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**Editor's comment:** Alpha-synuclein (AS) aggregates are detected post-mortem in the brain or the peripheral autonomic nervous system (pANS), in some subjects without parkinsonism or dementia, and are thought to mark early, pre-clinical stages of neurodegeneration. Similar aggregates were detected in asymptomatic living subjects, in the abdomino-pelvic pANS.
In this issue, Eduardo Tolosa and colleagues extend these concepts by documenting AS aggregates in the epicardial pANS in seven out of 91 biopsies from subjects without parkinsonism, undergoing elective cardiac surgery. Interestingly, non-motor features typically seen in patients with synucleinopathies (constipation or vivid dreams) were also noted. Follow-up of this cohort will be important to monitor the possible evolution of motor and non-motor symptoms and signs of synucleinopathies.

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# Alpha-synuclein aggregates in epicardial fat tissue in living subjects without parkinsonism
## *[Universally Available]*

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ABSTRACT

*Background:* In Parkinson's disease (PD), alpha-synuclein (AS) aggregates occur frequently in peripheral autonomic nervous system (pANS). Their presence in asymptomatic subjects suggests incidental Lewy-body disease (iLBD) that is thought to reflect pre-clinical PD. Cardiac involvement has been detected in *post-mortem* studies in both, PD and also in iLBD. *In vivo* documentation of cardiac AS pathology is lacking.
*Objective:* To prospectively assess the presence of AS aggregates in epicardial fat tissue from *living* subjects without parkinsonism undergoing elective cardiac surgery.
*Material and methods:* Epicardial fat tissue obtained during cardiac surgery from 91 subjects was studied by histology and immunohistochemistry. Areas more likely to contain pANS elements were selected. PD-related motor and non-motor symptoms (NMS) were assessed immediately before or after surgery.
*Results:* Small autonomic nerves, ganglia and/or tyrosine-hydroxylase positive fibres were identified in epicardial fat in each of the 91 subjects (62 male/29 female, mean age 67 years). AS aggregates were detected in 7 subjects (7.7%), and were more frequent in those aged above 70 years. In AS-positive subjects constipation and acting dreams were significantly more frequent than in the AS-negative ones.
*Conclusion:* AS aggregates occur in epicardial pANS in subjects without parkinsonism, suggesting the diagnosis of iLBD. The presence in some of these subjects of *non-motor symptoms* such as acting dreams and constipation known to occur in premotor PD supports this interpretation. Adequate follow-up of the subjects in this study will indicate the time, if any, to progression to motor PD.


## 1. Introduction

The presence of intraneuronal aggregates of the presynaptic protein alpha-synuclein (AS) in Lewy-bodies and neurites constitutes the pathological hallmark of Parkinson's disease (PD). Their presence in the peripheral autonomic nervous system (pANS) has

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been documented in the past years in PD and other Lewy-body disorders (LBD) in *post-mortem* [1–4] and *in vivo* studies [5,6].

There is increasing evidence that in PD, before the substantia nigra degenerates and parkinsonism appears, deposits of abnormal AS occur in lower brainstem areas and central olfactory pathways [7]. These extranigral lesions are thought to constitute the neural substrate of what is considered "premotor PD" [8] and, in *post-mortem* studies, to represent incidental Lewy-body disease (iLBD). Although it is unclear whether iLBD always evolves to motor PD, it is considered on the bases of pathological, inmunohistochemical and biochemical data [9,10], that affected subjects would have had a higher risk to develop classical motor PD [11].

Cardiovascular dysautonomic symptoms including orthostatic hypotension and abnormalities in R–R-interval [12] have been the focus of recent attention in PD. Of great interest are imaging studies with $^{123}$I-metaiodobenzylguanidine (MIBG) that have documented changes indicative of cardiac postganglionic sympathetic denervation in most patients with PD. At what time in the natural history of PD do these abnormalities occur is unclear but pathological and imaging studies suggest that it may occur in the early [13], even premotor phase of PD [14–18].

