

## Differential expression of miRNA 155 and miRNA 146a in Parkinson's disease patients

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### ABSTRACT

Parkinson's disease is a neurodegenerative disorder and its etiology is unknown, numerous studies show how different environmental factors can influence the development of disease. miRNAs are involved in several pathologies and their dysregulation contribute to different pathologies, also in neurodegenerative such as Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotrophic lateral sclerosis. In this study, we profiled the expression of different candidate miRNAs: miR-155, miR-26a, miR-146a, and miR132, in PBMCs of L-dopa treated Parkinson patients and unaffected controls (HCs). We investigated the expression of miRNAs by RT-real time PCR, the results were subjected to statistical analysis. miRNA-155-5p was generally up-regulated in PD patients compared to HCs whereas miRNA-146a-5p was down-regulated in PD patients in comparison to HCs. It is interesting to point out that the expression of miR-155-5p was modified by levodopa treatment, in fact a down-regulation of miR-155-5p in PD patients with the highest dosage was observed.

In conclusion, miRNA 155 could not only be a promising target for the anti-inflammatory therapy in PD but also a good candidate as a disease progression biomarker. The role of levodopa in modulating the levels of miRNA 155 requires further studies.

### 1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons, causing symptoms such as muscle rigidity, resting tremor, bradykinesia, and postural instability [1]. The etiology is unknown, but the interaction between genetic and environmental factors seems to be crucial in causing the disease. Several genes including  $\alpha$ -synuclein, Parkin, PINK and others are involved in pathogenesis of PD [2]. Numerous studies show how different environmental factors such as nutrition, exposure to metals and pesticides can influence the development of the disease [3–8]. Alpha-synuclein ( $\alpha$ -syn), one of the most abundant proteins in Lewy bodies and Lewy neuritis, plays a leading role in initiation and progression of Parkinson-like neurodegeneration [9]. Neuroinflammation has been increasingly studied as a chief mediator in the pathogenesis and progression of PD [10]. miRNAs, small non coding RNA, are involved in several pathologies since their activity consists in controlling the genetic expression and their dysregulation contribute to different pathologies, including PD [11]. miRNA could be perfect candidates as biomarkers for diseases

in which they are altered. Furthermore, they could be potentially used in order to monitor the progression of the disease. Peripheral blood mononuclear cells (PBMCs) share more than the 80% of the transcriptome with other tissues, including the SNC, so peripheral blood could be considered a great source of biomarkers being also widely available [12]. Several studies show how a dysregulation of miRNA is involved in the pathogenesis of different neurodegenerative diseases like: Alzheimer's disease, Parkinson's disease, Huntington's disease and Amyotrophic lateral sclerosis. The molecular mechanisms underlying the pathological implications of misregulated miRNA expression and the regulation of the key genes involved in neurodegenerative disorders remain largely unknown [13–16]. Since most PD symptoms are caused by a lack of dopamine in the striatum, many Parkinson's drugs are aimed at either temporarily replenishing or mimicking the action of dopamine, Levodopa is most commonly used drug [17].

In this study, we profiled the expression of different candidate PD miRNAs in PBMCs of L-dopa-treated PD patients and unaffected controls. We tested different miRNA such as miR-155, miR-26a, miR-146a and miR-132. We have selected these miRNAs because they are

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**Table 1**  
Demographic and clinical characteristics of study participants.

Group	Age, Mean $\pm$ SD	Sex, females/males	Disease duration, Mean $\pm$ SD	H&Y score, Median $\pm$ SD	Levodopa mg, Mean $\pm$ SD
PD(#37)	71,3 $\pm$ 9,6	0.8	8.3 $\pm$ 4.8	3.3 $\pm$ 1.2	458,3 $\pm$ 227,71
HC(#43)	60 $\pm$ 13.14	1.7			
p value	0.0031	0.8036			

commonly studied in neurodegenerative diseases, but to date only few studies have been conducted in PD patients except for miR-155 that has only been studied in a mouse model of Parkinson disease [18–23]. Our primary aim is to investigate the potential of circulating miRNAs as non-invasive diagnostic candidate biomarkers of PD patients.

