Original Articles

Salivary ATP13A2 is a potential marker of therapy-induced motor complications and is expressed by inclusions in submandibulary glands in Parkinson’s disease

Emilio Fernández-Espejo a,b, Ana L. Gavito b,c, Juan Suárez b,c,d, Eduardo Tolosa e,f,g, Dolores Vilas h, Iban Aldecoa i,j, Joan Berenguer k, Antonio Córdoba-Fernández l, Fátima Damas-Hermoso m, Fernando Rodríguez de Fonseca b,c,*

a Instituto de Estudios Campogibraltareños (IECG), 11027 Algeciras, Cádiz, Spain
b Red Andaluza de Investigación Clínica y Translacional en Neurología (Neuro-RECA), Laboratorio de Medicina Regenerativa, Hospital Regional Universitario, 29010 Málaga, Spain
c Departamento de Anatomía Patológica, Centro de Diagnóstico Biomédico, Hospital Clínico, 08036 Barcelona, Spain
d Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, 28031 Madrid, Spain
e Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, 08036 Barcelona, Spain
f Servicio de Neurología, Hospital Universitari Germans Trias i Pujol, 08916 Badalona, Barcelona
h Banco de Tejidos Neurológicos del Biobanco, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain
i Servicio de Radiología, Hospital Clínico, 08036 Barcelona, Spain
j Departamento de Podología, Universidad de Sevilla, 41009 Sevilla, Spain
k Unidad de Parkinson y movimientos anormales, Servicio de Neurología, Hospital Clínic, 08036 Barcelona, Spain
l Unidad de Neurociencias, Hospital Universitario de Valme, 41014 Sevilla, Spain
m Servicio de Neurología, Hospital Universitari Germans Trias i Pujol, 08916 Badalona, Barcelona

ABSTRACT

Background: ATP13A2 holds promise as biomarker for Parkinson’s disease (PD). No study has examined how salivary ATP13A2 is related to motor features in idiopathic PD.

Methods: Salivary ATP13A2 concentration was evaluated with ELISA, and statistical correlations of ATP13A2 level with PD parameters were examined. The dose intensity of the dopaminergic medication regimen was expressed as levodopa equivalent daily dose (LEDD). ATP13A2 expression on histological sections of submandibular glands was evaluated using immunohistochemistry.

Results: Salivary ATP13A2 was undetectable in many subjects (28 % of patients, 43.7 % of controls). However, all the patients with motor complications (n = 28) showed quantifiable levels of ATP13A2, that positively correlated with MDS-UPDRS (total, parts III and IV), and LEDD (p < 0.05). Dyskinetic patients showed the highest LEDD values (p < 0.05). The histological study revealed: a) ATP13A2 staining was very intense in duct cells and vascular endothelium, and b) two patterns of ATP13A2-positive deposits are observed: rounded inclusions of 10–20 µm in diameter located in the interlobular tissue of the patients, and amorphous aggregates inside duct lumen in controls and patients.

Conclusions: The sensitivity of the ELISA assay was a major limitation for quantifying ATP13A2. However, salivary ATP13A2 was detected in all patients with motor complications, where a direct relationship among ATP13A2 concentration, levodopa equivalent daily dose, and MDS-UPDRS was found. Therefore, salivary ATP13A2 might be a reliable index of therapy-induced motor complications. ATP13A2 was expressed by rounded inclusions in the submandibulary gland of patients. This is the first description of ATP13A2-positive inclusions outside the nervous system.

* Corresponding author at: Instituto de Investigación Biomédica de Málaga, 29010 Málaga, Spain.
E-mail address: fernando.rodriguez@ibima.eu (F. Rodríguez de Fonseca).

https://doi.org/10.1016/j.prdoa.2022.100163
Received 22 February 2022; Received in revised form 1 August 2022; Accepted 19 August 2022
Available online 28 August 2022

2590-1125/© 2022 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Parkinson's disease (PD) is characterized by progressive degeneration of dopaminergic neurons of the substantia nigra. Many factors such as neuroinflammation, redox balance, mitochondria bioenergetics, autophagy, exosomal α-synuclein release, and aging are involved in its pathogenesis [1–5]. The enzyme ATP13A2 (ATPase Cation Transporting 13A2) participates in most of these mechanisms [6–11]. In addition, ATP13A2 mutation is involved in monogenic PD, underlying an autosomal recessive form of early-onset parkinsonism or Kufor-Rakeb syndrome, and ATP13A2 is noted in Lewy inclusions in the brain of patients with idiopathic PD [6,8,10,11,12]. Therefore, the ATPase enzyme holds promise as biomarker for diagnosis of idiopathic Parkinson’s disease.

