 (2017) 94–99.