## 2. Materials and methods

### 2.1. Samples

The peripheral blood of Sardinian PD patients, enrolled at the Neurology Clinic of the University Hospital of Sassari, Italy, and Healthy Controls (HCs) provided by a family physician of Li Punti district, Sassari, Italy, were collected. The diagnosis of PD was based on the established criteria [24]. The cohort included 37 PD patients (M/F = 0.8, mean age 71.3  $\pm$  9.6, mean disease duration 8.3  $\pm$  4.8 years, mean Hoehn-Yahr scale 3.3  $\pm$  1.2) and 43 HCs (M/F = 1.7, mean age 60  $\pm$  13.14), Table 1. Immediately after collection, PBMCs were isolated from 10 ml of blood by density gradient centrifugation on Ficoll-Paque Plus, (GE Healthcare Bioscience, Sweden), washed twice in phosphate-buffered saline (PBS), counted and stored at  $-80^{\circ}\text{C}$  with RNA later (Sigma) until further use.

The study was approved by ethics committee of the Azienda Sanitaria Locale 1, Sassari, Italy (Prot. N 22, 2015). The patients and the volunteers gave written informed consent.

### 2.2. miRNAs cDNA synthesis and real-time PCR

Purification of total RNA containing miRNA from PBMCs was performed using miRNeasy Mini kit (Qiagen, USA) according to the manufacturer's recommendations. Quality of extracted RNA was determined according to 260/280 absorbance ratio, measured by Nano Drop spectrometer (Thermo Scientific, USA). 500 ng/RNA were used in reverse-transcription reaction.

cDNA synthesis for miR-155, miR-132, miR-146a and miR-26a was fulfilled using a miScript II RT Kit (Qiagen) according to the manufacturer's leaflet. MiRNAs quantification was performed with Custom miScript miRNA PCR Array.

### 2.3. Heat maps

We performed heat maps using GeneGlobe Data Analysis Center (Qiagen). The heat map provides a visualization of the fold changes in expression between the selected groups for every gene in the array in the context of the array layout. The table provides the fold regulation data used for the map as well as the Comments associated with each one. The color of the square denotes the relative up- or down-regulation of the miRNA in that sample. In addition, it produces dendrograms for the rows and columns, which are computed using hierarchical clustering. The ordering of the rows and columns is the most compatible with the dendrograms.

### 2.4. Statistical analysis

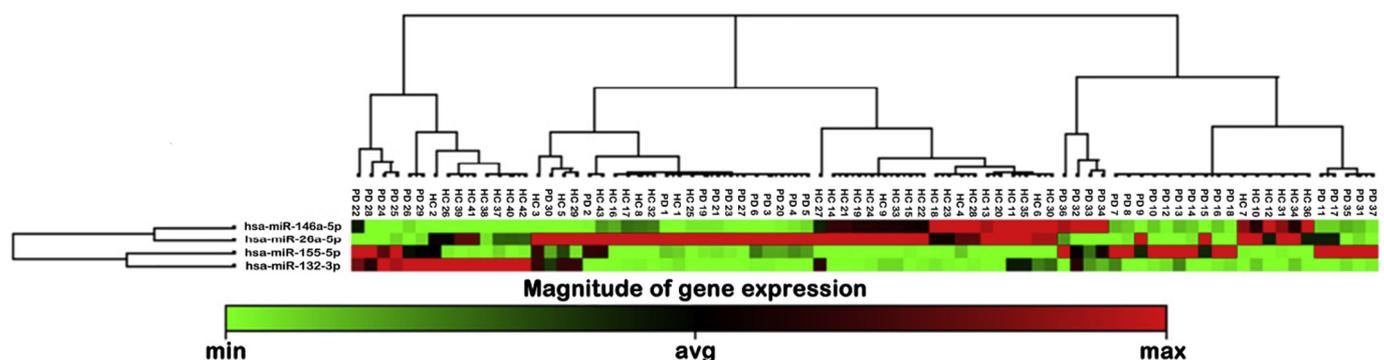
miRNAs data analysis was performed using the  $\Delta\Delta\text{CT}$  method by Qiagen miRNA detection software and final data were normalized for small nuclear RNA, miR-155 (median Ct = 24.86  $\pm$  0.614) PPC (median Ct = 21.27  $\pm$  0.302), RNU6-6P (median Ct = 23.34  $\pm$  0.116), SNORD68 (median Ct = 22.54  $\pm$  0.211) expression levels as endogenous controls.

