Prevalencia, fenotipo clínico y neuropatológico del parkinsonismo asociado a mutaciones en el gen LRRK2

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Tesis Doctoral

PREVALENCIA, FENOTIPO CLÍNICO Y NEUROPATOLÓGICO DEL PARKINSONISMO ASOCIADO A MUTACIONES EN EL GEN LRRK2

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EDUARD TOLOSA SARRÓ, Catedrático de la Universitat de Barcelona,

Certifica que la memoria titulada “PREVALENCIA, FENOTIPO CLÍNICO Y NEUROPATOLÓGICO DEL PARKINSONISMO ASOCIADO A MUTACIONES EN EL GEN LRRK2”, presentada por Carles Gaig Ventura, ha estado realizada bajo mi dirección, y considero que reúne las condiciones necesarias para ser defendida ante el tribunal correspondiente para optar al Grado de Doctor en Medicina y Cirugía.

Eduard Tolosa Sarró

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Agraïments

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ÍNDICE

1. INTRODUCCIÓN

La enfermedad de Parkinson (EP) es una de las enfermedades neurodegenerativas más prevalentes, afectando al 1% de las personas mayores de 60 años y a más del 3% de las mayores de 75 años [De Rijk et al., 1995; Benito-León et al., 2003]. Desde un punto de vista motor la EP se caracteriza clínicamente por la presencia de temblor en reposo, bradicinesia, rigidez muscular e inestabilidad postural. La presencia de estos síntomas cardinales, junto con la respuesta al tratamiento con levodopa u otros fármacos antiparkinsonianos, son esenciales para realizar el diagnóstico clínico de la enfermedad [Hughes et al., 1992]. Además de estos síntomas, los pacientes con EP presentan con frecuencia síntomas no motores diversos como trastornos cognitivos y psiquiátricos (demencia, depresión, ansiedad, alucinaciones, psicosis), disfunción autonómica (estreñimiento, disfunción genitourinaria, hipotensión ortostática), alteraciones del sueño (insomnio, somnolencia diurna excesiva, trastorno de conducta de sueño REM) o alteraciones sensitivas (pérdida del sentido del olfato, dolor) [Chaudhuri et al., 2006]. Anatomopatológicamente la EP se define por la pérdida de neuronas dopaminérgicas junto con la presencia de agregados de sinucleína en forma de cuerpos y neuritas de Lewy en las neuronas restantes de la sustancia negra [Hughes et al., 1992; Spillantini et al., 1997]. La pérdida neuronal y los agregados de sinucleína también se producen en otras estructuras del tronco cerebral como el locus ceruleus o el núcleo dorsal del vago, y a menudo afecta al sistema límbico y la corteza cerebral [Braak et al., 2006].

La EP es una enfermedad idiopática, cuya etiología y fisiopatología están aún por esclarecer en la mayoría de los casos. Durante mucho tiempo se creyó que los factores ambientales jugaban un papel mucho más relevante que los factores genéticos en la etiopatogenia de esta enfermedad [Calne et al., 1983; Lewin., 1985]. Esta visión venía respaldada por la consideración clásica que la EP era una enfermedad eminentemente esporádica, si bien en el siglo XIX y a principios del siglo XX autores como Leroux (discípulo de Charcot), Gowers o Mjönes, ya describieron casos familiares de EP [Leroux., 1880; Gowers., 1902; Mjönes.,1949], o trabajos como los de Martin y colaboradores, que en los años setenta ya mostraron que hasta un 27% de los pacientes con EP presentaban antecedentes familiares de la enfermedad en comparación con el 15% de los controles [Martin et al., 1973]. Por otro lado la existencia de casos de parkinsonismo postencefalítico, en especial tras la pandemia de gripe del año 1918 [Poskanzer et al., 1963; Howard et al., 1987], así como la descripción de pacientes que habían desarrollado un parkinsonismo tras exposición a tóxicos como el MPTP (1-metil-4-fenil-tetrahidropiridina) [Langston et al., 1983; Snyder et al., 1985] o el Manganeso [Huang et al., 1989; Meco et al., 1994], o la posterior descripción de diversos factores ambientales asociados a un mayor riesgo de desarrollar una EP, como la exposición a pesticidas, el consumo de agua de pozo o vivir en un ambiente rural [Golbe et al., 1990 (a); Semchuk et al., 1991; Butterfield et al., 1993; Hubble et al., 1993], respaldaban fuertemente la existencia de algún factor ambiental, quizás tóxico o vírico, en la etiopatogenia de esta enfermedad.

Esta visión “ambientalista” de la etiopatogenia de la EP cambio en los años noventa. Se describieron diversas familias con varios miembros afectos por la EP [Golbe et al., 1990 (b); Golbe et al., 1993; Wszolek et al., 1995; Denson et al., 1995; Wszolek et al., 1997; Hasegawa et al., 1997] y se

observó que entre un 10% y un 24% de los pacientes con EP presentaban antecedentes familiares de la enfermedad [Payami et al., 1994; Bonifati et al., 1995]. Además, también se constató que la presencia de antecedentes familiares de EP era un factor de riesgo para desarrollar la enfermedad [Payami et al., 1994; Taylor et al., 1999; Rocca et al., 2004], y en algunos estudios realizados en parejas de gemelos se demostró la existencia de una concordancia para la EP en aquellos gemelos monocigotos con una EP de inicio temprano, antes de los 50 años, en comparación con los gemelos dicigotos o los que tenían un inicio más tardío de la enfermedad [Tanner et al., 1999; Wirdefeldt et al., 2004]. Todo ello sugería que los factores genéticos podían jugar un papel tan relevante como los factores ambientales en la etiopatogenia de la EP, y de forma especial en aquellos casos con una historia familiar de la enfermedad positiva.

1.1 Genética y enfermedad de Parkinson

En los últimos años una intensa labor investigadora ha llevado al descubrimiento de mutaciones en diferentes genes que determinan algunas formas de EP monogénica o de herencia mendeliana (Tabla 1), y por otro lado a la descripción de diversas variantes genéticas que podrían representar un factor de riesgo para el desarrollo de la EP esporádica (Tabla 2) [Huang et al., 2004; Gasser., 2007].

Hasta finales del año 2007 se habían identificado 13 locus genéticos o cromosómicos asociados a formas familiares o monogénicas de EP (PARK-1 a PARK-13). En nueve de estos locus se ha podido identificar el gen cuya mutación es la responsable de la enfermedad [Huang et al., 2004; Gasser., 2007]. Los primeros genes identificados fueron el gen de la alfa-sinucleína (PARK-1) [Polymeropoulos et al., 1997] y el gen de la parkina (PARK-2) [Kitada et al., 1998]. Posteriormente se identificaron los otros genes asociados a estas formas mendelianas o monogénicas de EP: el gen UCHL1 (PARK-5) [Leroy et al., 1998], PINK1 (PARK-6) [Valente et al., 2004], DJ-1 (PARK-7) [Bonifati et al., 2003], LRRK2 (PARK-8) [Paisán-Ruiz et al., 2004; Zimprich et al., 2004 (a)], ATP13A2 (PARK-9) [Ramirez et al., 2006; Di Fonzo et al., 2007], GIGYF2 o TNRC15 (PARK-11) [Pankratz et al., 2003; Lautier et al., 2008] y HTRA2 (PARK-13) [Strauss et al., 2005] (Tabla 1).