Recently, we examined the association between serum and cerebrospinal fluid (CSF) ATP13A2 levels with clinical features in PD patients [13]. The findings revealed that ATP13A2 concentration in serum, but not in the CSF, positively correlated with the dose intensity of the dopaminergic medication, expressed as levodopa equivalent daily dose (LED) [13]. These findings suggest that serum ATP13A2 content might be an index of the intensity of dopaminergic medication in patients with idiopathic PD.

Salivary ATP13A2 content and its expression in salivary glands in patients with idiopathic PD is unknown. Saliva is an interesting biofluid that is being studied in PD because of its easy accessibility, and the occurrence of Lewy pathology in salivary glands is well known [14–20]. To date, the salivary ATP13A2 levels and its correlation with clinical features in idiopathic PD has not been assessed, nor the presence of ATP13A2-expressing inclusions in salivary glands. The objectives of this study are: (a) to study the concentration and histological expression of ATP13A2 in the saliva and submandibular glands in patients with idiopathic PD and control subjects, and (b) to explore the relationship of salivary ATP13A2 level with clinical features of the disease. For histological analysis, the submandibular gland was selected because it is the most active salivary gland [16].

2. Patients and methods

2.1. Participants, and demographic and clinical information

Patients were enrolled at Hospital Valme and Hospital Macarena (Sevilla, Spain) and were diagnosed of PD by expert physicians. Patients fulfilled the Movement Disorders Society criteria of PD [14,21], and they presented a reliable loss of dopamine-transporter binding signal on basal ganglia as evaluated with dopamine transporter single-photon emission computed tomography (DAT-SPECT) [22]. All SPECT scans were performed, quantitatively analyzed, and visually assessed by expert physicians at the Service of Nuclear Medicine [22]. Patients had an age-at-PD onset from 45 to 75 years, and those subjects with atypical deficits, Deep Brain Stimulation (DBS), or first-degree relatives with PD were discarded. Control participants were recruited from volunteers, and they were group-matched by age and sex to PD subjects. Controls were excluded if they had a first-degree family member with a neurological disorder.

Exclusion criteria of all participants included liver, renal, hematological, and cardiovascular dysfunctions, as well as malabsorption, morbid obesity, autoimmune diseases, acquired immunodeficiency syndrome, cancer, and infectious conditions because lysosomal biomarkers can be affected in these diseases [23]. Morbid obesity was diagnosed when BMI was higher than 35 kg/m². In addition, all participants were non-alcohol drinkers, non-smokers, and non-coffee drinkers, according to established criteria [13,24,25].

Standard demographic information was obtained from patients, including age, gender, body mass index, and years of education. Clinical data included the modified Hoehn and Yahr staging, the International Parkinson and Movement Disorder Society-Sponsored revision of the Unified Parkinson’s Disease Rating Scale (MDS-UPDRS), the modified Schwab-England scale, age of onset of PD, disease duration, and dopaminergic treatment. Regarding the medication regimen, patients were treated not only with levodopa, but also with dopamine agonists and supportive medication that enhance dopaminergic effect. For this reason, the antiparkinsonian medication regimen was expressed as levodopa equivalent daily dose or LEDD (mg), following an established formula [26,27].

2.2. Saliva collection and ELISA analysis

Three ml of saliva were collected in 5-ml polypropylene tubes (Eurotube DeltaLab, Barcelona, Spain). Saliva was centrifuged at 2500 rpm during 10 min, and then the liquid portion was immediately frozen at −80 °C in 0.5-ml aliquots. Hemoglobin concentration in a fresh 0.5-ml saliva aliquot was quantified as recommended [7], and those saliva samples with hemoglobin concentration higher than 1200 mg/ml were discarded. Saliva aliquots were unfrozen and sonicated with homogenizing solution (150 mM NaCl, 50 mM HEPES, 1 mM phenylmethylsulfonyl fluoride, 0.6 µm leupeptin, 1 % Triton X-100, pH 7.4). ATP13A2 content was evaluated with a commercially available Enzyme-linked Immunosorbent Assay (ELISA) kit (Human ATP13A2 ELISA kit, Sandwich ELISA, Catalog #LS-F10891, Lifespans Biosciences Inc., USA), following manufacturer’s instructions. Sensitivity of the ELISA kit was ~ 230 pg/ml. Each sample was analyzed in duplicate without diluting the saliva.

