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Autonomic involvement in Parkinsonian carriers of *PARK2* gene mutations

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ABSTRACT

Background and objectives: The objective of this study was to assess the presence of autonomic nervous system dysfunction in *PARK2* mutation carriers.
Patients and methods: We performed a cross-sectional analysis of 8 *PARK2* carriers (age: 60.1 ± 12.8 years) and 13 individuals with idiopathic PD (iPD) (age: 59.2 ± 8.9 years). Autonomic dysfunction was measured using the SCOPA-AUT questionnaire, non-invasive autonomic tests and responses of noradrenaline and vasopressin levels to postural changes. Myocardial sympathetic denervation was assessed with metaiodobenzylguanidine (MIBG) scintigraphy. This damage was further investigated in postmortem epicardial tissue of one *PARK2* carrier and three control cases (two PD patients and one subject without PD).
Results: The prevalence of autonomic symptoms and orthostatic hypotension (OH) was lower in *PARK2* mutation carriers than in iPD patients (SCOPA OUT: 3.4 ± 4.8 vs. 14.7 ± 7.2, $p < 0.001$; OH: present in three iPD patients but none of the *PARK2* mutation carriers). Second, sympathetic myocardial denervation was less severe in *PARK2* mutation carriers compared to controls, both in MIBG scintigraphy (late H/M uptake ratio: 1.52 ± 0.35 vs. 1.32 ± 0.25 $p < 0.05$) and in postmortem tissue study. Interestingly, axonal alpha-synuclein deposits were absent in epicardial tissue of the *PARK2* mutation carrier while they were present in the two PD patients.
Interpretation: Our study supports the view that autonomic nervous system dysfunction and myocardial sympathetic denervation are less pronounced in *PARK2* mutation carriers than in individuals with iPD, suggesting that the involvement of small peripheral sympathetic nerve fibers is a minor pathological hallmark in *PARK2* carriers.

1. Introduction

Mutations in the *PARK2* gene explain up to half of the cases with recessively inherited Parkinson's disease (PD), and 15% of sporadic PD with onset before 45 years of age [1]. Compared to idiopathic PD (iPD), patients with *PARK2* gene mutations have significantly earlier

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and more symmetrical onset, more frequent dystonia, slower progression, and a tendency toward a greater response to low doses of levodopa with early fluctuations and dyskinesia [2]. Heterozygous *PARK2* mutations are relatively frequent and it has been speculated that they are associated with PD [3]. The majority of PD patients with mutations in the *PARK2* gene do not exhibit the classical neuropathological markers of PD; however, in a few cases, pathological analysis of *PARK2* mutation carriers has revealed Lewy bodies in the substantia nigra and locus coeruleus, and alpha-synuclein immunopositive inclusion bodies in the brain [4–7].

Autonomic nervous system involvement in *PARK2* mutation is a matter of discussion. In early reports, it was observed that patients with *PARK2* mutations showed some autonomic symptoms resembling those observed in PD [8,9]. However, recent studies have shown that cardiovascular dysautonomia is not associated with the *PARK2* phenotype [10,11]. In addition, a number of case reports in PARK2-positive PD patients have noted normal 123I-MIBG scans [12,13,8] and preserved TH-immunoreactive fibers in the epicardium [12]. While these findings support the view that small myelinated and unmyelinated autonomic nerve fibers are preserved in *PARK2* mutation carriers, this hypothesis contrasts with the presence of parkin protein in the axoplasm of bovine myelinated nerve fibers and Schwann cells [14] and with the presence of peripheral neuropathy in *PARK2* mutation carriers [15].

The main objective of this study was to assess the overall level of autonomic nervous system dysfunction and the specific involvement of the neurocirculatory system, including myocardial sympathetic denervation, in 8 *PARK2* mutation carriers compared to 13 iPD patients. We performed a comprehensive assessment of autonomic symptoms, quantified hemodynamic dysfunction by non-invasive tests and plasma neurohormone levels, and carried out myocardial MIBG scintigraphy in all patients. In addition, we assessed epicardial denervation in the postmortem tissue from one *PARK2* mutation carrier, two patients with PD (one with iPD and one symptomatic alpha synuclein mutation carrier) and one subject without PD.

2. Patients and Methods

2.1. Study population

We included 8 *PARK2* gene mutation carriers (60.1 ± 12.8 years; with a disease duration of 27.1 ± 11.4 years; 4 men) and 13 control patients with iPD (age: 59.2 ± 8.9 years, with a disease duration of 6.5 ± 5.1 years; 8 men). Patients classified as idiopathic had no family history of PD. All participants fulfilled the UK Parkinson's disease Society Brain Bank criteria for the clinical diagnosis of PD (except for family history of PD in PARK2 carriers). They were consecutively recruited in the Movement Disorders and Autonomic Unit at Cruces University Hospital. We excluded patients with diabetes or with myocardial perfusion defects observed in the 99mTc-MIBI SPECT, and any patients who were not able to complete the tests properly due to physical or cognitive disability. We also excluded one patient with one heterozygous *PARK2* mutation and another with a heterozygous compound *PARK2* mutation that developed parkinsonian symptoms when exposed to clebopride, which resolved when the drug was discontinued. All procedures were carried out with the adequate understanding and written consent of the patients involved, and with the approval of the local ethics committee.

