Relationship of irisin with disease severity and dopamine uptake in Parkinson's disease patients

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Abstract

Background: This study was designed to investigate the relationship of irisin with the severity of Parkinson’s disease (PD) and dopamine (DOPA) uptake in patients with PD and to understand the role of irisin in PD and its potential value as a drug therapy.

Methods: The plasma levels of irisin and α-syn were measured by ELISA. Motor and nonmotor symptoms were assessed with related scales. DOPA uptake was measured with DOPA PET/MRI.

Results: The plasma level of α-syn and irisin in patients with PD increased and decreased gradually with the progression of the disease, respectively. There was a negative correlation between plasma α-syn and irisin levels in patients with PD. The level of irisin in plasma was negatively correlated with UPDRS-III scores and positively correlated with MOCA scores. The SORs of the ipsilateral and contralateral caudate nucleus, anterior putamen and posterior putamen in the high-Irisin group was significantly higher than those in the low-Irisin group, and irisin levels in the caudate nucleus, anterior putamen, and posterior putamen contralateral to the affected limb were lower than those on the ipsilateral side. The level of irisin was positively correlated with the SORs of the ipsilateral and contralateral caudate nucleus and putamen in PD patients.

Conclusions: Irisin plays a neuroprotective role by decreasing the level of α-syn. Irisin is negatively correlated with the severity of motor symptoms and cognitive impairment. More importantly, irisin can improve DOPA uptake in the striatum of patients with PD, especially on the side contralateral to the affected limb.

1. Introduction

Parkinson’s disease (PD) is the second most common neurodegenerative disease and is characterized by both motor and nonmotor dysfunction[1]. The main pathological characteristics of PD consist of the loss of dopaminergic neurons and the formation of Lewy body pathology [mainly α-synuclein (α-syn)] in the substantia nigra[2, 3]. Clinically, there are two types of treatments for PD: surgical therapy (deep brain stimulation) and medication (levodopa and other dopamine receptor agonists) [4]. While these treatments are very effective initially, their effectiveness diminishes over time, and a range of side effects emerges [5]. The currently existing medication treatments provide only symptomatic relief and do not control or prevent disease progression[6, 7]. As such, treatments that slow the progression or inhibit the underlying drivers of PD pathogenesis are urgently needed.

Irisin is an exfoliated extracellular domain of a transmembrane protein called FNDC5 that is secreted from skeletal muscle and increases with exercise[8, 9]. Exercise enhances the activity and expression of some receptors, such as peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) [10]. This increased expression results in the production of the FNDC5 protein[11]. Splitting this protein leads to the production of irisin[12]. Some studies have reported that factors such as irisin have a neuroprotective effect and can improve the functioning of the nervous system after central nervous
system (CNS) injury[13, 14]. Irisin has been observed to prevent the loss of dopamine neurons and the reduction of striatal dopamine levels and to improve motor dysfunction[15, 16].

To better explore the mechanism of action of irisin in PD and its potential value as a drug therapy, we investigated the correlation between irisin and α-syn. To further evaluate the relationship between irisin and PD motor and nonmotor symptom scales. Finally, the relationship between irisin and DOPA uptake in the striatum of PD patients was further clarified by DOPA PET/MRI.

2. Methods

2.1 Participant inclusion criteria

A total of 100 PD patients were recruited from the Department of Neurology, Henan Provincial People’s Hospital, between March 2020 and October 2022. Patients were diagnosed by two experienced neurologists according to the 2015 MDS clinical diagnostic criteria for Parkinson’s disease[17]. Patients with (1) atypical or secondary PD, (2) cardiovascular or cerebrovascular diseases, such as myocardial infarction or cerebral infarction, and (3) acute or chronic infections or surgical procedures within the previous 3 months were excluded. A total of 70 healthy volunteers participated in this study. All subjects signed written informed consent before participation.

2.2 Clinical Characteristics

General clinical data, such as sex, age, education levels and body mass index (BMI), were recorded. The levodopa equivalent daily dose (LEDD) was assessed according to the levodopa conversion formula [18]. In brief, 100 mg levodopa = 133 mg entacapone = 1 mg pramipexole = 5 mg ropinirole = 10 mg selegiline = 1 mg rasagiline = 100 mg amantadine. Disease duration was defined as the time between presentation with initial motor symptoms and the present study. Hoehn and Yahr (H-Y) staging was used to divide PD into stages 0 ~ 5 [19] (stages 1 ~ 2 are the early stages, and stages 2.5 ~ 5 are the middle and late stages). Motor symptoms were evaluated by Part III of the Unified Parkinson’s Disease Rating Scale (UPDRS)[20]. Both the H-Y classification and the UPDRSIII were used to evaluate disease severity. Cognition was examined by the Montreal Cognitive Assessment (MoCA)[21]. All of the assessments were completed once during a patient’s “on” period.