Aparte de todos estos genes, también se han descrito otros 3 locus cromosómicos asociados a la EP (PARK-3, PARK-10 y PARK-12) y que sugieren la existencia de al menos otros tres genes, aún sin descubrir [Gasser et al., 1998; Hicks et al., 2002; Pankratz et al., 2003]. Más recientemente se han identificado dos nuevos genes, a los que se ha propuesto asignar los locus PARK-14 (gen de la fosfolipasa A2 o PLA2G6) y PARK-15 (gen FBXO7) [Paisán-Ruiz et al., 2009; Shojaee et al., 2008; Di Fonzo et al., 2009], si bien estos genes se han identificado en familias con un parkinsonismo atípico con signos piramidales y distonía associados.

Además existen otros cuatro genes que se han asociado a formas monogénicas o familiares de parkinsonismo: el gen del neurofilamento M [Lavedan et al., 2002], el gen de la sinfilina-1 [Marx et al., 2003], el gen NR4A2/Nurr1 [Le et al., 2003; Grimes et al., 2006] y el gen POLG/DNA polimerasa-γ

Tabla 1. Locus Cromosómicos y genes asociados a formas mendelianas o monógenicas de EP

Locus Cr.	Gen Herencia	Edad Inicio (años)	Fenotipo Clínico	Fenotipo Patológico	Observaciones
PARK-1 (4q21)	Sinucleína AD	40-45	Similar a la EP clásica pero edad de inicio más precoz y progresión más rápida En ocasiones disautonomía, demencia precoz (fenotipo de demencia por cuerpos de Lewy)	Patología tipo Lewy (Cuerpos y neuritas de Lewy)	Infrecuente: Tan sólo descritas 3 mutaciones puntuales y diversos casos con duplicaciones y triplicaciones del gen
PARK-2 (6q25)	Parkina AR	< 40 (7-58)	Inicio precoz. Parkinsonismo simétrico, lentamente progresivo. Buena respuesta a la levodopa pero aparición de discinecias tempranas. Distonía en pie. Hiperreflexia en piernas. En ocasiones inicio tardío con fenotipo similar a la EP clásica	▪ Ausencia de cuerpos de Lewy en la mayoría de casos ▪ Algunos casos con patología tipo Lewy	Múltiples mutaciones descritas Causa más frecuente de parkinsonismo de inicio temprano (<40 años)
PARK-3 (2p13)	No conocido AD penetrancia reducida	Locus detectado en un estudio de ligamiento realizado en múltiples familias alemanas. Otros estudios han replicado este ligamiento. El fenotipo clínico es similar a la EP esporádica y el examen anatomopatológico muestra patología de Lewy			
PARK-4 (4p15)	Triplicaciones de la Sinucleína AD	La descripción de este locus se considera en la actualidad errónea. Aunque inicialmente se localizó una región del cromosoma 4 asociada a la EP familiar diferente a la descrita para el locus PARK-1, posteriormente se ha observado que en realidad se trata de una triplicación del gen de la sinucleína. Por lo tanto, el locus PARK-4 no debería considerarse como un locus genético asociado a la EP			
PARK-5 (4p14)	UCHL1 AD	50	Similar a la EP esporádica con temblor de reposo y buena respuesta a la levodopa	Desconocido	Tan sólo descrita una familia La mutación se encontró al secuenciar directamente este gen (sin estudios de ligamiento previos), pues codifica una proteina que forma parte del sitema ubiquitina-proteosoma
PARK-6 (1p35-36)	PINK1 AR	35-45	Parkinsonismo de inicio precoz. Lentamente progresivo. Buena respuesta a la levodopa. Distonía en pie infrecuente	Desconocido	Varias mutaciones descritas Prevalencia del 1-2% de las EP de inicio precoz
PARK-7 (1p36)	DJ-1 AR	20-40	Parkinsonismo de inicio precoz. Lentamente progresivo. Buena respuesta a la levodopa. Distonía en pie	Desconocido	Pocas mutaciones descritas Infrecuente (<1%)
PARK-8 (12p11-q13)	LRRK2 AD	50-70	Muy similar a la EP clásica: Inicio tardío. Temblor de reposo frecuente. Parkinsonismo asimétrico. Excelente respuesta a la levodopa con desarrollo de las complicaciones motoras clásicas. En ocasiones demencia, oftalmoparesia supranuclear o signos de motoneurona inferior	Heterogéneo: ▪ Patología tipo Lewy ▪ Depósitos de proteína tau (ovillos neurofibrilares) ▪ Inclusiones ubiquitina positivas ▪ Degeneración nigral sin inclusiones específicas	Diversas mutaciones descritas: la más frecuente es la G2019S Mutaciones en este gen se encuentran en 5%-6% de los casos de EP familiares y en un 1-2% de los casos esporádicos

Locus Cr.	Gen Herencia	Edad Inicio	Fenotipo Clínico	Fenotipo Patológico	Observaciones
PARK-9 (1p36)	ATP13A2 AR	20-40	Síndrome de Kufor-Rakeb (en una familia Jordana): Parkinsonismo con espasticidad, demencia y parálisis supranuclear de la mirada. Inicio durante la segunda década de la vida Recientemente se han encontrado mutaciones en diversos casos con un fenotipo más similar a la EP, aunque con un inicio temprano	Desconocido	El fenotipo del Síndrome de Kufor-Rakeb es muy diferente a la EP, por lo que inicialmente se creyó que podría no ser adecuado incluirlo en esta clasificación
PARK-10 (1p32)	Desconocido AD?	Este locus se hallo mediante estudios de ligamiento de todo el genoma humano realizados en múltiples familias con EP. Se desconoce el posible gen asociado a este locus. No hay un fenotipo clínico ni una edad de inicio definida			
PARK-11 (2q34)	GIGYF2 (TNRC15) AD	45-65	Muy similar a la EP idiopática	Desconocido	Este locus se halló mediante estudios de ligamiento de todo el genoma humano realizados en pacientes con EP familiar Posteriormente un estudio ha identificado diversas mutaciones en este gen, que podrían explicar hasta un 5% de los casos de EP familiar
PARK-12 (Zq21)	Desconocido Ligado al Cr X	Este locus se halló mediante estudio de ligamiento de todo el genoma humano realizado en múltiples familias con EP. Se desconoce el posible gen asociado a este locus. No hay un fenotipo clínico ni una edad de inicio definida			
PARK-13 (Xp12)	HTRA2 AD?	50-75	Indistinguible de la EP esporádica	Desconocido	Tan solo una mutación descrita en 4 pacientes con una EP esporádica La mutación se encontró al secuenciar directamente el gen (sin estudios de ligamiento previos), pues se había visto que en ratones las mutaciones en este gen causaban neurodegeneración con un fenotipo parkinsoniano
PARK-14 (22q13.1)	PLA2G6 AR	<40	Parkinsonismo con distonia con buena respuesta a la levodopa	Patología tipo Lewy y tau difusa	Descrita en dos familias de origen pakistaní
PARK-15 (22q12-q13)	FBXO7 AR	<40	Síndrome parkinsoniano y parimidal	No descrito	Enfermedad rara descrita en familias iraníes. Posteriormente en familias europeas
Genes sin locus PARK asignado y cuya relación con la EP es controvertida					
(8p21)	Neurofilamento M	16	Parkinsonismo juvenil con demencia tardía	Desconocido	Tan solo 1 familia descrita
(5q23.1-q23.3)	Sinfilina-1	66-71	Indistinguible de la EP clásica	Desconocido	Se ha identificado una única mutación en 2 pacientes
(2q22-q23)	NR4A2/Nurr1	45-67	Indistinguible de la EP esporádica	Patología tipo Lewy	Descritas 3 mutaciones en 11 familias
(15q25)	POLG/DNA polimerasa γ	20-26	Parkinsonismo con distonía y neuropatía periférica axonal	Desconocido	Descrita una única mutación en una familia