We have assessed the presence of AS aggregates in epicardial fat in living subjects undergoing cardiac surgery in order to investigate involvement of cardiac pANS in subjects without parkinsonism. The presence of such AS aggregates would support early cardiac involvement in LBD and suggest that these subjects are in a stage of premotor PD.

## 2. Material and methods

Unselected patients without clinically manifest motor symptoms of PD and with cardiac disease undergoing elective cardiac operations at the Department of Cardiovascular Surgery, Hospital Clinic, Barcelona (CAM, EQ) between January 2009 and June 2011 were enrolled in this study. All individuals were appropriately informed about the study and gave their written consent. The project has been approved by the Institutional Ethical Committee.

### 2.1. General demographic characteristics

Recorded characteristics of participants included age, gender and cardiovascular risk factors such as high blood pressure, diabetes mellitus, obesity (measured by body mass index BMI), smoking, and type of heart disease (mostly ischaemic, valvular or both). Potential post-surgical complications were recorded, including neurological (post-surgical delirium/confusion, seizures), cardiac (arrhythmia, low cardiac output requiring circulatory support, pericardial effusion), renal (acute renal insufficiency), pulmonary (postoperative respiratory insufficiency requiring prolonged mechanical ventilation, pneumonia, pneumothorax), and surgical complications (e.g. infection, thrombosis, bleeding) as well as fatal events (death).

### 2.2. Clinical evaluation

Patients were examined by a neurologist from the Movement Disorders Unit of the Hospital Clinic Barcelona (ET, JN, SR) focusing on motor and non-motor features of Parkinson's disease. Most patients were visited before the surgical procedure to avoid potentially post-surgical confounders. In cases where it was not possible due to patient's state, evaluation was performed as early as possible after the surgical procedure. Neurologists were blinded to pathological results.

Examination consisted on a daily activities evaluation (using unified Parkinson's Disease Rating Scale – UPDRS II scale), motor exploration (UPDRS III scale) and application of a reduced version of a questionnaire of non-motor problems in Parkinson's disease (NMSQuest) [19]. Eleven non-motor symptoms-related items from the NMSQuest were chosen which included one related to hyposmia, four to autonomic dysfunction (gastrointestinal symptoms, genitourinary symptoms, and cardiovascular dysfunction), three related to cognitive problems, and three to sleep disturbances (for details see also Table 1).

In the first 20 subjects studied (pilot phase of the study) no information on non-motor symptoms was obtained. In eight additional cases clinical data were not available (fatal event before clinical evaluation in two patients, six patients could not be evaluated before nor after surgery because they were transferred to another hospital).

**Table 1**
Demographic characteristics of study subjects.

| Variable | Total $n = 91$ | AS (+) $n = 7$ | AS (−) $n = 84$ | *P*-value |
|---|---|---|---|---|
| Age, years (mean ± SD) | 67 (±11.08) | 70.7 (±12.8) | 66.7 (±10.95) | 0.197 |
| Gender male/female | 62/29 | 3/4 | 59/25 | 0.203 |
| BMI (kg/m$^2$) (mean ± SD) | 27.9 (±4.47) | 25.9 (±4.99) | 28.1 (±4.40) | 0.265 |
| HBP *n* (%) | 58/89 (65.2) | 3/7 (42.9) | 55/82(67.1) | 0.188 |
| DM *n* (%) | 25/89 (28.1) | 0/7 | 25/82(30.5) | 0.09 |
| Hyperlipidemia *n* (%) | 46/88 (52.3) | 3/7 (42.9) | 43/81 (53.1) | 0.449 |
| Smoking *n* (%) | 34/87 (46.2) | 2/6(33.3) | 32/81(39.5) | 0.564 |
| Heart attack *n* (%) | 18/88 (20.5) | 0/7 | 18/81 (22.2) | 0.189 |
| Heart disease | | | | |
| Valvular *n* (%) | 43/86 (50) | 4/7 (57.1) | 39/79 (49.4) | 0.438 |
| Ischaemic *n* (%) | 23/86 (26.7) | 1/7 (14.3) | 22/79 (27.8) | 0.431 |
| Valvular + ischaemic *n* (%) | 18/86 (20.9) | 2/7 (28.6) | 16/79 (20.3) | 0.420 |
| Others *n* (%) | 2/86 (2.4) | 0/7 | 2/79 (2.6) | 0.851 |
| Neurological complications *n* (%) | 5/64 (7.8) | 0/7 | 5/58 (8.6) | 0.99 |
| **Other complications** | | | | |
| Renal *n* (%) | 7/64 (10.9) | 0/5 | 7/59 (11.9) | 0.99 |
| Respiratory *n* (%) | 6/64 (9.4) | 1/6 (16.7) | 5/58 (8.6) | 0.46 |
| Cardiac *n* (%) | 18/64 (28.1) | 1/6 (16.7) | 17/58(29.3) | 0.667 |
| Surgical *n* (%) | 15/64 (23.4) | 1/6 (16.7) | 14/58(24.1) | 0.99 |
| Death *n* (%) | 5/91 (5.5) | 0/7 | 5/84 (6) | 0.99 |