## 3. Results

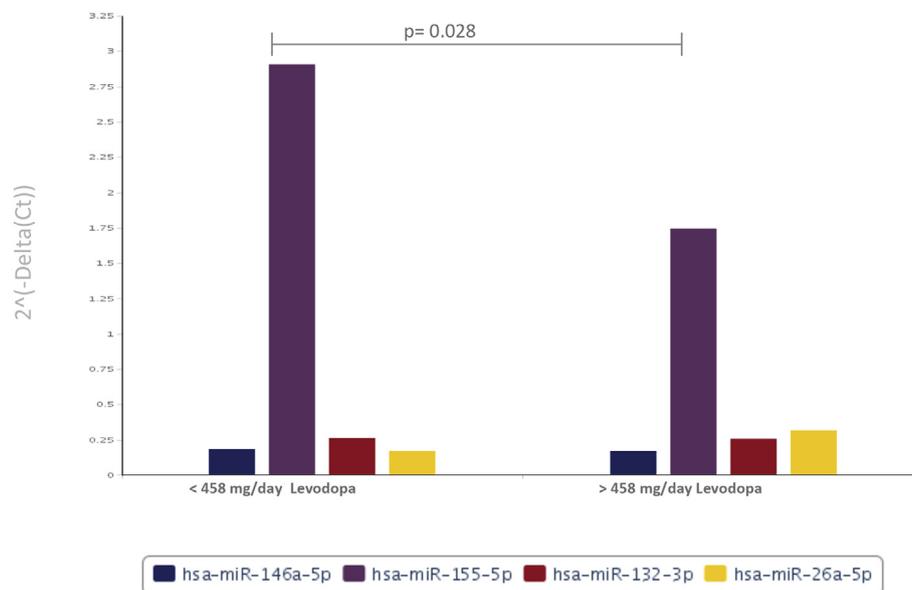
### 3.1. miRNA expression in patients with PD and their matched controls

microRNAs derived from PBMC samples of PD patients and HCs were extracted and the total miRNA isolation was analyzed for the expression of miRNA 155-5p, 146a-5p, 132-3p and 26a-5p.

The analysis of different miRNA expression showed that miRNA-155-5p (fold change = 27.18;  $p > .000001$ ) were generally up-regulated in PD patients compared to HCs whereas miRNA-146a-5p (fold change =  $-1.76$ ;  $p = .0015$ ) were down-regulated in PD patients in comparison to HCs. Other miRNA (miRNA-132-5p and miRNA-26a-5p) did not show a different expression between PD patients and HCs (Fig. 1).



**Fig. 1.** Heat map of microRNA (miRNA) microarray expression data from plasma samples of PD patients (PD 37) and Healthy controls (HCs 43). The miRNA species are shown on the left. Cluster analysis classified the samples into groups based on the miRNA expression levels in each sample. The dendrogram shows different expression levels of miRNA among samples. Red indicates high expression of miRNA, and green indicates relatively low expression of miRNA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 2.** The Column Chart showing the average miRNA expression in different grouped of PD patients. A) The PD patients are divided according to different dosage of Levodopa. B) The PD patients are divided according to different degree of disability. C) The PD patients are divided according to positivity of UL42 peptide.

### 3.2. miRNA expression in PD patients with different Levodopa dosage

Patients were classified into two groups based on the median daily dosage of levodopa (L-dopa): lower of 458 mg per day in the first group and > 458 mg per day in the second group. The statistical analysis with Mann Whitney Test showed no difference in the distribution of the two groups regarding to age and sex, with the respective  $p$  values of  $p > 0,7569$  for the age, and  $p > 0,7569$  for the sex. We analyzed the expression of different miRNA to verify if it can be modified by levodopa treatment. Upon quantification of the miRNAs 155-5p, 146a-5p, 132-3p and 26a-5p expression in the two groups we observed a down-regulation of miR-155-5p in PD patients with the highest dosage (fold change =  $-1.67$ ;  $p = .029$ ). The other miRNA did not show a different expression pattern in the different groups (Fig. 2A).