2.3. Immunohistochemical study of the submandibular gland

Histological slides containing 5-µm sections of human submandibular gland tissue were obtained from the IDIBAPS Biobank (Institut d’Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona). Submandibular gland tissue had been obtained through transcutaneous core needle biopsy with ultrasound guidance in patients with Parkinson’s disease (n = 6) and healthy controls (n = 6), as explained elsewhere [17]. Histological sections were deparaffinized and then stained against ATP13A2 alone, or in combination with Dehalogenase type 1 or DEHAL1 (also known as Lodostyrosine deiodinase or IYD). This latter enzyme was chosen because it is selectively expressed by excretory duct cells, not by secretory acinar cells (Dr. Fernández-Espejo, personal observation). The antibodies used were as follows: mouse monoclonal antibody to ATP13A2 (ATP13A2 (4B7) Antibody, sc-293367, Santa Cruz Biotechnology, Inc.), and IYD polyclonal antibody (ThermoFisher Scientific, cat. #PA5-63757, produced in rabbit). Sections were incubated in the primary antibody, diluted 1:100, for 24 h at 4 °C. The next day the sections were incubated in the respective secondary antibody for 90 min: biotinylated goat anti-mouse IgG (1:500; cat. no. B7264, Sigma) or biotinylated donkey anti-rabbit IgG (1:500; cat. no. RPN1004, Amer sham, Little Chalfont, England). The sections were then incubated in ExtrAvidin peroxidase (Sigma) diluted 1:2000, in darkness at room temperature for 1 h. Finally, immunoreactivity was revealed with 0.05 % diaminobenzidine (DAB; Sigma) diluted in 0.1 M phosphate-buffered saline (PBS) or DAB and 0.05 % nickel ammonium sulfate diluted in PBS. Peroxidase reaction was activated after the addition of 0.03 % H₂O₂.

All sections were reviewed by researchers blinded to the clinical information (E.F.E. and J.S.). Sections with positive immunoreactivity were visualized using a standard optical microscope (Nikon Instruments Europe B.V., Amstelveen, The Netherlands) coupled to the NIS-Elements Imaging Software 3.00 (Nikon). We screened 4–6 serial sections per subject with ATP13A2 immunostaining. The intensity of immunoreactive ATP13A2 staining within different cell regions of the submandibular gland was assessed in contiguous tissue sections according to a five-point rating scale: no expression (0), mild (1), moderate (2), intense (3), and very intense (4). The submandibular gland is an exocrine organ that contain secretory acinar cells arranged in lobules. These lobules are separated by interlobular connective tissue that contain excretory ducts, blood vessels, adipocytes, and autonomic nerves supplying the gland.
Finally, the presence of ATP-positive aggregates was also analyzed, and the density degree of immunoreactive inclusions was assessed according to a five-point rating scale: not detectable (0), mild (1), moderate (2), frequent (3), and very frequent (4).

2.4. Statistics

Data are given as mean ± standard deviation of the mean or SD. Two groups were compared using the Student’s t test (parametric distribution), or the Mann-Whitney U test (non-parametric distribution). Dichotomous variables were compared with the χ² test. Correlations between two dependent variables were carried out with the Pearson’s test. Data normalization was verified using the Shapiro-Wilk test. Correlation was adjusted for age, gender, BMI, and education. Data were analyzed with Minitab19 statistical package (AddLink Software Científico, Spain).

3. Results

3.1. Demographic and clinical parameters

Sixty-eight patients with idiopathic PD and 36 controls were enrolled at Hospital Valme and Hospital Macarena, but eight saliva samples (4 patients, 4 controls) were discarded due to high hemoglobin concentration or technical problems. Hence, the final number was 64 patients and 32 controls. First, we verified that basic demographic features were not found to be different between controls and patients with idiopathic PD. Demographic and clinical parameters of the patients and controls are shown in Table 1.

3.2. Salivary ATP13A2

Salivary ATP13A2 was undetectable in 18 out of 64 patients (28 %), and in 14 out of 32 control participants (43.7 %). The detection threshold of the ELISA assay (~230 pg/ml) was a limitation for quantifying ATP13A2 levels in the saliva.

In the cohort of patients, a significant correlation was found between salivary ATP13A2 level and MDS-UPDRS part-IV score (r = 0.344, p = 0.0053), a scale which allows evaluating motor complications in PD patients that include motor fluctuations and dyskinesias. In addition, all the patients with motor complications (n = 28) showed quantifiable levels of salivary ATP13A2 (Table 1, Fig. 1). For these reasons, the group of patients with motor complications was further studied. Main clinical parameters of PD patients with and without motor complications are shown in Table 1. Individual values of salivary ATP13A2 content in patients with motor complications and controls are illustrated in Fig. 1.