EP: Enfermedad de Parkinson; Locus Cr: Locus cromosómico; AD: Autosómico dominante; AR: Autosómico recesivo

Tabla 2. Polimorfismos o variantes genéticas que se han asociado a la EP

Variante Genética / Gen	Riesgo para la EP
Evidencia Alta	
"*Dinucleotide repeat sequence*" (Rep 1) de la región promotora del gen de la sinucleína	Aumentado (odds ratio 1,5)
Haplotipo H1/H1 del gen tau (MAPT)	Aumentado (odds ratio 1,5)
Variante S18Y del gen UCHL1	Disminuido (odds ratio 0,7)
Variantes G2385R y R1628P en el gen LRRK2	Aumentado (odds ratio 2,2) (solo en poblaciones asiáticas)
Evidencia Incierta	
Mutaciones heterocigotas del gen de la glucocerebrosidasa	Aumentado
Incremento en el numero de repeticiones CAG de la ataxia espinocerbelosa-2	Aumentado
Polimorfismo Val66Met del gen BDNF	Aumentado
Polimorfismo Val158Met de gen de la COMT	Aumentado
Variante GSTP1 del glutation transferasa	Aumentado
Incremento de las repeticiones dinucleotidas GT en el intrón 2 del gen MAO-B	Aumentado
Polimorfismos Taq1A y Taq1B en el gen del receptor dopaminérgico D2	Aumentado
Polimorfismo 10398G del gen de la NADH deshidrogenasa 3	Disminuido

EP: Enfermedad de Parkinson [Huang et al., 2004; Tan et al, 2007; Charles et al., 2007; Gan-Or et al., 2008]

[Davidzon et al., 2006]. Sin embargo, a estos genes no se les ha asignado ningún locus PARK pues la patogenicidad de las mutaciones y su relación con la EP es aún controvertida (Tabla 1). Esto es debido a que en muchos de ellos, como el gen Nurr1 o sinfilina-1, la identificación del gen mutado no se realizó mediante estudios previos de ligamiento genético en familias con EP, si no secuenciando directamente en pacientes con EP genes que se creen que pueden ser importantes para el correcto funcionamiento o desarrollo de las neuronas dopaminérgicas de la sustancia negra, o bien por que son genes que interactúan con otros genes, como la sinucleína o la parkina, que están claramente relacionados con formas genéticas de parkinsonismo [Le et al., 2003; Marx et al., 2003]. En muchos de estos genes tampoco se ha demostrado que la mutación segregué con la enfermedad dentro de una misma familia, o bien los pacientes con la supuesta mutación presentan una enfermedad con un fenotipo clínico muy diferente a la EP clásica. Además, otros estudios no han confirmado la presencia de mutaciones en algunos de estos genes en pacientes con EP, o bien se ha observado que las mutaciones pueden ser en realidad variantes genéticas presentes en la población normal [Zimprich et al., 2003; Tan et al., 2004].

Muchas de estas críticas también son aplicables a algunos de los genes a los que se ha asignado un locus PARK como los genes HTRA2 (PARK-13), UCHL-1 (PARK-5) o FBXO7 (PARK-15). Por ejemplo, el gen UCHL1 (ubiquitin carboxil-terminal hidrolasa L-1) codifica una proteína que forma parte del sistema ubiquitina-proteosoma, como la parkina [Leroy et al., 1998]. En el año 1998 se identificó una mutación al secuenciar directamente este gen en 2 hermanos alemanes con una EP iniciada a los 50 años, pero estudios posteriores no ha sido capaces de identificar otras familias o pacientes con mutaciones en el gen UCHL-1 [Harhangi et al., 1999; Maraganore et al., 1999]. Así

pues, tan sólo 5 genes están claramente asociados a formas monogénicas o de herencia mendeliana de EP o parkinsonismo: los genes de la sinucleína (PARK-1), la parkina (PARK-2), el PINK1 (PARK-6), el DJ-1 (PARK-7) y el LRRK2 (PARK-8) [Gasser., 2007; Klein et al., 2007].

1.2 El gen LRRK2 (PARK-8) y la enfermedad de Parkinson

La mayoría de genes que causan una EP familiar con herencia mendeliana son una causa infrecuente de parkinsonismo. Además la mayor parte de ellos provocan habitualmente una enfermedad que puede presentar ciertas características clínicas que difieren de la EP idiopática, como por ejemplo un inicio precoz, antes de los 40-45 años, y una progresión más lenta, como ocurre con los genes de la parkina o PINK1, o bien causan una enfermedad más agresiva, como en muchos casos con mutaciones puntuales o multiplicaciones del gen de la sinucleína. Por otro lado, algunas de estas formas monogénicas de parkinsonismo presentan una neuropatología diferente a la EP clásica, sin la presencia de patología tipo Lewy (cuerpos y neuritas de Lewy), tal y como ocurre en la mayoría de casos con mutaciones en el gen de la parkina.

La presente tesis se centrará en el gen LRRK2 (leucine-rich repeat kinase 2, PARK-8), en particular en la frecuencia de las mutaciones en este gen entre los pacientes con EP y en sus aspectos clínicos y neuropatológicos. Las mutaciones en este gen son una causa relativamente frecuente de EP, y a diferencia de los otros genes asociados a formas familiares o mendelianas de parkinsonismo, causan una enfermedad con un fenotipo clínico que parece ser mucho más similar a la EP clásica con una edad de inició más tardía, alrededor de los 55-60 años [Zimprich et al., 2004 (a); Paisán-Ruiz et al., 2004].

