Estudio del sistema nervioso autónomo en la enfermedad de Parkinson y otras alfasinucleinopatías

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Estudio del sistema nervioso autónomo en la enfermedad de Parkinson y otras alfasinucleinopatías

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INFORME DEL DIRECTOR DE TESIS

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El Dr. Eduard Tolosa Sarró, Consultor Senior del Servicio de Neurología del Hospital Clínic de Barcelona y Catedrático de Neurología de la Facultad de Medicina de la UB,

CERTIFICA:

Que la tesis doctoral “Estudio del sistema nervioso autónomo en la enfermedad de Parkinson y otras alfasinucleinopatías”, presentada por Judith Navarro Otano para optar al grado de Doctor por la Universidad de Barcelona se ha realizado bajo mi dirección y cumple todos los requisitos necesarios para ser defendida ante el Tribunal de evaluación correspondiente.

Que los artículos que conforman esta tesis y sus factores de impacto son:

- Multiple organ involvement by alpha-synuclein pathology in Lewy body disorders. Movement Disorders. FI: 5.634
- Alpha-synuclein aggregates in epicardial fat tissue in living subjects without parkinsonism. Parkinsonism and Related Disorders. FI: 3.274
- ^{123}I-MIBG cardiac uptake, smell identification and ^{123}I-FP-CIT SPECT in the differential diagnosis between vascular parkinsonism and Parkinson's disease. Parkinsonism and Related Disorders. FI: 4.126

Que estos artículos no han sido incluidos en ninguna otra tesis doctoral ni serán incluidos en el futuro.

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I.-Listado de abreviaturas

LISTADO DE ABREVIATURAS

AS	Alfa Sinucleína
DLB	Demencia con cuerpos de Lewy(*Dementia with Lewy bodies*)
FC	Frecuencia cardiaca
HO	Hipotensión ortostática
iLBD	Enfermedad por cuerpos de Lewy incidentales(*Incidental Lewy body disease*)
MIBG	Metaiodobenzilguanidina
MSA	Atrofia multisistémica*(Multisystem Atrophy*)
MSA-C	Atrofia multisistémica variante cerebelosa
MSA-P	Atrofia multisistémica variante parkinsoniana
PA	Presión arterial
PAF	Fallo autonómico puro (*Pure Autonomic Failure*)
pAS	Alfa Sinucleína fosforilada
PD	Enfermedad de Parkinson (*Parkinson's Disease*)
SNAP	Sistema nervioso autónomo periférico
SNC	Sistema nervioso central
SNE	Sistema nervioso entérico
SNPS	Sistema nervioso parasimpático
SNS	Sistema nervioso simpático
TCSR	Trastorno de conducta de fase REM del sueño

LISTADO DE ABREVIATURAS

II.- Introducción

INTRODUCCIÓN

1. Las sinucleinopatías y el sistema nervioso autónomo

En su forma nativa, la alfa sinucleína (AS) es una proteína soluble que se encuentra en el citosol y en las terminaciones presinápticas. Se puede detectar de manera fisiológica en distintas cantidades en la mayor parte de los tipos neuronales. Hasta ahora se desconoce con exactitud cuál es su función aunque se ha postulado que interviene en la plasticidad neuronal (Kaplan, Ratner et al. 2003), en el funcionamiento del aparato de Golgi (Cooper, Gitler et al. 2006) y en el tráfico de vesículas (George, Rey et al. 2013). En aquellas enfermedades que se asocian a agregados de AS esta proteína presenta una serie de cambios post-transcripcionales como fosforilación que la hace más propensa a la agregación con otras moléculas de AS y otras proteínas como la ubiquitina (Beyer and Ariza 2013).En el año 1912, Friedrich Lewy describió la presencia en pacientes con enfermedad de Parkinson de cuerpos de inclusión intracelulares, que hoy conocemos como cuerpos de Lewy. En 1997 distintos grupos de estudio descubrieron que el mayor componente presente en los cuerpos de Lewy era la proteína AS (Spillantini, Schmidt et al. 1997; Trojanowski and Lee 1998). A nivel de las prolongaciones de las neuronas los agregados de AS se organizan en forma de neuritas de Lewy. La formación de los cuerpos de inclusión se ha relacionado con plegamiento anómalo, agregación anormal, sobreexpresión y/o eliminación ineficiente de AS (Braak and Del Tredici-Braak 2013). En las enfermedades por depósito de sinucleína no todas las neuronas tienen la misma susceptibilidad para desarrollar agregados anómalos. Diversos estudios han sugerido que son las neuronas de proyección con axón largo y poco mielinizado las más vulnerables al desarrollo de cuerpos y neuritas de Lewy (Braak and Del Tredici 2009).

Las **sinucleinopatías** son enfermedades neurodegenerativas que se caracterizan por la presencia de agregados anómalos de AS intraneuronales o gliales. En la enfermedad de Parkinson (PD), la demencia con cuerpos de Lewy (DLB) y el fallo autonómico puro (PAF) los agregados de AS se organizan en forma de cuerpos de Lewy. Además, se ha descrito la presencia de patología tipo Lewy a nivel del sistema nervioso central y periférico en sujetos sin clínica sugestiva de enfermedad neurodegenerativa, siendo conocida actualmente esta entidad como "enfermedad por cuerpos de Lewy incidentales" (iLBD). La AS puede también agregarse anómalamente en forma de inclusiones en la oligodendroglía y en algunas neuronas como se observa en la atrofia multisistémica (MSA).

Clínicamente, las sinucleinopatías se caracterizan por la presencia de los llamados síntomas motores (parkinsonismo…) junto con los no motores, entre los que se encuentran los síntomas de disautonomía, el deterioro cognitivo, la hiposmia, las alteraciones del sueño… La combinación de estos dos grupos de síntomas en distintas proporciones conforma el amplio espectro clínico de estas enfermedades. Así, en uno de sus extremos clínicos se encontraría el fallo autonómico puro, en el que se observa disautonomía sin ningún síntoma motor mientras que en el otro extremo podríamos encontrar sujetos con enfermedad de Parkinson con gran afectación motora pero disautonomía subclínica.

En la PD se produce en el sistema nervioso central un proceso patológico progresivo que afecta de forma preferencial a determinadas áreas cerebrales siguiendo un patrón caudo-rostral. La neurodegeneración se iniciaría en las neuronas de los núcleos más caudales del tronco cerebral (núcleo dorsal del vago y estructuras olfativas anteriores) y ascendería por el tronco cerebral hasta alcanzar estructuras límbicas y en fases avanzadas áreas neocorticales, siguiendo el estadiaje propuesto por Braak (Braak, Del Tredici et al. 2003). Se afectarían en el proceso tanto el sistema dopaminérgico nigro-estriatal como otros sistemas extranigrales (por ejemplo los noradrenérgicos, serotoninérgicos o colinérgicos) (Parkkinen, Pirttila et al. 2008; Beach, Adler et al. 2009).

	PD	DLB	PAF	iLBD
Disautonomía	-/+	+	+	-?
Parkinsonismo	+	+	-	-
Demencia	-/+	+	-	-

En el texto que sigue a continuación nos centraremos principalmente en dos enfermedades asociadas a patología de tipo Lewy: la enfermedad de Parkinson y la demencia con cuerpos de Lewy. En estas enfermedades los depósitos de AS se encuentran tanto a nivel del sistema nervioso central como del sistema nervioso autónomo (SNA) (Beach, Adler et al. 2010). Desde un punto de vista anatómico y funcional el SNA se divide en sistema nervioso autónomo simpático (SNS), sistema nervioso autónomo parasimpático (SNPS) y sistema nervioso entérico (SNE). SNS y SNPS tienen núcleos neuronales localizados a nivel del SNC y otros localizados a nivel periférico. El estudio del SNA periférico (SNAP) es accesible *in vivo* a través de tejido biópsico de diversas estructuras como el colon (SNE), la piel (SNS) o las glándulas salivares (SNPS) (Lebouvier, Coron et al. 2010; Miki, Tomiyama et al. 2010; Wang, Gibbons et al. 2013; Adler, Dugger et al. 2014; Donadio, Incensi et al. 2014).

2. El sistema nervioso autónomo periférico en la enfermedad de Parkinson

La enfermedad de Parkinson se define clásicamente como un cuadro clínico en el que predominan las alteraciones **motoras** (síndrome rígido-acinético y temblor de reposo) (Hughes, Daniel et al. 1992) con una degeneración preferente del sistema nigroestriado que condiciona un déficit dopaminérgico estriatal. Así, desde el punto de vista anatomopatológico la PD se ha relacionado con pérdida neuronal y alteración conformacional y agregación de AS en el sistema nervioso central.

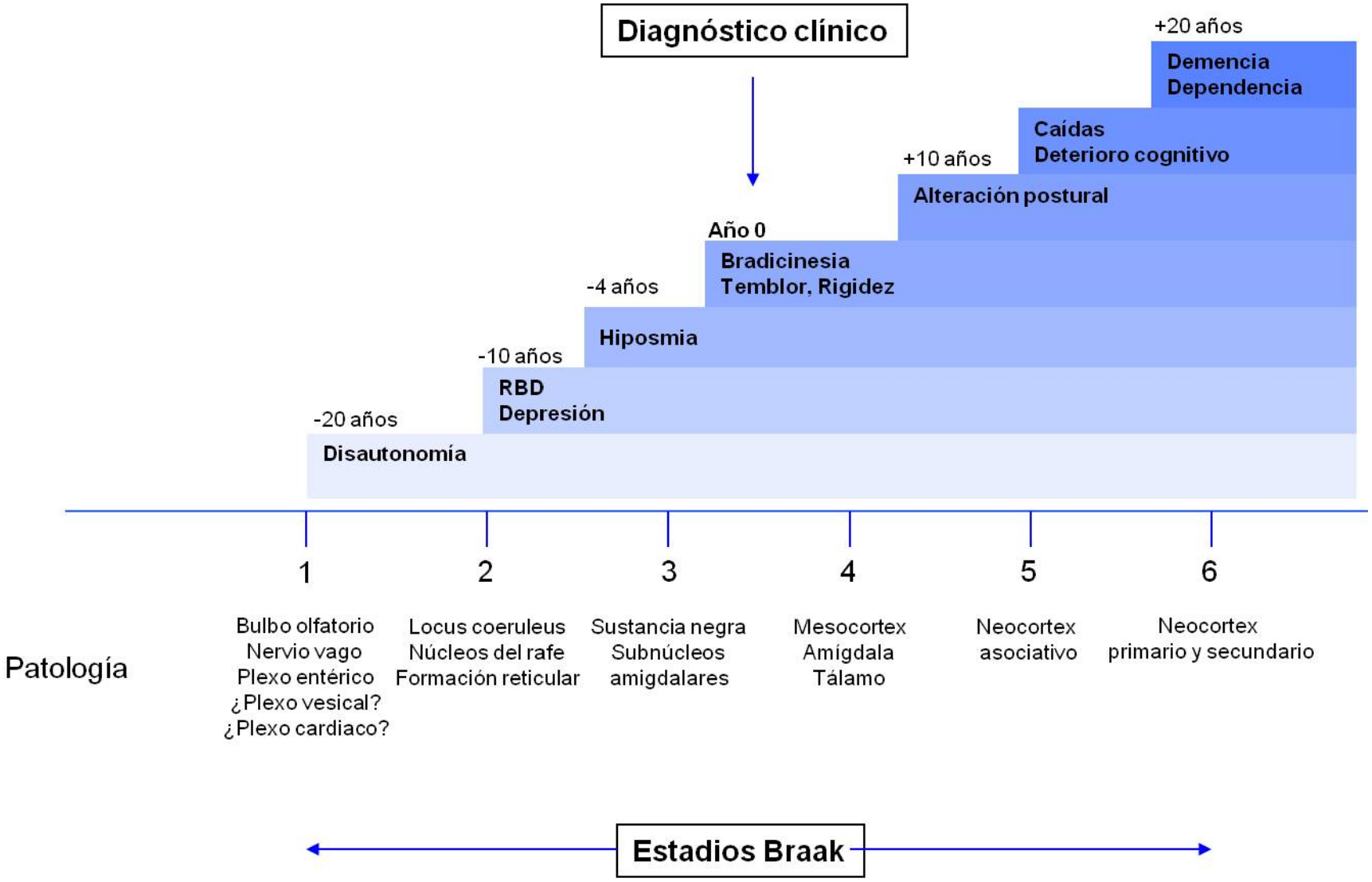
Sin embargo, en los últimos años se ha modificado esta imagen de la PD como una alteración primaria del sistema nervioso central, expandiéndose hasta el punto de que hoy se la considera una patología multisistema. Se habla así pues, añadidos a los síntomas motores, de síntomas no motores de la PD (Adler 2005; Tolosa, Gaig et al. 2009), ocasionados por la alteración del sistema nervioso no sólo central sino también periférico. La importancia del reconocimiento de los síntomas no motores de la PD radica en que muchos de ellos aparecen antes de que se presenten los síntomas motores más clásicos (ver figura 1). Su detección podría ayudar a diagnosticar más precozmente la PD. Además, el conocimiento de los primeros síntomas de la enfermedad podría ayudar en la búsqueda de su origen. Los síntomas no motores de la PD incluyen alteraciones cognitivas, hiposmia, alteraciones psiquiátricas, trastornos sensitivos, trastornos del sueño **y disfunción autonómica**.

Los síntomas de disfunción autonómica son frecuentes e invalidantes en el Parkinson. Estos síntomas son causa de importante discapacidad para el paciente (Martinez-Martin, Rodriguez-Blazquez et al. 2011) e incluyen problemas cardiovasculares, gastrointestinales, genitourinarios y alteraciones de la sudoración.

El sustrato de los síntomas de disfunción autonómica de la PD no está claro y probablemente se deba en parte a la afectación del núcleo dorsal del vago, el núcleo ambiguo y otros núcleos neuronales centrales (Braak, de Vos et al. 2006; Fujishiro, Frigerio et al. 2008; Orimo, Uchihara et al. 2008; Djaldetti, Lev et al. 2009).

A continuación se exponen los principales signos y síntomas de disautonomía que aparecen en la PD agrupados por sistemas y un breve resumen de la literatura disponible sobre este tema.

Figura 1



Adaptado de “A timeline for Parkinson’s Disease”, Hawkes CH, Del Tredici K, Braak H. Parkinsonism and Related Disorders2010;16:79–84

2.1 Sistema cardiovascular en la enfermedad de Parkinson

Se piensa que la disautonomía cardiovascular es una de las primeras alteraciones que ocurren en la PD. La afectación de este sistema, incluso en estadios premotores de la enfermedad, está apoyada por observaciones clínicas, estudios funcionales, estudios de imagen y hallazgos anatomopatológicos. Los estudios funcionales que muestran una disminución en la inervación cardiaca tienen una correlación anatomopatológica. Estudios *post-mortem* de tejido cardíaco (músculo miocárdico, tejido graso epicárdico, plexos cardíacos, etc.) han demostrado una pérdida de inervación autonómica y agregados anómalos de AS en los pacientes con PD (Wakabayashi, Takahashi et al. 1993; Wakabayashi and Takahashi 1997).

A continuación se resumen los principales síntomas relacionados con la disfunción del sistema cardiovascular que presentan los pacientes con PD.

a) Hipotensión ortostática

Uno de los síntomas de disautonomía cardiovascular más prevalente en la PD es la hipotensión ortostática (HO). La HO se define como una caída en la presión arterial sistólica de al menos 20mmHg o en la diastólica de al menos 10mmHg dentro de los tres primeros minutos tras levantarse el sujeto. Se considera también que existe HO si la caída de presión aparece dentro de los primeros 40 minutos de un test de mesa basculante con una inclinación de al menos 60º (Freeman, Wieling et al. 2011). Aunque a veces la HO puede ser asintomática, los pacientes suelen referir sensación de “cabeza hueca”, mareo, presíncope, síncope o alteraciones visuales que reflejan hipoperfusión cerebral.

En la PD, un reciente metanálisis incluyendo datos de 25 estudios previos ha calculado una prevalencia de HO determinada mediante medida de presión arterial de alrededor del 30.1%, con un rango de entre 9.6% y 64.9% (Velseboer, de Haan et al. 2011). Otro estudio reciente, valorando más de 1000 pacientes con PD, encuentra una prevalencia de HO de un 17.6% (Ha, Brown et al. 2011). Estos datos refuerzan la hipótesis de que en la PD hasta un 20% de los sujetos con HO pueden ser asintomáticos (Senard, Rai et al. 1997; Allcock, Kenny et al. 2006; Jamnadas-Khoda, Koshy et al. 2009).

b) Hipotensión postprandial

La hipotensión postprandial se define como una caída en la presión arterial sistólica de al menos 20mmHg o cifras tensionales sistólicas menores de 90mmHg durante las 2 horas que siguen a una comida (Luciano, Brennan et al. 2010). Se ha relacionado con alteraciones en los mecanismos de compensación que se deberían activar en repuesta a la acumulación de sangre a nivel del lecho esplácnico. Aunque no hay estudios poblacionales que cuantifiquen la prevalencia y relevancia clínica de la hipotensión postprandial en la PD, en laboratorios de disautonomía se ha encontrado una prevalencia cercana al 60% (Chaudhuri, Ellis et al. 1997).

c) Hipertensión supina

La hipertensión supina se ha relacionado con alteración en el baroreflejo y con efectos adversos del tratamiento de la HO en la PD (Sharabi and Goldstein 2011). Se define como cifras mayores de 150/90mmHg durante el sueño nocturno. La prevalencia de hipertensión supina en la PD es todavía desconocida, con cifras barajadas entre 0 y 100% (Plaschke, Trenkwalder et al. 1998; Ejaz, Sekhon et al. 2006; Schmidt, Berg et al. 2009; Sommer, Aral-Becher et al. 2011; Kim, Oh et al. 2012). Aunque la mayor parte de las veces es asintomática, la falta de caída de presión arterial nocturna se relaciona con un aumento del riesgo cardiovascular, hipertrofia miocárdica, infartos de miocardio y cerebrales (Schmidt, Berg et al. 2009) aunque esta relación ha sido poco estudiada en la PD (Huang, Chen et al. 2013). Estudios recientes han relacionado los patrones anómalos de presión nocturna con un mayor deterioro cognitivo tanto en los pacientes con sinucleinopatías como en los sujetos sin este diagnóstico (Guo, Tabara et al. 2010; Kim, Oh et al. 2012).

2.2 Tracto gastrointestinal en la enfermedad de Parkinson

Se cree que el tracto gastrointestinal se encuentra alterado en la PD desde el inicio más temprano (ver figura 1). Esta hipótesis está basada en hallazgos histopatológicos, estudios epidemiológicos y funcionales. Algunos autores apoyan la teoría de que los depósitos de AS se irían propagando desde el nivel digestivo, desencadenados por un patógeno desconocido en superficies mucosas (Braak, de Vos et al. 2006; Hawkes, Del Tredici et al. 2009). Después de que la neurodegeneración sea iniciada por este patógeno, viajaría desde la mucosa digestiva vía nervio vago hacia el núcleo motor dorsal del vago, donde los depósitos de AS y la

pérdida neuronal son hallazgos *post-mortem* frecuentes en la PD. Desde este núcleo, se distribuiría siguiendo un patrón ascendente por el troncoencéfalo y el cortex cerebral (Braak, Del Tredici et al. 2003). La idea de que el nervio vago juega un papel central en la patogénesis de la PD está también apoyada por el hallazgo en estudios *post-mortem* de un gradiente rostro-caudal de AS dentro del sistema gastrointestinal, imitando la inervación vagal (Wakabayashi, Takahashi et al. 1988; Beach, Adler et al. 2010).

Se han realizado biopsias *in vivo* a nivel de mucosa y submucosa de recto y colon en pacientes con PD es distintos estadios de evolución, con la idea de buscar agregados de AS que pudieran ser utilizados en el futuro como biomarcadores precoces (Lebouvier, Chaumette et al. 2008; Lebouvier, Neunlist et al. 2010; Shannon, Keshavarzian et al. 2011; Pouclet, Lebouvier et al. 2012).

Los síntomas autonómicos gastrointestinales relacionados con PD más importantes por su frecuencia y morbilidad son el estreñimiento y la gastroparesia.

a) Estreñimiento

El diagnóstico clínico de estreñimiento se basa en los criterios generales de Roma. Estos incluyen una frecuencia de evacuación menor de 3 evacuaciones / semana, esfuerzo para defecar o heces duras en más de 25% de las deposiciones con sensación de evacuación incompleta (Longstreth, Thompson et al. 2006). Aplicando los criterios de Roma, se ha calculado una prevalencia en torno al 70% de estreñimiento en la PD (Gage, Kaye et al. 2011). El estreñimiento en la PD se debe principalmente al retraso en el tránsito colónico y a disfunción anorrectal (Sakakibara, Odaka et al. 2003). En la PD el enlentecimiento del tránsito colónico puede reflejar afectación tanto a nivel central (alteración en el núcleo pontino de Barrington, coordinador de la actividad del colon (Pavcovich, Yang et al. 1998)) como a nivel periférico (afectación de plexos mientérico y submucoso (Lebouvier, Neunlist et al. 2010)).

b) Gastroparesia

La gastroparesia se define como un trastorno de la motilidad del estómago que implica retraso en el vaciamiento de sólidos y líquidos, sin evidencia de obstrucción mecánica. Los síntomas asociados más frecuentes son náuseas, vómitos y saciedad precoz (Hasler 2011). Los pacientes con PD pueden presentar además fluctuaciones motoras como síntomas de gastroparesia, ya que la medicación oral presentaría una

llegada errática al duodeno-yeyuno. Se ha calculado una prevalencia de gastroparesia en cualquier grado en la PD hasta de un 70 - 100% (Heetun and Quigley 2012).

La gastroparesia en la PD se relaciona con una inervación vagal anormal. Sin embargo, la edad avanzada puede jugar un papel como factor de confusión en los estudios poblaciones ya que algunos estudios han encontrado una relación entre la edad y la gastroparesia más fuerte que la existente entre PD y gastroparesia. Además, la levodopa por sí misma puede retrasar el vaciamiento gástrico en sujetos sanos, aunque su papel en la motilidad gástrica PD es desconocida (Robertson, Renwick et al. 1990).

2.3 Tracto génito-urinario en la enfermedad de Parkinson

Aunque la mayoría de los pacientes con PD presentan síntomas de disfunción genitourinaria una vez la enfermedad motora se ha presentado (Wenning, Scherfler et al. 1999), estos síntomas podrían llegar a representar hasta un 16% de las quejas iniciales en aquellos pacientes con inicio no motor de PD (O'Sullivan, Williams et al. 2008).

El aumento de frecuencia urinaria, la nicturia y la urgencia urinaria están presentes hasta en un 60% de los pacientes con PD. Sin embargo, como la PD se acompaña de alteraciones del sueño y se manifiesta principalmente en pacientes mayores de 60 años, la prevalencia de síntomas urinarios debidos primariamente a disautonomía puede estar sobrevalorada.

En estudios urodinámicos realizados en pacientes con PD se puede encontrar un patrón de vejiga neurógena, probablemente de origen multifactorial. Por una parte, se ha sugerido que el circuito ganglios basales-cortex frontal podría jugar un papel en la disfunción urinaria. Esta hipótesis se apoya en la descripción de mejoría de los síntomas urinarios tras realizarse estimulación cerebral profunda con disminución de actividad frontal en pacientes con PD. Además, a nivel central la micción está también regulada por neuronas localizadas en el centro pontino de la micción, hacia donde proyectan neuronas dopaminérgicas localizadas en el área tegmental ventral. La proyección de los ganglios basales es aquí inhibitoria, por lo que su afectación provocaría un aumento de actividad del núcleo de la micción (Sakakibara, Uchiyama et al. 2010; Yeo, Singh et al. 2012). Por otra parte, la afectación local de los plexos autonómicos urinarios, ya presente en estadios precoces de la enfermedad (Minguez-

Castellanos, Chamorro et al. 2007; Beach, Adler et al. 2010) puede influir en la sintomatología urinaria de la PD, al depender el almacenamiento urinario de reflejos sacros (Sakakibara, Kishi et al. 2011).

2.4 Sistema nervioso autónomo cutáneo en la enfermedad de Parkinson

Entre un 30 y un 64% de los pacientes con PD presentan hipohidrosis e hiperhidrosis como síntomas de alteración del sistema autonómico cutáneo (Swinn, Schrag et al. 2003; Martinez-Martin, Schapira et al. 2007). La hiperhidrosis es la queja más frecuente y se relaciona con una peor calidad de vida de los pacientes. Se localiza principalmente en cabeza, cuello y tronco. Algunos autores proponen que la hiperhidrosis es un mecanismo compensatorio a la hipohidrosis que se produciría en las extremidades por pérdida de inervación simpática glandular (Schestatsky, Valls-Sole et al. 2006).

El estudio de la inervación cutánea ha sido revolucionado en los últimos años con la llegada de la biopsia cutánea *in vivo* (Lauria, Cornblath et al. 2005; Donadio, Incensi et al. 2012). Mediante pequeños *punch* de entre 3 y 5mm de diámetro, bajo anestesia local, es posible acceder al SNA localizado principalmente alrededor de los anejos cutáneos. Esta técnica se ha usado recientemente en la PD, donde se ha sugerido una pérdida de inervación tanto autonómica (Dabby, Djaldetti et al. 2006; Wang, Gibbons et al. 2013) como sensitiva (Nolano, Provitera et al. 2008).

Junto con la pérdida de inervación, la piel puede presentar acúmulos de AS en la PD. En 2008 se demostró por primera vez presencia de AS en nervios cutáneos de la dermis e hipodermis (Ikemura, Saito et al. 2008) en muestras obtenidas *post-mortem*. Sin embargo, aunque un nuevo trabajo publicado en 2010 describía estos mismos agregados hasta en un 20% de pacientes con PD biopsiados en vida (Miki, Tomiyama et al. 2010), una extensa revisión de casos *post-mortem* reflejó la ausencia de estos agregados en los pacientes con PD (Beach, Adler et al. 2010). Recientemente un nuevo trabajo ha mostrado presencia de agregados de AS en el 100% de los sujetos biopsiados (tanto PD como controles) (Wang, Gibbons et al. 2013). El último trabajo sobre este tema publicado ha demostrado sin embargo que utilizando anticuerpos contra la forma fosforilada de AS es posible detectar agregados anómalos sólo en sujetos con PD (Donadio, Incensi et al. 2014).

3. ¿Cuándo se inicia la afectación del sistema nervioso autónomo en la enfermedad de Parkinson?

Hoy se cree que la afectación del SNAP ocurre de forma precoz e incluso premotora (Visser, Marinus et al. 2004). Como se ha introducido anteriormente, la detección *post-mortem* de depósitos anómalos de AS en pacientes sin sintomatología motora ha permitido acuñar el término de "enfermedad de cuerpos de Lewy incidentales "iLBD (Bloch, Probst et al. 2006). El hallazgo *post-mortem* de cuerpos de Lewy no se considera un simple marcador de envejecimiento celular. Por ejemplo en casos de iLBD los ganglios basales tienen menor actividad tirosina-hidroxilasa, similar a lo que ocurre en los sujetos con PD (Beach, Adler et al. 2008; Dickson, Fujishiro et al. 2008). Así pues, iLBD es considerada actualmente por muchos autores como PD prodrómica (DelleDonne, Klos et al. 2008). Sin embargo, hasta la fecha la mayoría de los trabajos sobre iLBD estaban basados en estudios de necropsia, en los que el seguimiento clínico de estos sujetos era obviamente imposible. Se han descrito casos en los que los depósitos de AS se encontraban tan solo a nivel periférico sin encontrarse a nivel central, lo que apoyaría la teoría de un inicio de la PD fuera del sistema nervioso central (Miki, Mori et al. 2009).