2.3 Sample collection

Between 07:30 and 08:30 am, after overnight fasting and before breakfast, peripheral blood from each subject was collected into EDTA-containing test tubes. Immediately after collection, blood was centrifuged (3000 rpm, 10 min) and preserved at -80°C until measurement. Participants were instructed to refrain from smoking, alcohol consumption, and vigorous activity for 24 h before the study.

2.4 Measurement of plasma irisin and αsyn levels

Plasma irisin levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (catalog number: E-EL-H5735c; Wuhan Elabscience Biotechnology Co., Ltd., Wuhan, China). The detection range of
this kit was 78.13–5000 pg/mL, with a sensitivity of 46.88 pg/mL, and the intra- and interassay variability were < 10%. Plasma α-syn levels were measured using an ELISA kit (catalog number: ZC-32191; Shanghai Zhuocai Biotechnology Co., Ltd., Shanghai, China). The detection range of this kit is 1.25-40 ng/mL, with a sensitivity of 0.1 ng/mL, and the intra- and interassay variability were < 10%. Samples were analyzed in duplicate on the same plate in accordance with the manufacturers’ instructions.

2.5 18F-DOPA PET/MRI imaging

18F-DOPA was prepared by the PET Center of Henan Provincial People's Hospital and was synthesized using a medical cyclotron (GE Minitrace, USA) and an automatic synthesis device (AllinOne, Belgium); the radiochemical purity of the product was > 95%. Subjects received an intravenous injection of 18F-DOPA (370 MBq). After approximately 90 min of quiet rest, PET/MRI was performed on the brain. The PET acquisition time was 30 min.

The following 3D-mode PET and MRI sequences were acquired: 3D T1-weighted imaging (repetition time (TR)/echo time (TE) = 7.2/3; matrix = 256 × 230; bandwidth = 250; layer thickness/interval = 1 mm/1.5 mm), 3D T2-weighted imaging (TR/TE = 2000/288.6; matrix = 256 × 230; bandwidth = 650; layer thickness/interval = 1 mm/1.5 mm), and 3D T2 fluid-attenuated inversion recovery (FLAIR; TR/TE = 6500/403.92; matrix = 256 × 230; layer thickness/interval = 1 mm/1.5 mm). The total collection time was approximately 20 min.

2.6 Image processing and analysis

Semiquantitative analysis of the striatal 18F-DOPA uptake index was conducted by an experienced deputy chief physician, who read the films while blinded to the patients’ diagnoses. The region of interest (ROI) was drawn on the fusion image on the PET workstation. First, the MRI images from the level of the striatum were selected and displayed, and the bilateral putamen and caudate nucleus were traced on each image. Next, these tracings were copied to the corresponding PET images to calculate the average radioactivity of the putamen and caudate nucleus on each side. In this study, the occipital lobe was selected as the reference area; three consecutive layers of the occipital lobe were selected. The ROI of the corresponding occipital lobe was drawn on each layer, and its average radioactivity was calculated. The striatal/occipital lobe uptake ratio (SOR) was used as the index of striatal 18F DOPA uptake [22].

2.7 Statistical analysis

The Kolmogorov–Smirnov test and Levene's test were used to check the data for normality and homogeneity of variance. Numerical variables that followed a normal distribution are expressed as the mean ± SD; those that did not follow a normal distribution are expressed as medians (interquartile ranges). Nominal variables are expressed as percentages. To compare two groups of normally distributed data, an independent-sample Student's t test was used. Multiple groups of data consistent with a normal distribution and homogeneity of variance were compared by one-way analysis of variance, and a post hoc least significant difference (LSD) test was used to further assess the differences through pairwise comparisons between groups. For variables that violated the assumption of normality or
homoscedasticity, the groups were compared using the nonparametric Mann–Whitney U test (for 2 groups) or Kruskal–Wallis test (for > 2 groups). Pearson's (or Spearman's) correlation analysis was conducted according to whether the variables were normally distributed. Statistical analysis was performed using SPSS version 22.0 (IBM Corporation, Armonk, NY, United States) and GraphPad Prism 7 (GraphPad Software, Inc., San Diego, CA, United States). A P value < 0.05 was considered statistically significant.