El locus genético en el cromosoma 12 donde se halla el gen LRRK2 fue descrito en el año 2002 en una familia japonesa (*"Sagamihara kindred"*) con EP de inicio tardío y un patrón de herencia autosómico dominante [Hasegawa et al., 1997; Funayama et al., 2002]. En esta familia la enfermedad se iniciaba alrededor de los 50 años de edad y la respuesta a la levodopa era buena aunque con frecuencia aparecían complicaciones motoras secundarias a la levodopaterapia. Si bien el fenotipo clínico era muy parecido a la EP idiopática, el examen postmortem de algunos miembros de esta familia mostró pérdida neuronal en la sustancia negra pero sin la presencia de cuerpos de Lewy u otras inclusiones positivas para sinucleína [Hasegawa et al., 1997; Funayama et al., 2002]. Posteriormente la existencia de este locus cromosómico fue confirmado en varia familias occidentales [Zimprich et al., 2004 (b)], y a finales del año 2004 el gen LRRK2 fue identificado de forma simultánea por dos grupos que estaban estudiando diversas familias de origen europeo con EP [Zimprich et al., 2004 (a); Paisán-Ruiz et al., 2004]. Algunas de estas familias en las que se identificaron las mutaciones en el gen LRRK2 eran de origen vasco. Finalmente en el año 2005 se confirmó que la familia japonesa en la cual se había descrito inicialmente el locus cromosómico PARK-8 era portadora de una mutación en el gen LRRK2 [Funayama et al., 2005].

1.2.1 Estructura genómica y dominios funcionales del gen LRRK2

El gen LRRK2 es extenso. Consta de 144Kb y 52 exones, y codifica una larga proteína de 2527 aminoácidos llamada dardarina en referencia a la palabra vasca "*dardara*", que significa temblor [Zimprich et al., 2004 (a); Paisán-Ruiz et al., 2004]. La proteína LRRK2 o dardarina se clasifica por sus características estructurales dentro del grupo recién descrito de las proteínas ROCO, de la superfamilia Ras guanosina trifosfatasa (Ras/GTPasa) [Bosgraaf et al., 2003]. Las proteínas de la familia ROCO parecen estar involucradas en diversas funciones como la regulación de la polaridad celular, la quimiotaxis, la regulación del citoesqueleto o la muerte celular programada [Bosgraaf et al., 2003; Marin., 2006]. La proteína LRRK2 o dardarina presenta diversos dominios de función (Figura 1). Los más relevantes son un dominio rico en repeticiones de leucina ("*leucin rich repeat*" o LRR), un dominio ROC (Ras/GTPasa), un dominio COR, un dominio WD40 (con repeticiones beta-transducina), y otro dominio catalítico tiroxina quinasa del tipo MAPKKK ("*mitogen-activated protein kinase kinase kinase*").

Todos estos dominios tendrían diferentes funciones relacionadas con la unión a sustratos, la interacción proteína-proteína o la fosforilación de sustratos proteicos. En la proteína LRRK2 existen dos dominios catalíticos, uno GTPasa en el domino ROC y otro del tipo MAPKKK quinasa [Zimprich et al., 2004 (a)]. El dominio GTPasa parece que podría autorregular la función de la proteína LRRK2 mediante la fosforilación del dominio MAPKKK quinasa, el cual fosforilaría otras proteínas y representaría la vía de salida de la actividad de la proteína. El dominio quinasa del tipo MAPKKK sugiere que la proteína LRRK2 podría tener un importante papel en las vías de señalización intracelular. Sin embargo la función fisiológica exacta del gen LRRK2, así como los sustratos para su actividad quinasa MAPKKK, estan aún por esclarecer [Anand et al., 2009].

Los estudios que han analizado los niveles de RNA y proteína LRRK2 han mostrado que su expresión génica y proteica ocurre en diversas regiones cerebrales, incluyendo las neuronas de la sustancia negra, y también en tejidos periféricos, aunque con niveles de expresión más bajos que en cerebro [Zimprich et al., 2004 (a); Melrose et al., 2006; Simón-Sánchez et al., 2006 (a); Galter et al., 2006; Greggio et al., 2006; Biskup et al., 2006]. La proteína LRRK2 parece tener una localización en el citosol, quizás asociada a estructuras membranosas del aparato de Golgi, de lisosomas, de membranas plasmáticas y mitocondriales, e incluso de vesículas sinápticas [Biskup et al., 2006]. Existen evidencias que sugieren que la proteína LRRK2 podría regular la longitud y la ramificación de las neuritas [MacLeod et al., 2006].

1.2.2 Mutaciones en el gen LRRK2

Hasta la fecha se han descrito más de 50 mutaciones o variantes genéticas en el gen LRRK2, siendo todas ellas mutaciones puntuales [Klein et al., 2007]. Sin embargo, la patogenicidad de muchas de ellas es incierta, pudiendo representar polimorfismos o genotipos, que sin ser patogénicos, si podrían conferir un mayor riesgo a desarrollar una EP. Al menos unas 19 variantes genéticas

parecen ser patogénicas (Figura 1, Tabla 3), y cinco de ellas, la R1441C, la R1441G, la Y1699C, la G2019S y la I2020T, lo son claramente dado que: 1) segregan con la enfermedad en las diversas familias estudiadas; 2) están ausentes o son muy infrecuentes en la población control, lo que indicaría una penetrancia reducida de la mutación; y 3) afectan a aminoácidos que están altamente conservados en diferentes especies de animales vertebrados [Zimprich et al., 2004 (a); Paisán-Ruiz et al., 2004; Funayama et al., 2005].

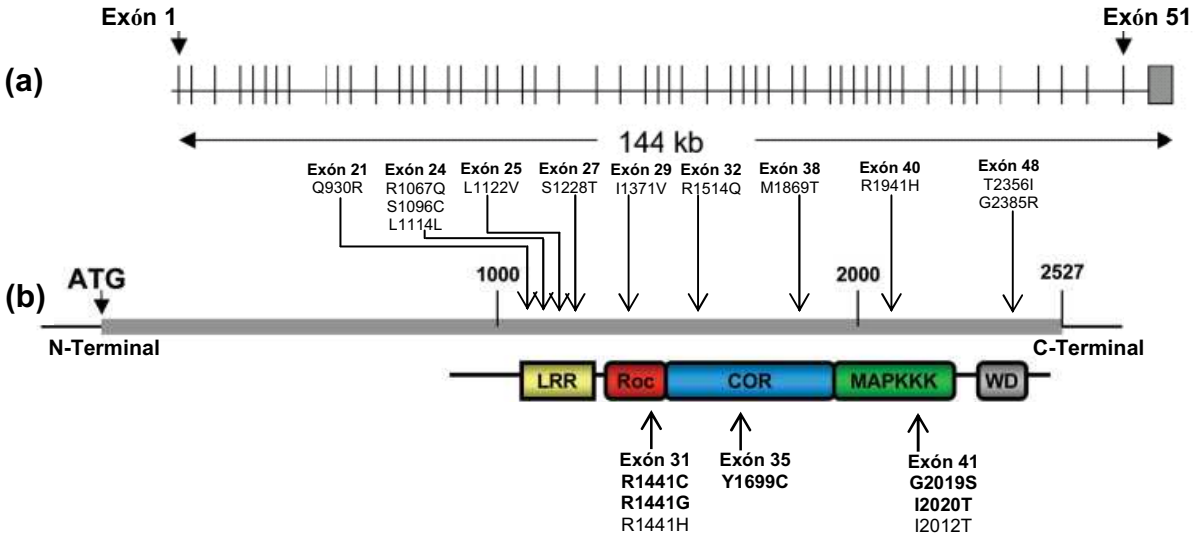

Figura 1. Estructura del gen LRRK2 (a) y dominios funcionales de la proteína dardarina (b). Mutaciones en el gen LRRK2: Localización exónica y en el dominio funcional (*en negrita las mutaciones que son claramente patogénicas*).