En los últimos años se está trabajando en detectar en vida a sujetos con cuerpos de Lewy incidentales sin clínica sugestiva de enfermedad neurológica para poder confirmar o descartar su progresión a una sinucleinopatía clínica (Minguez-Castellanos, Chamorro et al. 2007). La identificación de estos sujetos, probablemente con alto riesgo de desarrollar una sinucleinopatía, abriría además la puerta a estudios de neuroprotección precoz y a un mejor entendimiento de la historia natural de estas enfermedades.

3.1 Afectación premotora del sistema cardiovascular en la enfermedad de Parkinson

En la literatura se pueden encontrar casos de clínica de disautonomía cardiovascular que preceden al inicio motor de la PD entre 4 y 20 años (Kaufmann, Nahm et al. 2004; Goldstein, Sharabi et al. 2009; Goldstein, Holmes et al. 2012; Milazzo, Di Stefano et al. 2012). Estos síntomas precoces incluyen principalmente hipotensión ortostática y síncopes, pero también se ha descrito hipertensión paroxística. Además, el descubrimiento de casos de PD genéticamente determinados

ha permitido el estudio desde un punto de vista autonómico de los denominados "portadores asintomáticos" de la enfermedad, antes del inicio de los síntomas motores característicos (Tijero, Gomez-Esteban et al. 2010). Sujetos asintomáticos con disfunción autonómica cardíaca subclínica demostrada por una captación anormal de ^{123}I-metaiodobenzilguanidina (MIBG) presentan una vía dopaminérgica conservada en los ganglios basales, demostrada mediante ^{123}I-FP-CIT SPECT (Tijero, Gomez-Esteban et al. 2012). El hecho de que sujetos asintomáticos desde el punto de vista motor presenten disfunción cardíaca autonómica (clínica o subclínica) podría reforzar la idea de una afectación precoz del sistema cardiovascular en la PD.

Además de los sujetos portadores de mutaciones patógenas, otro grupo de sujetos considerados como de alto riesgo para el desarrollo de una sinucleinopatía (PD o DLB) y que permite el estudio *in vivo* de la integridad autonómica cardiaca está formado por los pacientes con trastorno de conducta del sueño REM (TCSR). Estudios observacionales han permitido el desarrollo de modelos estadísticos que estiman el riesgo de presentar una enfermedad neurodegenerativa en el 52.4% a los 12 años de haberse iniciado la clínica de TCSR (Postuma, Gagnon et al. 2009). El sistema cardiovascular autónomo ha sido recientemente estudiado en esta población de alto riesgo. Estudios funcionales, bien basados en exploraciones neurofisiológicas (Ferini-Strambi, Oldani et al. 1996; Lanfranchi, Fradette et al. 2007; Postuma, Lanfranchi et al. 2010; Sorensen, Kempfner et al. 2012) o en cuantificación de imágenes de captación cardíaca de ^{123}I-MIBG (Miyamoto, Miyamoto et al. 2006; Miyamoto, Miyamoto et al. 2008; Miyamoto, Miyamoto et al. 2011) han demostrado resultados anómalos en los pacientes con diagnóstico de TCSR.

Al igual que los estudios funcionales apoyan la hipótesis de una disfunción temprana del sistema autónomo cardíaco en la PD, varios estudios *post-mortem* han demostrado la presencia de agregados de AS cardíaca en sujetos sin síntomas clínicos de parkinsonismo (iLBD y TCSR)(Iwanaga, Wakabayashi et al. 1999; Okada, Ito et al. 2004; Orimo, Uchihara et al. 2008; Miki, Mori et al. 2009; Iranzo, Gelpi et al. 2014).

3.2 Afectación premotora del tracto gastrointestinal en la enfermedad de Parkinson

Se ha demostrado la presencia de agregados de AS en plexos digestivos en un 4% de sujetos sin PD conocida que se sometieron a cirugía oncológica. Estos casos "positivos" han sido seguidos en el tiempo y han presentado con mayor frecuencia que los casos negativos rasgos parkinsonianos y alteración en pruebas de disautonomía (Minguez-Castellanos, Chamorro et al. 2007). De manera retrospectiva, varios estudios han hallado depósitos anómalos de AS en SNE en piezas quirúrgicas del tracto gastrointestinal obtenidas años antes del inicio del parkinsonismo clínico, lo que podría apoyar de nuevo el inicio periférico de la enfermedad (Shannon, Keshavarzian et al. 2012; Ito, Takao et al. 2014).

Por otra parte, en los últimos años modelos animales han demostrado cómo un agente químico administrado externamente vía digestiva (la rotenona, inhibidora del complejo mitocondrial I) puede inducir no sólo la agregación de AS a nivel entérico sino también su propagación hacia la médula espinal y núcleos del troncoencéfalo como el núcleo motor dorsal del vago (Pan-Montojo, Anichtchik et al. 2010). El mismo grupo ha demostrado en este modelo animal que la propagación se detenía si se destruía la inervación entérica simpática y parasimpática. Tras esta destrucción, los núcleos del troncoencéfalo permanecían respetados a pesar de existir alteración a nivel entérico (Pan-Montojo, Schwarz et al. 2012). Se trataría de la demostración *in vivo* en un modelo animal de la teoría del inicio digestivo de la PD (Hawkes, Del Tredici et al. 2007).

La relación entre la disfunción gastrointestinal precoz y la aparición de parkinsonismo se ha apoyado también en estudios epidemiológicos. Por una parte, el grupo Honolulu-Asia Aging Study ha encontrado una correlación entre la frecuencia de movimientos intestinales en las etapas últimas de la vida y la presencia de cuerpos de Lewy incidentales en la sustancia negra y en el locus ceruleus (Abbott, Ross et al. 2007). También ha relacionado esta frecuencia con la densidad neuronal en la sustancia negra medida *post-mortem* (Petrovitch, Abbott et al. 2009). Por otra parte, estudios observacionales han sugerido que el estreñimiento puede ser una de las primeras manifestaciones disautonómicas de los futuros pacientes con PD y aparecer hasta 12 años antes (Abbott, Petrovitch et al. 2001; Gao, Chen et al. 2011). Así, un metanálisis reciente ha calculado en un 2.34 el riesgo relativo para desarrollar PD en sujetos con estreñimiento frente a sujetos no estreñidos (Noyce, Bestwick et al. 2012).

La idea de una afectación premotora del SNE también está apoyada por estudios en población con alto riesgo de desarrollar PD. Así, sujetos con TCSR presentan una respuesta anormal de liberación del péptido grelina (que podría ser neuroprotector) en el estado postprandial (Unger, Moller et al. 2011). Además, pruebas funcionales han evaluado la velocidad de vaciamiento gástrico mediante la prueba de aliento con ^{13}C-acetato, encontrándose reducida ya en pacientes con PD inicial no tratada (Tanaka, Kato et al. 2011).

4. Estudio *in vivo* del sistema nervio autónomo en la enfermedad de Parkinson

Hasta ahora en la introducción de esta defensa de tesis doctoral se ha resumido el conocimiento actual sobre la afectación del sistema nervioso autónomo en la enfermedad de Parkinson y la hipótesis de que esta afectación puede estar ya presente en un estadio premotor. Sin embargo, estas ideas no son solamente aplicables en el campo de la investigación, sino que el estudio del sistema nervioso autónomo tiene su aplicación en la práctica clínica diaria en el ámbito de las enfermedades neurodegenerativas.

Como hemos detallado, las entidades descritas hasta ahora en este proyecto de tesis doctoral se caracterizan desde un punto de vista anatomopatológico por el hallazgo de agregados de AS a nivel del sistema nervioso central y del SNA. Sin embargo, hay otras entidades que desde el punto de vista clínico pueden ser indistinguibles de la PD pero que en el estudio histológico no presentan estos agregados. Entre estas formas podemos encontrar los parkinsonimos farmacológicos, alteraciones de la marcha en contexto de hidrocefalia y el parkinsonismo vascular. La importancia de realizar un correcto diagnóstico radica en el hecho de que el pronóstico y el tratamiento van a ser distintos, evitando por ejemplo el uso de ciertos fármacos si se sospecha una sinucleinopatía de base.

Así, a pesar de que el parkinsonismo que acompaña a una enfermedad vascular cerebral pueda ser idéntico al observado en la PD (Zijlmans, Daniel et al. 2004), como se ha venido repitiendo en esta defensa de tesis la PD es mucho más que una enfermedad motora. El estudio de los síntomas no motores entre los que se encuentra la disautonomía podría ser una de las claves para diferenciar entre el parkinsonismo vascular y la PD.

5. El sistema nervioso autónomo en la demencia con cuerpos de Lewy

La DLB es una sinucleinopatía caracterizada por un deterioro cognitivo progresivo que interfiere con las actividades cotidianas del paciente (demencia) con especial afectación de la atención, funciones ejecutivas y visuoespaciales. Para poder diagnosticarla es necesaria además la presencia de fluctuaciones cognitivas tanto de atención como de nivel de alerta, alucinaciones visuales recurrentes y parkinsonismo. El hallazgo de dos de estas características clínicas junto con una demencia haría factible el diagnóstico de "probable DLB" mientras que con una el diagnóstico sería de "posible DLB". Se ha definido el punto de corte de un año de manera arbitraria para diferenciar entre la entidad llamada "demencia asociada a PD" y la DLB. Así pues, el término DLB se reserva para aquellos casos en los que la demencia precede o acompaña al parkinsonismo en un lapso de tiempo menor a un año (McKeith, Dickson et al. 2005).

Desde el punto de vista de la afectación del SNA, según los criterios actuales la disfunción autonómica grave y la disminución de la captación en el MIBG SPECT cardiaco se consideran criterios de soporte para el diagnóstico de DLB (McKeith, Dickson et al. 2005). La MIBG es un análogo de la guanitidina que es captada a nivel de las terminaciones postgangliónicas presinápticas. Se comporta como un análogo de noradrenalina, por lo que su unión a terminaciones nerviosas permite la valoración de la inervación simpática. Una vez inyectado, se realiza un SPECT con el que se puede calcular el ratio mediastino:corazón de captación de MIBG. La frecuencia de afectación del sistema nervioso autónomo en una serie con confirmación neuropatológica del diagnóstico de DLB es tan alta como el 98%. En esta serie, el síntoma más frecuente de disautonomía recogido era la incontinencia urinaria (98%) seguida por el estreñimiento (83%) y por hipotensión (66%) (Horimoto, Matsumoto et al. 2003).

Al ser tan frecuente la afectación del sistema nervioso autónomo en la DLB, hay autores que sugieren que el MIBG SPECT podría jugar un papel importante como herramienta de diagnóstico diferencial entre DLB y otras causas de demencia ya que en la clínica diaria puede ser complicado el diagnóstico diferencial inicial entre una enfermedad de Alzheimer (la causa de demencia más frecuente) y una DLB. En una revisión reciente se estudia el papel que juega el estudio *in vivo* del SNA mediante escintigrafía miocárdica con MIBG en el diagnóstico diferencial entre la DLB y otras causas de demencia. Después de revisar 8 estudios con un total de 346 pacientes, los autores concluyen que el MIBG SPECT tiene una sensibilidad del 98% (ausencia

práctica de falsos negativos) con una especificidad del 94% (muy pocos falsos positivos) para el diagnóstico diferencial entre la LBD y otras causas de demencia (Treglia and Cason 2012). A pesar de estos datos tan prometedores, el MIBG SPECT es una técnica relativamente nueva por lo que se desconoce su utilidad para el diagnóstico diferencial de otras entidades.

Al igual que se ha descrito en la PD, la disautonomía puede preceder a la DLB. (Kaufmann, Nahm et al. 2004). Recientemente, la presencia de disautonomía clínica se ha relacionado con una menor supervivencia de los pacientes con DLB (Stubendorff, Aarsland et al. 2012).

En cuanto a la neuropatología de la DLB, el estudio anatomopatológico del sistema nervioso central valora tanto los hallazgos de AS en forma de cuerpos y neuritas de Lewy como cambios compatibles con el diagnóstico de otros tipos de demencia. Se recomienda realizar una valoración semicuantitativa de la presencia de sinucleinopatía en distintas zonas cerebrales que permite la clasificación de la enfermedad en tres subtipos: predominio en troncoencéfalo, límbico y neocortical difuso. Mientras aquellos sujetos con el subtipo de predominio en troncoencéfalo tienen una probabilidad baja de ser diagnosticados de DLB, aquellos con los subtipos límibico y neocortical difuso tiene una probabilidad intermedio-alta. Sobre la afectación del SNA en la DLB la literatura es aun escasa. En el extenso trabajo realizado por el grupo de Beach y colaboradores (Beach, Adler et al. 2010) se estudiaron 9 sujetos con diagnóstico *post-mortem* de DLB. En ellos, la afectación por agregados de AS a nivel de la médula espinal era universal (9/9) seguida por la afectación de ganglios simpáticos (7/9), tracto gastrointestinal (5/9) y nervio vago (4/6).

En resumen en esta introducción hemos querido destacar la importancia del estudio del sistema nervioso autónomo en las sinucleinopatías. Sin embargo, todavía quedan preguntas sin respuesta tales como el momento en el que se inicia la afectación a este nivel, cómo es posible evaluarla, qué implicaciones prácticas podría tener etc. Con esta tesis pretendemos aumentar el conocimiento sobre estos y otros aspectos de la afectación del SNA en las sinucleinopatías.

III.- Hipótesis

HIPÓTESIS

En esta tesis se pretenden describir las características del sistema nervioso autónomo en la enfermedad de Parkinson y otras sinucleinopatías, partiendo de las siguientes hipótesis:

1.- En las enfermedades neurodegenerativas asociadas a cuerpos de Lewy a nivel del sistema nervioso central existe una afectación generalizada del SNAP.

2.- En la enfermedad de Parkinson la afectación del SNAP ya está presente en el momento de aparición de los síntomas motores clásicos y también en la fase premotora.

3.- La alteración en el SNAP permite la distinción entre enfermedad de Parkinson y otros parkinsonismos no asociados a cuerpos de Lewy.

HIPÓTESIS

IV.- Objetivos

1.- Estudiar la presencia, extensión y distribución de la patología por cuerpos de Lewy/agregados de AS en el sistema nervioso central y en el sistema nervioso autónomo periférico en tejidos *post-mortem* de individuos diagnosticados de una enfermedad con cuerpos de Lewy.

2.- Estudiar en vida la integridad del SNAP cardiaco y la presencia de agregados de AS en sujetos sin síntomas motores de parkinsonismo y correlacionar estos hallazgos con la presencia de síntomas no motores, incluyendo disautonomía.

3.- Valorar la utilidad del estudio funcional del SNAP cardiaco en el diagnóstico diferencial de la enfermedad de Parkinson.

V.-Resultados

Trabajo número1

Multiple organ involvement by alpha-synuclein pathology in Lewy-body disorders

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Afectación de múltiples órganos por patología tipo alfa-sinucleína en las enfermedades asociadas a cuerpos de Lewy

Las enfermedades asociadas a cuerpos de Lewy (LB) están caracterizadas por presentar agregados de alfa-sinucleína (AS) en el sistema nervioso central (CNS). La afectación del sistema nervioso autónomo periférico (pANS) es cada vez más reconocida, aunque está menos estudiada. El objetivo de este trabajo fue analizar de manera sistemática la distribución y gravedad de la patología por AS en el CNS y en el pANS. Se realizó un estudio histopatológico detallado *post-mortem* tanto de tejido cerebral como de tejidos periféricos provenientes de 28 donantes del banco de tejidos neurológicos (10 sujetos con enfermedad de Parkinson (PD), 5 con demencia con cuerpos de Lewy (DLB) y 13 con enfermedades no relacionadas con LB incluyendo parkinsonismos atípicos y demencias sin relación con LB. Se encontraron agregados de AS en el pANS de los 15 sujetos con enfermedades asociadas a LB (PD, DLB) en el ganglio estrellado y ganglios simpáticos (100%), nervio vago (86.7%), tracto gastrointestinal (86.7%), glándula suprarrenal y/o grasa cercana (53.3%), corazón (100%) y tracto génito-urinario (13.3%), al igual que en 1 caso con enfermedad por cuerpos de Lewy incidentales (iLBD). Se observó un gradiente descendente cráneo-caudal de agregados de AS tanto en la cadena simpática como en el tracto gastrointestinal. Los sujetos con DLB presentaban mayor cantidad de agregados de AS en el CNS que los sujetos con PD, pero esta diferencia no se observó en el pANS. No se encontraron agregados de AS en el pANS en los sujetos con enfermedad de Alzheimer (AD) independientemente de presentar o no agregados de AS en el CNS.

Resumen: Todos los sujetos con confirmación patológica de tener una enfermedad ligada a LB incluyendo 1 caso con iLBD tenían agregados de AS en el pANS con un gradiente descendente cráneo-caudal de patología en la cadena simpática y en el tracto gastrointestinal. No se detectó AS en el pANS en ningún caso con AD. Estos hallazgos podrían ayudar en la búsqueda de agregados de AS periférica *in vivo* para un llegar a un diagnóstico precoz de PD.

RESEARCH ARTICLE

Multiple Organ Involvement by Alpha-Synuclein Pathology in Lewy Body Disorders

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ABSTRACT: Lewy body (LB) diseases are characterized by alpha-synuclein (AS) aggregates in the central nervous system (CNS). Involvement of the peripheral autonomic nervous system (pANS) is increasingly recognized, although less studied. The aim of this study was to systematically analyze the distribution and severity of AS pathology in the CNS and pANS. Detailed postmortem histopathological study of brain and peripheral tissues from 28 brain bank donors (10 with Parkinson's disease [PD], 5 with dementia with LB [DLB], and 13 with non-LB diseases including atypical parkinsonism and non-LB dementia). AS aggregates were found in the pANS of all 15 LB disease cases (PD, DLB) in stellate and sympathetic ganglia (100%), vagus nerve (86.7%), gastrointestinal tract (86.7%), adrenal gland and/or surrounding fat (53.3%), heart (100%), and genitourinary tract (13.3%), as well as in 1 case of incidental Lewy body disease (iLBD). A craniocaudal gradient of AS burden in sympathetic chain and gastrointestinal tract was observed. DLB cases showed higher amounts of CNS AS aggregates than PD cases, but this was not the case in the pANS. No pANS AS aggregates were detected in Alzheimer's disease (AD) cases with or without CNS AS aggregates. All pathologically confirmed LB disease cases including 1 case of iLBD had AS aggregates in the pANS with a craniocaudal gradient of pathology burden in sympathetic chain and gastrointestinal tract. AS was not detected in the pANS of any AD case. These findings may help in the search of peripheral AS aggregates in vivo for the early diagnosis of PD. © 2014 International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease; dementia with Lewy bodies; Parkinson's disease with dementia; autonomic diseases; alpha-synuclein

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Abnormal aggregation of alpha-synuclein (AS) in the perikaryon, axons, and dendrites of neurons of different brain regions represents the molecular and pathological hallmark of Parkinson's disease (PD) and other Lewy body (LB) diseases. These aggregates have been increasingly found in tissues outside the central nervous system (CNS), mainly in the peripheral autonomic nervous system (pANS).[1-5] These observations have changed our understanding of PD from the conception of a disorder with selective involvement of nigrostriatal dopaminergic neurons to a much broader multisystem LB disorder, also characterized as "Parkinson complex"[6] or Lewy-complex.[7]

It is still unclear whether peripheral pathology precedes or occurs concomitantly to the central pathology and whether it progresses in a predictable way as has been proposed for Lewy pathology in the CNS.[7] It is a relatively frequent finding in subjects who have LB pathology at autopsy but have not shown parkinsonism during life (so-called incidental Lewy body disease [iLBD]) and in some such cases, postmortem studies have reported AS aggregates in pANS even without CNS involvement[8] (see Supporting Table 1), suggesting that pANS involvement might be an early, pre-CNS event in PD pathogenesis. A detailed revision of the peripheral autonomic involvement in neurodegenerative disorders in general and alpha-synucleinopathies in particular, has been published recently.[9]

In the present study we aimed to systematically assess the distribution and severity of AS pathology in central and peripheral structures of the nervous system in postmortem tissue of donors with clinical diagnosis of PD and dementia with Lewy bodies (DLB) and other non-LB related neurodegenerative diseases.

Materials and Methods

Consecutive brain donors of the Neurological Tissue Bank of the Biobank-Hospital Clinic-IDIBAPS with the diagnosis of PD, DLB, and other non-LB disorders (atypical parkinsonisms and non-LB dementia) were included in the study. Written informed consent was obtained from donors and/or next of kin and the study was approved by our Institutional Ethics Committee.

The following tissues were prospectively collected: brain and spinal cord, pituitary gland, vagus nerve at brainstem and thoracic level, stellate ganglion and paravertebral sympathetic ganglia of both sides, mesenteric sympathetic ganglion, adrenal glands and surrounding fat tissue, distal esophagus, stomach (cardias, corpus, and fundus), ileum, colon (transverse and descending), rectum, periprostatic plexus or uterus, urinary bladder, heart, abdominal skin, and psoas muscle.

Processing of Samples

Brain

The left brain hemisphere and hemicerebellum were sliced in the fresh state and frozen at −80°C. The right hemisphere, hemicerebellum, and alternate sections of brainstem and spinal cord were fixed in 10% buffered formaldehyde and multiple brain areas were embedded in paraffin for histopathological evaluation.

TABLE 1. Study subjects

Diagnosis (n)	Age (y)	Gender (% male)	PMI (h)	Disease duration (y)
PD (4)	79.75 (8.1)	25	9.58 (6)	11.5 (9)
PDD (6)	79.67 (6.1)	50	9.97 (5)	18 (8.8)
DLB (5)	80 (3.7)	20	14.23 (4.3)	4.6 (2.6)
AD (8)	86.13 (5.1)	25	10.67 (6.6)	8.75 (4.1)
Others (5)[a]	78.4 (10.2)	40	10.78 (5.2)	9.4 (6.4)

Values are mean (SD) except where indicated.
[a]Others: Progressive supranuclear palsy (1), vascular dementia (1), vascular dementia and Alzheimer-type pathology (1), frontotemporal lobar degeneration (1), hepatic encephalopathy + mild AD-related changes (1).
PMI, *postmortem* interval; PD, Parkinson's disease; PDD, Parkinson's disease-dementia; DLB, dementia with Lewy bodies; AD, Alzheimer's disease.

Peripheral Tissues

One half of the sampled tissues was fresh-frozen and stored at −80°C, the other half was fixed in 10% buffered formaldehyde solution, embedded in paraffin and cut into 5-μm-thick sections for histopathological assessment.

Brain and spinal cord sections were stained with hematoxylin and eosin (H&E) and by immunohistochemistry using an automated immunostainer (Dako Autostainer plus, Glostrup, Denmark) with the following monoclonal and polyclonal (pc) primary antibodies: anti-alpha-synuclein (Novocastra, Newcastle, UK; clone KM51), anti-phospho-alpha-synuclein (Wako Pure Chemical Industries LTD, Japan; phosphorylated at Ser 129), anti-bA4-amyloid (DAKO, Glostrup, Denmark, clone 6F/3D), anti-phosphorylated tau (Thermo Scientific, Rockford, IL, USA; clone AT8), anti-ubiquitin (DAKO, pc), anti-TDP-43 (Abnova, Taipei, Taiwan; clone 2E2-D3), anti-RD3 (Millipore, Temecula, CA, USA; clone 8E6/C11), anti-RD4 (Millipore, clone 1E1/A6), anti-p62 (BD Transduction Laboratories, NJ, USA; clone 3/p62 lck ligand).

Peripheral tissues were stained with H&E and by immunohistochemistry for detection of alpha-synuclein (clone KM51) and tyrosine-hydroxylase (TH) (Sigma-Aldrich, St. Louis, MO, USA; clone TH-16). In selected cases and areas, anti-phospho-alpha-synuclein (pAS), considered to be a more specific and sensitive marker of LB-related pathology, anti-TDP43, anti-hpTau, and anti-betaA4 antibodies were also applied in the same conditions as in the CNS.

Semiquantitative Evaluation

The presence of LBs in H&E-stained sections was evaluated as present or absent. The density of AS-immunoreactive LBs and neurites (using the KM51

antibody) was semiquantitatively assessed according to McKeith et al.[10] as: 0, absent; 1, isolated or mild (Fig. 3N); 2, moderate (Fig. 3F); and 3, abundant (Fig. 3B). Density of TH-immunoreactive nerve fibers and neurons was also semiquantitatively assessed in selected areas as: 3, normal (Fig. 3H); 2, moderately reduced (Fig. 3G); 1, severely reduced (Fig. 3K); and 0, absent. In selected cases, stellate ganglion was analyzed for the presence of AT8, betaA4, TDP43-immunoreactive structures and was indicated as present (1) or absent (0).

Other, non-LB related pathologies in the brain were evaluated according to their respective diagnostic criteria.

Comparative Studies and Statistical Analyses

Comparisons between central and peripheral pathology and clinical variables among the different disease groups were performed using Kruskal Wallis or Spearman correlation tests when appropriate.

Results

Patients

Twenty-eight adult donors were included between April 2009 and January 2010. Thirty-two percent were male. Mean age was 81.36 years (range, 62–93). The mean postmortem delay was 11.02 hours (range, 4.25-21.35).

Clinicopathological diagnoses comprised PD (n = 10; 6 with dementia), DLB (n = 5), AD (n = 8), progressive supranuclear palsy (PSP) (n = 1), vascular encephalopathy with small vessel disease (n = 2, one with associated AD neuropathological changes), and frontotemporal lobar degeneration with motor neuron disease and TDP43+ inclusions (n = 1). One case with cognitive impairment showed iLBD/AS pathology in brain stem nuclei and intermediolateral column of spinal cord (n = 1) (Table 1), along with mild AD neuropathological changes, and was considered an iLBD case.

Mean disease duration from the time of diagnosis to death was 10.5 years (range 1–30). Patients with LB pathology (PD and DLB, n = 15) had a mean age at the time of death of 79.8 years (range, 68–86) and a mean disease duration of 11.8 years (range, 1–30). DLB patients presented a shorter disease duration compared to PD (4.6 vs 15.4 years; Mann-Whitney U, $P = 0.012$), without differences in age at the time of death (80 vs 79.7 years).

Distribution of AS Aggregates in the CNS

All cases with the diagnosis of an LB disorder (n = 15) presented AS aggregates in the intermediolateral column of spinal cord, brainstem, limbic region, and variably in cortical areas. PD cases had a median Braak stage of 5 (range, 4–5)[7] and DLB cases were all classified as neocortical stage.[10] Data from a semiquantitative assessment are shown in Supporting Figure 1 and in Table 2. The highest AS density was found in the olfactory and limbic regions in both LB conditions, while there was less severe cortical involvement in PD than in DLB (Supporting Fig. 1). Overall, DLB patients showed a higher amount of CNS AS aggregates compared to PD (mean 2.24 vs 1.89; Mann-Whitney U, $P = 0.017$) (Fig. 1A). This difference was mainly due to a greater involvement of limbic and cortical structures (limbic 2.41 vs 2.0, Mann-Whitney U, $P = 0.022$; cortical 1.8 vs 1.23, Mann-Whitney U, $P = 0.022$). A correlation study showed a weak, nonsignificant negative relationship between mean AS load and disease duration.