After comparing the subgroup of patients with motor complications with control participants with quantifiable ATP13A2 levels (n = 18), ATP13A2 concentration was found to be significantly higher in PD patients with motor complications relative to controls (t = 5.9245, p < 0.0001), as shown in Table 1. Salivary ATP13A2 content in patients with motor complications significantly correlated with MDS-UPDRS part-IV (r = 0.566, p = 0.0016), MDS-UPDRS part-III (r = 0.388, p = 0.0413), total MDS-UPDRS (r = 0.391, p = 0.0396), and LEDD (r = 0.384, p = 0.0436), as shown in Fig. 1. Total MDS-UPDRS (parts I-III) is a scale that allows evaluating motor deficits and non-motor experiences of daily living. MDS-UPDRS part-III is the rating scale for motor deficits. LEDD also correlated with MDS-UPDRS part IV (all patients, r = 0.643, p < 0.001; patients with motor complications, r = 0.538, p = 0.0031). Statistical correlations of PD patients with motor complications are shown in Supplemental Table 1. Scores of disease severity, disease duration, and LEDD were significantly higher in patients with motor complications relative to patients without motor complications (Hoehn-Yahr, t = 4.002, p = 0.0002; MDS-UPDRS part-III, t = 11.4647, p < 0.0001; total MDS-UPDRS, t = 5.946, p < 0.0001; disease duration, t = 2.7328, p = 0.0082; LEDD, t = 4.0827, p = 0.0001, Table 1).

| Table 1 | Demographic and clinical parameters, as well as salivary ATP13A2 concentration in PD patients and control subjects. |
|---------|-----------------------------------------------------------------------------------------------------------------------------------|
|          | All patients (n = 64) | Patients without motor complications (n = 36) | Patients with motor complications (n = 28) | Controls (n = 32) |
| Demographic and clinical parameters | | | | |
| Age (years) | 65.5 ± 11.7 | 65 ± 12 | 66.5 ± 11 | 61.4 ± 10 |
| Gender, male n (%) | 33 (52) | 19 (53) | 14 (50) | 18 (56) |
| Body mass index | 24.3 ± 2.2 | 24.2 ± 2.1 | 24.4 ± 2.3 | 25.6 ± 2.6 |
| Education (years) | 18.1 ± 2.4 | 17.9 ± 2.5 | 18.2 ± 2.2 | 17.8 ± 1 |
| Levodopa equivalent daily dose (mg) | 682 ± 644 | 427 ± 520 | 1008 ± 618 ** | |
| Disease duration (years) | 13.3 ± 6.7 | 11.5 ± 5 | 15.6 ± 7 ** | |
| Age at PD onset (years) | 55.6 ± 11 | 56.5 ± 12 | 54.5 ± 9 | |
| Hoehn-Yahr stage | 2.2 ± 0.9 | 1.8 ± 0.7 | 2.6 ± 0.9 ** | |
| Modified Schwab-England scale | 81.8 ± 24 | 86 ± 20 | 76.8 ± 28 | |
| MDS-UPDRS part III (on) | 20.7 ± 15 | 13.1 ± 10 | 49 ± 15 *** | |
| Total MDS-UPDRS (on) | 34.6 ± 26 | 21 ± 14 | 52 ± 27 *** | |
| MDS-UPDRS part IV ATP13A2 content (pg/ml) | 1.7 ± 2.6 | 0 | 3.7 ± 2.9 | |
| Patients with motor complications (without or with dyskinesias) | | | | |
| Without dyskinesias ATP13A2 content (ng/ml) | 959 ± 370 | 1001 ± 304 | | |
| With dyskinesias ATP13A2 content (mg/ml) | 762 ± 394 | 1168 ± 699 | | |
| Levodopa equivalent daily dose (mg) | 30.5 ± 18 | 29.5 ± 13 | | |
| MDS-UPDRS part III (on) | 53.1 ± 31 | 50.5 ± 25 | | |
| Total MDS-UPDRS (on) | 1.7 ± 0.9 | 5.1 ± 3 ||

Mean ± SD, ** p < 0.01, *** p < 0.001 vs patients without motor complications; ## p < 0.0001 versus controls with quantifiable ATP13A2 levels; §§ p < 0.05, §§§ p < 0.01 versus patients without dyskinesias. Statistical comparisons were carried out with the χ² test (dichotomous variables) or the Student’s t-test or the Mann-Whitney U test (quantitative variables). Abbrev.: ATP13A2, ATPase Cation Transporting 13A2; nd, nondetectable; MDS-UPDRS, International Parkinson and Movement Disorder Society-Sponsored revision of the Unified Parkinson’s Disease Rating Scale; PD, Parkinson’s disease.

To further studying patients with motor complications, this group was divided into patients with motor fluctuations and without dyskinesias (n = 17), and patients with motor fluctuations and dyskinesias (n = 11). LEDD and MDS-UPDRS part-IV score were found to be
significantly higher in patients with dyskinesias relative to patients without dyskinesias (LEDD, $t = 2.0678$, $p = 0.0487$; MDS-UPDRS part-IV, $t = 4.4154$, $p = 0.0002$). Main clinical parameters of both subgroups are shown in Table 1.