Abbreviations: BMI: body mass index; HBP: high blood pressure; DM: diabetes mellitus.
*P*-value: Mann–Whitney test and two-tailed Fisher's exact test as appropriated.

### 2.3. Surgical procedure

During cardiac surgery, small fragments of epicardial fat tissue (0.5–2 cm in diameter) were obtained from the areas more likely to contain autonomic nervous tissue according to previous literature [20] and to our own experience in preliminary *post-mortem* studies (unpublished data). We did not expect to cause any additional risk to patients nor complicate the surgical procedure due to sampling. The areas from where samples were taken are routinely approached in cardiac surgery for different purposes like placement of cavitary vents, access to epicardial coronary arteries and routine cannulations. These areas included fat tissue from the following regions: cavoatrial junction, interatrial groove (Waterson–Sondergaard groove), right coronary artery along the acute cardiac margin, left anterior descending coronary artery along the interventricular septum, and fatty tissue surrounding the aortic root and ascending aorta and right superior pulmonary vein. Samples were taken before performing the epicardial (coronary bypass) or intracardiac procedure (valve replacement/repair) with the use of cardiopulmonary bypass, and were immediately placed in 4.5% buffered formaldehyde solution.

Special attention was paid to possible complications related to the site of fat sampling. Therefore care was taken to avoid potential bleeding from sampling sites with exhaustive regional check before closure of the chest.

### 2.4. Pathological studies

Formalin-fixed samples were embedded in paraffin. Five micrometer thick sections from each block were stained with haematoxylin–eosin for standard histopathological evaluation and by immunohistochemistry using antibodies directed against tyrosine-hydroxylase (mouse monoclonal, clone TH-16, dilution 1:3000, Sigma–Aldrich, St. Louis, MO, USA), alpha-synuclein (mouse monoclonal, clone KM51, dilution 1:500, Novocastra, Newcastle upon Tyne, UK), and anti-phosphorylated alpha-synuclein (mouse monoclonal, phosphorylated at Ser 129, dilution 1:1000, Wako Pure Chemical Industries LTD, Japan) at the Neurological Tissue Bank of the Biobanc-Hospital Clinic-IDIBAPS. Tissue section pretreatment for antigen retrieval was performed by boiling sections in 10 mM citrate buffer at pH6 for 10 min.

Detection of immunostaining was performed using the Envision® kit, and diaminobenzidine was used as chromogen.

On histological examination, presence of autonomic nervous tissue and detection of pathological AS and phospho-AS (pAS) aggregates in these structures were assessed (EG). In cases with positive AS aggregates, consensus evaluation was performed on a multiheaded microscope by ET, JN, CAM, and EG. To assess degeneration in those cardiac nerves with AS aggregates we performed immunohistochemistry using anti-TH, anti-AS and anti-pAS antibodies on serial sections in selected cases.

In addition, to investigate whether potential AS aggregation in our living subjects could be related to underlying heart disease, we analysed *post-mortem*