In 4 AD cases, mild to moderate concomitant AS immunoreactive (AS+) LBs and Lewy neurites (LNs) were observed in amygdala and/or olfactory bulb but not in other brain regions. In the 1 iLBD case, infrequent LN were observed in brainstem nuclei such as dorsal nucleus of the vagal nerve, formatio reticularis, locus coeruleus, and in the intermediolateral column of spinal cord, corresponding to Braak stage 2.

Distribution of AS/pAS Aggregates in Peripheral Organs

LB and AS/pAS aggregates in pANS were found in the 15 LB disorders and the single iLBD case in different grades of severity in the following distribution (see also Figs. 2 and 3, Supporting Fig. 2, and Table 2). No AS aggregates were detected in the pANS of non-LB diseases.

Paravertebral Sympathetic Ganglia

Sympathetic paravertebral ganglia were affected in all LB disease cases and were the structures with the highest AS/pAS burden. Affected ganglia showed some vacuolated neurons and variable loss of pigmented and nonpigmented neurons with increased cellularity and formation of Nageotte nodules (Figs. 2A, 3A). Abundant LB and LN-like structures were observed in H&E-stained sections as large, pale, or bright eosinophilic amorphous structures, in neuronal processes and in the perikaryon of sympathetic neurons (Fig. 2A). The highest density of LB and LN occurred at the periphery of the ganglia. LB and LN were immunoreactive for AS (Fig. 3B), pAS (not shown), and ubiquitin (Fig. 2I) and showed a peripheral rim stained by anti-neurofilaments (Fig. 2H). They were also faintly and irregularly stained by anti-hyperphosphorylated Tau (Fig. 2D) and nonspecifically by anti-TDP43 antibodies (Fig. 2F, arrows). No beta-A4 deposits were identified in any ganglion.

A gradient of severity of pathology was observed with the highest AS burden present in the stellate

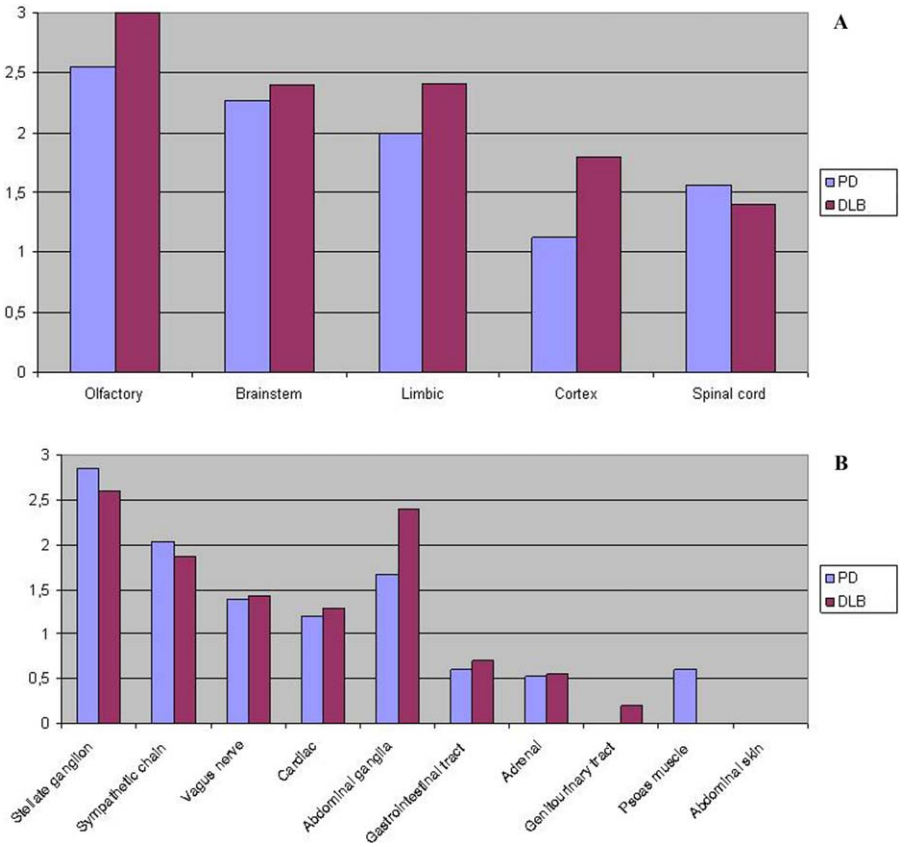

FIG. 1. AS density in CNS and pANS. Comparison of semiquantitatively assessed AS density in grouped CNS areas (**A**) and pANS (**B**) between PD and DLB. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ganglion and in the cervical sympathetic ganglia followed by a progressive reduction in AS density from upper thoracic to the lower thoracic and lumbar sympathetic ganglia. A reduction of TH immunoreactivity in some neuronal cell bodies and nerve fibers was also observed (Fig. 2B, 2G, and Fig. 3C).

These alterations (cell loss and AS aggregates) and the rostrocaudal gradient were seen in both, PD and DLB cases, but were more prominent in DLB cases.

Moreover, other sympathetic ganglia such as the mesenteric ganglion were also affected in PD and DLB cases and showed a similarly high density of AS aggregates as in the cervical sympathetic ganglia.

Heart

Multiple areas were sampled to include the regions more likely to contain AS aggregates. The most frequently involved areas were the anterior and lateral wall of the left ventricle. AS/pAS aggregates were detected as LN of variable thickness in the myocardium, in the intramyocardial nerve fibers (Fig. 3J) and also at perivascular location in 2 cases. Most frequently, however, AS/pAS+ LN and LB-like structures were identified in epicardial fat tissue in small nerves and small autonomic ganglia, both in neuronal processes and cell bodies (Fig. 3F). These changes were observed in all PD and DLB cases. They were also frequently observed in nerve branches around coronary arteries. Immunostaining for TH showed a reduction of the density of TH-positive fibers in AS+ cases compared to cases without AS aggregates between myocytes (Fig. 3K vs 3L) and in epicardial fat tissue fibers (Fig. 3G vs 3H), suggesting some degree of denervation, although these data were not quantified. A positive correlation between TH fiber density and density of AS aggregates, ie, less AS-aggregates in cases with less TH innervation, was observed (data not shown).

Vagus Nerve

AS aggregates were observed along nerve fibers most frequently at the brainstem level. At the thoracic level isolated positive fibers were seen in several LB disease cases.

Gastrointestinal System

We assessed distal esophagus, stomach (cardias, corpus, and fundus), ileum, colon (transverse and descending), and rectum. Delicate AS/pAS-immunoreactive neuronal processes were detected in the autonomic ganglia of the myenteric plexus in some cases

TABLE 2. AS aggregates in the CNS and pANS

	PD (n = 10)	DLB (n = 5)	AD (n = 8)	Others (n = 5)[a]
CNS region				
Olfactory	10/10, 100%	5/5, 100%	4/8, 50%	0/5, 0%
Brainstem	10/10, 100%	5/5, 100%	1/8, 12.5%	1/5, 20%
Limbic	10/10, 100%	5/5, 100%	3/8, 37,5%	0/5, 0%
Cortical	9/10, 90%	5/5, 100%	0/8, 0%	0/5, 0%
Spinal cord	10/10, 100%	5/5, 100%	0/8, 0%	1/5, 20%
pANS				
Stellate ganglion	10/10, 100%	5/5, 100%	0/8, 0%	1/5, 20%
Sympathetic chain	10/10, 100%	5/5, 100%	0/8, 0%	1/5, 20%
Abdominal ganglia	8/10, 80%	5/5, 100%	0/8, 0%	1/5, 20%
Vagus nerve	9/10, 90%	4/5, 80%	0/8, 0%	1/5, 20%
GI	8/10, 80%	5/5, 100%	0/8, 0%	1/5, 20%
Cardiac	10/10, 100%	5/5, 100%	0/8, 0%	1/5, 20%
GU	0/10, 0%	2/5,40%	0/8, 0%	0/5, 0%
Adrenal	6/10, 60%	2/5, 40%	0/6, 0%	0/5, 0%
Psoas	6/10, 60%	0/5, 0%	0/8, 0%	0/5, 0%
Skin	0/10, 0%	0/5, 0%	0/8, 0%	0/5, 0%

Values are absolute numbers (percentage).
[a]Others: Progressive supranuclear palsy (1), vascular dementia (1), vascular dementia and Alzheimer-type pathology (1), frontotemporal lobar degeneration (1), hepatic encephalopathy + mild AD-related changes (1).
AS, alpha-synuclein; CNS, central nervous system; pANS, peripheral autonomic nervous system; PD, Parkinson's disease; DLB, dementia with Lewy bodies; AD, Alzheimer's disease; GI, gastrointestinal system; Cardiac, heart, cardiac plexus and epicardial fat; GU, genitourinary system; Adrenal, adrenal gland.

(Fig. 3N), whereas in others, coarse LB-type inclusions were seen. AS/pAS aggregates were observed in distal esophagus, stomach, and colon, in all DLB cases and 80% of PD. We observed a gradient of severity of pathology with the highest AS burden present in distal esophagus and stomach, and the lowest in rectum. No AS/pAS aggregates were observed in lamina propria mucosae. The presence of AS aggregates was not related to the presence of inflammatory infiltrates.

Adrenal Glands

Isolated LB and/or LN were seen in the medulla of adrenal gland but were more frequently found in the autonomic ganglia embedded in the periadrenal fat tissue. TH immunoreactivity was very intense in adrenal medulla in both non-LB and LB disorders.

Vesicoprostatic/Vesicouterine Plexus

AS/pAS aggregates were not detected in any PD case and only in 2 DLB cases.

Psoas Muscle

Fine AS+ neurites were identified in 6 cases in nerve fibers crossing the muscle, but not in muscle cells themselves.

Abdominal Skin

No AS aggregates were detected in abdominal skin in any of the cases studied.

In PD and DLB cases there were significantly more aggregates in the CNS than in the pANS (Wilcoxon test, $P = 0.005$ and $P = 0.043$, respectively). No significant differences in the amount of peripheral AS between DLB and PD patients were detected (mean 1.11 vs 1.08, P = nonsignificant) (Fig. 1B). There was a weak, but nonsignificant relationship between AS density in the pANS and disease duration. No relationship between TH density and disease duration was found.

Discussion

In this study, all pathologically confirmed LB disorders had pANS involvement with AS/pAS aggregates, irrespective of the clinical diagnosis, including 1 iLBD case. These results are in accordance with previous studies showing that extensive AS pathology occurs in the pANS in PD, DLB, and iLBD.

As none of our cases with AD with or without associated olfactory and/or amygdala LB had AS aggregates in pANS, it might be suggested that CNS accumulation of AS in these cases may possibly represent a consequence of a more local effect of abnormal brain proteins such as beta-amyloid and tau, promoting AS fibrillation and its aggregation, rather than a "primary" phenomenon as observed in PD.

Organs invariably harboring AS pathology in our LB disease study subjects were the stellate ganglion and paravertebral sympathetic chain and the heart, with other systems more variably affected.

We observed a craniocaudal gradient of pathology burden involving paravertebral sympathetic chain and gastrointestinal tract; ie, more abundant AS aggregates in cervical and upper thoracic sympathetic ganglia than in lower lumbar ganglia, and more abundant AS

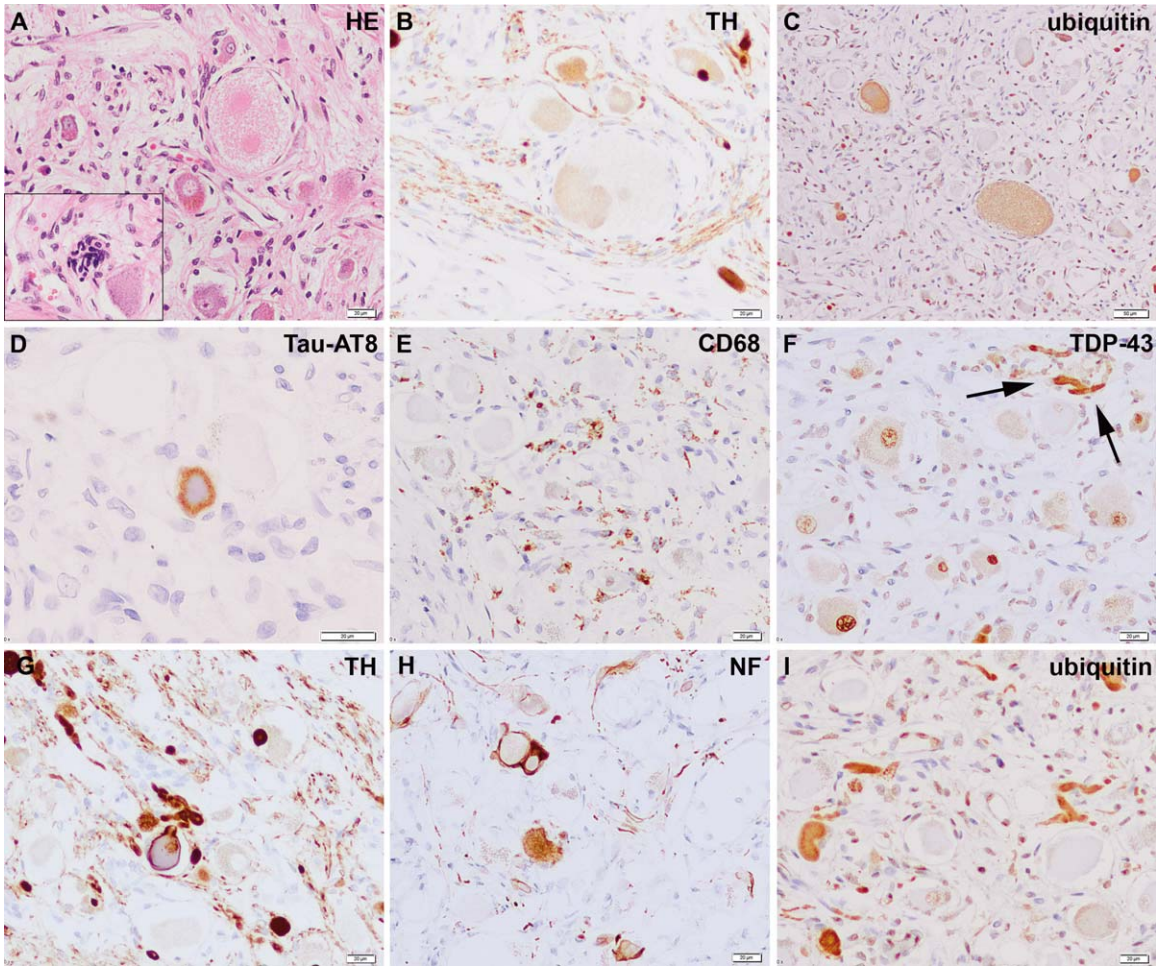

FIG. 2. Stellate ganglion. Histological images of an affected stellate ganglion in DLB showing some enlarged neurons (**A**, HE) that lost TH immunoreactivity (**B**, anti-TH) and are immunoreactive for ubiquitin (**C**, anti-ubiquitin). Some figures reminiscent of Nageotte nodules were also detected (**A**, inset). Some of the LB-like structures show a delicate rim of hpTau-immunoreactivity, as frequently seen in the CNS (**D**, AT8). Frequent CD68 positive microglia/macrophages were seen between neuronal bodies (**E**, anti-CD68). TDP43 immunohistochemistry showed physiological nuclear stain of sympathetic neurons and some unspecific stain of tortuous Lewy neurites (**F**, arrows). Some of the LB and LN were immunolabeled by anti-TH antibodies (**G**, center of the image), as did anti-neurofilaments (**H**) and anti-ubiquitin (**I**). Scale bars: 20 μm (**A**, **B**, **D**-**I**), 50 μm for (**C**). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

aggregates in esophagus and stomach than in colon. The rostrocaudal gradient within the gastrointestinal system has been also observed by others[5,11] and may reflect distribution of lesions in the territory of vagal innervation. The rostrocaudal gradient of pathology observed in the sympathetic chain might reflect greater involvement of cardiac than of the gastrointestinal autonomic system. In the heart, we observed a greater involvement with AS of the anterior wall of the left ventricle and of the epicardial autonomic tissue than of the myocardium, and a reduction of the density of AS aggregates with decreasing density of myocardial and epicardial TH-positive fibers, supporting a degeneration of sympathetic fibers as suggested by Orimo and colleagues.[1] Involvement of paravertebral sympathetic ganglia as well as of cardiac and gastrointestinal autonomic nervous system has been reported previously in postmortem studies (see Supporting Table 1) showing results comparable to ours. We did not assess submandibular or salivary glands as recently described,[5,12,13] neither endocrine or respiratory organs.[5] In contrast to the frequent involvement of vesicoprostatic plexus described, eg, by Minguez-Castellanos and colleagues[14] in asymptomatic subjects and Beach and colleagues,[5] we did not observe involvement of these structures in our postmortem material. AS/pAS aggregates in adrenal medulla were much less prominent than in periadrenal ganglia. Involvement of adrenal glands has been studied in detail by Fumimura and colleagues[3] and AS aggregates have been detected in about 26% of a series of 783 consecutive autopsies. Although they found involvement in all PD cases and 91% of DLB cases, the adrenal gland was not affected by AS aggregates in amygdala variant cases and LBD cases with concomitant AD pathology. Similarly, in our AD cases with amygdala-only LB we did not detect peripheral AS aggregates. Moreover, we could not detect AS

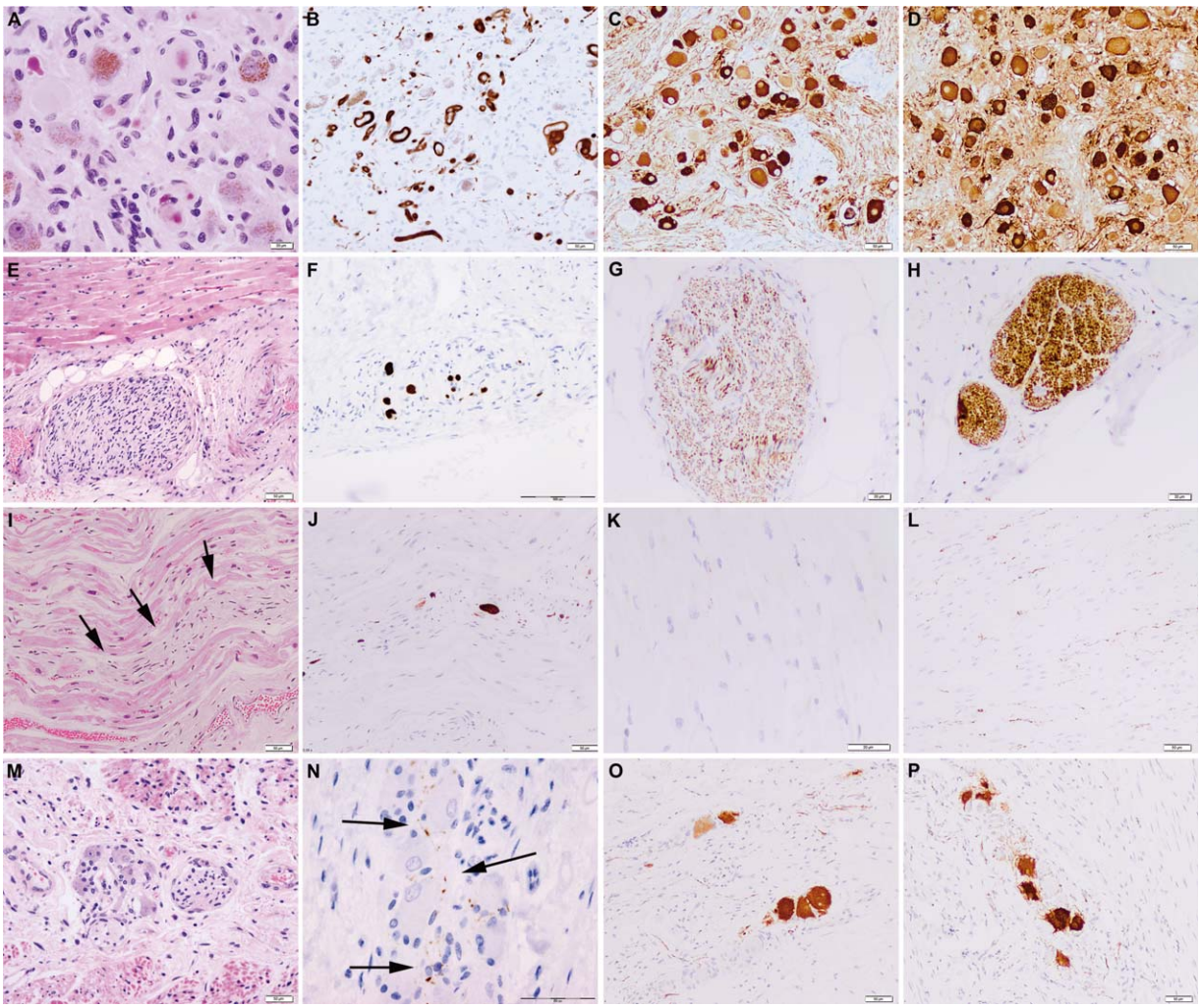

FIG. 3. Involvement of pANS. Representative images of pANS involvement of different organs in a DLB case and a non-DLB case. (**A**-**C**) represents stellate ganglion, (**E**-**G**) the epicardial fat tissue, (**I**-**K**) myocardial muscle and nerve fibers, and (**M**-**O**) myenteric plexus of esophagus in a DLB case compared to the same structures in (**D**), (**H**), (**L**), and (**P**) in a non-DLB case. **A:** Abundant LBs are already seen in HE stain between pigmented sympathetic neurons. These are strongly immunoreactive for AS (clone KM51), with a peripheral enhancement (**B**). Mild loss of TH immunoreactivity is observed in neuronal bodies and fibers between them (**C**) when compared to the density in an unaffected case (**D**). **E**-**H:** In epicardial fat tissue large autonomic nerves can be seen already in HE-stained sections (**E**). There, AS aggregates are seen along the nerve fibers as coarse deposits (**F**, clone KM51). There is a reduced density of TH-immunoreactive fibers (**G**) when compared to an unaffected case (**H**). **I**-**L:** In the myocardium, some large nerve fibers can be seen in HE-stained sections (**I**). Also these fibers may show coarse AS-aggregates in cases with shorter disease course (**J**). In these cases, there is a marked reduction of TH-immunoreactive profiles in the myocardium (**K**) when compared to an unaffected case (**L**). **M**-**P:** In the gastrointestinal system, autonomic ganglia of the myenteric plexus as seen in HE-stained sections (**M**) are usually the area where AS-aggregates can be found. These can be seen in some cases as delicate processes surrounding neuronal bodies (**N**, arrows). TH-immunoreactivity is only seen in few neurons (**O**), as seen also in nonaffected cases (**P**). Scale bars: 20 μm (**A**, **F**, **G**, **H**, **K**, **N**); 50 μm (**B**, **C**, **D**, **E**, **I**, **J**, **L**, **M**, **O**, **P**). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

aggregates in abdominal skin, described by Ikemura and colleagues[4] in 24% of LBD autopsy cases.

The cause of pANS involvement by LB-type pathology, as is the case for the selective involvement of some central neuronal systems, remains unclear. It has been speculated that peripheral toxins entering the body via the gastrointestinal tract could in part explain the prominent and early pANS involvement[15,16] in the LB disorders. The fact that the long parasympathetic neurons in the dorsal nucleus of the vagal nerve and the long sympathetic neurons in the paravertebral sympathetic ganglia are most severely affected supports Braak's consideration that the length of unmyelinated axons may stand behind this vulnerability.

Burden of AS may vary with disease progression both in the CNS and the peripheral nervous system. In the CNS, cases with shorter survival and more aggressive course show higher LB loads than cases with long clinical course.[17] In our study, higher AS load occurred in DLB brains when compared to PD cases, with or without dementia. DLB cases had shorter disease duration and the higher AS burden could reflect a more rapidly evolving process and, accordingly, a reduced AS turnover and increased deposition. However, differences in AS burden was not observed in the

pANS in these 2 disorders, maybe suggesting a similar peripheral AS turnover.

The results of our report are based on the study of subjects with late, very advanced disease. Distribution and severity of peripheral AS/pAS pathology may not be similar though in early PD subjects. It has been suggested that AS aggregation occurs at the axonal endings of neurons and later in the soma, progressively disappearing from the distal sites at later disease stages.[1] Should such developments take place, one could expect higher levels of AS/pAS in the distal end organs in early than in late disease stages. Postmortem studies in early PD are needed to validate this hypothesis. In iLBD, considered to represent an early, premotor stage of PD, AS aggregates have been described in the myenteric plexus of the esophagus and stomach, submandibular gland, paravertebral sympathetic ganglia, vagus nerve, sciatic nerve, and endocrine system.[5,12,18,19] In our single iLBD case, however, we did not observe higher AS load in peripheral organs.

The observed distribution of AS pathology in our subjects and in previous studies, suggests that the sympathetic cardiac and stellate/paravertebral ganglia would be informative targets for identifying AS aggregates in living subjects, and we have recently studied epicardial fat autonomic tissue obtained during surgery and observed AS/pAS in 8% of subjects without parkinsonism.[20] The obvious difficulties and risks involved in the access of such tissues through biopsy makes them unfeasible for diagnostic purposes. Cardiac sympathetic innervation, however, can be studied by functional imaging, using ligands such as ^{123}I-meta-iodobenzylguanidine (MIBG) single-photon emission computed tomography (SPECT),[21] and this imaging tool is being increasingly used for the differential diagnosis of atypical parkinsonism or in subjects at risk without parkinsonism.[22] In subjects with idiopathic rapid eye movement (REM) sleep behavior disorder, a symptom that is considered to represent prodromal phases of PD,[22-26] in vivo functional studies have shown involvement of pANS.[25,27,28] Moreover, recent studies have suggested that the identification of peripheral AS aggregates in pANS in vivo could be a diagnostic marker in the preclinical phase of subjects at high risk for PD.[29]

Several recent studies have studied colonic and salivary glands and identified AS in most of the tissues biopsied in advanced, early, and even premotor PD.[12,13,30-32] Due to its accessibility the distal gastrointestinal tract has been the focus of several recent studies on the presence of AS pathology in PD although diverging results in these tissues have been reported.[5,33,34] Our study suggests that AS aggregates may not always be present in these tissues in late stage of PD.