3.3. ATP13A2 immunohistochemical expression in the submandibular gland

The immunohistochemical study of the submandibulary gland revealed that ATP13A2 is expressed by most regions of the organ. A very intense ATP13A2 staining was observed in glandular duct cells (apical region and villi) and vascular endothelial cells, in both the patients and controls (see Fig. 2). Acinar cells and red blood cells showed diffuse and moderate ATP13A2 staining (Fig. 2). Mild intensity of ATP13A2 signal was observed in the interlobular connective tissue in the patients and controls, and adipocytes scarcely expressed ATP13A2. Duct cells showed a cytoplasmic gradation of immunoreactivity because the basal region of the cell presented less intense ATP13A2 expression than the apical region (Fig. 2).

Regarding ATP13A2-expressing inclusions, two patterns of deposits were observed: lumenal aggregates in duct cells, and rounded deposits in the interlobular tissue. First, in both patients and controls, deposits with amorphous shape and fibrils were observed inside lumen of gland ducts. These lumenal inclusions are found in 5/6 IPD patients and 4/6 controls. Second, the interlobular connective tissue of the patients, but not controls, contained aggregates of spheroid/ovoid shape (Fig. 2). Spheroid or ovoid shape might result from a different viewing angle. These rounded inclusions had a diameter of 10–20 µm and were found in 5/6 IPD patients. The intensity of ATP13A2 staining in regions of the submandibular gland along with density degree of ATP13A2-expressing inclusions in patients and controls are shown in Table 2.

There were no significant differences in age (patients with IPD, 66.8 ± 8 years; control participants, 63.5 ± 10 years), and gender between the patients and controls.

4. Discussion

The sensitivity of the ELISA assay was a major limitation for quantifying ATP13A2 levels in the saliva because this molecule was below the detection threshold in many subjects. However, all the patients with motor complications (motor fluctuations and dyskinesias) showed quantifiable levels of ATP13A2. In these patients, MDS-UPDRS and LEDD correlated with salivary ATP13A2 content. In other words, patients with motor fluctuations and dyskinesias were treated with a high-intensity dopaminergic regimen, as measured with LEDD, and presented high salivary ATP13A2 content in comparison with controls with quantifiable levels.

Motor fluctuations are variable motor responses to medication, and dyskinesias are involuntary random movements. Motor complications are attributed to the interaction of intense dopaminergic therapy, advanced nigrostriatal cell loss, aging and genetic factors [2,28,29]. A role of the pharmacological factor in the development of motor complications was corroborated in this study. Dyskinetic patients were those with the highest LEDD values. Patients with motor complications were at advanced stages of the disease, as revealed by the Hoehn-Yahr staging and disease duration in years. Therefore, quantifiable high level of salivary ATP13A2 in patients with motor complications could be accounted for by pharmacological effects of levodopa and dopaminergic agents and advanced disease.

Sustained dopaminergic stimulation is known to induce oxidative damage and inflammation in vascular endothelial cells [28], and the histological study showed very intense expression of ATP13A2 in vascular endothelial cells. High dopaminergic stimulation is also associated with excess of reactive oxidant species and lysosome dysfunction [29], mechanisms that are linked to ATP13A2 activity [2,6,7]. Another possibility is that we are measuring ATP13A2 coming from intestinal...
epithelial cells because ATP13A2 is highly expressed in intestinal cells [30]. However, the role of intestinal ATP13A2 in the saliva might be low, since patients’ salivary glands presented abundant rounded aggregates expressing ATP13A2, likely reflecting the glandular origin of salivary ATP13A2. To sum up, the presence of high salivary ATP13A2 level would reflect oxidative and inflammatory effects of dopaminergic therapy on vascular endothelial cells of submandibulary glands. The data allow proposing that salivary ATP13A2 might be a reliable marker for the occurrence of therapy-induced motor complications in PD patients. The relevance of salivary ATP123A2 as a marker of oxidative/
effects of dopaminergic medication and motor complications in PD patients is worthy of further investigation.

As regards the histological study, ATP13A2 was differentially expressed by most cell types in the submandibular gland. This finding fits well with the ubiquitous bodily distribution of the protein [12,13]. Tissue distribution and intensity of ATP13A2 staining seem to be similar in both the patients and controls. The most intense ATP13A2 staining was observed in glandular duct cells and the vascular endothelium. The strong expression of ATP13A2 in duct cells and vascular endothelium would indicate that this protein might subserve important functions in these regions. The expression of ATP13A2 in secretory acinar cells was found to be fainter than that in excretory duct cells. ATP13A2 is involved in several crucial steps of excretory and secretory activity such as cargo trafficking, handling of metals and lipids, endo-/lysosome function, and exosomal release [6–11].