3. Results

3.1 Clinical characteristics

No differences were found between the PD and control groups in age (60.80±9.94 vs. 62.89±6.98, P = 0.132), sex (64.0% vs. 36.0%, P = 0.147), BMI (24.55±3.20 vs. 23.78±3.21, P=0.128) or education (9, 6, 12 vs. 9, 6, 12, P=0.669). Plasma α-syn levels were significantly higher in the PD patients than in the healthy controls (3.25±0.56 ng/ml vs. 2.62±0.51 ng/ml, P<0.001) and plasma irisin levels were significantly lower in the PD patients than in the healthy controls (238.61±39.08 pg/ml vs. 251.77±31.77 pg/ml, P=0.021).

3.2 Plasma α-syn and irisin levels in controls and PD patients at different H-Y stages

Plasma α-syn levels were significantly higher in PD patients than in healthy controls. When the PD patients were divided into early-stage (n = 58) and middle-late-stage PD patients (n = 42) based on the H-Y classification, it was noted that plasma α-syn levels in early-stage PD patients were higher than those in healthy controls (P <0.001), and plasma α-syn levels in middle- to late-stage PD patients were higher than those in early-stage PD patients (P = 0.017) (Fig. 1A).

Plasma irisin levels were significantly lower in PD patients than in healthy controls. When the PD patients were divided into early-stage (n = 58) and middle-late-stage PD patients (n = 42) based on the H-Y classification, plasma irisin levels in early-stage PD patients were found to be lower than those in healthy controls (P=0.037), and plasma irisin levels in middle-late-stage PD patients were lower than those in early-stage PD patients (P =0.016) (Fig. 1B).

3.3 Correlation of plasma α-syn and irisin levels in PD Patients

We found a negative correlation between plasma α-syn and irisin levels in PD patients (r = -0.605, P < 0.001) (Fig. 2).

3.4 Correlation of plasma irisin levels and clinical characteristics in PD patients

Correlation analysis showed that plasma irisin levels were negatively correlated with disease duration (r = -0.309, P =0.002) and UPDRS III scores (r = -0.524, P 0.001) and positively correlated with MOCA scores (r = 0.327, P =0.001). The LEDD were not related to plasma irisin levels (P > 0.05).

3.5 The relationship between irisin level and striatal DOPA uptake in patients with PD
With the mean irisin concentration of all PD patients as the cut-off point, PD patients were divided into a high-Irisin group and a low-Irisin group.

We obtained corresponding DOPA PET/MRI scans for all 100 of the enrolled PD patients; however, corresponding DOPA PET/MRI scans could be obtained for only 28 of the healthy controls.

### 3.5.1 Visual analysis

¹⁸F-DOPA PET images of typical cases in the healthy control group, the high-Irisin group and the low-Irisin group are shown in Figure 3. We found that the concentrations of ¹⁸F-DOPA in the caudate nucleus, the anterior putamen and the posterior putamen were higher in the healthy controls than in the PD group. Compared with the high-Irisin group, the low-Irisin group had reduced concentrations of ¹⁸F-DOPA in the caudate nucleus, the anterior putamen and the posterior putamen bilaterally.

### 3.5.2 Semiquantitative analysis

The SORs of the contralateral caudate nucleus, anterior putamen and posterior putamen in the healthy control group were significantly higher than those in the PD group (3.44±0.60 vs. 2.68±0.47, t=-7.080, P=0.001; 3.62±0.74 vs. 2.42±0.40, t=-11.465, P=0.001; 3.30±0.81 vs. 1.90±0.29, t=-14.409, P=0.001). And the SORs of the ipsilateral caudate nucleus, anterior putamen and posterior putamen in the healthy control group were significantly higher than those in the PD group (3.29±0.63 vs. 2.84±0.40, t=-4.597, P=0.001; 3.51±0.74 vs. 2.54±0.36, t=-9.790, P=0.001; 3.29±0.84 vs. 2.06±0.28, t=-12.374, P=0.001). Additionally, the SORs of the contralateral caudate nucleus, anterior putamen and posterior putamen in the high-Irisin group were significantly higher than those in the low-Irisin group (2.91±0.43 vs. 2.43±0.39, t=-5.919, P=0.001; 2.59±0.32 vs. 2.23±0.38, t=-5.140, P=0.001; 2.00±0.30 vs. 1.79±0.24, t=-3.923, P=0.001). On the ipsilateral side, the SORs of the caudate nucleus, anterior putamen and posterior putamen in the high-Irisin group were significantly higher than those in the low-Irisin group (2.96±0.40 vs. 2.71±0.37, t=-3.250, P=0.002; 2.64±0.35 vs. 2.42±0.36, t=-3.277, P=0.001; 2.13±0.26 vs. 2.00±0.28, t=-2.495, P=0.014) (Fig. 4).