In conclusion, we observed AS/pAS in pANS in all LB disease cases studied, reinforcing the view that pANS involvement is an integral part of these disorders. pANS involvement, on the other hand, did not occur in AD cases despite the frequent presence of concomitant CNS LBs. The extensive involvement of the pANS in LB disorders suggests that tissue-based and functional studies of this system may prove useful in the future for an accurate diagnosis of LB diseases in vivo. ■

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References

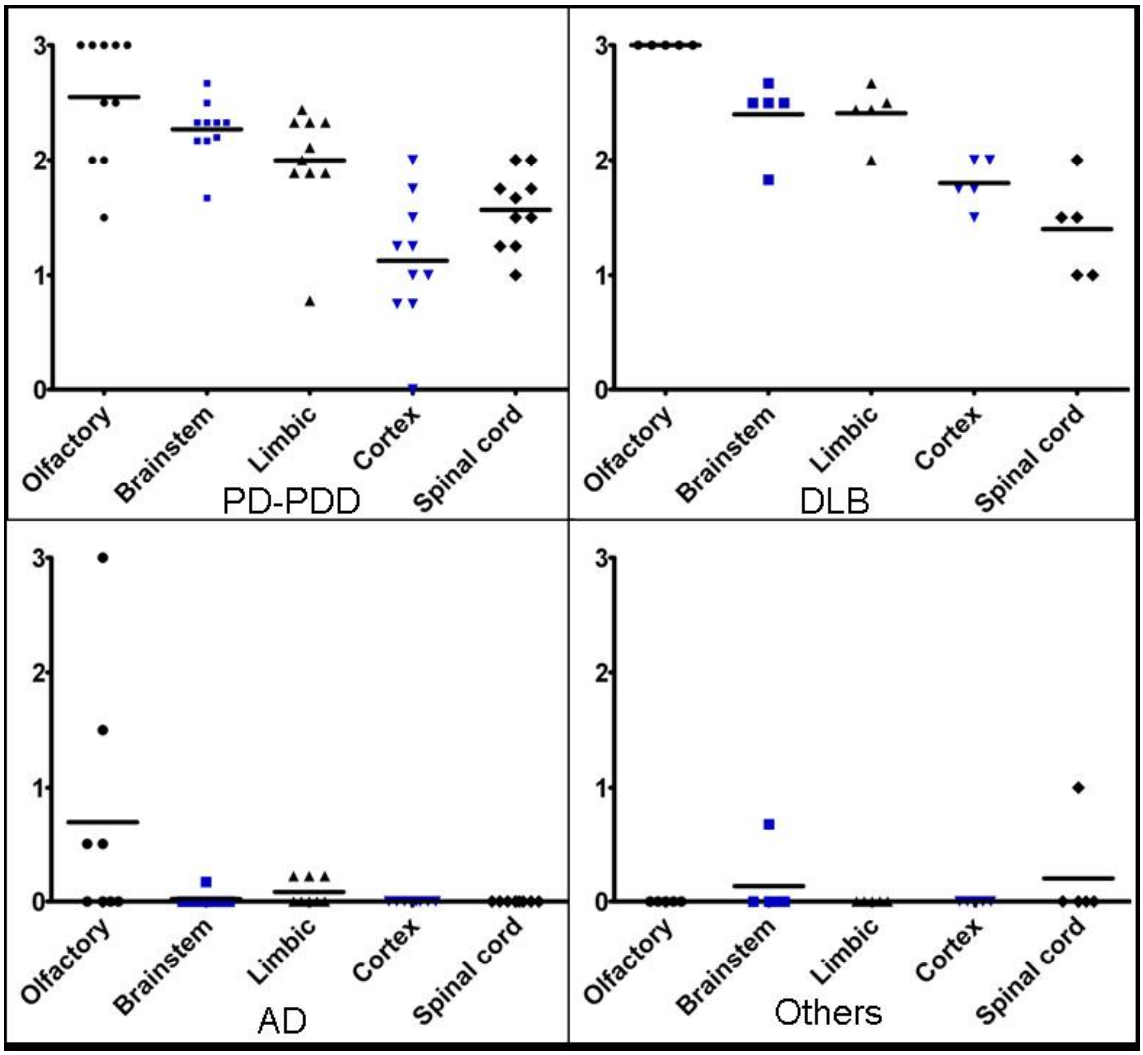
1. Orimo S, Uchihara T, Nakamura A, et al. Axonal alpha-synuclein aggregates herald centripetal degeneration of cardiac sympathetic nerve in Parkinson's disease. Brain 2008;131(Pt 3):642-650.
2. Wakabayashi K, Takahashi H. Neuropathology of autonomic nervous system in Parkinson's disease. Eur Neurol 1997;38(Suppl 2):2-7.
3. Fumimura Y, Ikemura M, Saito Y, et al. Analysis of the adrenal gland is useful for evaluating pathology of the peripheral autonomic nervous system in Lewy body disease. J Neuropathol Exp Neurol 2007;66:354-362.
4. Ikemura M, Saito Y, Sengoku R, et al. Lewy body pathology involves cutaneous nerves. J Neuropathol Exp Neurol 2008;67:945-953.
5. Beach TG, Adler CH, Sue LI, et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol 2010;119:689-702.
6. Langston JW. The Parkinson's complex: parkinsonism is just the tip of the iceberg. Ann Neurol 2006;59:591-596.
7. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197-211.
8. Miki Y, Mori F, Wakabayashi K, Kuroda N, Orimo S. Incidental Lewy body disease restricted to the heart and stellate ganglia. Mov Disord 2009;24:2299-2301.
9. Wakabayashi K, Mori F, Tanji K, Orimo S, Takahashi H. Involvement of the peripheral nervous system in synucleinopathies, tauopathies and other neurodegenerative proteinopathies of the brain. Acta Neuropathol 2010;120:1-12.
10. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005;65:1863-1872.
11. Wakabayashi K, Takahashi H, Takeda S, Ohama E, Ikuta F. Parkinson's disease: the presence of Lewy bodies in Auerbach's and Meissner's plexuses. Acta Neuropathol 1988;76:217-221.
12. Del Tredici K, Hawkes CH, Ghebremedhin E, Braak H. Lewy pathology in the submandibular gland of individuals with incidental Lewy body disease and sporadic Parkinson's disease. Acta Neuropathol 2010;119:703-713.
13. Cersosimo MG, Perandones C, Micheli FE, et al. Alpha-synuclein immunoreactivity in minor salivary gland biopsies of Parkinson's disease patients. Mov Disord 2011;26:188-190.
14. Minguez-Castellanos A, Chamorro CE, Escamilla-Sevilla F, et al. Do alpha-synuclein aggregates in autonomic plexuses predate Lewy body disorders?: a cohort study. Neurology 2007;68:2012-2018.
15. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. Neuropathol Appl Neurobiol 2007;33:599-614.
16. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: the dual hit theory revisited. Ann N Y Acad Sci 2009;1170:615-622.

17. Halliday G, Hely M, Reid W, Morris J. The progression of pathology in longitudinally followed patients with Parkinson's disease. Acta Neuropathol 2008;115:409-415.
18. Bloch A, Probst A, Bissig H, Adams H, Tolnay M. Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. Neuropathol Appl Neurobiol 2006;32:284-295.
19. Del Tredici K, Duda JE. Peripheral Lewy body pathology in Parkinson's disease and incidental Lewy body disease: four cases. J Neurol Sci 2011;310(1–2):100-106.
20. Navarro-Otano J, Gelpi E, Mestres CA, et al. Alpha-synuclein aggregates in epicardial fat tissue in living subjects without parkinsonism. Parkinsonism Relat Disord 2013;19:27-31.
21. Orimo S, Suzuki M, Inaba A, Mizusawa H. (123)I-MIBG myocardial scintigraphy for differentiating Parkinson's disease from other neurodegenerative parkinsonism: a systematic review and meta-analysis. Parkinsonism Relat Disord 2012;18:494-500.
22. Miyamoto T, Miyamoto M, Suzuki K, Nishibayashi M, Iwanami M, Hirata K. 123I-MIBG cardiac scintigraphy provides clues to the underlying neurodegenerative disorder in idiopathic REM sleep behavior disorder. Sleep 2008;31:717-723.
23. Iranzo A, Lomena F, Stockner H, et al. Decreased striatal dopamine transporter uptake and substantia nigra hyperechogenicity as risk markers of synucleinopathy in patients with idiopathic rapid-eye-movement sleep behaviour disorder: a prospective study. Lancet Neurol 2010;9:1070-1077.
24. Postuma RB, Gagnon JF, Vendette M, Montplaisir JY. Markers of neurodegeneration in idiopathic rapid eye movement sleep behaviour disorder and Parkinson's disease. Brain 2009;132(Pt 12):3298-3307.
25. Sorensen GL, Kempfner J, Zoetmulder M, Sorensen HB, Jennum P. Attenuated heart rate response in REM sleep behavior disorder and Parkinson's disease. Mov Disord 2012;27:888-894.
26. Iranzo A, Tolosa E, Gelpi E, et al. Neurodegenerative disease status and post-mortem pathology in idiopathic rapid-eye-movement sleep behaviour disorder: an observational cohort study. Lancet Neurol 2013;12:443-453.
27. Miyamoto T, Miyamoto M, Inoue Y, Usui Y, Suzuki K, Hirata K. Reduced cardiac 123I-MIBG scintigraphy in idiopathic REM sleep behavior disorder. Neurology 2006;67:2236-2238.
28. Postuma RB, Aarsland D, Barone P, et al. Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. Mov Disord 2012;27:617-626.
29. Cersosimo MG, Benarroch EE. Autonomic involvement in Parkinson's disease: pathology, pathophysiology, clinical features and possible peripheral biomarkers. J Neurol Sci 2012;313(1–2):57-63.
30. Lebouvier T, Neunlist M, Bruley des Varannes S, et al. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PLoS One 2010;5:e12728.
31. Shannon KM, Keshavarzian A, Mutlu E, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. Mov Disord 2012;27:709-715.
32. Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH. Is alpha-synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3 cases. Mov Disord 2012;27:716-719.
33. Gold A, Turkalp ZT, Munoz DG. Enteric alpha-synuclein expression is increased in Parkinson's disease but not Alzheimer's disease. Mov Disord 2013;28:237-240.
34. Bottner M, Zorenkov D, Hellwig I, et al. Expression pattern and localization of alpha-synuclein in the human enteric nervous system. Neurobiol Dis 2012;48:474-480.

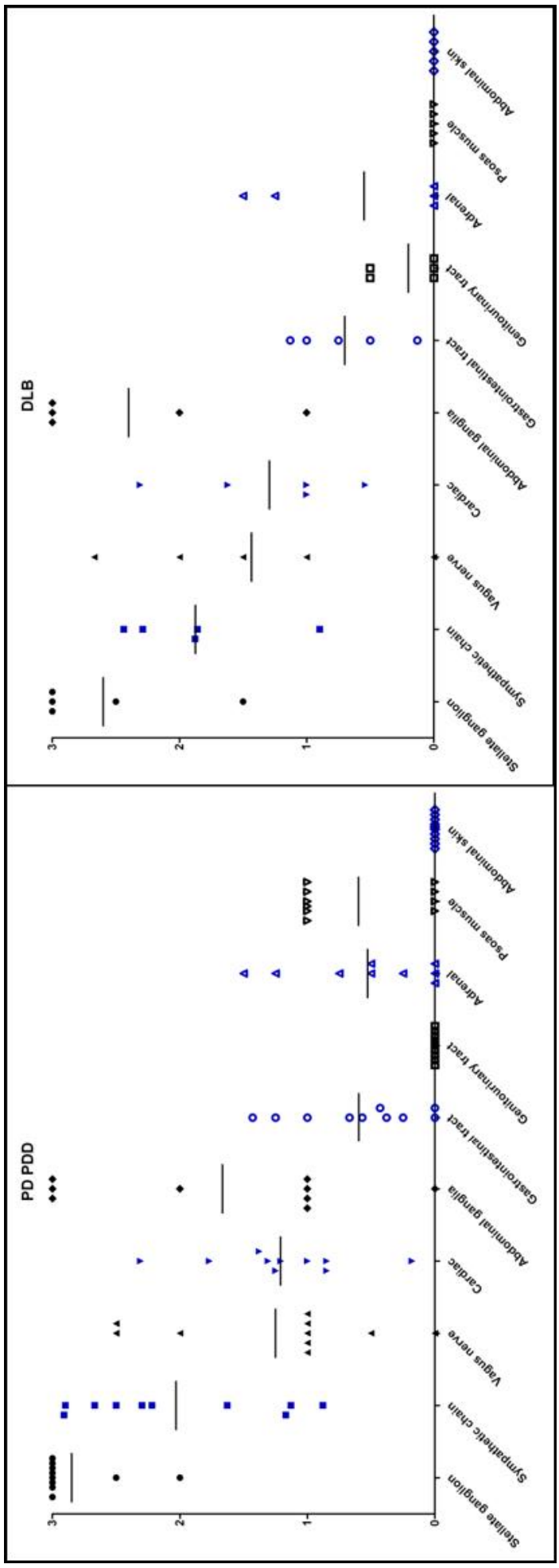
Supporting Data

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Supplementary Figure 1: Semiquantitative AS-assessment Results of semiquantitative assessment of AS-aggregates in grouped areas of the CNS of all study subjects that include PD, DLB, AD and others. Grouped areas refer to “olfactory” (olfactory bulb and tract), “brainstem” (dorsal nucleus of vagus nerve, formatio reticularis, locus coeruleus, raphe, substantia nigra, periaqueductal gray matter, colliculi), “limbic” (amygdala, entorhinal and transentorhinal region, hippocampus, basal nucleus of Meynert, cingulum, insula), “cortical” (frontal, temporal, parietal and occipital cortex) and “spinal cord” (cervical, thoracic, lumbar). Semiquantitative assessment of AS density was performed as follows: 0: absent, 1: isolated or mild, 2: moderate, and 3: abundant



Supplementary Figure 2: Semiquantitative AS-assessment Semiquantitative assessment of AS density in different peripheral organs of PD and DLB cases. Each sign corresponds to an individual subject.



PD	iLBD	DLB	*In vivo*	*Post mortem*	Distribution of LB/AS pathology		Reference
					Sympathetic chain		
X				X	Stellate ganglion, symp chain	[1]Den Hartog 1960
X				X	Sympathetic ganglia	[2]Rajput 1976
X				X	Sympathetic ganglia	[3]Wakabayashi 1990
X	X			X	Paravertebral and celiac	[4]Wakabayashi 1997
	X			X	Sympathetic ganglia	[5]Iwanaga 1999
	X			X	Sympathetic ganglia	[6]Bloch 2006
X				X	Sympathetic ganglia	[7]Mitsui 2006
	X			X	Sympathetic ganglia and stellate g	[8]Orimo 2007
	X			X	Stellate ganglia	[9]Miki 2009
X	X	X		X	Sympathetic ganglia	[10]Beach 2010
X	X			X	Sympathetic ganglia	[11]Del Tredici 2010

					Vagus nerve		
	X			X		Vagus nerve	[6]Bloch 2006
X	X	X		X		Vagus nerve	[10]Beach 2010
X	X			X		Vagus nerve	[11]Del Tredici 2010
					Cardiac		
X				X		Interartrial groove, around coronaries arteries, myocardium	[4]Wakabayashi 1997
X	X			X		Cardiac plexus	[5]Iwanaga 1999
X	X					Sinoatrial nodal ganglion	[12]Okada 2004
X				X		Cardiac plexus	[7]Mitsui 2006
X	X			X		Cardiac symp	[13]Orimo 2008
	X			X		Cardiac symp	[9]Miki 2009
		X		X		Cardio	[10]Beach 2010
	X		X			Cardiac symp	[14]Navarro-Otano 2012

					Gastrointestinal tract		
X				X		Oesophagus, colon	[15]Qualman 1984
	X			X		Oesophagus	[15]Qualman 1984
X			X			Colon	[16]Kupsky 1987
X				X		Oesophagus, stomach, small intestine, colon, rectum	[17]Wakabayashi 1988
	X			X		Oesophagus, small intestine	[17]Wakabayashi 1988
X				X		-------	[3]Wakabayashi 1990
X	X			X		Oesophagogastric junction	[12]Okada 2004
	X			X		Oesophagus	[6]Bloch 2006
X				X		Oesophagus, stomach	[18]Braak 2006
	X		X			Digestive tract	[19]Minguez-Castellanos 2007
X	X	X		X		GI-tract (submandibular gland,	[10]Beach 2010

						oesophagus, stomach, duodenum, jejunum, ileum, colon, rectum). Not divided in subgroups	
X	X			X		Submandibular gland	[11]Del Tredici 2010
X			X			Colonic biopsies (submucosa)	[20]Lebouvier 2010
X			X			Sigmoid colon (lam. propria)	[21]Shannon 2011
X			X			Minor salivary gland	[22]Cersosimo 2011
					Genitourinary tract		
X				X		Ganglia near urinary bladder	[4]Wakabayashi 1997
	X		X			Vesicoprostatic	[19]Minguez-Castellanos 2007
X		X		X		TGU	[10]Beach 2010
					Adrenal gland		
X				X		Autonomic ganglia around adrenals	[1]Den Hartog 1960
X				X		Adrenal medulla	[4]Wakabayashi 1997

X	X			X		Adrenal medulla	[12]Okada 2004
X	X	X		X		Adrenal glands	[23]Fumimura 2007
					Skin		
X	X	X		X		Cutaneus nerves abdominal flexor arm	[24]Ikemura 2008
X			X			Skin thoracic	[25]Miki 2010
					Others		
X	X	X		X		Sciatic nerve	[10]Beach 2010
X	X	X		X		Endocrine	[10]Beach 2010
X		X		X		Respiratory	[10]Beach 2010
	X			X		Spinal cord	[26]Tamura 2012

Table 1 Legend: Literature review of *in vivo* and *postmortem* studies of pANS involvement in Parkinson's disease (PD), incidental Lewy-body disease (iLBD) and dementia with Lewy bodies (DLB)

1. den HJW, Bethlem J. The distribution of Lewy bodies in the central and autonomic nervous systems in idiopathic paralysis agitans. J Neurol Neurosurg Psychiatry 1960; 23:283-290.
2. Rajput AH, Rozdilsky B. Dysautonomia in Parkinsonism: a clinicopathological study. J Neurol Neurosurg Psychiatry. 1976; 39(11):1092-1100.
3. Wakabayashi K, Takahashi H, Ohama E, Ikuta F. Parkinson's disease: an immunohistochemical study of Lewy body-containing neurons in the enteric nervous system. Acta Neuropathol 1990; 79(6):581-583.
4. Wakabayashi K, Takahashi H. Neuropathology of autonomic nervous system in Parkinson's disease. Eur Neurol 1997; 38 Suppl 2:2-7.
5. Iwanaga K, Wakabayashi K, Yoshimoto M, Tomita I, Satoh H, Takashima H, et al. Lewy body-type degeneration in cardiac plexus in Parkinson's and incidental Lewy body diseases. Neurology 1999 12; 52(6):1269-1271.
6. Bloch A, Probst A, Bissig H, Adams H, Tolnay M. Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. Neuropathol Appl Neurobiol 2006; 32(3):284-295.
7. Mitsui J, Saito Y, Momose T, Shimizu J, Arai N, Shibahara J, et al. Pathology of the sympathetic nervous system corresponding to the decreased cardiac uptake in 123I-metaiodobenzylguanidine (MIBG) scintigraphy in a patient with Parkinson disease. J Neurol Sci 2006; 243(1-2):101-104.
8. Orimo S, Takahashi A, Uchihara T, Mori F, Kakita A, Wakabayashi K, et al. Degeneration of cardiac sympathetic nerve begins in the early disease process of Parkinson's disease. Brain Pathol 2007; 17(1):24-30.
9. Miki Y, Mori F, Wakabayashi K, Kuroda N, Orimo S. Incidental Lewy body disease restricted to the heart and stellate ganglia. Mov Disord. 2009; 24(15):2299-22301.
10. Beach TG, Adler CH, Sue LI, Vedders L, Lue L, White Iii CL, et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol. 2010; 119(6):689-702.
11. Del Tredici K, Hawkes CH, Ghebremedhin E, Braak H. Lewy pathology in the submandibular gland of individuals with incidental Lewy body disease and sporadic Parkinson's disease. Acta Neuropathol. 2010; 119(6):703-713.
12. Okada Y, Ito Y, Aida J, Yasuhara M, Ohkawa S, Hirokawa K. Lewy bodies in the sinoatrial nodal ganglion: clinicopathological studies. Pathol Int 2004; 54(9):682-687.
13. Orimo S, Uchihara T, Nakamura A, Mori F, Kakita A, Wakabayashi K, et al. Axonal alpha-synuclein aggregates herald centripetal degeneration of cardiac sympathetic nerve in Parkinson's disease. Brain 2008; 131(Pt 3):642-650.
14. Navarro-Otano J, Gelpi E, Mestres CA, Quintana E, Rauek S, Ribalta T, et al. Alpha-synuclein aggregates in epicardial fat tissue in living subjects without parkinsonism. Parkinsonism Relat Disord 2012 Aug 1.

15. Qualman SJ, Haupt HM, Yang P, Hamilton SR. Esophageal Lewy bodies associated with ganglion cell loss in achalasia. Similarity to Parkinson's disease. Gastroenterology 1984; 87(4):848-856.
16. Kupsky WJ, Grimes MM, Sweeting J, Bertsch R, Cote LJ. Parkinson's disease and megacolon: concentric hyaline inclusions (Lewy bodies) in enteric ganglion cells. Neurology 1987; 37(7):1253-1255.
17. Wakabayashi K, Takahashi H, Takeda S, Ohama E, Ikuta F. Parkinson's disease: the presence of Lewy bodies in Auerbach's and Meissner's plexuses. Acta Neuropathol 1988; 76(3):217-221.
18. Braak H, de Vos RA, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci Lett 2006; 396(1):67-72.
19. Minguez-Castellanos A, Chamorro CE, Escamilla-Sevilla F, Ortega-Moreno A, Rebollo AC, Gomez-Rio M, et al. Do alpha-synuclein aggregates in autonomic plexuses predate Lewy body disorders?: a cohort study. Neurology 2007 Jun 5;68(23):2012-8.
20. Lebouvier T, Neunlist M, Bruley des Varannes S, Coron E, Drouard A, N'Guyen JM, et al. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PLoS One 2010; 5(9):e12728.
21. Shannon KM, Keshavarzian A, Mutlu E, Dodiya HB, Daian D, Jaglin JA, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. Mov Disord 2011 Jul 15.
22. Cersosimo MG, Perandones C, Micheli FE, Raina GB, Beron AM, Nasswetter G, et al. Alpha-synuclein immunoreactivity in minor salivary gland biopsies of Parkinson's disease patients. Mov Disord. 2011; 26(1):188-190.
23. Fumimura Y, Ikemura M, Saito Y, Sengoku R, Kanemaru K, Sawabe M, et al. Analysis of the adrenal gland is useful for evaluating pathology of the peripheral autonomic nervous system in lewy body disease. J Neuropathol Exp Neurol 2007 May;66(5):354-62.
24. Ikemura M, Saito Y, Sengoku R, Sakiyama Y, Hatsuta H, Kanemaru K, et al. Lewy body pathology involves cutaneous nerves. J Neuropathol Exp Neurol 2008; 67(10):945-953.
25. Miki Y, Tomiyama M, Ueno T, Haga R, Nishijima H, Suzuki C, et al. Clinical availability of skin biopsy in the diagnosis of Parkinson's disease. Neurosci Lett. 2010; 469(3):357-359.
26. Tamura T, Yoshida M, Hashizume Y, Sobue G. Lewy body-related alpha-synucleinopathy in the spinal cord of cases with incidental Lewy body disease. Neuropathology. 2012; 32(1):13-22.

Trabajo número 2

Alpha-synuclein aggregates in epicardial fat tissue in living subjects without parkinsonism

Navarro-Otano J, Gelpi E, Mestres CA, Quintana E, Rauek S, Ribalta T, Santiago V, Tolosa E

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Agregados de alfa-sinucleína en el tejido graso epicárdico de sujetos vivos sin parkinsonismo

Antecedentes: En la enfermedad de Parkinson (PD) los agregados de alfa-sinucleína (AS) se encuentran frecuentemente en el sistema nervioso autónomo periférico (pANS). Su presencia en sujetos asintomáticos sugiere el diagnóstico de enfermedad por cuerpos de Lewy incidentales (iLBD), entidad que se cree que refleja un estado preclínico de PD. La afectación cardiaca por agregados de AS se ha comprobado en estudios *post-mortem* tanto en sujetos con PD como en iLBD. Sin embargo, no se tienen datos sobre la afectación cardíaca por AS *in vivo*.

Objetivos: Valorar de manera prospectiva la presencia de agregados de AS en la grasa epicárdica de sujetos vivos sin parkinsonismo que van a ser sometidos a cirugía cardíaca electiva.

Material y métodos: Mediante histología e inmunohistoquímica se estudiaron muestras de tejido graso epicárdico obtenido durante cirugía cardíaca de 91 sujetos. Se seleccionaron las áreas donde era más probable encontrar componentes del pANS. Inmediatamente antes o después de la cirugía se valoró la presencia de síntomas motores y no motores relacionados con PD.

Resultados: En cada uno de los 91 sujetos biopsiados (62 hombres / 29 mujeres, edad media de 67 años) se identificaron pequeños nervios autonómicos, ganglios y/o fibras tirosín-hidroxilasa positivas. Se encontraron agregados de AS en 7 sujetos (7.7%), sobre todo en aquellos mayores de 70 años. Los sujetos con agregados de AS comparados con los individuos sin estos agregados presentaban de manera significativa más quejas de estreñimiento y de sueños vivos.

Conclusiones: Los agregados de AS ocurren en el pANS epicárdico de sujetos sin parkinsonismo, sugiriendo el diagnóstico de iLBD. La presencia en algunos de estos sujetos de síntomas no-motores como sueños vivos y estreñimiento que aparecen también en la PD premotora apoya esta interpretación. El seguimiento adecuado de los sujetos de este estudio podría indicar el tiempo de progresión a PD motora, si se diera esta progresión.

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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.

Vincenzo Bonifati, MD, PhD, Associate Editor; Professor, Erasmus Medical Center, Rotterdam, The Netherlands

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*

cardiac tissue from left ventricle wall and adjacent epicardial fat tissue of a small autopsy group without known cardiologic or neurologic clinical symptoms, except one case with mild cognitive impairment without parkinsonism.

We used brain tissue from a brain donor with Parkinson's disease as positive control for AS and pAS immunoreactivity, a paravertebral sympathetic ganglion as positive control for TH-immunoreactivity, and omitted the primary antibody for control of antibody specificity.

2.5. Statistical analyses

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) (PASW Statistics Version 18.00). Comparisons between groups were performed using Mann–Whitney test and two-tailed Fisher's exact test as appropriated. A *p*-value lower than 0.05 was considered statistically significant.

3. Results

3.1. Study subjects

Ninety-one adult patients (older than 18 years of age) were enrolled in the study. Demographic data of the study participants are shown in Table 1. Seventy-one percent were male. Mean age was 67 years (range 31–84). Cardiovascular risk factors and type of cardiac disease for which the patients were listed for an operation are presented in Table 1.

None of the patients were thought to have parkinsonism on neurological examination (Table 2). One patient with the highest UPDRS III (12) had severe generalised arthrosis. Forty patients had symptoms known to occur in premotor PD: hyposmia in 20%, constipation in 11.5% and acting dreams in 12.9%.

Post-surgery, neither anatomical nor functional cardiac complications occurred that could be related to the sampling of fat tissue.