Two patterns of deposits expressing ATP13A2 were observed in the submandibular gland: amorphous aggregates in duct lumen, and rounded inclusions in the connective tissue. ATP13A2-positive deposits with amorphous shape and fibrils were observed inside lumen of gland ducts in both the patients and control participants. Lumenal aggregates were observed in all subjects suggest that they are not of pathological significance, and it is possible that they represent an aging or involutional change in salivary ducts. ATP13A2 is involved in several crucial steps of excretory and secretory activity such as cargo trafficking, handling of metals and lipids, endo-/lysosome function, and exosomal release [6–11].

Table 2
Table 2 Demographic, clinical, and neuropathological data from all cases studied with idiopathic PD and controls.

| Case | Gender | Age (y) | H- Y duration (y) | Disease | Intensity of ATP13A2 staining | Density of ATP13A2-positive inclusions (location) |
|------|--------|---------|------------------|---------|-------------------------------|---------------------------------------------|
|      |        |         |                  |         | Duct cells (basal-apical cytoplasm) | Vascular endothelial cells | Acinar cells & Red blood cells | Interlobular connective tissue | Rounded inclusions (inter-lobular tissue) | Lumenal aggregates (Ducts) |
| PD1  | F      | 76      | 2                | 6       | 3-4                           | 4                             | 2                            | 1                            | 1                           | 1                         |
| PD2  | M      | 73      | 2.5              | 8       | 3-4                           | 4                             | 2                            | 1                            | 1                           | 1                         |
| PD3  | M      | 56      | 2                | 6       | 3-4                           | 4                             | 2                            | 2                            | 1                           | 1                         |
| PD4  | M      | 55      | 2                | 7       | 3-4                           | 4                             | 2                            | 2                            | 1                           | 1                         |
| PD5  | M      | 70      | 1.5              | 5       | 3-4                           | 4                             | 2                            | 1                            | 0                           | 1                         |
| PD6  | F      | 71      | 2.5              | 7       | 3-4                           | 4                             | 2                            | 1                            | 1                           | 1                         |
| CT1  | M      | 66      | 3.4              |         | 4                             | 4                             | 2                            | 1                            | 1                           | 1                         |
| CT2  | F      | 55      | 3.4              |         | 4                             | 4                             | 2                            | 1                            | 0                           | 0                         |
| CT3  | F      | 57      | 3.4              |         | 4                             | 4                             | 2                            | 1                            | 0                           | 0                         |
| CT4  | F      | 66      | 3.4              |         | 4                             | 4                             | 2                            | 2                            | 0                           | 0                         |
| CT5  | M      | 77      | 3.4              |         | 4                             | 4                             | 2                            | 1                            | 0                           | 0                         |
| CT6  | M      | 60      | 3.4              |         | 4                             | 4                             | 2                            | 1                            | 0                           | 1                         |

The intensity of immunoreactive ATP13A2 staining within different cell regions of the submandibular gland was assessed in contiguous tissue sections according to a five-point rating scale: no expression (0), mild (1), moderate (2), intense (3), and very intense (4). The presence of aggregates was also analyzed, and the density of immunoreactive inclusions within different regions of the submandibular glands was assessed according to a five-point rating scale: not detectable (0), mild (1), moderate (2), frequent (3), and very frequent (4). Abbrev.: PD, Parkinson’s disease; CT, control; F, female; M, male; H-Y, Hoehn-Yahr stage; y, years.

5. Ethics approval

All protocols were approved by the internal ethics and scientific boards of Hospital Universitario Valme (CEI Valme, #10/05/2018 & #1694-M1-19), Hospital Universitario Macarena (CEI #19/05/2010 & #2149-29/10/2013), Comité de Ética de Investigación de la Junta de Andalucía (PEIBA, CEI Sevilla-Sur #20150520554-1 & #2017121418738), and University of Seville (CEE-27/05/2010 & SeccionInvestigacion/Gd-21/06/2010). The subjects’ consent was obtained according to the Declaration of Helsinki (BMJ 1991; 302: 1194).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Acknowledgements

The authors are most grateful to all the subjects who participated in this study. The authors thank Mara Gerra, Silvia Castellano (Universidad de Sevilla), and Antonio Vargas (Instituto de Investigación Biomédica de Málaga) for their technical assistance; Dr. Ángel Martín de Pablos for his excellent clinical work (Hospital Macarena, Sevilla); Drs. Guillermo Izquierdo, Miguel Ángel Rico and Eva Cuartero for allowing the use of the facilities of Hospital Macarena and Hospital Valme; Dr. Cinta Calvo-Morón (Servicio de Medicina Nuclear, Sevilla) and Ana Santurrián (Universidad de Cantabria) for Dat-SCAN studies, and Dr. María-Isabel García-Sánchez (Biobanco Hospitalario Macarena, National Biobank Network, Instituto de Salud Carlos III) for the storage of samples. Some experiments were performed at Laboratorio de Neurología Molecular, Universidad de Sevilla.