We also found that the levels of irisin in the contralateral caudate nucleus, anterior putamen and posterior putamen of the affected limb were lower than those in the ipsilateral side of the affected limb, and the difference was statistically significant (2.68±0.47 vs. 2.84±0.40, t=-2.558, P=0.011; 2.42±0.40 vs. 2.54±0.36, t=-2.197, P=0.029; 1.90±0.29 vs. 2.07±0.28, t=-4.063, P=0.001).

### 3.5.3 Correlation Analysis

We found a positive correlation between plasma irisin levels and the SORs of the contralateral caudate nucleus, anterior putamen and posterior putamen in PD patients; the same was true for the ipsilateral side (Fig. 5).

### 4. Discussion
The main finding of this study is the relationship of plasma irisin levels with disease severity and dopamine uptake in patients with PD. Kim et al. [15] showed that irisin prevented pathological α-syn-induced neurodegeneration, loss of dopamine neurons and reduction in striatal dopamine in a mouse model of PD. These findings highlight the potential of irisin in the treatment of PD.

We found that the level of plasma irisin in patients with PD was significantly lower than that in healthy controls and gradually decreased with the progression of the disease. Upon exploring the neuroprotective role of irisin in PD, we further concluded that irisin had a significant negative correlation with α-syn. Accumulation of misfolded pathological α-syn is involved in the pathogenesis of PD, and available evidence suggests that in a mouse model, irisin can prevent the formation of pathological α-syn and protect neurons from its toxic effects[3, 15]. This study offers mechanistic evidence that the amelioration of α-syn pathology by irisin takes place via at least three pathways: 1. Irisin prevents the formation of pathologic α-syn and protects neurons against α-syn neurotoxicity. 2. Irisin can prevent the accumulation of pathological α-syn by reducing its internalization and aggregation. 3. Irisin prevents the pathological transmission of α-syn by promoting its endolysosomal degradation. Irisin is highly conserved between species and has 100% sequence identity between mice and humans [12]. In our study, we found that α-syn increased as irisin decreased in patients with PD. On the basis of our findings, we hypothesized that irisin may act on α-syn and thus exert neuroprotective effects in patients with PD.

In the present study, we showed that plasma irisin levels were negatively correlated with disease duration and UPDRS-III scores. Irisin largely prevented the behavioral deficits induced by α-syn preformed fibrils, as determined by the pole test and the grip strength test [15]. Higher plasma α-syn levels were significantly associated with worse UPDRS Part III motor scores and more advanced stages according to the modified H-Y classification [23].

Cognitive dysfunction is common in patients with PD [24, 25]. Recent studies have shown that irisin helps to preserve cognitive function through various mechanisms. Irisin is processed from the type I membrane protein encoded by the FNDC5 gene and is then secreted into the blood, where it circulates to several systems and passes through the blood–brain barrier (BBB) [12]. FNDC5 and irisin are expressed in many tissues, especially in the hippocampus and hypothalamus, and are important for memory and cognition [13, 26]. FNDC5 gene expression is elevated following the increase in PGC-1α expression induced by exercise in both central and peripheral organs, which stimulates the expression of BDNF in the brain [12]. Irisin, a myokine derived from FNDC5, also acts through PGC-1α and passes through the BBB to increase BDNF expression and enhance learning, memory, and mood [12]. FNDC5/irisin acts as a messenger of muscle–brain crosstalk, influencing neurogenesis in people with cognitive impairment, particularly through the neuroprotective effects of BDNF [27]. In the CNS, irisin causes neurogenesis and particularly the differentiation of stem cells into neurons in the hippocampus[28]. At the same time, our study confirms that irisin was negatively associated with cognitive severity in patients with PD.

In this study, we found for the first time that the SOR of the ipsilateral and contralateral caudate, anterior putamen, and posterior putamen was significantly higher in the high-Irisin group than in the low-Irisin
group among patients with PD. The levels of Irisin in the caudate nucleus, anterior putamen and posterior putamen contralateral to the affected limb were lower than those on the ipsilateral side. In PD patients, the plasma levels of Irisin were positively correlated with the SORs in the ipsilateral and contralateral caudate nucleus, anterior putamen and posterior putamen. \(^{18}\)F-DOPA, a radioactive marker similar to levodopa, binds specifically to presynaptic receptors when it enters the brain, and \(^{18}\)F-DOPA PET imaging is used to assess the function of central dopaminergic neurons as presynaptic cells [29]. The \(^{18}\)F-DOPA used in this study was produced by fully automatic synthesis, which made the process easy to carry out. Additionally, \(^{18}\)F-DOPA has the advantages of a long physical half-life, ease of use and good imaging performance. In a mouse model of PD, irisin injection reduced the loss of DA neurons in the striatum, and irisin was found to rescue both dopamine transporter (DAT)-positive and tyrosine hydroxylase (TH)-positive fibers as determined by immunoblotting. At the same time, irisin successfully prevented the accumulation of pathological \(\alpha\)-syn [15]. Interestingly, administration of irisin over 7 days may increase PGC-1\(\alpha\) expression in different areas of the brain, especially in the striatum and substantia nigra pars compacta, to protect dopaminergic neurons from degeneration [30].