3.2. Autonomic nervous tissue in epicardial fat

In all but one individual, small nerve fibres and/or autonomic ganglia were detected in three or four of the total epicardial fat tissue samples obtained in each case (Fig. 1A, C). Fat from the cavoatrial junction and interatrial groove were the regions where larger ganglia and thicker, partly tyrosine-hydroxylase positive nerve fibres were observed (Fig. 1D).

Table 2
Results of motor and non-motor evaluations.

Clinical variables	Total $n = 91$	AS (+) $n = 7$	AS (−) $n = 84$	*P*-value
UPDRS II median (range)	0 (0–2)	0 (0–0)	0 (0–2)	0.26
UPDRS III median (range)	1 (0–12)[c]	0 (0–2)	1 (0–12)	0.15
Hyposmia *n* (%)[a]	12/60 (20)	1/6 (16.7)	11/54 (20.4)	0.99
Constipation *n* (%)	7/61 (11.5)	3/7 (42.9)	4/54 (7.4)	0.03[b]
Urinary urgency *n* (%)	19/61 (31.1)	1/7 (14.3)	18/54 (33.3)	0.42
Impaired memory *n* (%)	11/61 (18)	3/7 (42.9)	8/54 (14.8)	0.10
Lack of interest *n* (%)	3/54 (5.6)	1/4 (25)	2/50 (4)	0.21
Hallucinations *n* (%)	4/55 (7.3)	2/6 (33.3)	2/49 (4.1)	0.06
Clinical orthostatic hypotension *n* (%)	8/55 (14.5)	1/6 (16.7)	7/49 (14.3)	0.99
Problems in getting asleep *n* (%)	18/59 (30.5)	1/6 (16.7)	17/53 (32.1)	0.66
Vivid dreams *n* (%)	11/62 (17.7)	3/7 (42.9)	8/55 (14.5)	0.10
Acting dreams *n* (%)	8/62 (12.9)	3/7 (42.9)	5/55 (9.1)	0.04[b]
Swelling of legs *n* (%)	3/49 (6.1)	1/4 (25)	2/45 (4.4)	0.23
Any non-motor symptom *n* (%)	40/62 (64.5)	6/7 (85.7)	34/55 (61.8)	0.41

AS (+) subjects with alpha-synuclein aggregates; AS (−) subjects without alpha-synuclein aggregates.

[a] Percentages are represented in relation to the total of subjects with a valid response in each item.

[b] Statistically significant differences between AS (+) and AS (−) groups. Mann–Whitney test and two-tailed Fisher's exact test as appropriated.

[c] One patient with UPDRS III of 12 had severe arthrosis.

3.3. Alpha-synuclein aggregates

In seven out of 91 subjects (7.7%), abnormal AS/pAS aggregates were detected in at least two samples from the same individual. These aggregates were observed predominantly along nerve fibres (Fig. 1F, J) and in between neurons of autonomic ganglia (Fig. 1E). Here, in addition, isolated neurons showed diffuse dot-like perikaryal immunoreactivity. This type of immunoreactivity has been described by some authors as an early step in the morphogenesis of Lewy-bodies in the CNS [21]. Similarly to what has been described in *post-mortem* brains, phospho-AS showed more diffuse, dot-like immunoreactivity along nerve fibres than observed by KM51 antibody (Fig. 1G, K, N). pAS is considered a more specific and sensitive marker of LB-related pathology, probably representing a pathological change that precedes LB-related neuronal degeneration [22]. We observed that a proportion of cases containing AS aggregates in epicardial nerve fibres showed reduced TH-immunoreactivity (Fig. 1H, L) in some nerve fibres when compared to cases without AS aggregates (Fig. 1D), although this was not a consistent finding. No quantitative assessment was performed due to the small sample sizes.

3.4. AS aggregates and clinical correlation

Among the seven patients with abnormal AS aggregates in epicardial fat tissue, the most common reported non-motor symptoms were constipation in three, impaired memory in three, and vivid dreams and acting dreams in three that clinically suggested REM sleep behaviour disorder (RBD). The youngest patient (45 years) had no symptoms suggestive of premotor PD (Table 3). One of the patients with constipation, hallucinations, RBD and memory complaints had the highest amount of AS aggregates in epicardial fat tissue (case 5).

3.5 Post-mortem cohort

We studied *post-mortem* cardiac tissue of 14 consecutive autopsy cases without known cardiac disease (43% male; mean age 68 years, range 46–85). We found AS/pAS positive aggregates in cardiac autonomic nerves in one subject (7%) who had mild cognitive impairment without parkinsonism according to clinical records. No detailed analysis of AS distribution in the brain was available in this case.

4. Discussion

Our results show that abnormal, phosphorylated AS aggregates can be detected *in vivo* in neurons and nerve fibres of the epicardial ANS in subjects without parkinsonism. AS inclusions have been described in *post-mortem* studies in the cardiac pANS of patients with PD [3,13] and also in subjects without parkinsonism or dementia thought to have iLBD [13,23–25]. They are also analogous to those described *in vivo* in abdominopelvic plexus in asymptomatic subjects [26]. In this study subjects with incidental AS-positive aggregates were thought to have pre-clinical PD or pre-clinical dementia with Lewy-bodies (DLB) like those with iLBD. ILBD is a term usually applied *post-mortem*, but that could also be applied *in vivo* to subjects without parkinsonism but documented AS in the nervous system. The percentage of our non-parkinsonian study subjects with incidental AS aggregates in epicardial pANS (7.7%) is not dissimilar from the percentages of iLBD encountered in *post-mortem* [1,11,13] and *in vivo* studies [26], with a tendency to

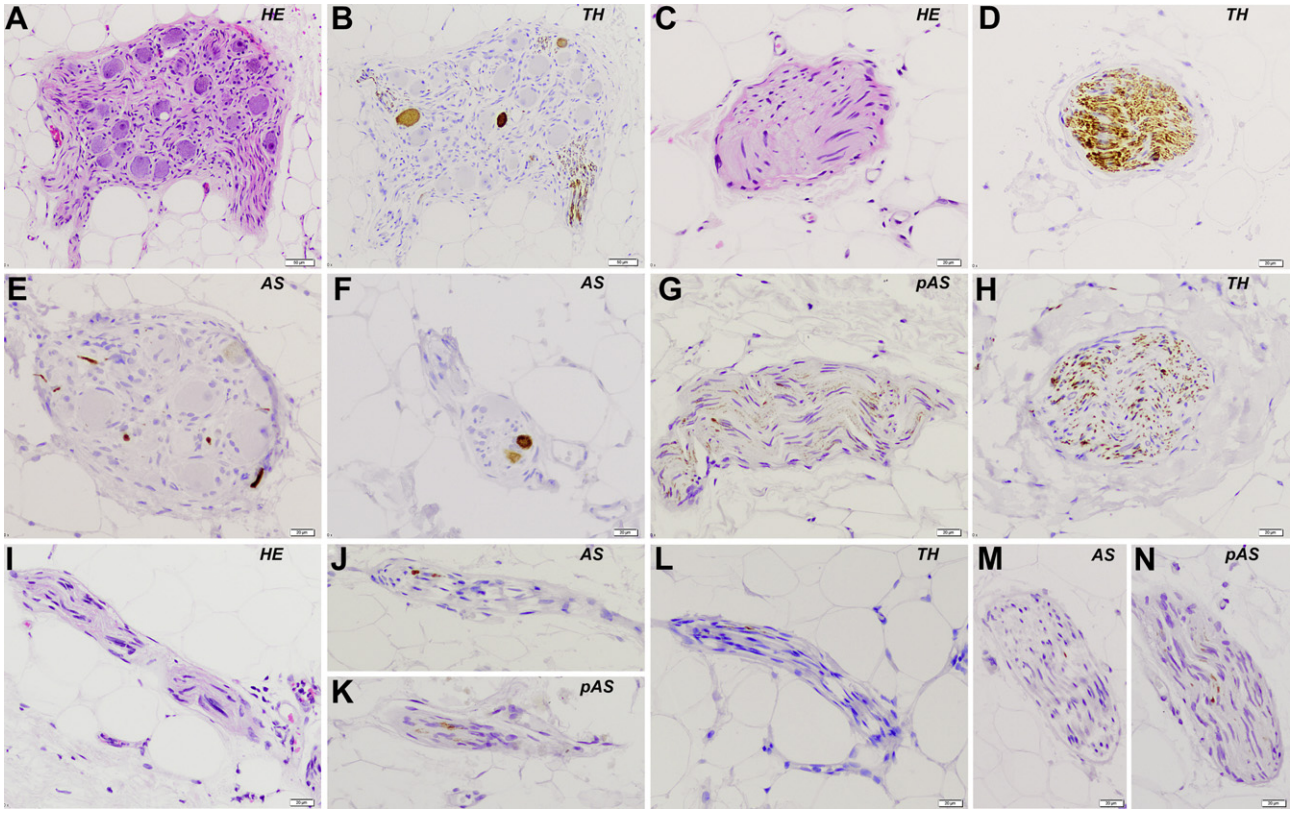

Fig. 1. Alpha-synuclein aggregates in epicardial fat tissue. First row (A–D) illustrates histological findings in normal appearing epicardial fat tissue in a case without AS aggregates. Haematoxylin–eosin stain of epicardial fat tissue containing small autonomic ganglia (A) and small nerves (C). Some nerve fibres are strongly immunoreactive for tyrosine-hydroxylase (TH) (D) whereas in autonomic ganglia, only few neurons and delicate nerve fibres show TH-immunoreactivity (B). Middle and lower rows (E–N) depict AS-positive cases. AS aggregates are detected in autonomic ganglia (E) as fine elongated processes between satellite and Schwann cells, as well as in small nerves (F) as relatively compact aggregates. Using anti-phosphorylated AS (pAS) antibodies, more diffuse and dot-like immunoreactivity along nerve fibres can be detected in some larger fibres (G). This can also be appreciated, in adjacent tissue sections, in small nerve fibres (I–J–K and M–N), where more diffuse pAS than AS immunoreactivity is observed. In addition, a reduction of TH-immunoreactivity was observed in some larger fibres (H) and in smaller nerve fibres (L) containing AS/pAS aggregates (J, K), but this was not a regular finding. Scale bars: A, B: 50 μm; C–N: 20 μm.

increase with age (from 4.3% in the 7th decade to 11.8% in the 8th and 9.1% in the 9th) as also observed in previous studies [11,13,24]. We believe that our study subjects bearing AS aggregates in epicardial pANS could be considered iLBD and as such to suffer from premotor PD. These subjects could also be considered to represent pre-clinical DLB, as might be suggested in one of our study subject who suffered from hallucinations, RBD and memory complaints but did not fulfill criteria of dementia at the moment of clinical assessment.

Table 3
Clinical characteristics of subjects with AS aggregates in epicardial fat tissue.

Case	1	2	3	4	5	6	7
Age	77	78	62	75	80	45	78
Gender	f	m	f	m	f	f	m
Number of AS (+) samples/total samples	3/6	2/5	3/5	3/6	3/5	3/6	3/5
UPDRS II	n.a.	0	0	0	n.a.	0	0
UPDRS III	0	1	0	0	n.a.	0	2
Hyposmia	No	Yes	No	No	n.a.	No	No
Constipation	Yes	Yes	No	No	Yes	No	No
Urinary urgency	No	No	No	Yes	No	No	No
Memory	No	Yes	No	Yes	Yes	No	No
Lack of interest	n.a.	n.a.	No	Yes	n.a.	No	No
Hallucinations	n.a.	No	No	Yes	Yes	No	No
Clinical orthostatism	No	No	No	Yes	n.a.	No	No
Getting asleep problems	No	n.a.	Yes	No	No	No	No
Vivid dreams	No	Yes	No	Yes	Yes	No	No
Acting dreams	No	Yes	No	Yes	Yes	No	No
Swelling of legs	n.a.	n.a.	No	No	n.a.	No	Yes

f: Female, m: male, n.a.: not available.

Cardiovascular dysautonomic symptoms are a focus of attention in PD but the clinical correlation of cardiac sympathetic denervation is still unclear. Although cardiovascular examinations are frequently normal even in advanced cases and in those with MIBG abnormalities, retrospective studies had found a higher prevalence of diastolic hypertension, angina, arrhythmia, and heart infarct during the decade before onset of motor symptoms when compared with a control group [27]. Consistent data are even less available for iLBD. Retrospective analysis of *post-mortem* confirmed iLBD cases showed no significant differences compared to controls concerning high blood pressure, coronary artery disease, peripheral vascular disease [10,28] or cardiac arrhythmia, as a possible consequence of sympathetic denervation [9]. Also Beach et al found that supraventricular arrhythmias and hypertension were common in iLBD cases but they were also common in control cases [10].

Even if we can not exclude the possibility that in our study patients AS aggregates in cardiac autonomic nerves might be related to hypoxic damage, the presence of these aggregates in patients with both, ischaemic and non-ischaemic cardiac pathology and in *post-mortem* cases without cardiac disease suggests that probably ischaemia is not a major AS aggregation trigger in these cases. Furthermore, other studies have evaluated the presence of AS

in diabetic patients and in patients with recent and old myocardial infarction and observed no increase of cardiac AS pathology [13].

A fraction of cases with AS aggregates showed reduced TH-immunoreactivity in some nerve fibres. To draw firm conclusions concerning the presence and/or severity of nerve fibre degeneration in these small tissue samples is difficult. On the one hand, the number and thickness of epicardial nerve fibres is variable and there is a mixture of sympathetic and parasympathetic fibres. Furthermore, reduced TH-immunoreactivity was not a consistent finding, and its assessment is especially difficult in small tissue samples and remains subjective.

As we have studied only cardiac ANS, our results do not clarify whether heart involvement is the only site of AS pathology in our study subjects. The presence in some of our AS-positive study subjects of symptoms that are known to antedate in some cases motor PD such as acting dreams (suggestive of RBD [29,30]), hyposmia and constipation, could suggest that central nervous system and/or other sites of the ANS are already affected.

In summary, we have shown that abnormal AS aggregates are present in epicardial pANS obtained during cardiac surgery in a substantial number of living subjects without parkinsonism. We believe that these subjects could represent instances of pre-clinical LBD, the same way as subjects without parkinsonism shown to have Lewy-bodies in the central nervous system are thought to have iLBD, a prelude for the development of the motor syndrome of PD. Extensive NMS evaluation including objective assessment of smell and cardiac dysautonomia and follow-up studies are in progress to assess the possible development of both, non-motor and motor PD-related symptoms in the subjects of our study.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

[1] Beach TG, Adler CH, Sue LI, Vedders L, Lue L, White Iii CL, et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol 2010;119:689–702.
[2] Wakabayashi K, Takahashi H. Neuropathology of autonomic nervous system in Parkinson's disease. Eur Neurol 1997;38(Suppl. 2):2–7.
[3] Orimo S, Uchihara T, Nakamura A, Mori F, Kakita A, Wakabayashi K, et al. Axonal alpha-synuclein aggregates herald centripetal degeneration of cardiac sympathetic nerve in Parkinson’s disease. Brain 2008;131:642–50.
[4] Ikemura M, Saito Y, Sengoku R, Sakiyama Y, Hatsuta H, Kanemaru K, et al. Lewy body pathology involves cutaneous nerves. J Neuropathol Exp Neurol 2008;67:945–53.
[5] Miki Y, Tomiyama M, Ueno T, Haga R, Nishijima H, Suzuki C, et al. Clinical availability of skin biopsy in the diagnosis of Parkinson's disease. Neurosci Lett 2010;469:357–9.
[6] Lebouvier T, Neunlist M, Bruley des Varannes S, Coron E, Drouard A, N'Guyen JM, et al. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PLoS One 2010;5:e12728.
[7] Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson’s disease. Neurobiol Aging 2003;24:197–211.
[8] Tolosa E, Gaig C, Santamaria J, Compta Y. Diagnosis and the premotor phase of Parkinson disease. Neurology 2009;72:S12–20.
[9] Dickson DW, Fujishiro H, DelleDonne A, Menke J, Ahmed Z, Klos KJ, et al. Evidence that incidental Lewy body disease is pre-symptomatic Parkinson's disease. Acta Neuropathol 2008;115:437–44.
[10] Beach TG, Adler CH, Sue LI, Peirce JB, Bachalakuri J, Dalsing-Hernandez JE, et al. Reduced striatal tyrosine hydroxylase in incidental Lewy body disease. Acta Neuropathol 2008;115:445–51.
[11] Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson’s disease. J Neurol Neurosurg Psychiatry 1988;51:745–52.
[12] Oka H, Toyoda C, Yogo M, Mochio S. Cardiovascular dysautonomia in de novo Parkinson’s disease without orthostatic hypotension. Eur J Neurol 2011;18:286–92.
[13] Iwanaga K, Wakabayashi K, Yoshimoto M, Tomita I, Satoh H, Takashima H, et al. Lewy body-type degeneration in cardiac plexus in Parkinson’s and incidental Lewy body diseases. Neurology 1999;52:1269–71.
[14] Fujishiro H, Frigerio R, Burnett M, Klos KJ, Josephs KA, Delledonne A, et al. Cardiac sympathetic denervation correlates with clinical and pathologic stages of Parkinson's disease. Mov Disord 2008;23:1085–92.
[15] Goldstein DS, Sharabi Y, Karp BI, Bentho O, Saleem A, Pacak K, et al. Cardiac sympathetic denervation preceding motor signs in Parkinson disease. Cleve Clin J Med 2009;76(Suppl. 2):S47–50.
[16] Goldstein DS, Holmes C, Sewell L, Park MY, Sharabi Y. Sympathetic noradrenergic before striatal dopaminergic denervation: relevance to Braak staging of synucleinopathy. Clin Auton Res 2012;22:57–61.
[17] Kaufmann H, Nahm K, Purohit D, Wolfe D. Autonomic failure as the initial presentation of Parkinson disease and dementia with Lewy bodies. Neurology 2004;63:1093–5.
[18] Orimo S, Takahashi A, Uchihara T, Mori F, Kakita A, Wakabayashi K, et al. Degeneration of cardiac sympathetic nerve begins in the early disease process of Parkinson's disease. Brain Pathol 2007;17:24–30.
[19] Chaudhuri KR, Martinez-Martin P, Schapira AH, Stocchi F, Sethi K, Odin P, et al. International multicenter pilot study of the first comprehensive self-completed nonmotor symptoms questionnaire for Parkinson’s disease: the NMSQuest study. Mov Disord 2006;21:916–23.
[20] Kawano H, Okada R, Yano K. Histological study on the distribution of autonomic nerves in the human heart. Heart Vessels 2003;18:32–9.
[21] Kuusisto E, Parkkinen L, Alafuzoff I. Morphogenesis of Lewy bodies: dissimilar incorporation of alpha-synuclein, ubiquitin, and p62. J Neuropathol Exp Neurol 2003;62:1241–53.
[22] Saito Y, Kawashima A, Ruberu NN, Fujiwara H, Koyama S, Sawabe M, et al. Accumulation of phosphorylated alpha-synuclein in aging human brain. J Neuropathol Exp Neurol 2003;62:644–54.
[23] Markesbery WR, Jicha GA, Liu H, Schmitt FA. Lewy body pathology in normal elderly subjects. J Neuropathol Exp Neurol 2009;68:816–22.
[24] Bloch A, Probst A, Bissig H, Adams H, Tolnay M. Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. Neuropathol Appl Neurobiol 2006;32:284–95.
[25] Miki Y, Mori F, Wakabayashi K, Kuroda N, Orimo S. Incidental Lewy body disease restricted to the heart and stellate ganglia. Mov Disord 2009;24:2299–301.
[26] Minguez-Castellanos A, Chamorro CE, Escamilla-Sevilla F, Ortega-Moreno A, Rebollo AC, Gomez-Rio M, et al. Do alpha-synuclein aggregates in autonomic plexuses predate Lewy body disorders? A cohort study. Neurology 2007;68:2012–8.
[27] Goner EG, van't Hof M, Berger HJ, van Weel C, Horstink MW. Symptoms and duration of the prodromal phase in Parkinsons disease. Mov Disord 1997;12:871–6.
[28] Frigerio R, Fujishiro H, Maraganore DM, Klos KJ, DelleDonne A, Heckman MG, et al. Comparison of risk factor profiles in incidental Lewy body disease and Parkinson disease. Arch Neurol 2009;66:1114–9.
[29] Iranzo A, Molinuevo JL, Santamaria J, Serradell M, Marti MJ, Valldeoriola F, et al. Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study. Lancet Neurol 2006;5:572–7.
[30] Postuma RB, Gagnon JF, Vendette M, Fantini ML, Massicotte-Marquez J, Montplaisir J. Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder. Neurology 2009;72:1296–300.

Trabajo número 3

^{123}I-MIBG cardiac uptake, smell identification and ^{123}I- FP-CIT SPECT in the differential diagnosis between vascular parkinsonism and Parkinson's disease

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Captación cardíaca de ^{123}I-MIBG, identificación olfativa y SPECT con ^{123}I-FP-CIT en el diagnóstico diferencial entre parkinsonismo vascular y enfermedad de Parkinson

Antecedentes: El parkinsonismo vascular (VP) puede ocurrir como una entidad clínico-patológica definida aunque es también muy frecuente la presencia de daño vascular en la enfermedad de Parkinson (PD) idiopática. El diagnóstico diferencial entre estas dos entidades tiene implicaciones tanto terapéuticas como pronósticas, pero continúa siendo un reto ya que la utilidad de las pruebas que apoyarían el diagnóstico es todavía controvertida.

Objetivo: Comprobar el valor clínico del SPECT cardíaco con ^{123}I-meta-yodo-benzil-guanidina (^{123}I-MIBG), función olfativa y SPECT cerebral con ^{123}I-FP-CIT como herramientas de apoyo en el diagnóstico diferencial entre VP y PD.

Métodos: Estudio transversal de 15 pacientes consecutivos con sospecha de VP, 15 pacientes con PD y 9 sujetos sanos. En todos ellos se realizaron ^{123}I-MIBG SPECT cardíaco (cálculo del ratio corazón-mediastino) y test de olfacción (test de identificación de los olores de la universidad de Pennsylvania-UPSIT). En los pacientes con sospecha de VP se realizó además un ^{123}I-FP-CIT-SPECT cerebral.

Resultados: El ratio corazón-mediastino fue significativamente menor en los pacientes con sospecha de VP (media 1.45) y en los PD (media 1.16) que en el grupo control (media 1.69) ($p = 0.017$ y $p < 0.0001$). Los pacientes con VP presentaban un ratio mayor que los pacientes con PD ($p=0.001$). El grupo control presentaba de manera significativa mayor puntuación en el UPSIT (media 30.71) en comparación con los VP (media 18.33) y los PD (media 15.29) ($p=0.001$ para ambos grupos). Aquellos pacientes con VP que tenían un ^{123}I-MIBG no sugestivo de PD tendían a alcanzar puntuaciones de UPSIT más altas ($p=0.006$). Los hallazgos del ^{123}I-FP-CIT SPECT fueron heterogéneos (7/15 VP normales, 3/15 alterados sugestivos de PD y 5/15 alterados no típicos de PD).

Conclusiones: El uso del ^{123}I-MIBG SPECT cardíaco y en menor medida del UPSIT podría ayudar en el diagnóstico diferencial entre los pacientes con VP y PD en los que el diagnóstico queda incierto a pesar de estudios con ^{123}I-FP-CIT SPECT.

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^{123}I-MIBG cardiac uptake, smell identification and ^{123}I-FP-CIT SPECT in the differential diagnosis between vascular parkinsonism and Parkinson's disease

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ABSTRACT

Vascular parkinsonism (VP) may occur as a distinct clinicopathological entity but the comorbid presence of vascular damage in Parkinson's disease (PD) is very frequent too. This differential diagnosis has therapeutic and prognostic implications but remains challenging as the usefulness of a number of supporting tools is still controversial.

Objective: To ascertain the clinical value of cardiac ^{123}I-meta-iodobenzylguanidine (^{123}I-MIBG) SPECT, olfactory function and ^{123}I-FP-CIT SPECT as supporting tools in the differential diagnosis between VP and PD.

Methods: Cross-sectional study of 15 consecutive patients with suspected VP, 15 PD patients and 9 healthy subjects. Cardiac ^{123}I-MIBG SPECT (heart-to-mediastinum ratio) and olfactory testing (University of Pennsylvania Smell Identification Test-UPSIT) were performed in all of them. ^{123}I-FP-CIT SPECT was performed in VP-suspected patients.

Results: Heart-to-mediatinum ratio was significant lower in suspected VP (mean 1.45) and PD (mean 1.16) compared to control group (mean 1.69) ($p = 0.017$ and $p < 0.0001$). VP patients presented a higher ratio than PD patients ($p = 0.001$). Control group presented a significant higher UPSIT score (mean 30.71) when compared to both VP (mean 18.33) and PD (mean 15.29) ($p = 0.001$ for both groups). Those VP with a cardiac ^{123}I-MIBG non suggestive of PD were more likely to have a higher UPSIT score ($p = 0.006$). ^{123}I-FP-CIT SPECT imaging was heterogeneous (7/15 VP normal, 3/15 abnormal suggestive of PD and 5/15 abnormal but atypical for PD).

Conclusions: The use of cardiac ^{123}I-MIBG SPECT and to a lesser extent UPSIT could assist the differential diagnosis between VP and PD in subjects in which the diagnosis remains uncertain despite ^{123}I-FP-CIT SPECT imaging.

1. Introduction

Vascular parkinsonism (VP) in the absence of Lewy body pathology is a distinct clinicopathological entity first described by Critchley in 1929 [1]. The most important concern while making a diagnosis of VP is its differentiation from Parkinson's disease (PD) because of therapeutic and prognostic implications. Although a pure vascular etiology exists for a subset of patients with parkinsonism, comorbid vascular lesions are more common, and have been reported in 19–50% of patients with Lewy body-confirmed PD [2,3]. Clinical criteria for the diagnosis of VP have been developed [4,5]; however, the differential diagnosis with PD is often difficult due to the overlap in clinical presentation and the common co-occurrence of vascular damage in PD. Although some clinical clues were thought to be indicative of VP [5], post-mortem studies have shown that cerebrovascular disease can display an indistinguishable clinical pattern from PD [6,7].

In recent decades new tools have been tested in the differential diagnosis of PD from secondary parkinsonisms. As motor PD is

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clinically manifest when neurodegeneration reaches the substantia nigra, ^{123}I-FP-CIT SPECT showing a decrease of dopamine function at this level has been used as a supporting tool in diagnosis of PD [8]. This technique, though, is not fully specific. For instance, in VP patients ^{123}I-FP-CIT SPECT imaging can be altered as a consequence of the underlying ischemic damage [9].

The assessment of cardiac innervation using ^{123}I-meta-iodobenzylguanidine (MIBG) SPECT has also been used in the differential diagnosis of PD. It is used to assess the presynaptic cardiac sympathetic innervation [10] which it is thought to be decreased even at an early stage of PD [11]. However, the diagnostic accuracy and efficacy of ^{123}I-MIBG in daily clinical practice remains unclear.

The UPSIT (University of Pennsylvania Smell Identification Test) is a rapid method to assess human olfactory function and can be used as a supporting tool of the diagnosis of PD [12]. Hyposmia has been described in PD even at an early stage [13].