Las mutaciones en el gen LRRK2 actuarian mediante un mecanismo de ganancia de función, lo que concordaría con el patrón de herencia dominante. Sin embargo, el mecanismo a través del cual estas mutaciones en el gen LRRK2 acaban provocando la enfermedad es desconocido. En la actualidad se cree que las mutaciones en el gen LRRK2 podrían actuar incrementando la actividad fosforiladora de la proteína dardarina [West et al., 2005; Gloeckner et al., 2006; Greggio et al., 2006]. Es bien conocido que el acumulo de fosfoproteínas es un evento relevante en la fisiopatogenia de diversas enfermedades neurodegenerativas como la EP, la parálisis supranuclear progresiva (PSP) o la enfermedad de Alzheimer [Bueé et al., 2000; Fujiwara et al., 2002; Chen et al., 2005]. Pero que sustratos proteicos se hiperfosforilarían debido al incremento de la actividad quinasa de la proteína LRRK2, y como ello induciría neurodegeneración y muerte neuronal, es aún desconocido. Una posibilidad es que la proteína LRRK2 mutada hiperfosforilará mediante su dominio MAPKKK diversos sustratos como la sinucleína o la proteína tau, provocando su agregación y depósito neuronal y la consecuente muerte neuronal. También pueden existir otros posibles mecanismos a través de los cuales las mutaciones del gen LRRK2 podrían causar neurodegeneración y muerte neuronal. Por ejemplo, existen evidencias que indican que la proteína LRRK2 o dardarina podría regular la longitud

Tabla 3. Mutaciones descritas en el gen LRRK2

Cambio de Nucleótido	Exón	Sustitución de aminoácido	Aminoácido conservado entre las especies vertebradas	Dominio	Número de probandos (familias) descritas	Segregación familiar demostrada	Población identificada
2789A>G	21	Q930R	Si	LRR	1	NR	Europea
3200G>A	24	R1067Q	Si	LRR	1	NR	Asiática
3287C>G	24	S1096C	Solo en mamíferos	LRR	1	NR	Europea
3342A>G	24	L1114L	NA	"Splicing"	3	Si **	Europea
3364A>G	25	I1122V	Si	LRR	1	Si	Europea
3683G>C	27	S1228T	Si (excepto en pollo)	LRR	1	NR	Europea
4111A>G	29	I1371V	Si	ROC	1	NR	India
4321C>T	**31**	**R1441C**	**Si**	**ROC**	**Múltiples**	**Si**	Europea
4321C>G	**31**	**R1441G**	**Si**	**ROC**	**Múltiples**	**Si**	**Vasca**
4322G>A	31	R1441H	Si	ROC	2	NR	Europea, Asiática
4541G>A	32	R1514Q	Solo en mamíferos	COR	1	NR	Europea
5096A>G	**35**	**Y1699C**	**Si**	**COR**	**2**	**Si**	**Europea**
5606T>C	38	M1869T	Si	COR	2	NR	Europea
5822G>A	40	R1941H	Si	MAPKKK	1	NR	Europea
6035T>C	41	I2012T	Si	MAPKKK	1	Si	Asiática
6055G>A	**41**	**G2019S**	Si	**MAPKKK**	**Múltiples***	**Si**	**Europea, Norte-africana**
6059T>C	**41**	**I2020T**	**Si**	**MAPKKK**	**3**	**Si**	**Europea, Asiática**
7067C>T	48	T2356I	No	WD40	1	NR	Europea
7153G>A	48	G2385R	No	WD40	1	NR	Asiática

En negrita mutaciones que son claramente patogénicas

* Mutaciones detectadas también en controles, probablemente relacionado con la penetrancia reducida de la mutación.

LRR: Dominio "*leucin rich repeat*"; ROC: dominio ROC-Ras/GTPasa; MAPKKK: Dominio quinasa tipo MAPKKK ("*mitogen-activated protein kinase*")

** Mutaciones que segregán con la enfermedad en algunas familias, mientras en otras no.

NR: No reportado; NA: No aplicable.

Referencias: [Zimprich et al., 2004 (a); Paisán-Ruiz et al., 2004; Funayama et al., 2005; Kachergus et al., 2005; Aasly et al., 2005; Hernandez et al., 2005 (a); Berg et al., 2005; Khan et al., 2005; Mata et al., 2005 (a); Farrer et al., 2005; Zabetian et al., 2005; Lu et al., 2005; Skipper et al., 2005; Paisán-Ruiz et al., 2005 (a)]

y la ramificación de las neuritas, estando quizás esta función alterada en los casos con mutación [Biskup et al., 2006; McLeod et al., 2006].

La mayoría de mutaciones en el gen LRRK2 se localizan en los dos dominios catalíticos de la proteína: el MAPKKK y el ROC con actividad GTPasa (Figura 1 y Tabla 3). Como se ha comentado previamente, la actividad quinasa del dominio tipo MAPKKK es capaç de fosforilar diversos sustratos y su actividad estaría regulada por el dominio ROC GTPasa [Ito et al., 2007]. En el dominio quinasa MAPKKK se afectan especialmente los codones Glicina-2019 e Isoleucina-2020, que es donde se hallan las mutaciones G2019S y la I2020T, las cuales incrementarian de forma anormal la actividad quinasa de este dominio, y por lo tanto la fosforilación de diversos sustratos proteicos [Zimprich et al., 2004 (a); Kachergus et al., 2005; Toft et al., 2005 (a); Greggio et al 2006; West et al., 2005; Gloeckner et al., 2006; West et al., 2007]. En el dominio ROC con actividad GTPasa las mutaciones afectan principalmente al codón Arginina-1441. Este codón parece ser especialmente importante para el buen funcionamiento de la proteína LRRK2 y además es muy susceptible (es un "*hot spot*") a presentar mutaciones, pues se han encontrado 3 mutaciones en él, la R1441G, la R1441C y la R1441H [Zimprich et al., 2004 (a); Paisán-Ruiz et al., 2004; Zabetian et al., 2005]. Las mutaciones en este dominio ROC disminuirían la actividad quinasa de la GTPasa, lo que posiblemente modificaría la actividad quinasa del dominio MAPKKK [Lewis et al., 2007].