Our study has some limitations. First, the number of enrolled subjects was small, and the small sample size limits the generalization of the results to all PD patients. Second, the design of this study is only cross-sectional. In the future, we will conduct a longitudinal follow-up study to further explore the relationship between Irisin and PD. Finally, at present, DOPA PET MRI is available only in tertiary hospitals and research institutions, which currently limits the scale of its use for PD diagnosis.

However, this study is noteworthy because it establishes a previously unreported relationship of irisin with disease severity and dopamine uptake in PD. The basis of this association is still unclear, but it still reveals that irisin plays an important role in the occurrence and development of PD and has great potential for its treatment.

5. Conclusions

This study was designed to investigate the relationship of irisin with the severity of Parkinson’s disease (PD) and dopamine (DOPA) uptake in patients with PD and to understand the role of irisin in PD and its potential value as a drug therapy. Our study conclusions that irisin plays a neuroprotective role by decreasing the level of \(\alpha\)-syn. Irisin is negatively correlated with the severity of motor symptoms and cognitive impairment. More importantly, irisin can improve DOPA uptake in the striatum of patients with PD, especially on the side contralateral to the affected limb. Irisin plays an important role in the occurrence and development of PD and has great potential for its treatment.

Declarations

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Declaration of competing interest

None.

Data Availability statement

The data used to support the results of this study were partially reflected in the article and could be obtained from the corresponding authors upon request if further need arises.

Ethics approval and consent to participate

This research has been approved by the Ethics Committee of Henan Provincial People's Hospital. All subjects agreed to participate and signed a written informed consent form.

Author Contributions

Xiaoxue Shi: Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

Qi Gu: Formal analysis, Writing - review & editing.

Chang Fu: Formal analysis, Writing - review & editing.

Jianjun Ma: Conceptualization, Resources, Supervision, Project administration, Writing - review & editing.

Dongsheng Li: Formal analysis, Investigation.


Siyuan Chen: Validation.

Zonghan She: Formal analysis, Investigation.

Xuelin Qi: Formal analysis, Investigation.

Xue Li: Formal analysis, Investigation.

Shaopu Wu: Formal analysis, Investigation.

Li Wang: Formal analysis, Investigation.

Consent to participate and publish

Informed consent was obtained from all individual participants included in the study.
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References


**Figures**

**A**

![Graph A](image)

**B**

![Graph B](image)

**Figure 1**

Distribution of plasma α-syn and irisin levels in PD patients classified by H-Y stages. (A) Distribution of plasma α-syn levels in different H-Y stages. (B) Distribution of plasma irisin levels in different H-Y stages.
Correlations between plasma α-syn and irisin levels in PD patients.
Figure 3

$^{18}$F-DOPA PET images of healthy volunteers in the control group $^{18}$F-DOPA PET imaging in high-Irisin group of patients with PD $^{18}$F-DOPA PET imaging in low-Irisin group of patients with PD.
Figure 4

(A) The SORs of the contralateral caudate nucleus in high-Irisin and low-Irisin in PD patients. (B) The SORs of the ipsilateral caudate nucleus in high-Irisin and low-Irisin in PD patients. (C) The SORs of the contralateral anterior putamen in high-Irisin and low-Irisin in PD patients. (D) The SORs of the ipsilateral anterior putamen in high-Irisin and low-Irisin in PD patients. (E) The SORs of the contralateral posterior
putamen in high-Irisin and low-Irisin in PD patients. (F) The SORs of the ipsilateral posterior putamen in high-Irisin and low-Irisin in PD patients.

Figure 5

(A) Correlations between the SORs of the contralateral caudate nucleus and plasma irisin levels in PD patients. (B) Correlations between the SORs of the ipsilateral caudate nucleus and plasma irisin levels in
PD patients. (C) Correlations between the SORs of the contralateral anterior putamen and plasma irisin levels in PD patients. (D) Correlations between the SORs of the ipsilateral anterior putamen and plasma irisin levels in PD patients. (E) Correlations between the SORs of the contralateral posterior putamen and plasma irisin levels in PD patients. (F) Correlations between the SORs of the ipsilateral posterior putamen and plasma irisin levels in PD patients.