The objective of the present study was to analyze the usefulness of ^{123}I-MIBG cardiac imaging as a supporting tool and to compare the diagnostic value of this technique with that of the UPSIT score and ^{123}I-FP-CIT SPECT in the differential diagnosis between VP and PD in daily clinical practice.

2. Methods

2.1. Design

This was a cross-sectional comparative study. All individuals were informed about the study and gave their written consent. The project was approved by the Institutional Ethical Committee.

2.2. Subjects of the study

From January 2008 to December 2009 we included consecutive patients with clinically suspected VP according to an expert neurologist opinion applying previously published criteria [5] who were referred to our Movement Disorders Unit due to parkinsonism or gait disturbance. As reference group we prospectively included PD patients diagnosed according to accepted clinical criteria [6]. PD patients were recruited in our Unit in a consecutive manner among those followed in our institution who were visited the same days as the suspected VP patients. The study was designed in a 1:1 manner regarding VP and PD patients. We included also control subjects without any neurological disorder which were recruited among relatives of VP and PD patients.

A 1.5 T cerebral MRI was programmed in all patients of the study. Exclusion criteria for entering the study have been described elsewhere [14]. Briefly, subjects presenting previous history of diabetes mellitus, peripheral neuropathy, cardiopathy or coronariopathy, receiving any medication known to modify the ^{123}I-MIBG uptake, proven iodine allergy, suffering from rhinologic disorders known to impair the sense of smell and subjects with moderate to severe cognitive impairment were excluded.

After giving their informed consent for genetic studies, all PD patients were analyzed for the possible existence of the LRRK2 G2019S and codon 1441 mutations which were ruled out in all of them. LRRK2 mutation-carrier patients are known to have different performance score at ^{123}I-MIBG cardiac gammagraphy compared with idiopathic PD [14].

2.3. Clinical assessment

2.3.1. General demographic and clinical characteristics

Recorded characteristics of all participants included age, gender, presence of cardiovascular risk factors (high blood pressure, dyslipidemia, smoking, peripheral vascular disease…) and history of clinical cerebrovascular disease (clinical stroke, transient ischemic accident, intracranial hemorrhage).

Both VP and PD patients were asked about age at onset and at diagnosis, chronology of the presentation of parkinsonism, site of involvement of first parkinsonian motor symptoms, cardinal motor symptom and other parkinsonian symptoms at onset, progression of parkinsonian symptoms and response to levodopa. Levodopa response was codified as good, partial and absent.

All VP and PD patients were assessed through the Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn & Yahr staging system at the first visit when entering the study after the usual morning dose of antiparkinsonian medication. Antiparkinsonian and other pharmacological treatments were recorded and levodopa equivalent daily dose was calculated [15].

2.3.2. ^{123}I-MIBG cardiac gammagraphy

The acquisition technique was described elsewhere previously by our group [14]. Briefly, anterior planar images of the chest were obtained and data were collected for 30 min, and 4 h after injection of 111 MBq of ^{123}I-MIBG a static image was obtained with a 128 9128 matrix using a double-head gamma camera system (Siemens E-CAM Dual-head, Erlangen, Germany). The organ uptake of ^{123}I-MIBG was determined by setting the regions of interest (ROI), which were manually drawn around the left cardiac ventricle, and the upper mediastinum. The average counts per pixel in the heart and mediastinum were determined within each ROI to calculate the heart-to-mediastinum (H/M) ratio at 4 h (H/M late ratio). Nuclear medicine specialists were blinded to the status of the subject. Our group has recently published a diagnostic cut-off of 1.43 at 4 h for Spanish population [16] that was used in the present study as the reference ratio for abnormal ^{123}I-MIBG binding.

2.3.3. Olfaction testing

Olfaction was evaluated through the UPSIT (Spanish version).

2.3.4. DaT-SPECT

^{123}I-labeled N-(3-fluoropropyl)-2β-carbometh-oxy-3β-(4-iodophenyl) nortropane (FP-CIT, DaTSCAN; Amersham Health) was used to visualize dopaminergic nerve terminals. Results were classified by a nuclear medicine specialist blinded to the status of the subject. DaT-SPECT images where categorized as 1) normal, 2) decreased uptake with a pattern typical for PD (symmetric or asymmetric uptake reduction in the putamen with activity confined to caudate nucleus or absent uptake bilaterally affecting both putamen and caudate nucleus [17]) or 3) decreased uptake pattern non-typical of PD (as local or patchy defect where cerebral MR imaging showed an ischemic lesion).

2.4. Statistical analyses

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) (PASW Statistics Version 18.00). Since many variables were not normally distributed and the sample was relatively small, non-parametric tests were used to assess differences between groups. Global comparison of age at evaluation (expressed in years) late H/M ratio and UPSIT score were established by Kruskal Wallis test. Further pair-wise comparisons (VP vs. PD, VP vs. controls, PD vs. controls) were calculated by means of Mann Whitney test. Differences in age at disease onset and disease duration expressed both as well in years, UPDRS part III score and levodopa equivalent daily dose (expressed in mg) were also assessed between VP and PD applying Mann–Whitney *U* test. Differences regarding qualitative variables (gender, presence/absence of different cardiovascular risk factors, response to levodopa and

presence of abnormal MR imaging) were assessed by means of Fisher's exact test. To evaluate the diagnostic utility of H/M late ratio and UPSIT score in the diagnosis of VP, we calculated the area under the receiver-operating characteristic (ROC) curve. All estimates were calculated with 95% confidence intervals. All analyses were two-tailed and a significance level of <0.05 was used.

3. Results

3.1. Study subjects

Fifteen patients with suspected clinical diagnosis of VP, fifteen consecutive patients with the diagnosis of PD and nine healthy control subjects were enrolled in the study. Demographic data and cardiovascular risk factors of the study participants are shown in Table 1. We did not find a significant difference regarding age at evaluation between VP or PD and control group (mean VP age 73.4 years, PD 66.2 years and controls 68.11 years) although VP patients were older than PD patients (p 0.04). VP were older than PD patients at onset of motor symptoms (mean 67.0 vs 54.53 years, $p = 0.005$). As anticipated, suspected VP patients had a higher prevalence of cardiovascular risk factors compared to PD patients (See also Table 1).

The VP group compared to PD patients presented a shorter disease duration (mean 6.6 vs. 11 years, $p = 0.001$) with higher UPDRSIII score (mean 35.07 vs. 19.83, $p = 0.023$) and Hoehn & Yahr stage ($p = 0.001$). As a group, VP subjects were taken a lower daily dose of levodopa equivalent (mean 522.73 mg vs. 1281, $p = 0.002$) (see data in Table 1).

Cerebral MR imaging was obtained in 14/15 VP patients and in 12/15 PD patients. One clinical VP patient did not tolerate the MR and cranial CT was performed instead whereas 3 PD patients declined cerebral imaging. The cranial imaging showed significant abnormalities due to vascular damage in all the patients classified as VP and in 6 PD patients ($p = 0.003$). The abnormalities found in MR imaging of VP patients were classified as diffuse (14/14, mainly perivascular white matter changes, subcortical white matter changes, multiple subcortical infarcts and multiples infarcts lacunar in the basal ganglia) with or without focal lesions (5/14, mainly in globus pallidus, thalamus and frontal lobe). The cranial CT scan of the VP patient who did not tolerate MR imaging showed focal left frontal chronic stroke and diffuse perivascular and subcortical white matter changes. Regarding PD patients, the cranial imaging was classified as abnormal in 6/12 cases (50%), with focal lesions in 1/12 and diffuse ones in 5/12.

3.2. H/M ratio

H/M late ratio was significant lower in suspected VP (mean 1.45) and PD (mean 1.16) compared to control group (mean 1.69, $p = 0.017$ and $p < 0.0001$). VP patients presented an H/M late ratio higher than PD patients ($p = 0.001$, see Fig. 1 and Table 1). The estimated area under the ROC curve to discriminate between VP and PD was 0.849 (95% C.I. 0.7–0.998) for H/M late ratio, see figure e-1.

Characteristics of VP patients with normal H/M late ratio (non suggesting subjacent PD, 7/15) and with a low uptake (suggesting PD, 8/15) are presented in Table 2. Subjects with normal H/M late ratio compared to those with a low ratio were prone to present a longer disease duration (mean 9.29 vs. 4.25 years, $p = 0.682$) with the same severity evaluated by UPDRSIII score (mean 35.17 vs. 35, $p = 0.99$) and Hoehn–Yahr scale (median 3 vs. 3, $p = 0.693$). Receiving the same equivalent amount of levodopa (mean 490 vs. 550 mg, $p = 0.305$), none of the VP patients with a normal H/M late ratio presented any response to medication whereas 28.6% of VP patients with a low ratio presented a good response ($p = 0.462$). One patient with clinical suspicion of VP did not receive any amount of levodopa due to gastric intolerance and was therefore excluded from this analysis.

3.3. UPSIT

Control group presented a significant higher UPSIT score when compared to both VP and PD (mean UPSIT score: control 30.71, VP 18.33, PD 15.29, $p = 0.001$ for both groups). There were no differences in the score between VP and PD ($p = 0.128$) (see Table 1 and Fig. 2a). The estimated area under the ROC curve to discriminate between VP and PD was 0.676 (95% C.I. 0.463–0.889) for the UPSIT score, see figure e-2.Those VP with a normal H/M late ratio (non suggestive of PD) when compared to VP with a low ratio were more likely to have a higher UPSIT score (mean UPSIT score 22.4 *vs.* 15.43, $p = 0.006$)(see Table 2 and Fig. 2b).

Table 1
Demographic data, clinical data, late myocardial ^{123}I-MIBG H/M ratio, UPSIT score and RM imaging.

	VP $n = 15$	PD $n = 15$	Controls $n = 9$	p VP vs. PD	p VP vs. control	p PD vs. control
Sex (male, *n*, %)	12 (80%)	10 (66.7%)	6 (66.7%)	0.682[a]	0.685[a]	0.999[a]
Age (years), mean ± SD	73.4 ± 8.3	66.2 ± 9.5	68.11 ± 8.2	0.04[b]	0.135[b]	0.834[b]
Dyslipidemia (*n*, %)	3/15 (20%)	2/14(14.3%)	–	0.999[a]	–	–
High blood pressure (*n*, %)	12/15 (80%)	3/14(21.4%)	–	0.003[a]	–	–
Smokers (*n*, %)	13/15 (86.7%)	0/13(0%)	–	$p < 0.0001$[a]	–	–
Peripheral vascular disease (*n*, %)	3/15 (20%)	0/14 (0%)	–	0.224[a]	–	–
Clinical cerebrovascular disease (n, %)	7/15 (46.7%)	1/14 (7.1%)	–	0.035[a]		
Age at onset (years), mean ± SD	67 ± 12.3	54.53 ± 10.9	n.a.	0.005[b]	–	–
Disease duration (years), mean ± SD	6.6 ± 9.3	11 ± 4.6	n.a.	0.001[b]	–	–
UPDRS part III (On) score, mean ± SD	35.07 ± 15.9	19.83 ± 11.2	n.a.	0.023[b]	–	–
Hoehn & Yahr (On) stage, median (interquartile range)	3 (3–4)	2 (2–2.5)	n.a.	0.001[b]	–	–
Levodopa equivalent daily dose (mg), mean ± SD	522.73 ± 217.2	1281 ± 632.7	n.a.	0.002[b]	–	–
Levodopa response (good, *n*, %)	2/14, 14.3%	15/15, 100%	n.a.	$p < 0.0001$[a]		
Late H/M ratio, mean ± SD	1.45 ± 0.25	1.16 ± 0.12	1.69 ± 0.14	0.001[b]	0.017[b]	$p < 0.0001$[b]
UPSIT score, mean ± SD	18.33 ± 4.44	15.29 ± 5.7	30.71 ± 5.02	0.128[b]	0.001[b]	0.001[b]
MR, abnormal (*n*,%)	14 (100%)	6 (50%)	n.a.	0.003[a]	n.a.	n.a.

n.a. Not applicable.
[a] Fisher's exact test.
[b] Mann–Whitney U.

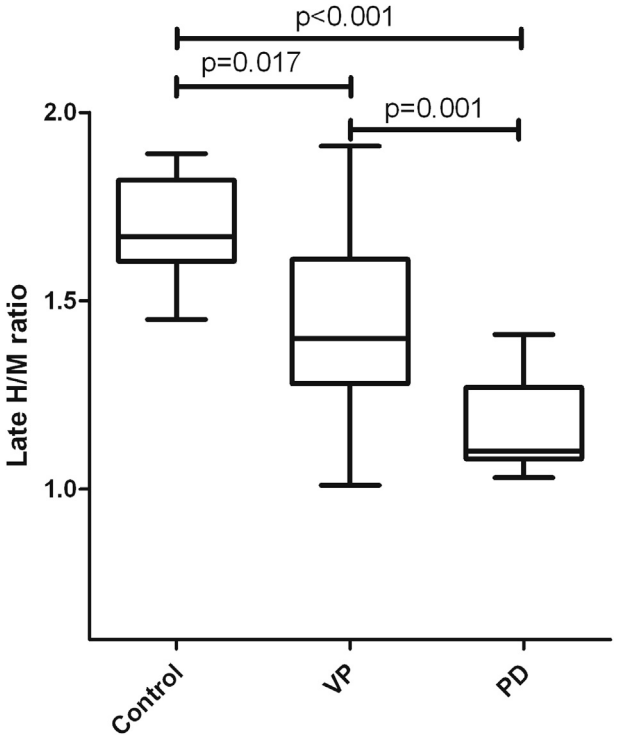

Fig. 1. Box plot showing H/M late ratios for ^{123}I-MIBG cardiac uptake in controls, patients with clinically diagnosed vascular parkinsonism (VP) and patients with idiopathic Parkinson's disease (PD). Whiskers represent minimum and maximum values.

3.4. ^{123}I-FP-CIT SPECT

Suspected VP patients were divide as those with normal ^{123}I-FP-CIT-SPECT imaging ($n = 7$), those with abnormal ^{123}I-FP-CIT-SPECT imaging "typical" of PD ($n = 3$) and those with abnormal ^{123}I-FP-CIT-SPECT imaging "not suggestive or atypical" for PD ($n = 5$) by a nuclear medicine specialist. Characteristics of the three VP groups regarding UPSIT score, disease duration, UPDRSIII score, Hoehn–Yahr score and response to Levodopa are shown in Table e1.

4. Discussion

^{123}I-MIBG cardiac imaging helped to define two different subgroups in patients with suspected VP. The group showing an abnormal ^{123}I-MIBG uptake suggesting PD had a better response to levodopa and a lower UPSIT score compared to those with a normal ^{123}I-MIBG uptake, non suggestive of PD.

We also found a subgroup of patients with parkinsonism and normal or non PD-suggestive ^{123}I-FP-CIT-SPECT imaging who showed abnormal ^{123}I-MIBG cardiac uptake ($n = 6$). We hypothesized that this finding might be due to the presence of vascular lesions in addition to PD in a very early phase. The decrease of cardiac sympathetic innervation is an early finding in PD [11] and has been proposed as a valuable tool in the diagnostic of the disease, even at a pre-motor stage [18,19]. This idea is also supported by post-mortem pathological studies showing degeneration of cardiac sympathetic nerve in incidental Lewy body disease with preserved dorsal vagal nucleus and sympathetic ganglia [11]. A recent study has also described in vivo abnormal aggregates of alpha synuclein in epicardial fat tissue in subjects without clinical parkinsonism, supporting so an early involvement of cardiac autonomic innervation [20]. Besides, a recent report pointed out the high specificity of ^{123}I-MIBG cardiac imaging in diagnosing PD among other neurodegenerative diseases [21] and how can accurately distinguish between PD and multiple system atrophy, and between Alzheimer's disease and dementia with Lewy bodies [22]. Our results would strengthen the utility of ^{123}I-MIBG cardiac imaging as a supporting tool in the differential diagnosis of VP vs PD. However, we have to acknowledge that there are cases of PD with normal ^{123}I-MIBG cardiac uptake some years after disease onset. Several recent meta-analysis showed a pooled sensitivity to differentiate PD from other parkinsonisms (both neurodegenerative and not) by the H/M ratio of 89.7% [21] and 88% [22]. Therefore, normal ^{123}I-MIBG cardiac uptake can not rule out PD.

Olfactory function evaluation has also been postulated as a useful tool in differentiating VP from PD. A previous study had specifically evaluated smell function in VP founding a score significantly higher than in PD in a very well clinical defined cohort of VP patients [23]. Interestingly, our suspected VP patients presented an intermediate UPSIT score between control subjects and PD. However, when analyzing VP patients regarding their ^{123}I-MIBG uptake, we found that those patients with low ^{123}I-MIBG uptake, suggesting subjacent PD, presented a significant lower UPSIT score compared to those with a non-PD suggesting ^{123}I-MIBG.

Another diagnostic tool used in the differential diagnosis of parkinsonism is ^{123}I-FP-CIT-SPECT. Despite having widely experience in its use as supporting tool of PD, the value of ^{123}I-FP-CIT-SPECT in the differential diagnosis between VP and PD is still controversial. In a recent multicentre study using this technique in 158 patients with parkinsonism and cerebrovascular lesions it was found that imaging was normal in 30.4% of patients [24].

Table 2
Demographical and complementary data of VP patients with normal MIBG compared to those with abnormal uptake.

	VP patients with normal H/M ratio $n = 7$	VP patients with low H/M ratio $n = 8$	p
Sex (male, *n*, %)	6 (85.7)	6 (75%)	0.999[a]
Age (years), mean ± SD	70.43 ± 9.6	76±6.4	0.223[b]
Dyslipidemia (*n*, %)	2(28.6%)	1(12.5%)	0.569[a]
High blood pressure (*n*, %)	6(85.7%)	6(75%)	0.999[a]
Smokers (*n*, %)	6(85.7%)	7(87.5)	0.999[a]
Peripheral vascular disease (*n*, %)	1(14.3%)	2(25%)	0.999[a]
Clinical cerebrovascular disease (*n*, %)	3(42.9%)	4(50%)	0.999[a]
Age at onset (years), mean ± SD	62.57 ± 15.6	70.88 ± 7.5	0.322[b]
Duration from PK diagnosis (years), mean ± SD	9.29 ± 13.41	4.25 ± 2.7	0.682[b]
UPDRS part III score, mean ± SD	35.17 ± 19.2	35 ± 14.5	0.999[b]
Hoehn & Yahr stage, median (interquartile range)	3 (3–4)	3 (3–4)	0.693[b]
Levodopa equivalent daily dose (milligrams), mean ± SD	490 ± 292.4	550 ± 154.9	0.305[b]
Levodopa response (good, *n*, %)	0(0%)	2(28.6%)	0.462[a]
DAT-SPECT (suggestive of PD *n*, %)	1(14.3%)	2 (25%)	0.999[a]
UPSIT score, mean ± SD	22.4 ± 2.3	15.43 ± 2.9	0.006[b]

[a] Fisher's exact test.
[b] Mann–Whitney U.

J. Navarro-Otano et al. / Parkinsonism and Related Disorders 20 (2014) 192–197

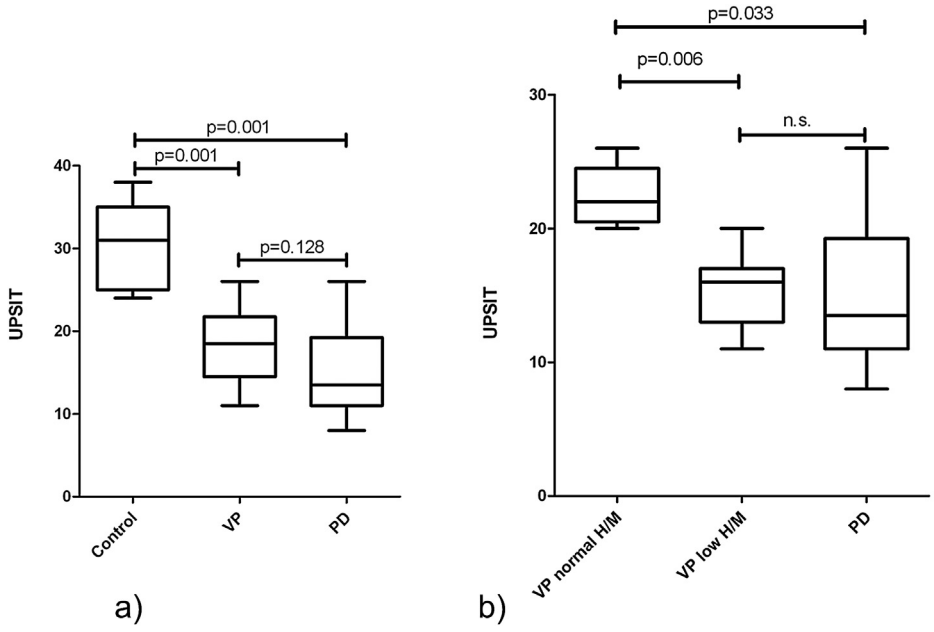

Fig. 2. a) Box plot showing UPSIT scores in controls, patients with clinically diagnosed vascular parkinsonism (VP) and patients with idiopathic Parkinson's disease (PD). Whiskers represent minimum and maximum values. b) Box plot showing UPSIT scores in: VP with normal H/M late ratio (not suggestive of PD) – VP with low H/M late ratio (suggestive of PD) – Idiopathic PD Whiskers represent minimum and maximum values.

Similarly, other study with PD and VP patients showed that qualitatively ^{123}I-FP-CIT SPECT images were normal in 32.5% of patients with VP while, as expected, they were abnormal in all patients with PD [25]. Besides, ^{123}I-FP-CIT-SPECT asymmetry index has been postulated to differentiate Parkinson's disease from VP but the degree of sensitivity for this assessment is as low as 50% [26]. This could point out that, as represented in our study, although ^{123}I-FP-CIT SPECT imaging could still be though as the first available test when the clinical diagnosis is not clear, doubts could remain after this test. In this group of patients, ^{123}I-MIBG imaging could support or dismiss the possibility of VP. As presented in this study, combination of both tests, if needed, permits a more suitable diagnosis.

Several studies tried to clinically characterize patients with VP. It has been demonstrated that the onset of motor symptoms is asymmetric in 59% of patients with VP, being chronic levodopa response positive in almost a half of them [24]. Also, a case–control study has analyzed the differences in the clinical features in patients with VP and with PD. It was found that patients with VP had a higher age at symptom onset and lower disease duration than patients with PD. The most frequent symptom at onset was gait disorder in VP and tremor in PD. Gait disorder, postural instability and falls were more frequent in VP. Rest and mixed tremor were more prevalent in PD. Of the patients who received levodopa treatment in the VP group, only about half had a good response [25]. Our data would support those of previous studies, as our VP patients were also older at disease presentation than PD patients, and presented shorter disease duration. However, in our study, only 2/14 of clinical suspected VP patients who received levodopa presented a good response to this drug whereas as expected 100% of clinical PD patients presented this good response.

The pathogenesis of VP is not completely understood. Bilateral diffuse white matter lesions cause parkinsonism because of damage to the net thalamo-cortical drive, which decreases the ultimate influence of basal ganglia on higher centers of motor planning and execution [27]. Sustaining this idea, a recent multicentre study found that the presence of white matter lesions in nondisabled elderly people was associated with a history of falls, and correlated with mobility impairments, such as decreased walking speed, decreased balance, and reduced physical activity levels [28]. RM ischemic lesions are frequently found in the daily practice and are reported in 40% of patients with idiopathic PD [3]. Besides, in a study with diffusor tensor imaging in patients with VP it has been found that the disruption of the microstructural organization of frontal lobe white matter is associated with the severity of VP [29].

One of the limitations of this study is the fact that ^{123}I-MIBG SPECT study is not reliable in patients suffering from diabetes mellitus where the cardiac sympathetic nervous system is usually involved resulting in a lower H/M ratio [30]. However, although diabetes mellitus is a well known cerebrovascular risk factor, observational studies pointed out that its prevalence in a group of PD and suspected VP is as low as 15% [24]. We acknowledge also that we have to take with caution our results as the relatively small sample size is a limitation. These results are exploratory and require validation in independent patient groups. Furthermore due to the sample size, we could not completely role out the possibility of type II error.

In summary, considering that results of ^{123}I-FP-CIT-SPECT uptake may be confusing, we suggest that the use of ^{123}I-MIBG SPECT H/M late ratios, and to a lesser extent, the UPSIT scores may be supportive to discriminate idiopathic and vascular parkinsonism in patients in which diagnosis uncertainty persists after clinical examination and MR imaging. Regarding this information, it is likely that there exists a subgroup of patients with PD and additional cerebrovascular damage. In this group of patients, treatment with antiparkinsonian drugs should be maintained and even augmented along their disease.

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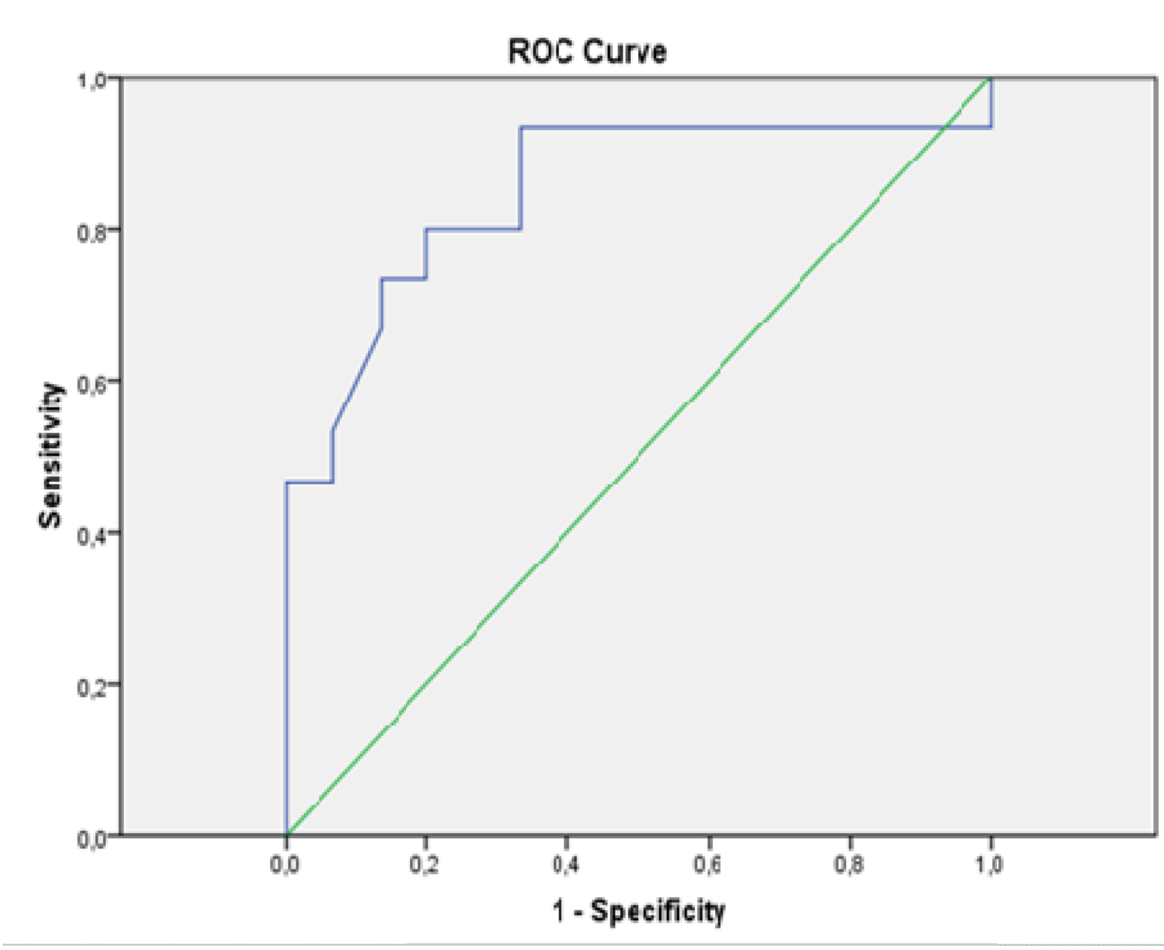
Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2013.10.025.