1.2.3 Frecuencia de las mutaciones en el gen LRRK2

En uno de los dos trabajos iniciales en los que se identificaron las mutaciones en el gen LRRK2 se analizaron 34 familias con EP y un patrón de herencia autosómico dominante. En cinco (14,7%) de ellas se identificaron mutaciones en este gen [Zimprich et al., 2004 (a)]. En el otro trabajo se observó que de 137 pacientes con EP estudiados, 30 de ellos con antecedentes familiares de la enfermedad y 107 esporádicos, 11 (8% de todos los casos: 6 (20%) casos familiares y 5 (3,6%) esporádicos) eran portadores de una mutación en el gen LRRK2 [Paisán-Ruiz et al., 2004]. Estos resultados ya sugerían que las mutaciones en el gen LRRK2 podían ser muy frecuentes en pacientes con EP, estando presentes entre el 10 y el 20% de las familias con EP autosómica dominante, así como en un porcentaje significativo de casos esporádicos de la enfermedad. Estudios posteriores han confirmado este relativa alta frecuencia de mutaciones en el gen LRRK2 en casos de EP familiar y esporádica [Berg et al., 2005; Gilks et al., 2005; Di Fonzo et al., 2006], si bien estudios que analicen de forma sistemática todo el gen LRRK2 en una larga cohorte de pacientes con EP aún no existen en la actualidad dada la dificultad que representa estudiar un gen de este tamaño, con 52 exones y 144kb de extensión.

De las cinco mutaciones en el gen LRRK2 que son claramente patogénicas, una de ellas, la mutación G2019S, es particularmente frecuente (Figura 2). Nichols y colaboradores analizarón la presencia de la mutación G2019S en 767 pacientes procedentes de 358 familias con EP de los Estados Unidos, e identificarón esta mutación en el 5% de los pacientes y en el 6% de las familias. Las características clínicas de todos estos pacientes portadores de la mutación eran las típicas de la

EP idiopática [Nichols et al., 2005]. En otro estudio realizado por Di Fonzo y colaboradores, se estudio la presencia de la mutación G2019S en 61 familias italianas, portuguesas y brasileñas con EP y patrón de herencia autosómico dominate, y en 4 (6,6%) de ellas se identificó la mutación [Di Fonzo et al., 2005]. Gilks y colaboradores analizarón la presencia de esta mutación en el gen LRRK2 en 482 pacientes ingleses con EP esporádica y encontrarón la mutación en 8 (1,6%) de ellos [Gilks et al., 2005]. Así pues, todos estos estudios señalan que la mutación G2019S es frecuente en diversas poblaciones occidentales, estando presente en el 5-6% de los casos con EP familiar y herencia autosómica dominante y en el 1-2 % de los casos esporádicos [Nichols et al., 2005; Di Fonzo et al., 2005; Gilks et al., 2005].

Más recientemente se ha observado que la mutación G2019S pueden ser especialmente frecuente en los pacientes con EP del Norte de África. Lesage y colaboradores estudiaron la presencia de la mutación G2019S en 198 pacientes con EP familiar autosómica dominante [Lesage et al., 2005 (a)]. Ciento setenta y cuatro de estos pacientes eran franceses mientras que 17 eran de origen norte-africano, concretamente de Marruecos, Túnez o Algeria. En el grupo de pacientes franceses encontraron 5 (2,9%) casos con la mutación, y sorprendentemente, en el grupo de pacientes del Norte de África, 7 de 17 (41%) eran portadores de la mutación. En diversos estudios se ha observado que pacientes con EP portadores de la mutación G2019S y pertenecientes a familias aparentemente no relacionadas y de países tan dispares como los Estados Unidos, Noruega, Irlanda, Polonia, o el Norte de África, comparten todos ellos un mismo haplotipo entorno al gen LRRK2 [Kachergus et al., 2005; Lesage et al., 2005 (b)]. Esta observación sugiere que las mutaciones en

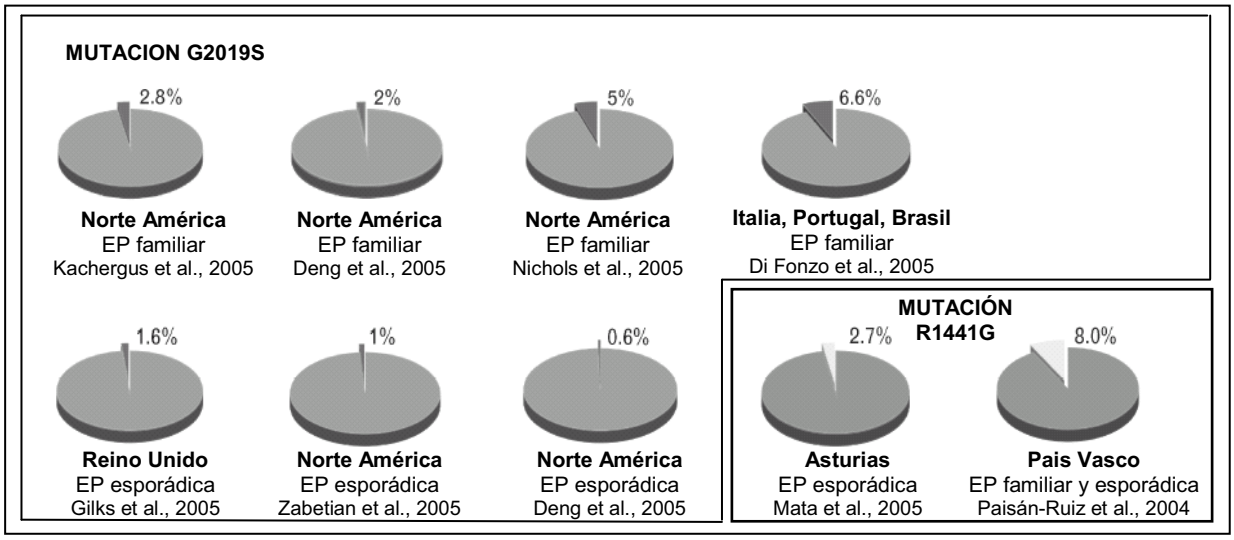

Figura 2. Frecuencia de las mutaciones G2019S y R1441G del gen LRRK2 en diversos estudios realizados en diferentes poblaciones.

estas diferentes familias se originaron a partir de un fundador común muchos años atrás, o lo que es lo mismo, que la mutación G2019S se originó en un sujeto y que a lo largo del tiempo, probablemente siglos, la mutación se ha ido difundiendo a través de diferentes poblaciones.