Fundings

This work was supported by grants to E.F.-E. from Sociedad Andaluza de Neurología, Sevilla, Spain (SUBAIA2015/006), and to F.R.D.F. from Proyectos Retos Colaboración, Ministerio de Ciencia e Innovación, Spain, and European Regional Development Funds, European Union (ERDF-EU, RTC2019-007329-1), EU-LAC-HEALTH Project, European Union (EULACHHEALTH H2020) FATZHEIMER Project, European Union (EULACHHEALTH H2020), Red de Investigación en Atención Primaria en Adicciones, convocatoria RICORS2021, Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación, Spain, and European Regional Development Funds, European Union (ERDF-EU; RD21/0009/0003); Instituto de Salud Carlos III, Spain, and ERDF-EU, European Union (P119/01577); Consejería de Economía, Conocimiento y Universidad, Junta de Andalucía, Spain (Grant P18-TF-5194), and Consejería de Salud y Familia, Junta de Andalucía, Spain (Neuro-RECA, RIC-0111-2019). The funding sources had no further role in study design; in the collection, analysis, and interpretation of data; in writing the report; and in deciding to submit the paper for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.prdoa.2022.100163.

References

[1] K.A. Jellinger, Neuropathological spectrum of synucleinopathies, Mov. Disord. 18 (16) (2003) 22–27.
[2] C.W. Olanow, M.B. Stern, K. Sethi, The scientific and clinical basis for the treatment of Parkinson's disease, Neurology 72 (4) (2009) S1–S136.
[3] J. Navarro-Yepes, M. Burns, A. Anandhan, O. Khallamouch, L.M. del Razo, B. Quintanilla-Vega, A. Pappa, M.I. Panayiotidis, R. Franco, Oxidative stress, redox signaling, and autophagy: cell death versus survival, Antioxid. Redox Signal. 21 (1) (2014) 66–85.
[4] P.P. Michel, E.C. Hirsch, S. Hunot, Understanding Dopaminergic Cell Death Pathways in Parkinson Disease, Neuron 90 (2016) 675–691.
[5] J.I. Shido, S.H. Snyder, B.D. Paul, Redox Mechanisms in Neurodegeneration: From Disease Outcomes to Therapeutic Opportunities, Antioxid. Redox Signal. 30 (11) (2019) 1450–1499.
[6] B. Behay, A. Ramirez, M. Martinez-Vicente, C. Perier, M.H. Canron, E. Doudnikoff, et al., Loss of P-type ATPase ATP13A2/PARK9 function induces general lysosomal deficiency and leads to Parkinson disease neurodegeneration, Proc. Natl. Acad. Sci. USA 109 (2012) 9611–9616.
[7] A.M. Gondón, J. Zhu, B. Van Houten, C.T. Chu, ATP13A2 regulates mitochondrial bioenergetics through macroautophagy, Neurobiol. Dis. 45 (2012) 962–972.
[8] D. Ramonet, A. Podbielska, K. Stafl, S. Sonnay, A. Trancikova, E. Taška, et al., PARK9-associated ATP13A2 localizes to intracellular acidic vesicles and regulates cation homeostasis and neuronal integrity, Hum. Mol. Genet. 21 (2012) 1725–1743.
[9] P.J. Schultein, S.M. Fleming, A.K. Clippinger, J. Lewis, T. Tsunemi, B. Giasson, et al., Atg13a2-deficient mice exhibit neuronal ceroid lipofuscinosis, limited α-synuclein accumulation and age-dependent sensorimotor deficits, Hum. Mol. Genet. 22 (2013) 2067–2082.
[10] K.E. Murphy, L. Cottle, A.M. Gbyders, A.A. Cooper, G.M. Halliday, ATP13A2 (PARK9) protein levels are reduced in brain tissue of cases with Lewy bodies, Acta Neuropathol. Comm. 1 (2013) 11.
[11] A. Ramírez, A. Heimbach, J. Gründemann, B. Stiller, D. Halliday, L.P. Gid, et al., Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase, Nat. Genet. 38 (2016) 1184–1191.
[12] L. Marulli, J.A. Viçcarra, A. Sturchio, A.K. Dwivedi, E.G. Keeling, D. Patel, et al., When does postural instability appear in monogenic parkinsonisms? An individual-patient meta-analysis, J. Neurol. 268 (2021) 3203–3211.