References

[1] Critchley M. Arteriosclerotic parkinsonism. Brain 1929;52:23–83.
[2] Jellinger KA. Prevalence of cerebrovascular lesions in Parkinson's disease. A postmortem study. Acta Neuropathol 2003;105:415–9.
[3] Kalra S, Grosset DG, Benamer HT. Differentiating vascular parkinsonism from idiopathic Parkinson's disease: a systematic review. Mov Disord 2010;25:149–56.
[4] Winikates J, Jankovic J. Clinical correlates of vascular parkinsonism. Arch Neurol 1999;56:98–102.
[5] Zijlmans JC, Daniel SE, Hughes AJ, Revesz T, Lees AJ. Clinicopathological investigation of vascular parkinsonism, including clinical criteria for diagnosis. Mov Disord 2004;19:630–40.
[6] Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatr 1992;55:181–4.
[7] Murrow RW, Schweiger GD, Kepes JJ, Koller WC. Parkinsonism due to a basal ganglia lacunar state: clinicopathologic correlation. Neurology 1990;40:897–900.
[8] Kagi G, Bhatia KP, Tolosa E. The role of DAT-SPECT in movement disorders. J Neurol Neurosurg Psychiatry 2010;81:5–12.
[9] Lorberboym M, Djaldetti R, Melamed E, Sadeh M, Lampl Y. ^{123}I-FP-CIT SPECT imaging of dopamine transporters in patients with cerebrovascular disease and clinical diagnosis of vascular parkinsonism. J Nucl Med 2004;45:1688–93.
[10] Kline RC, Swanson DP, Wieland DM, Thrall JH, Gross MD, Pitt B, et al. Myocardial imaging in man with I-123 meta-iodobenzylguanidine. J Nucl Med 1981;22:129–32.
[11] Orimo S, Takahashi A, Uchihara T, Mori F, Kakita A, Wakabayashi K, et al. Degeneration of cardiac sympathetic nerve begins in the early disease process of Parkinson's disease. Brain Pathol 2007;17:24–30.
[12] Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania smell identification test: a standardized microencapsulated test of olfactory function. Physiol Behav 1984;32:489–502.
[13] Katzenschlager R, Lees AJ. Olfaction and Parkinson's syndromes: its role in differential diagnosis. Curr Opin Neurol 2004;17:417–23.
[14] Valldeoriola F, Gaig C, Muxi A, Navales I, Paredes P, Lomena F, et al. ^{123}I-MIBG cardiac uptake and smell identification in parkinsonian patients with LRRK2 mutations. J Neurol 2011;258:1126–32.
[15] Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord 2010;25:2649–53.
[16] Muxi A, Paredes P, Navales I, Valldeoriola F, Gaig C, Lomena F, et al. Diagnostic cutoff points for (1)(2)(3)I-MIBG myocardial scintigraphy in a Caucasian population with Parkinson's disease. Eur J Nucl Med Mol Imaging 2011;38:1139–46.
[17] Benamer TS, Patterson J, Grosset DG, Booij J, de Bruin K, van Royen E, et al. Accurate differentiation of parkinsonism and essential tremor using visual assessment of [^{123}I]-FP-CIT SPECT imaging: the [^{123}I]-FP-CIT study group. Mov Disord 2000;15:503–10.
[18] Goldstein DS, Sharabi Y, Karp BI, Bentho O, Saleem A, Pacak K, et al. Cardiac sympathetic denervation preceding motor signs in Parkinson disease. Cleve Clin J Med 2009;76(Suppl. 2):S47–50.
[19] Goldstein DS, Holmes C, Sewell L, Park MY, Sharabi Y. Sympathetic noradrenergic before striatal dopaminergic denervation: relevance to Braak staging of synucleinopathy. Clin Auton Res 2012;22:57–61.
[20] Navarro-Otano J, Gelpi E, Mestres CA, Quintana E, Rauek S, Ribalta T, et al. Alpha-synuclein aggregates in epicardial fat tissue in living subjects without parkinsonism. Parkinsonism Relat Disord 2013;19:27–31. discussion 27.
[21] Orimo S, Suzuki M, Inaba A, Mizusawa H. (123)I-MIBG myocardial scintigraphy for differentiating Parkinson's disease from other neurodegenerative parkinsonism: a systematic review and meta-analysis. Parkinsonism Relat Disord 2012;18:494–500.
[22] King AE, Mintz J, Royall DR. Meta-analysis of ^{123}I-MIBG cardiac scintigraphy for the diagnosis of Lewy body-related disorders. Mov Disord 2011;26:1218–24.
[23] Katzenschlager R, Zijlmans J, Evans A, Watt H, Lees AJ. Olfactory function distinguishes vascular parkinsonism from Parkinson's disease. J Neurol Neurosurg Psychiatry 2004;75:1749–52.
[24] Antonini A, Vitale C, Barone P, Cilia R, Righini A, Bonuccelli U, et al. The relationship between cerebral vascular disease and parkinsonism: the VADO study. Parkinsonism Relat Disord 2012;18:775–80.
[25] Benitez-Rivero S, Marin-Oyaga VA, Garcia-Solis D, Huertas-Fernandez I, Garcia-Gomez FJ, Jesus S, et al. Clinical features and ^{123}I-FP-CIT SPECT imaging in vascular parkinsonism and Parkinson's disease. J Neurol Neurosurg Psychiatry 2013;84:122–9.
[26] Contrafatto D, Mostile G, Nicoletti A, Dibilio V, Raciti L, Lanzafame S, et al. [(123) I]FP-CIT-SPECT asymmetry index to differentiate Parkinson's disease from vascular parkinsonism. Acta Neurol Scand 2012;126:12–6.
[27] Thompson PD, Marsden CD. Gait disorder of subcortical arteriosclerotic encephalopathy: Binswanger's disease. Mov Disord 1987;2:1–8.
[28] Baezner H, Blahak C, Poggesi A, Pantoni L, Inzitari D, Chabriat H, et al. Association of gait and balance disorders with age-related white matter changes: the LADIS study. Neurology 2008;70:935–42.
[29] Wang HC, Hsu JL, Leemans A. Diffusion tensor imaging of vascular parkinsonism: Structural changes in cerebral white matter and the association with clinical severity. Arch Neurol 2012:1–9.
[30] Scott LA, Kench PL. Cardiac autonomic neuropathy in the diabetic patient: does ^{123}I-MIBG imaging have a role to play in early diagnosis? J Nucl Med Technol 2004;32:66–71.

Supplementary figure 1: To evaluate the diagnostic utility of H/M late ratio in the diagnosis of VP, we calculated the area under the receiver-operating characteristic (ROC) curve, under the nonparametric assumption. All estimates were calculated with 95% confidence intervals. The estimated area under the receiver-operating characteristic (ROC) curve for VP was 0.849 (95% C.I. 0.7 to 0.998) for H/M late ratio.



Supplementary figure 2: To evaluate the diagnostic utility of UPSIT score in the diagnosis of VP, we calculated the area under the receiver-operating characteristic (ROC) curve, under the nonparametric assumption. All estimates were calculated with 95% confidence intervals. The estimated area under the receiver-operating characteristic (ROC) curve for VP was 0.676 (95% C.I. 0.463 to 0.889) for the UPSIT score.

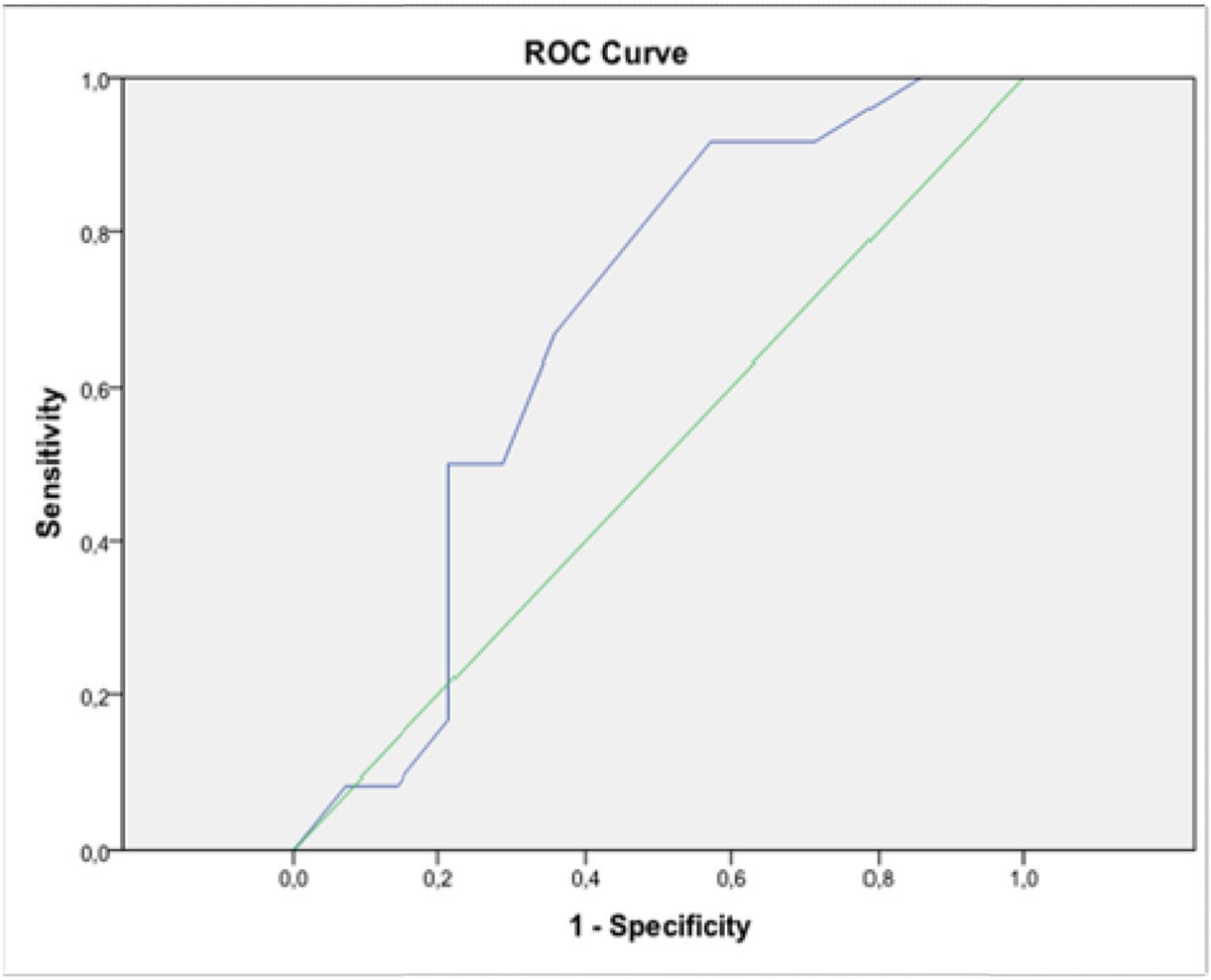


Table e1. Demographical and complementary data of VP patients and different DaT-SPECT pattern

	Normal DaT-SPECT imaging n= 7	Abnormal DaT-SPECT imaging suggestive of PD n= 3	Abnormal DaT-SPECT imaging non suggestive of PD n= 5
Age (years), mean ± SD	72.86 ±8.9	71.00±10.4	75.6±7.4
Age at onset (years), mean ± SD	68.0±7.5	58.00±23.5	71±9.3
Disease duration (years), mean ± SD	4.71±2.1	14.33±21.4	4.6±3.8
UPDRS part III score, mean ± SD	36.14±16.7	38.33±24.6	30.75±10.1
Hoehn & Yahr stage, median(range)	3 (3-4)	4 (3-4)	3 (3-3.75)
Levodopa response (good, n, %)	0, 0%	2 (66.7%)	0, 0%
UPSIT score, mean ± SD	17.5±4.7	19.00±4.24	19.25±5.1
Late H/M ratio, mean ± SD	1.42±0.3	1.46±0.3	1.5±0.2

VI.- Síntesis de resultados y discusión

Los trabajos presentados como parte de la memoria de esta tesis doctoral pretenden resaltar la importancia del estudio del sistema nervioso autónomo en las sinucleinopatías. Aunque las sinucleinopatías son enfermedades clínicamente heterogéneas, el sistema nervioso autónomo se encuentra afectado en mayor o menor medida en todas ellas. Los resultados de esta tesis doctoral apuntan igualmente hacia la idea de que esta afectación es precoz. Además, en esta tesis se defiende que el estudio del sistema nervioso autónomo periférico es útil para realizar el diagnóstico diferencial entre la enfermedad de Parkinson y parkinsonismos que no se asocian a depósitos de alfa-sinucleína.

Todavía está sin respuesta la pregunta de si en la enfermedad de Parkinson la afectación del SNAP y del sistema nervioso central se dan a la vez o si una antecede a la otra. Se desconoce también si esta afectación periférica ocurre de una manera secuencial siguiendo esquemas definidos, como se ha sugerido que sucede a nivel central. Así, en el sistema nervioso central está admitido que la patología por AS se distribuye generalmente siguiendo los estadios de Braak, de una manera ascendente a partir del núcleo motor dorsal del vago y de los núcleos olfatorios anteriores a través del locus coeruleus, sustancia negra y mesocortex hasta alcanzar en los últimos estadios el neocortex de asociación y el primario (Braak et al. 2003). El hecho de que a nivel central el núcleo del nervio vago sea de las primeras estructuras afectadas, abre la puerta a la hipótesis de que la patología siga una vía ascendente a través del nervio vago desde estructuras periféricas con inervación parasimpática. En cuanto a la vía simpática, el hecho de que a nivel de la médula espinal la afectación por AS se localice en estructuras autonómicas simpáticas podría indicar que existe un camino ascendente vía sistema nervioso autónomo simpático (Tamura et al. 2012). Estas hipótesis apoyarían la teoría de la doble puerta de entrada o *dual-hit*, en la que se defiende que un patógeno desconocido que accede vía mucosa nasal y vía mucosa digestiva sería capaz de desencadenar cambios moleculares periféricos que determinarían la formación de agregados anómalos de AS (Hawkes et al. 2007) que progresarían hasta alcanzar el sistema nervioso central.

En el **trabajo número 1** presentado como parte de esta tesis doctoral estudiamos la afectación de estructuras periféricas inervadas por el SNAP y la correlación de la patología periférica con la afectación a nivel central. Así, en este primer trabajo se presenta el estudio *post-mortem* llevado a cabo en sujetos afectos de distintas sinucleinopatías (PD, DLB, iLBD) y otras enfermedades no relacionadas con agregados de AS. Como se había sugerido con anterioridad (Beach et al. 2010), encontramos una afectación universal del SNAP por depósitos anómalos de AS en los

sujetos con PD, DLB y en nuestro único caso de iLBD. Dentro del SNAP, todos nuestros sujetos con PD y DLB presentaban agregados de AS a nivel del ganglio estrellado y cadena simpática paravertebral así como a nivel cardiaco. A nivel del sistema nervioso central, como es de esperar todos los sujetos con diagnóstico de PD y DLB presentaban afectación por AS con un estadio de Braak clasificado en entre 4 y 5 (mediana 5) en el caso de PD y como neocortical en los DLB. En la mitad de los casos de sujetos con enfermedad de Alzheimer que fueron incluidos en el trabajo se encontraron a nivel del sistema nervioso central pequeños depósitos de AS en forma de cuerpos y neuritas de Lewy restringidos a amígdala y bulbo olfatorio. Sin embargo, hay que destacar que ninguno de los casos con diagnóstico de enfermedad de Alzheimer presentaba afectación periférica por AS. Este hallazgo reforzaría la idea de que la afectación del SNAP en las sinucleinopatías es una parte intrínseca del proceso patológico que las diferencia de otros tipos de enfermedades neurodegenerativas.

Estudiando más en detalle la afectación del SNAP *post-mortem*, encontramos un patrón de afectación con gradiente ascendente, con un mayor acúmulo de AS en las regiones más rostrales. Si estudiamos el sistema nervioso simpático centrándonos en la cadena simpática paravertebral (afectada en el 100% de nuestros casos con PD y DLB) encontramos cómo la máxima cantidad de AS se detecta a nivel del ganglio estrellado. La afectación por AS es más escasa conforme avanzamos por la cadena simpática hacia regiones más distales. De la misma manera, el análisis de la afectación del sistema nervioso entérico nos muestra un gradiente de afectación descendente craneo-caudal, con mayor afectación en las regiones inervadas principalmente por el sistema nervioso parasimpático a través del nervio vago.

En el trabajo 1 se valoró de manera semicuantitativa la presencia de inmunoreactividad para la enzima tirosín-hidroxilasa como marcador de integridad de la inervación autonómica a nivel cardiaco. Así, se observó que al menos a este nivel (donde la afectación por acúmulos de AS se presentaba en el 100% de nuestros sujetos con sinucleinopatías) la pérdida de inervación se correlacionaba con un descenso en el número de agregados de AS hallados. Al igual que se ha descrito en la sustancia negra y otras regiones del sistema nervioso central, aquí también podríamos pensar que el daño celular que se ha desencadenado por el acúmulo de AS ha sido tan profundo que ha producido la destrucción de la terminación nerviosa, lo que podría conllevar la desaparición de los agregados de AS contenidos en ella. Si esta neurodegeneración avanzase de manera retrógrada por los nervios autonómicos, encontraríamos el patrón hallado en nuestro estudio. Así, la patología ascendente por el sistema nervioso simpático conllevaría que el mayor número de agregados se

dieran a nivel de los ganglios más craneales de la cadena simpática paravertebral. De la misma manera, el ascenso de la patología por el SNE y el SNPS propiciaría que los agregados de AS fueran encontrándose cada vez en localizaciones más craneales del tracto digestivo y del nervio vago, mientras que en las regiones más caudales "la batalla estaría ya perdida" y tan sólo quedaría muerte celular y denervación. Esta hipótesis que se sugiere en nuestro trabajo se ha podido comprobar *in vivo* en modelos animales como se ha explicado en la introducción de esta defensa de tesis doctoral.

La cuestión de si existe una relación entre la afectación por agregados de AS central y periférica no está cerrada. En nuestros sujetos estudiados no encontramos una relación directa entre la cantidad de AS que se encontraba a nivel del SNAP y el estadiaje calculado a nivel del sistema nervioso central. Los pacientes con DLB, con una supervivencia más corta que el resto, presentaban mayor concentración de AS a nivel del sistema nervioso central, pero esta diferencia no se encontró a nivel del SNAP, sugiriendo tal vez que la velocidad de formación de los agregados varía entre el sistema nervioso central y el periférico.

En nuestro estudio el 100% de los pacientes con confirmación *post-mortem* de PD, DLB, nuestro único caso con iLBD y ninguno del resto de sujetos estudiados presentan agregados anómalos de AS a nivel del ganglio estrellado, cadena simpática y plexo cardíaco. Este hecho haría pensar que el acceso a estos plexos podría proporcionar un biomarcador 100% sensible y específico de sinucleinopatía. Además, el encontrar AS periférica en el sujeto con Parkinson pre-motor, es decir el sujeto clasificado como iLBD, podría apoyar la hipótesis de que esta afectación ocurre en el Parkinson de manera precoz antes de los síntomas motores más clásicos (Beach et al. 2010, Bloch et al. 2006, Del Tredici et al. 2010, Minguez-Castellanos et al. 2007). El acceso *in vivo* a estas estructuras con el fin puramente diagnóstico de una enfermedad neurodegenerativa no es aceptable, pero dentro del ámbito de la investigación algunas de estas partes quedan rutinariamente expuestas en el curso de las cirugías programadas.

Sabiendo por el trabajo 1 que el plexo cardíaco está claramente afectado al menos en los sujetos con PD en estadios finales y basándonos en los datos de la literatura previa se diseñó el **segundo trabajo** que conforma la defensa de esta tesis doctoral. En él, bajo la hipótesis de que los plexos cardíacos se afectan también de manera precoz en el curso de una sinucleinopatía, se recogieron muestras de grasa epicárdica durante cirugías cardíacas programadas en sujetos sin parkinsonismo. En

un primer momento se diseñó un estudio piloto en el que muestras de grasa epicárdica obtenidas en el curso de cirugías cardíacas rutinarias eran evaluadas para asegurar la presencia en las mismas de estructuras nerviosas. Una vez comprobado que era posible el estudio *in vivo* de tejido nervioso epicárdico (rico en terminaciones nerviosas autonómicas) se llevó a cabo el estudio a mayor escala. Para asegurar que en caso de encontrar agregados anómalos estaríamos delante de un sujeto afecto por iLBD todos los pacientes que participaron en este estudio fueron valorados desde el punto de vista neurológico antes de la cirugía. A estos sujetos se les administró una batería de preguntas enfocadas a detectar síntomas sugestivos de afectación no motora típica de una sinucleinopatía en fase premotora. De esta manera, logramos obtener muestras de grasa epicárdica de un total de 91 sujetos. Como se había demostrado en el estudio piloto, en las muestras fue posible detectar estructuras nerviosas, bien en forma de pequeños ganglios autonómicos o bien en forma de pequeños nervios. En 7 de estos 91 sujetos, lo que representa un 7.7% de la muestra, se detectaron en estructuras nerviosas agregados de AS y AS fosforilada.

Una de las principales novedades que aporta este estudio es el hecho de obtener las muestras en sujetos sanos vivos sin diagnóstico de enfermedad neurodegenerativa. Con esto, se busca el estudio *in vivo* del sujeto con iLBD. El estudio del SNAP, al ser por definición más accesible que el sistema nervioso central, nos permite el acceso *in vivo* a estructuras nerviosas. Este acercamiento había sido utilizado con anterioridad por el grupo de Minguez-Castellanos y colaboradores (Minguez-Castellanos et al. 2007). Este grupo estudió piezas quirúrgicas obtenidas en el curso de cirugía oncológica abdomino-pélvica. Tras estudiar 100 casos el porcentaje de sujetos con agregados patológicos de AS fue similar al observado por nosotros a nivel del plexo cardíaco (9% en su muestra frente al 7.7% de la nuestra). Una de las mayores ventajas que aportan los estudios *in vivo* es la posibilidad de realizar el seguimiento clínico de los sujetos estudiados. En nuestros sujetos se ha iniciado el seguimiento clínico de aquellos con agregados anómalos de AS a nivel del plexo cardíaco y de un grupo ajustado por edad y sexo de sujetos sin estos agregados. En nuestro estudio inicial en el momento de obtener las muestras de grasa epicárdica observamos diferencias entre el grupo de sujetos con y sin agregados anómalos de AS en plexo cardíaco. Así, el grupo de sujetos con agregados presentaba más frecuentemente síntomas de disautonomía gastrointestinal (estreñimiento), clínica neuropsiquiátrica (quejas subjetivas de memoria, pérdida de interés en las actividades diarias y el entorno y alucinaciones visuales) y clínica sugestiva de trastorno de la conducta del sueño REM (sueños vivos y actividad motora durante el sueño), todos

ellos hallazgos no motores típicos de la PD. El sujeto más joven no refería por anamnesis la presencia de ningún síntoma no motor asociado a la PD mientras que el sujeto con el máximo acúmulo de AS a nivel de grasa epicárdica tenía una clínica florida de estreñimiento, alucinaciones, quejas de memoria y trastorno de conducta de fase REM del sueño.

En el seguimiento de estos sujetos (trabajo en preparación) uno de los 7 sujetos con agregados anómalos de AS murió por causas cardíacas a los pocos meses de la cirugía (no se dispone de autopsia). De los 6 sujetos restantes, 2 de ellos han declinado continuar en el estudio prospectivo por lo que no disponemos de más datos clínicos. Contamos con los datos de los 4 sujetos restantes con agregados anómalos de AS y con los datos de 6 sujetos control. El primero de los resultados a destacar es que el sujeto con más agregados de AS a nivel cardiaco es el único que ha desarrollado un cuadro neurodegenerativo compatible con demencia florida y cuadro parkinsoniano asociado. Un segundo sujeto con agregados de AS presentaba en el momento del seguimiento quejas de memoria y alteración en las pruebas básicas de despistaje de memoria con diagnóstico de deterioro cognitivo leve. El tercer sujeto del que se dispone de datos, el más joven de todos, presenta en la exploración parkinsonismo leve unilateral, si bien este hallazgo puede estar motivado por secuelas de un accidente cerebral isquémico. A favor de esta teoría iría el hecho de que no presenta ningún síntoma no motor de los que se han comentado en esta defensa de tesis doctoral. En contra de esta hipótesis tenemos el hecho de que este paciente ha puntuado por debajo de la media de nuestros controles en el test de hiposmia realizado (22 puntos frente a una media de 30.71±5.02-ver trabajo 3), si bien de nuevo podría estar falseado este resultado ya que el ictus afectó también a áreas del lenguaje. El último paciente del que disponemos de datos de seguimiento permanece totalmente asintomático. En cuanto a los sujetos sin agregados de AS a nivel cardiaco ninguno de ellos presentaba deterioro cognitivo en el momento de la exploración. Curiosamente, uno de los sujetos sin agregados de AS presentaba en la exploración signos parkinsonianos sutiles sin acompañarse de ninguna queja subjetiva por parte del sujeto. El seguimiento a más largo plazo de estos sujetos podría ayudar a aclarar la secuencia temporal de afectación del SNAP-sistema nervioso central en la PD y DLB.

Los resultados de estos dos primeros trabajos presentados sugieren que el estudio del SNAP cardiaco podría ser útil en la valoración de las sinucleinopatías. En el trabajo número 1 el plexo cardiaco era uno de los sistemas que ineludiblemente se encontraba afectado en todos los sujetos con diagnóstico anatomopatológico de

sinucleinopatía. En el trabajo número 2 se refuerza la hipótesis de que la afectación del plexo cardiaco puede ser además un evento precoz en el desarrollo de una sinucleinopatía. Hoy contamos con herramientas que nos permiten estudiar funcionalmente la afectación del plexo cardiaco mediante pruebas de medicina nuclear con análogos sintéticos con comportamiento similar al de la noradrenalina que de manera selectiva marcan la inervación simpática presináptica. Nos planteamos expandir el estudio del SNAP cardíaco *in vivo* con la idea de que la alteración del SNAP cardíaco podría ayudar a diagnosticar una sinucleinopatía.