## 4. Discussion

miRNAs are more important in post-transcriptional regulation of target gene expression and each of them acts on the expression of a specific target. The maturation of miRNA is a highly controlled process and they undergo further post-transcriptional control. Abnormal miRNA expression may play a role in the pathogenesis of different diseases [25]. miRNAs appear to be suitable tools for the diagnosis, prognosis, and therapy of several disorders on the basis of their functional roles in diverse biological pathways.

Dysregulation of miRNA has been implicated in several neurological disorders, such as neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis and Huntington's disease. Numerous reports demonstrate the role of miRNAs in Parkinson's disease. Studies conducted to PD patients compared with healthy controls on the expression of different miRNA revealed that miR-34c-5p and miR-637 were significantly down-regulated in the amygdala of PD patients [26]. Other authors reported that miR-133b was down-regulated in the midbrain of PD patients compared with healthy controls (HCs) [27]. Several studies indicated miR-335,  $-374a/b$ ,  $-199$ ,  $-126$ ,  $-151-5p$ ,  $29b/c$ ,  $-147$ ,  $-28-5p$ ,  $-30b/c$ ,  $-301a$ , and  $-26a$  to be decreased in PBMCs of PD patients compared with controls [28].

In the present study, we investigated the expression of four miRNA (155-5p, 146a-5p, 132-3p and 26a-5p) in PD patients compared to HCs and analyzed their possible association to inflammation and

neurodegeneration. Our data showed an up-regulation of miR-155-5p in PD patients respect to HCs whereas miRNA 146a was under regulated. miR-155 in fact, it is up-regulated in the inflammatory processes with a particular ability to suppress the expression of anti-inflammatory molecules such as SOCS-1 and SOCS-3 [29]. Numerous studies indicate miR-155 to be over-expressed in inflammatory disorders of the central nervous system such as multiple sclerosis and amyotrophic lateral sclerosis [30]. In addition, studies on animal models of the two diseases document how the inhibition of this miRNA through the use of complementary oligonucleotides can reduce disease-related dysfunctions [31]. For the first time we observed an overexpression of miRNA 155 in PBMCs of PD patients. Indeed we confirmed what has been previously reported in an in vivo mouse model of PD produced by adeno-associated-virus-mediated expression of  $\alpha$ -syn. Suggesting that miR-155 has a central role in the inflammatory response to  $\alpha$ -syn in the brain [22].

In this context, our findings suggest that miR-155 is altered also in PD and, indirectly, could be a further evidence that inflammation may play an important role in the pathogenesis of this disease. It is also reported in the literature that, besides being miRNA a key regulator of neuroinflammation, it may be crucial in regulating neuroinflammation following the production of  $\alpha$ -syn oligomers. Indeed, in miR-155<sup>(-/-)</sup> mice there is a marked reduction in inflammation induced by  $\alpha$ -syn fibrils [22].

Regarding miRNA 146a, a downregulation was observed. The same data has been reported by Ma et al. [32]. A previous study conducted by Jayadev et al. in 2013 reported that miR-146a, a negative regulator of the monocyte pro-inflammatory response, is constitutively down-regulated in mice microglia with dysfunctional presenilin 2 (PS2) [33] whose mutations were shown to condition autosomal dominant AD [34]. MiRNA 146a acts regulating negatively the powerful pro-inflammatory pathway mediated by nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB), a transcription factor able to regulate inflammation, immunity and cell survival [35] and attenuating the proinflammatory response. Our results on miRNA 146a are in accordance with the above mentioned studies, where a downregulation is observed of this miRNA.

We also report that the expression of miR-155 is reduced in patients receiving a higher dosage of Levodopa. It remains to be seen whether the drug can somehow reduce the levels of this miRNA or indirectly it can reduce the inflammation. In contrast to many studies reporting that levodopa treatment can increase the levels of different miRNA

[36], we observed a consistent decrease in miRNA 155 in patients with higher doses of Levodopa. The alterations of miR-155 levels in untreated PD patients needs to be verified before and after therapy in order to assess a modulatory potential of this drug. We did not find any difference in expression levels of miRNA 155 when compared to the degree of disability.

In conclusion, miRNA 155 could not only be a promising target for the anti-inflammatory therapy in PD but also its evaluation can help the disease progression. The role of levodopa in modulating the levels of miRNA 155 requires further studies.

#### Conflict of interest

The authors have no conflicting financial interests.

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