Otra mutación patogénica en el gen LRRK2 particularmente relevante es la mutación R1441G. Esta mutación ha sido tan solo descrita en pacientes con EP de España, y en concreto del País Vasco (Figura 2). En uno de los trabajos originales que describió las mutaciones en el gen LRRK2, se identifico la mutación R1441G en 4 familias con EP autosómica dominante que aparentemente no estaban relacionadas entre si [Paisán-Ruiz et al., 2004]. Posteriormente en este mismo estudio se analizó la presencia de esta mutación en 30 pacientes con EP familiar y 107 casos esporádicos, todos ellos de origen vasco. En 6 (20%) de los casos familiares y en 5 (4,6%) de los esporádicos se identificó la mutación R1441G, señalando que esta mutación es muy frecuente en pacientes con EP tanto familiar como esporádica del País Vasco y sugiriendo un efecto fundador en esta población. En otro estudio, se observó que 5 (2,7%) de 225 pacientes con EP esporádica e inicio tardío de Asturias eran portadores de la mutación R1441G [Mata et al., 2005 (b)]. Si bien estos pacientes asturianos no estaban relacionados entre si, todos ellos compartían el mismo haplotipo en el cromosoma 12q12 que los pacientes vascos con la mutación, sugiriendo un fundador común de origen Vasco y una posterior dispersión de la mutación a esta región vecina del norte de España [Paisán-Ruiz et al., 2004; Mata et al., 2005 (b)].

La presencia de casos de EP con la mutación G2019S o R1441G del gen LRRK2 sin antecedentes familiares de la enfermedad, junto con la presencia de un haplotipo ancestral común en los portadores de estas mutaciones, sugieren una penetrancia reducida de estas mutaciones en el gen LRRK2, que al menos en parte explicaría la presencia de estas mutaciones en casos esporádicos de EP [Kachergus et al., 2005; Gilks et al., 2005; Mata et al., 2005 (b)]. Kachergus y colaboradores estudiaron 42 sujetos pertenecientes a 13 familias diferentes con EP y la mutación G2019S del gen LRRK2 [Kachergus et al., 2005]. De estos 42 sujetos, 22 eran portadores de la mutación G2019S, y tan sólo 7 de ellos tenian un diagnóstico clínico de EP. En este estudio se observó que la penetrancia de la mutación G2019S era dependiente de la edad, pues se incrementaba del 17% a los 50 años al 85% a los 70 años de edad [Kachergus et al., 2005]. Aparte de la penetrancia reducida, otras posibles explicaciones sobre la presencia de mutaciones en el gen LRRK2 en casos aparentemente esporádicos de EP podrían ser que las mutaciones se hubieran generado de “novo”, la muerte de los padres o abuelos en una edad temprana antes de que hubierán podido desarrollar la enfermedad, la adopción o falsa paternidad, o bien la ausencia de un diagnóstico de EP en un familiar realmente afectado por la enfermedad.

1.2.4 Fenotipo clínico del parkinsonismo asociado a mutaciones en el gen LRRK2

Las características clínicas del parkinsonismo asociado a mutaciones en el gen LRRK2 parecen ser indistinguibles de la EP clásica, tanto por la edad de inicio que habitualmente es tardía, a partir de los 50 años de edad, como por la presencia de los signos parkinsonianos cardinales (temblor en reposo, bradicinesia y rigidez), que frecuentemente son asimétricos y responden bien al tratamiento con levodopa [Zimprich et al., 2004 (a); Paisán-Ruiz et al., 2004]. La edad de inicio media es entre los 55 y 65 años, sin bien es variable, con algunos casos que se inician tempranamente, antes los 45 años de edad, y otros más tardíamente, a partir de los 70 u 80 años [Hasegawa et al.,

1997; Paisán-Ruiz et al., 2005 (a); Di Fonzo et al., 2005; Nichols 2005 et al., Gilks et al., 2005; Aasly et al., 2005; Hernandez et al., 2005 (a)].

En la familia japonesa (*"Sagamihara kindred"*) en la que inicialmente se identificó el locus PARK-8 en el cromosoma 12, y que es portadora de la mutación I2020T, los sujetos afectos presentaban un parkinsonismo con buena respuesta a la levodopa y sin la presencia de signos atípicos para la EP clásica [Hasegawa et al., 1997; Funayama et al., 2002; Funayama et al., 2005]. En esta familia, con 10 sujetos con una EP confirmada y 26 miembros que posiblemente padecieron esta enfermedad a lo largo de 5 generaciones, la edad de inicio media era de 54 años, si bien algunos casos iniciaron la enfermedad más tempranamente, a los 39 años de edad, y otros más tardíamente, a los 74 años. Los síntomas motores parkinsonianos en esta familia eran asimétricos, y tal y como ocurre en la EP idiopática, las respuesta a la levodopa era excelente si bien desarrollaban las complicaciones motoras secundarias a levodopa (fluctuaciones y discinesias) tras 6-8 años de evolución de la enfermedad [Hasegawa et al., 1997].

En los pacientes vascos con la mutación R1441G descritos por Paisán-Ruiz y colaboradores, la edad de inicio era entre los 50 y 70 años, siendo el síntoma inicial más frecuente el temblor de reposo asimétrico [Paisán-Ruiz et al., 2004; Paisán-Ruiz et al., 2005 (b)]. El curso de la enfermedad era lentamente progresivo, siendo el temblor el síntoma predominante. La respuesta al tratamiento dopaminérgico era excelente, si bien muchos de los pacientes al cabo de los años presentaban fluctuaciones motoras y discinesias. La aparición de demencia era rara, aún después de una duración de la enfermedad de más de 15 años en algunos casos.

En la familia D, descrita por Wszolek y colaboradores y portadora de la mutación R1441C, localizada en el mismo codón que la mutación vasca, el temblor de reposo no era muy prominente. El cuadro clínico de los miembros afectos de esta familia era sin embargo indistinguible de la EP clásica, excepto en un caso que presentaba una paresia supranuclear de la mirada [Wszolek et al., 1995; Wszolek et al., 2004; Zimprich et al., 2004 (a)]. En otra familia descrita por estos mismos autores, la familia A, portadora de la mutación Y1699C en el gen LRRK2, de los 8 miembros afectos por la enfermedad, dos presentaban amiotrofia mientras otros dos habían desarrollado una demencia [Wszolek et al., 1997; Zimprich et al., 2004 (a)].

Los pacientes reportados con la mutación G2019S en el gen LRRK2, la mayoría presentan un parkinsonismo similar a la EP esporádica [Di Fonzo et al., 2005; Nichols 2005 et al., Gilks et al., 2005; Aasly et al., 2005; Hernandez et al., 2005 (a); Deng et al., 2005]. La edad de inicio es habitualmente entre los 50 y los 70 años, y la mayoría de pacientes presentan un inicio asimétrico con temblor de reposo, bradicinesia o rigidez, aislados o en combinación. Ocasionalmente algunos pacientes presentan distonía focal del pie como síntoma inicial [Gilks et al., 2005; Aasly et al., 2005]. La respuesta a la levodopa es buena, desarrollando fluctuaciones motores y discinesias tras varios años de tratamiento, tal y como ocurre en la EP clásica. Algún estudio ha sugerido que los pacientes con la mutación G2019S podrían tener una enfermedad menos agresiva, con una progresión más lenta,

comparado con otros pacientes con EP familiar [Nichols et al., 2005]. Otros trabajos sugieren que en estos pacientes con la mutación G2019S, la demencia podría ser una complicación infrecuente, incluso en casos con una duración de la enfermedad de más de 30 años [Di Fonzo et al., 2005]. Estos datos sugieren la posibilidad que los pacientes con la mutación G2019S del gen LRRK2 pueden presentar una enfermedad más benigna que los pacientes con EP sin esta mutación.