Con esta hipótesis se desarrolló el **trabajo número 3** que muestra la utilidad en la práctica clínica del estudio del sistema nervioso autónomo en el diagnóstico diferencial de los parkinsonismos. En el día a día uno de los problemas a los que nos enfrentamos los neurólogos es el de diagnosticar en vida una enfermedad neurodegenerativa cuyo diagnóstico definitivo en este momento es neuropatológico *post-mortem*. Con los criterios actuales de diagnóstico clínico, en manos de neurólogos especializados en trastornos del movimiento hasta en un 10% de los casos el diagnóstico del neurólogo no se corresponde con el diagnóstico del estudio neuropatológico (Hughes et al. 2001). Dada la alta prevalencia de las enfermedades cerebrovasculares, una de las entidades que entraría dentro del diagnóstico diferencial de la PD sería el parkinsonismo de causa vascular por lesión en los ganglios basales (Jellinger 2003, Kalra et al. 2010, Zijlmans et al. 2004). Como se ha discutido en esta defensa de tesis, la afectación del SNA cardiaco podría ser un hecho precoz y casi universal en los pacientes con enfermedad de Parkinson. Para comprobar si el análisis funcional de la inervación autonómica cardíaca podía ayudar en el diagnóstico diferencial de los parkinsonismos se diseñó un estudio en el que evaluamos el resultado de ^{123}I-MIBG SPECT cardiaco, test de olfacción y ^{123}I-FP-CIT-SPECT en sujetos con parkinsonismo vascular y otros con enfermedad de Parkinson. Este estudio se realizó en 15 sujetos con sospecha clínica de parkinsonismo vascular, 15 con sospecha clínica de PD y 9 sujetos sanos. Encontramos que aquellos sujetos inicialmente clasificados como parkinsonismo vascular presentaban un índice de captación de ^{123}I-MIBG cardiaco en la gammagrafía intermedio entre el grupo control y el grupo de sujetos con PD.

Dentro del trabajo describimos cómo los sujetos con diagnóstico clínico de parkinsonismo vascular y con una captación alterada en el ^{123}-I MIBG clínicamente se asemejaban más a los sujetos con enfermedad de Parkinson. Así por ejemplo estos sujetos con alteración en la gammagrafía cardíaca respondían mejor a la medicación antiparkinsoniana y en las pruebas complementarias realizadas tenían peor

puntuación en el test estandarizado para el estudio de la hiposmia (UPSIT). En conjunto, esto nos llevaría a pensar que además del daño cerebrovascular estos sujetos presentan una enfermedad neurodegenerativa tipo enfermedad de Parkinson. Aunque el DaT-SPECT continúa siendo una herramienta muy útil en el diagnóstico diferencial de los parkinsonismos, en casos como el parkinsonismo vascular en el que esta prueba puede estar alterada por daño isquémico subyacente la oportunidad de realizar un ^{123}I-MIBG SPECT cardiaco puede servir de apoyo para el diagnóstico final.

En este tercer trabajo encontramos también un grupo de sujetos con parkinsonismo en el que el MIBG alterado sugería una enfermedad de Parkinson pero que mostraban integridad a nivel de la vía nigroestriatalmedida mediante ^{123}I-FP-CIT-SPECT. Desde un punto de vista teórico estos sujetos podrían recordar a aquellos casos de enfermedad de Parkinson que han debutado clínicamente con una disautonomía cardíaca, con captación cardiaca alterada de ^{123}I-MIBG y vía nigroestriatal íntegra en un inicio (Goldstein et al. 2009, Kaufmann et al. 2004).

En resumen en esta defensa de tesis doctoral se ha querido ahondar en la importancia del SNAP en la enfermedad de Parkinson y en el concepto de que las sinucleinopatías son mucho más que trastornos del sistema nervioso central porque también afectan extensamente al sistema nervioso autónomo periférico. La idea de que enfermedad de Parkinson es igual a degeneración nígrica con parkinsonismo aislado ha quedado superada. La importancia del sistema nervioso autónomo en la enfermedad de Parkinson radica en que su estudio puede aportar biomarcadores y puede ser de ayuda en el diagnóstico precoz y diferencial de la enfermedad. Los artículos presentados en esta defensa de tesis doctoral intentan subrayar esta importancia, pudiendo servir como base para investigaciones futuras dentro de las que se incluiría la búsqueda del lugar más adecuado para realizar una biopsia en vida con la idea de detectar AS anómala y llegar a un diagnóstico precoz

VII.-Conclusiones

CONCLUSIONES

1. En las enfermedades asociadas a agregados patológicos de alfa sinucleína en forma de cuerpos y neuritas de Lewy a nivel del sistema nervioso central existe depósito de estos agregados en el sistema nervioso autónomo periférico que no aparece en otras enfermedades neurodegenerativas.

2. El plexo cardíaco, ganglio estrellado y cadena simpática ganglionar se encuentran afectados por depósitos de alfa sinucleína en todos los sujetos con confirmación neuropatológica de enfermedad de Parkinson, demencia con cuerpos de Lewy y cuerpos de Lewy incidentales.

3. La distribución de agregados de alfa sinucleína en cadena simpática y tracto gastrointestinal presenta un gradiente descendente cráneo-caudal.

4. Un 8% de sujetos asintomáticos presentan agregados de alfa sinucleína a nivel del plexo cardiaco. Ello sugeriría que estos sujetos podrían encontrarse en una etapa pre-motora de una sinucleinopatía.

5. El estudio funcional de la inervación autonómica cardiaca mediante SPECT miocárdico con ^{123}I-MIBG podría ser de utilidad en el estudio del parkinsonismo vascular cuando se sospeche una enfermedad de Parkinson coexistente.

CONCLUSIONES

VIII.- Bibliografía

BIBLIOGRAFÍA

Abbott RD, Ross GW, Petrovitch H, Tanner CM, Davis DG, Masaki KH, Launer LJ, Curb JD, White LR. Bowel movement frequency in late-life and incidental Lewy bodies. Mov Disord 2007;22: 1581-1586.

Abbott RD, Petrovitch H, White LR, Masaki KH, Tanner CM, Curb JD, Grandinetti A, Blanchette PL, Popper JS, Ross GW. Frequency of bowel movements and the future risk of Parkinson's disease. Neurology 2001;57: 456-462.

Adler CH. Nonmotor complications in Parkinson's disease. Mov Disord 2005;20 Suppl 11: S23-29.

Adler CH, et al. Submandibular gland needle biopsy for the diagnosis of Parkinson disease. Neurology 2014;82: 858-864.

Allcock LM, Kenny RA, Burn DJ. Clinical phenotype of subjects with Parkinson's disease and orthostatic hypotension: autonomic symptom and demographic comparison. Mov Disord 2006;21: 1851-1855.

Beach TG, et al. Reduced striatal tyrosine hydroxylase in incidental Lewy body disease. Acta Neuropathol 2008;115: 445-451.

Beach TG, et al. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol 2010;119: 689-702.

Beach TG, et al. Unified staging system for Lewy body disorders: correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. Acta Neuropathol 2009;117: 613-634.

Beyer K, Ariza A. alpha-Synuclein posttranslational modification and alternative splicing as a trigger for neurodegeneration. Mol Neurobiol 2013;47: 509-524.

Bloch A, Probst A, Bissig H, Adams H, Tolnay M. Alpha-synuclein pathology of the spinal and peripheral autonomic nervous system in neurologically unimpaired elderly subjects. Neuropathol Appl Neurobiol 2006;32: 284-295.

Braak H, Del Tredici K, eds. 2009. Neuroanatomy and Pathology of Sporadic Parkinson's Disease: Springer. Colección Advances in Anatomy, embryology and cell biology.

Braak H, Del Tredici-Braak K. 2013. Development of Parkinson’s disease-related pathology in the enteric and central nervous system. Pages 531-547 in Mathias CJ, Bannister R, eds. Autonomic Failure. Oxford: Oxford University Press.

Braak H, de Vos RA, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. Neurosci Lett 2006;396: 67-72.

Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24: 197-211.

Cooper AA, et al. Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science 2006;313: 324-328.

Chaudhuri KR, Ellis C, Love-Jones S, Thomaides T, Clift S, Mathias CJ, Parkes JD. Postprandial hypotension and parkinsonian state in Parkinson's disease. Mov Disord 1997;12: 877-884.

Dabby R, Djaldetti R, Shahmurov M, Treves TA, Gabai B, Melamed E, Sadeh M, Avinoach I. Skin biopsy for assessment of autonomic denervation in Parkinson's disease. J Neural Transm 2006;113: 1169-1176.

Del Tredici K, Hawkes CH, Ghebremedhin E, Braak H. Lewy pathology in the submandibular gland of individuals with incidental Lewy body disease and sporadic Parkinson's disease. Acta Neuropathol 2010;119: 703-713.

DelleDonne A, et al. Incidental Lewy body disease and preclinical Parkinson disease. Arch Neurol 2008;65: 1074-1080.

Dickson DW, et al. Evidence that incidental Lewy body disease is pre-symptomatic Parkinson's disease. Acta Neuropathol 2008;115: 437-444.

Djaldetti R, Lev N, Melamed E. Lesions outside the CNS in Parkinson's disease. Mov Disord 2009;24: 793-800.

Donadio V, Incensi A, Giannoccaro MP, Cortelli P, Di Stasi V, Pizza F, Jaber MA, Baruzzi A, Liguori R. Peripheral autonomic neuropathy: diagnostic contribution of skin biopsy. J Neuropathol Exp Neurol 2012;71: 1000-1008.

Donadio V, Incensi A, Leta V, Giannoccaro MP, Scaglione C, Martinelli P, Capellari S, Avoni P, Baruzzi A, Liguori R. Skin nerve alpha-synuclein deposits: A biomarker for idiopathic Parkinson disease. Neurology. 2014; 82:1362-1369.

Ejaz AA, Sekhon IS, Munjal S. Characteristic findings on 24-h ambulatory blood pressure monitoring in a series of patients with Parkinson's disease. Eur J Intern Med 2006;17: 417-420.

Ferini-Strambi L, Oldani A, Zucconi M, Smirne S. Cardiac autonomic activity during wakefulness and sleep in REM sleep behavior disorder. Sleep 1996;19: 367-369.

Fujishiro H, Frigerio R, Burnett M, Klos KJ, Josephs KA, Delledonne A, Parisi JE, Ahlskog JE, Dickson DW. Cardiac sympathetic denervation correlates with clinical and pathologic stages of Parkinson's disease. Mov Disord 2008;23: 1085-1092.

Gage H, Kaye J, Kimber A, Storey L, Egan M, Qiao Y, Trend P. Correlates of constipation in people with Parkinson's. Parkinsonism Relat Disord 2011;17: 106-111.

Gao X, Chen H, Schwarzschild MA, Ascherio A. A prospective study of bowel movement frequency and risk of Parkinson's disease. Am J Epidemiol 2011;174: 546-551.

George S, Rey NL, Reichenbach N, Steiner JA, Brundin P. alpha-Synuclein: the long distance runner. Brain Pathol 2013;23: 350-357.

Goldstein DS, Holmes C, Sewell L, Park MY, Sharabi Y. Sympathetic noradrenergic before striatal dopaminergic denervation: relevance to Braak staging of synucleinopathy. Clin Auton Res 2012;22: 57-61.

Goldstein DS, Sharabi Y, Karp BI, Bentho O, Saleem A, Pacak K, Eisenhofer G. Cardiac sympathetic denervation preceding motor signs in Parkinson disease. Cleve Clin J Med 2009;76 Suppl 2: S47-50.

Guo H, Tabara Y, Igase M, Yamamoto M, Ochi N, Kido T, Uetani E, Taguchi K, Miki T, Kohara K. Abnormal nocturnal blood pressure profile is associated with mild cognitive impairment in the elderly: the J-SHIPP study. Hypertens Res 2010;33: 32-36.

Ha AD, Brown CH, York MK, Jankovic J. The prevalence of symptomatic orthostatic hypotension in patients with Parkinson's disease and atypical parkinsonism. Parkinsonism Relat Disord 2011;17: 625-628.

Hasler WL. Gastroparesis: pathogenesis, diagnosis and management. Nat Rev Gastroenterol Hepatol 2011;8: 438-453.

Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. Neuropathol Appl Neurobiol 2007;33: 599-614.

Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: the dual hit theory revisited. Ann N Y Acad Sci 2009;1170: 615-622.

Heetun ZS, Quigley EM. Gastroparesis and Parkinson's disease: a systematic review. Parkinsonism Relat Disord 2012;18: 433-440.

Horimoto Y, Matsumoto M, Akatsu H, Ikari H, Kojima K, Yamamoto T, Otsuka Y, Ojika K, Ueda R, Kosaka K. Autonomic dysfunctions in dementia with Lewy bodies. J Neurol 2003;250: 530-533.

Huang YP, Chen LS, Yen MF, Fann CY, Chiu YH, Chen HH, Pan SL. Parkinson's disease is related to an increased risk of ischemic stroke-a population-based propensity score-matched follow-up study. PLoS One 2013;8:e68314.

Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease. Neurology 2001;57: 1497-1499.

Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55: 181-184.

Ikemura M, et al. Lewy body pathology involves cutaneous nerves. J Neuropathol Exp Neurol 2008;67: 945-953.

Iranzo A, Gelpi E, Tolosa E, Molinuevo JL, Serradell M, Gaig C, Santamaria J. Neuropathology of prodromal Lewy body disease. Mov Disord 2014;29: 410-415.

Ito S, Takao M, Hatsuta H, Kanemaru K, Arai T, Saito Y, Fukayama M, Murayama S. Alpha-synuclein immunohistochemistry of gastrointestinal and biliary surgical specimens for diagnosis of Lewy body disease. Int J Clin Exp Pathol 2014;7: 1714-1723.

Iwanaga K, Wakabayashi K, Yoshimoto M, Tomita I, Satoh H, Takashima H, Satoh A, Seto M, Tsujihata M, Takahashi H. Lewy body-type degeneration in cardiac plexus in Parkinson's and incidental Lewy body diseases. Neurology 1999;52: 1269-1271.

Jamnadas-Khoda J, Koshy S, Mathias CJ, Muthane UB, Ragothaman M, Dodaballapur SK. Are current recommendations to diagnose orthostatic hypotension in Parkinson's disease satisfactory? Mov Disord 2009;24: 1747-1751.

Jellinger KA. Prevalence of cerebrovascular lesions in Parkinson's disease. A postmortem study. Acta Neuropathol 2003;105: 415-419.

Kalra S, Grosset DG, Benamer HT. Differentiating vascular parkinsonism from idiopathic Parkinson's disease: a systematic review. Mov Disord 2010;25: 149-156.

Kaplan B, Ratner V, Haas E. Alpha-synuclein: its biological function and role in neurodegenerative diseases. J Mol Neurosci 2003;20: 83-92.

Kaufmann H, Nahm K, Purohit D, Wolfe D. Autonomic failure as the initial presentation of Parkinson disease and dementia with Lewy bodies. Neurology 2004;63: 1093-1095.

Kim JS, Oh YS, Lee KS, Kim YI, Yang DW, Goldstein DS. Association of cognitive dysfunction with neurocirculatory abnormalities in early Parkinson disease. Neurology 2012;79: 1323-1331.

Lanfranchi PA, Fradette L, Gagnon JF, Colombo R, Montplaisir J. Cardiac autonomic regulation during sleep in idiopathic REM sleep behavior disorder. Sleep 2007;30: 1019-1025.

Lauria G, Cornblath DR, Johansson O, McArthur JC, Mellgren SI, Nolano M, Rosenberg N, Sommer C. EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy. Eur J Neurol 2005;12: 747-758.

Lebouvier T, Coron E, Chaumette T, Paillusson S, Bruley des Varannes S, Neunlist M, Derkinderen P. Routine colonic biopsies as a new tool to study the enteric nervous system in living patients. Neurogastroenterol Motil 2010;22: e11-14.

Lebouvier T, Chaumette T, Damier P, Coron E, Touchefeu Y, Vrignaud S, Naveilhan P, Galmiche JP, Bruley des Varannes S, Derkinderen P, Neunlist M. Pathological lesions in colonic biopsies during Parkinson's disease. Gut 2008;57: 1741-1743.

Lebouvier T, Neunlist M, Bruley des Varannes S, Coron E, Drouard A, N'Guyen JM, Chaumette T, Tasselli M, Paillusson S, Flamand M, Galmiche JP, Damier P, Derkinderen P. Colonic biopsies to assess the neuropathology of Parkinson's disease and its relationship with symptoms. PLoS One 2010;5:e12728 .

Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterology 2006;130: 1480-1491.

Luciano GL, Brennan MJ, Rothberg MB. Postprandial hypotension. Am J Med 2010;123: 281 e281-286.

Martinez-Martin P, Rodriguez-Blazquez C, Kurtis MM, Chaudhuri KR. The impact of non-motor symptoms on health-related quality of life of patients with Parkinson's disease. Mov Disord 2011;26: 399-406.

Martinez-Martin P, et al. Prevalence of nonmotor symptoms in Parkinson's disease in an international setting; study using nonmotor symptoms questionnaire in 545 patients. Mov Disord 2007;22: 1623-1629.

McKeith IG, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005;65: 1863-1872.

Miki Y, Mori F, Wakabayashi K, Kuroda N, Orimo S. Incidental Lewy body disease restricted to the heart and stellate ganglia. Mov Disord 2009;24: 2299-2301.

Miki Y, Tomiyama M, Ueno T, Haga R, Nishijima H, Suzuki C, Mori F, Kaimori M, Baba M, Wakabayashi K. Clinical availability of skin biopsy in the diagnosis of Parkinson's disease. Neurosci Lett 2010;469: 357-359.

Milazzo V, Di Stefano C, Servo S, Zibetti M, Lopiano L, Maule S. Neurogenic orthostatic hypotension as the initial feature of Parkinson disease. Clin Auton Res 2012;22: 203-206.

Minguez-Castellanos A, Chamorro CE, Escamilla-Sevilla F, Ortega-Moreno A, Rebollo AC, Gomez-Rio M, Concha A, Munoz DG. Do alpha-synuclein aggregates in autonomic plexuses predate Lewy body disorders?: a cohort study. Neurology 2007;68: 2012-2018.

Nolano M, Provitera V, Estraneo A, Selim MM, Caporaso G, Stancanelli A, Saltalamacchia AM, Lanzillo B, Santoro L. Sensory deficit in Parkinson's disease: evidence of a cutaneous denervation. Brain 2008;131: 1903-1911.

Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, Lees AJ, Schrag A.. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Ann Neurol. 2012;72: 893-901

O'Sullivan SS, Williams DR, Gallagher DA, Massey LA, Silveira-Moriyama L, Lees AJ. Nonmotor symptoms as presenting complaints in Parkinson's disease: a clinicopathological study. Mov Disord 2008;23: 101-106.

Okada Y, Ito Y, Aida J, Yasuhara M, Ohkawa S, Hirokawa K. Lewy bodies in the sinoatrial nodal ganglion: clinicopathological studies. Pathol Int 2004;54: 682-687.

Orimo S, Uchihara T, Nakamura A, Mori F, Kakita A, Wakabayashi K, Takahashi H. Axonal alpha-synuclein aggregates herald centripetal degeneration of cardiac sympathetic nerve in Parkinson's disease. Brain 2008;131: 642-650.

Pan-Montojo F, Anichtchik O, Dening Y, Knels L, Pursche S, Jung R, Jackson S, Gille G, Spillantini MG, Reichmann H, Funk RH. Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. PLoS One 2010;5: e8762.

Pan-Montojo F1, Schwarz M, Winkler C, Arnhold M, O'Sullivan GA, Pal A, Said J, Marsico G, Verbavatz JM, Rodrigo-Angulo M, Gille G, Funk RH, Reichmann H Environmental toxins trigger PD-like progression via increased alpha-synuclein release from enteric neurons in mice. Sci Rep 2012;2: 898.

Parkkinen L, Pirttila T, Alafuzoff I. Applicability of current staging/categorization of alpha-synuclein pathology and their clinical relevance. Acta Neuropathol 2008;115: 399-407.

Pavcovich LA, Yang M, Miselis RR, Valentino RJ. Novel role for the pontine micturition center, Barrington's nucleus: evidence for coordination of colonic and forebrain activity. Brain Res 1998;784: 355-361.

Petrovitch H, Abbott RD, Ross GW, Nelson J, Masaki KH, Tanner CM, Launer LJ, White LR. Bowel movement frequency in late-life and substantia nigra neuron density at death. Mov Disord 2009;24: 371-376.

Plaschke M, Trenkwalder P, Dahlheim H, Lechner C, Trenkwalder C. Twenty-four-hour blood pressure profile and blood pressure responses to head-up tilt tests in Parkinson's disease and multiple system atrophy. J Hypertens 1998;16: 1433-1441.

Postuma RB, Lanfranchi PA, Blais H, Gagnon JF, Montplaisir JY. Cardiac autonomic dysfunction in idiopathic REM sleep behavior disorder. Mov Disord 2010;25: 2304-2310.

Postuma RB, Gagnon JF, Vendette M, Fantini ML, Massicotte-Marquez J, Montplaisir J. Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder. Neurology 2009;72: 1296-1300.

Pouclet H, Lebouvier T, Coron E, Des Varannes SB, Neunlist M, Derkinderen P. A comparison between colonic submucosa and mucosa to detect Lewy pathology in Parkinson's disease. Neurogastroenterol Motil. 2012;24: e202-205

Robertson DR, Renwick AG, Wood ND, Cross N, Macklin BS, Fleming JS, Waller DG, George CF. The influence of levodopa on gastric emptying in man. Br J Clin Pharmacol 1990;29: 47-53.

Sakakibara R, Uchiyama T, Yamanishi T, Kishi M. Genitourinary dysfunction in Parkinson's disease. Mov Disord 2010;25: 2-12.

Sakakibara R, Kishi M, Ogawa E, Tateno F, Uchiyama T, Yamamoto T, Yamanishi T. Bladder, bowel, and sexual dysfunction in Parkinson's disease. Parkinsons Dis 2011: 924605.

Schestatsky P, Valls-Sole J, Ehlers JA, Rieder CR, Gomes I. Hyperhidrosis in Parkinson's disease. Mov Disord 2006;21: 1744-1748.

Schmidt C, Berg D, Prieur S, Junghanns S, Schweitzer K, Globas C, Schols L, Reichmann H, Ziemssen T. Loss of nocturnal blood pressure fall in various extrapyramidal syndromes. Mov Disord 2009;24: 2136-2142.

Senard JM, Rai S, Lapeyre-Mestre M, Brefel C, Rascol O, Rascol A, Montastruc JL. Prevalence of orthostatic hypotension in Parkinson's disease. J Neurol Neurosurg Psychiatry 1997;63: 584-589.

Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH. Is alpha-synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3 cases. Mov Disord 2012;27: 716-719.

Shannon KM, Keshavarzian A, Mutlu E, Dodiya HB, Daian D, Jaglin JA, Kordower JH. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. Mov Disord. 2012;27: 709-15

Sharabi Y, Goldstein DS. Mechanisms of orthostatic hypotension and supine hypertension in Parkinson disease. J Neurol Sci 2011;310: 123-128.

Sommer S, Aral-Becher B, Jost W. Nondipping in Parkinson's disease. Parkinsons Dis 2011: 897586.

Sorensen GL, Kempfner J, Zoetmulder M, Sorensen HB, Jennum P. 2012. Attenuated heart rate response in REM sleep behavior disorder and Parkinson's disease. Mov Disord. 2012;27: 888-94.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997;388: 839-840.

Stubendorff K, Aarsland D, Minthon L, Londos E. The impact of autonomic dysfunction on survival in patients with dementia with Lewy bodies and Parkinson's disease with dementia. PLoS One 2012;7: e45451.

Swinn L, Schrag A, Viswanathan R, Bloem BR, Lees A, Quinn N. Sweating dysfunction in Parkinson's disease. Mov Disord 2003;18: 1459-1463.

Tamura T, Yoshida M, Hashizume Y, Sobue G. Lewy body-related alpha-synucleinopathy in the spinal cord of cases with incidental Lewy body disease. Neuropathology 2012;32: 13-22.

Tijero B, Gomez-Esteban JC, Llorens V, Lezcano E, Gonzalez-Fernandez MC, de Pancorbo MM, Ruiz-Martinez J, Cembellin JC, Zarranz JJ. Cardiac sympathetic denervation precedes nigrostriatal loss in the E46K mutation of the alpha-synuclein gene (SNCA). Clin Auton Res 2010;20: 267-269.

Tijero B, Gómez-Esteban JC, Lezcano E, Fernández-González C, Somme J, Llorens V, Martínez A, Ruiz-Martínez J, Foncea N, Escalza I, Berganzo K, Aniel-Quiroga MA, Ruiz V, Terán N, Kaufmann H, Zarranz JJ. Cardiac sympathetic denervation in symptomatic and asymptomatic carriers of the E46K mutation in the alpha synuclein gene. Parkinsonism Relat Disord. 2013;19: 95-100.

Tolosa E, Gaig C, Santamaria J, Compta Y. Diagnosis and the premotor phase of Parkinson disease. Neurology 2009;72: S12-20.

Treglia G, Cason E. Diagnostic performance of myocardial innervation imaging using MIBG scintigraphy in differential diagnosis between dementia with lewy bodies and other dementias: a systematic review and a meta-analysis. J Neuroimaging 2012;22: 111-117.

Trojanowski JQ, Lee VM. Aggregation of neurofilament and alpha-synuclein proteins in Lewy bodies: implications for the pathogenesis of Parkinson disease and Lewy body dementia. Arch Neurol 1998;55: 151-152.

Unger MM, et al. Postprandial ghrelin response is reduced in patients with Parkinson's disease and idiopathic REM sleep behaviour disorder: a peripheral biomarker for early Parkinson's disease? J Neurol 2011;258: 982-990.

Velseboer DC, de Haan RJ, Wieling W, Goldstein DS, de Bie RM. Prevalence of orthostatic hypotension in Parkinson's disease: a systematic review and meta-analysis. Parkinsonism Relat Disord 2011;17: 724-729.

Visser M, Marinus J, Stiggelbout AM, Van Hilten JJ. Assessment of autonomic dysfunction in Parkinson's disease: the SCOPA-AUT. Mov Disord 2004;19: 1306-1312.

Wakabayashi K, Takahashi H, Takeda S, Ohama E, Ikuta F. 1988. Parkinson's disease: the presence of Lewy bodies in Auerbach's and Meissner's plexuses. Acta Neuropathol 76: 217-221.

Wang N, Gibbons CH, Lafo J, Freeman R. alpha-Synuclein in cutaneous autonomic nerves. Neurology 2013;81: 1604-1610.

Wenning GK, Scherfler C, Granata R, Bosch S, Verny M, Chaudhuri KR, Jellinger K, Poewe W, Litvan I. Time course of symptomatic orthostatic hypotension and urinary incontinence in patients with postmortem confirmed parkinsonian syndromes: a clinicopathological study. J Neurol Neurosurg Psychiatry 1999;67: 620-623.

Yeo L, Singh R, Gundeti M, Barua JM, Masood J. Urinary tract dysfunction in Parkinson's disease: a review. Int Urol Nephrol 2012;44: 415-424.

Zijlmans JC, Daniel SE, Hughes AJ, Revesz T, Lees AJ. Clinicopathological investigation of vascular parkinsonism, including clinical criteria for diagnosis. Mov Disord 2004;19: 630-640.