2.2. Clinical assessment of PD

At study inclusion, we collected a set of clinical and demographic data including gender, age, disease duration, age at disease onset and scores on the Unified Parkinson Disease Rating Scale (UPDRS I-IV) and the 12-item Brief Identification Smell Test (BSIT; Sensonic®). We classified the BSIT result as normal or abnormal following the norms for age and gender provided by the manufacturer.

2.3. Molecular genetic analysis

PARK2 mutations were identified on DNA obtained from peripheral lymphocytes. Single-strand conformation polymorphism (SSCP) analysis of the 12 exons was followed by nucleotide sequencing whenever an SSCP band shift was observed. To detect alterations in gene dosage (deletions and duplications), a quantitative RTPCR assay was performed [10].

2.4. Study of the autonomic nervous system

Assessments were made early in the morning, after 12 h of fasting and overnight bed rest. Participants were not allowed to consume drugs or foods capable of modifying blood catecholamine levels within the previous 24 h. For the analysis of noradrenaline (NA) and vasopressin levels, peripheral blood samples were collected (30 ml from arm venipuncture) after the patient had rested for at least 40 min in the supine position, and again after standing for 3 min. NA levels were measured by high-performance liquid chromatography (HPLC) with electrochemical detection, while plasma vasopressin was measured by radioimmunoassay (RIA).

We then analyzed heart rate and blood pressure variability during various physical maneuvers using a continuous monitoring device, the Task Force® Monitor (CNSystems, Graz, Austria): a 20-min tilt table test (TTT) at an inclination of 60°; the Valsalva maneuver (VM) (40 mmHg for at least 15 s); isometric contraction (handgrip isometer); and deep breathing (6 inspiration/expiration cycles) (I/E). Finally, we administered the SCOPA-AUT, a brief questionnaire concerning autonomic symptoms classified into 6 dimensions [16].

2.5. Myocardial MIBG scintigraphy

All participants underwent myocardial MIBG scintigraphy with a gamma camera no more than 6 months before study inclusion. We analyzed the early (15 min) and late (4 h) heart–to-mediastinum uptake ratios. Participants also underwent 99mTc-MIBI (6-methoxy isobutyl isonitrile) SPECT to rule out myocardial perfusion defects.

2.6. Postmortem histopathological studies of epicardial and brain tissues

In order to further characterize epicardial sympathetic nerve involvement in *PARK2* carriers, we performed immunohistochemical analysis of postmortem myocardial and epicardial tissue from one *PARK2* mutation carrier. Brain tissue from this patient was also studied to evaluate the presence of Lewy Body pathology. As a reference, we studied post mortem epicardial and brain samples from one subject without PD and from two PD patients: one iPD patient (with no family history of PD) and another PD patient with a specific mutation (E46K) in the alpha synuclein gene (*SNCA*), a condition associated with an aggressively progressing Lewy Body disease, with marked autonomic dysfunction and cardiac denervation. Epicardial samples were sliced into 5-μm-thick sections, which were then deparaffinized and subjected to routine histological staining (hematoxylin-eosin) and standard immunohistochemical processing with monoclonal antibodies against neurofilaments (NF), tyrosine hydroxylase (TH) (TH16; Sigma, St. Louis, MO, USA) (1:3000), phosphorylated alpha synuclein and alpha synuclein (Zymed®). Regarding brain samples, blocks were taken from representative cortical and subcortical regions in accordance with standardized protocols. The paraffin-embedded sections were stained with routine histological methods and processed for immunocytochemistry with antibodies against glial fibrillary acidic protein (1:5000; Dako, Carpinteria, CA), phosphorylated NF (1:40, Zymed, San Francisco, CA), tau (1:400; Sigma, St. Louis, MO), ubiquitin (Z0458, 1:400; Dako), and alpha synuclein (1:200, without formic acid pretreatment; Zymed).

2.7. Statistical analysis

Descriptive data were expressed using the mean and standard deviation for the quantitative variables, and percentages for the qualitative variables. The Mann–Whitney U non-parametric test was used to compare mean ranks. All the analyses were carried out with the statistical software package SPSS 13 for Windows (SPSS, Chicago, IL).

3. Results

Table 1 shows an individual description of the main characteristics of the 8 *PARK2* mutation carriers in terms of demographics, family history, genetics and duration of PD, as well as a summary of the main outcomes from autonomic nervous system and myocardial SPECT MIBG assessments. Of these patients, three carried homozygous and five compound heterozygous mutations; and, while two had no family history of PD, the remaining six mutations occurred in three sibling sets, one of which was the product of a consanguineous marriage.

There were no statistically significant differences between *PARK2* mutation carriers and iPD patients in terms of age or motor status (UPDRS III scale) at inclusion. Disease onset was before the age of 50 years in all *PARK2* mutation carriers and occurred significantly earlier than in individuals with iPD. Consequently, disease duration was longer in *PARK2* mutation carriers (27.1 ± 13.4 years in *PARK2* mutation carriers vs. 6.5 ± 5.1 years in iPD patients; $p < 0.001$) (Table 2).