[13] E. Fernández-Espío, E. Rodríguez de Fonseca, J. Suárez, R. González-Aparicio, A. Santurrián, ATP13A2 levels in serum and cerebrospinal fluid in patients with idiopathic Parkinson’s disease, Parkinsonism Relat. Disord. 88 (2021) 3–9.
[14] J.G. Goldman, H. Andrews, A. Amara, A. Naito, R.N. Alcalay, L.M. Shaw, P. Taylor, T. Xie, P. Tuite, C. Henchcliffe, P. Hogarth, S. Frank, M.H. Saint-Hilaire, M. Frasier, V. Arnedo, A.N. Reimer, M. Sutherland, C. Swanson-Fischer, K. Gwinn, U.J. Kang, Fox Investigation of New Biomarker Discovery, Cerebrospinal fluid, plasma, and saliva in the BioFIND study: Relationships among biomarkers and Parkinson’s disease Features, Mov. Disord. 33 (2021) 282–288.
[15] K. Del Tredici, H. Braak, Lewy pathology and neurodegeneration in pre-motor Parkinson’s disease, Mov. Disord. 27 (2012) 597–607.
[16] E. Fernández-Espío, F. Rodríguez de Fonseca, J. Suárez, E. Tolosa, D. Vilas, I. Aldekoa, et al., Native α-synuclein, 3-nitrotyrosine-proteins, and patterns of nitro-α-synuclein-immunoreactive inclusions in the saliva and submandibular gland in Parkinson’s disease, Antioxidants 10 (2021) 715.
[17] D. Vilas, A. Irango, E. Tolosa, I. Aldekoa, J. Berenguer, I. Villarcea, C. Martí, M. Serradell, E. Tolosa, A. Martínez-Arcos, T. Simuni, N. Seedorff, C. Caspall-García, et al., Basic clinical features do not predict dopamine transporter binding in idiopathic REM behavior disorder, N.P.J. Parkinsons Dis. 5 (2019) 2.
[18] U.J. Kang, J.G. Goldman, R.N. Alcalay, T. Xie, P. Tuite, C. Henchcliffe, et al., The BioFIND study: Characteristics of a clinically typical Parkinson’s disease biomarker cohort, Mov. Disord. 31 (2016) 924–932.
[19] L.M. Chahine, A. Irango, A. Fernández-Arcos, T. Simuni, N. Seedorff, C. Caspall-García, et al., Clinical diagnostic features do not predict dopamine transporter binding in idiopathic REM behavior disorder, N.P.J. Parkinsons Dis. 5 (2019) 2.
[20] A. Martín-de-Pablos, A. Córdoba-Fernández, E. Fernández-Espío, Analysis of neurotrophic and antioxidant factors related to midbrain dopamine neuronal loss and brain inflammation in the cerebrospinal fluid of the elderly, Exp. Gerontol. 110 (2018) 54–60.
[21] M.A. Hernández, B. Takkouch, F. Caamaño-Iorma, J.J. Gestal-Otero, A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson’s disease, Ann. Nutr. 52 (2002) 276–284.
[22] J.H. O’Keefe, S.K. Bharti, H.R. Patil, J.J. DiNiccolantonio, S.C. Lucan, C.J. Lavie, Effects of habitual coffee consumption on cardiometabolic disease, cardiovascular health, and all-cause mortality, J. Am. Coll. Cardiol. 62 (12) (2013) 1043–1051.
[23] D. Verber, D. Novak, M. Borovj, J. Dugonik, D. Fliser, EUQDopa: A responsive web application for the levodopa equivalent dose calculator, Comput. Methods Programs Biomed. 196 (2020) 105633.
[24] C.L. Tomlinson, R. Stowe, S. Patel, C. Rick, R. Gray, C.E. Clarke, Systematic review of levodopa dose equivalency reporting in Parkinson’s disease, Mov. Disord. 25 (2010) 2649–2655.
[25] C.M. Chen, J.L. Liu, Y.R. Wu, Y.C. Chen, H.S. Cheng, M.L. Cheng, D.T. Chiu, Increased oxidative damage in peripheral blood correlates with severity of Parkinson’s disease, Neurobiol. Dis. 33 (2009) 4294–35.
[26] P. Jenner, Molecular mechanisms of L-DOPA-induced dyskinesia, Nat. Rev. Neurosci. 9 (9) (2008) 665–677.
[27] The protein atlas. https://www.proteinatlas.org/ENS00000159363-ATP13A2.