Así pues, el parkinsonismo asociado a mutaciones en el gen LRRK2 parece ser indistinguible de la EP idiopática desde un punto de vista clínico, si bien algunos casos pueden presentar signos atípicos para la EP clásica como paresia supranuclear de la mirada o signos de motoneurona inferior [Zimprich et al., 2004 (a); Paisán-Ruiz et al., 2004]. Esta similitud clínica es congruente con el hecho que en pacientes con parkinsonismo y mutaciones en el gen LRRK2, el patrón de disfunción dopaminérgica nigroestriatal, estudiado mediante tomografía por emisión de positrones (PET) con ^{18}Fluoro-dopa [Hernandez et al., 2005 (a); Paisán-Ruiz et al., 2005 (b); Adams et al., 2005; Khan et al., 2005], o bien con tomografía computadorizada por emisión de fotón único (SPECT) del transportador de la dopamina (DAT) [Isaias et al., 2006], muestra un gradiente caudado-putaminal y una asimetría similar al observado en la EP esporádica.

1.2.5 Neuropatología del parkinsonismo asociado a mutaciones en el gen LRRK2

Aunque la similitud del fenotipo clínico del parkinsonismo asociado a mutaciones en el gen LRRK2 con la EP idiopática podría sugerir también un fenotipo anatomopatológico idéntico, en los pocos casos de paciente con parkinsonismo y mutaciones en este gen en los que se ha podido realizar el estudio postmortem, los hallazgos neuropatológicos han sido sorprendentemente heterogéneos [Zimprich et al., 2004(a); Wszolek et al., 2004; Hasegawa et al., 1997; Funayama et al., 2005; Gilks et al., 2005; Ross et al., 2006 (a); Giasson et al., 2006; Rajput et al., 2006]. Hasta la fecha, se ha descrito la anatomía patológica subadyacente de 4 mutaciones en el gen LRRK2 (I2020T, R1441C, Y1699C y G2019S) en poco más de 20 casos (Tabla 4). La neuropatología del parkinsonismo asociado a la mutación de origen vasco R1441G es desconocida, no habiéndose descrito ningún caso hasta finales del año 2006.

La pérdida de neuronas pigmentadas y la gliosis en la sustancia negra es un hallazgo histopatológico universal en todos los casos con parkinsonismo y mutaciones en el gen LRRK2 examinados hasta el momento. Algunos casos presentan patología tipo Lewy, con la presencia de cuerpos y neuritas de Lewy positivas para sinucleína, tal y como ocurre en la EP clásica. Otros casos presentan depósitos de proteína tau en forma de ovillos neurofibrilares, muy parecidos a los observados en la PSP, o bien inclusiones intracitoplasmáticas o intranucleares positivas para ubiquitina que recuerdan a las observadas en la degeneración lobar frontotemporal con inclusiones de ubiquitina (DLFT-U). Otros casos presentan degeneración nigral sin ningún depósito proteico o hallazgo anatomopatológico distintivo [Hasegawa et al., 1997; Wszolek et al., 2004; Zimprich et al., 2004 (a); Funayama et al., 2005], tal y como sucede en la mayor parte de casos de parkinsonismo asociado a mutaciones en el gen de la parkina [Mori et al., 1998].

El estudio postmortem de 4 miembros con EP pertenecientes a la familia japonesa en la que inicialmente se identificó el locus PARK-8 en el cromosoma 12 (*"Sagamihara kindred"*, mutación I2020T), mostró en todos ellos pérdida neuronal leve-moderada en la sustancia negra y muy leve en el locus ceruleus sin la presencia de cuerpos de Lewy ni otras inclusiones histológicas distintivas [Hasegawa et al.,1997; Funayama et al., 2005]. En la familia D (mutación R1441C) descrita por

Tabla 4. Hallazgos neuropatológicos en casos con parkinsonismo y mutaciones en el gen LRRK2 reportados hasta finales del año 2006

Mutación en el gen LRRK2	Número de casos reportados [Referencias]	Neuropatología			
		Patología tipo LB	Taupatía (PSP "*like*")	DLFT-U	Degeneración nigral inespecífica
G2019S	15 [Gilks et al., 2006 ; Giasson et al., 2006 ; Ross et al., 2006 (a) ; Rajput et al., 2006]	13	1	-	1
I2020T	4 [Hasegawa et al.,1997 ; Funayama et al., 2002]	-	-	-	4
R1441C	4 [Wszolek et al., 2004]	2	1	1	
R1441G	-	-	-	-	-
Y1699C	3 [Wszolek et al., 2004 ; Khan et al., 2005]	1	-	2	-

LB: Lewy bodies; PSP: Parálisis supranuclear progresiva; DLFT-U: Degeneración lobar frontotemporal con inclusiones ubiquitina positivas

Wszolek, el examen neuropatológico se realizó en 4 miembros, y los hallazgos fueron heterogéneos [Wszolek et al., 2004; Zimprich et al., 2004 (a)]. Dos casos presentaban patología tipo Lewy: en un caso los cuerpos y neuritas de Lewy estaban limitados a los núcleos del tronco cerebral, mientras que en el otro caso la patología tipo Lewy afectaba tanto a tronco como a estructuras límbicas y corticales. Otro miembro de esta familia presentaba inclusiones de proteína tau en forma de ovillos neurofibrilares como los observados en la PSP, aunque con una distribución en las áreas cerebrales diferente a la observada en esta taupatía. Este paciente presentó en vida un parkinsonismo con paresia supranuclear de la mirada. El último caso examinado en la familia D no mostró patología tipo Lewy ni ovillos neurofibrilares, sino que evidenció la presencia de inclusiones intracitoplasmáticas e intranucleares inmunoreactivas para ubiquitina. La inclusiones intranucleares ubiquitina positivas en este caso recordaban a los cuerpos de Marinesco [Wszolek et al., 2004; Zimprich et al., 2004 (a)].

Inclusiones similares, ubiquitina positivas, también se han observado en 2 pacientes portadores de la mutación Y1699C y pertenecientes a la familia A [Wszolek et al., 2004; Zimprich et al., 2004 (a)]. Uno de ellos mostraba una enfermedad de Alzheimer concomitante mientras que el otro caso, que en vida presentó signos de enfermedad de motoneurona, el examen postmortem identificó

una leve pérdida neuronal con gliosis y esferoides axonales en el asta anterior de la medula espinal. En otro paciente con la mutación Y1699C pero que no pertenecía a la familia A, el estudio anatomopatológico evidenció la presencia de cuerpos y neuritas de Lewy en la sustancia negra y en otros núcleos del tronco cerebral, y en menor grado en áreas corticales [Khan et al., 2005].

En pocos pacientes con la mutación G2019S se ha podido estudiar la histología subadyacente. Gilks y colaboradores examinaron 3 pacientes con esta mutación, y los 3 mostraron pérdida neuronal en la sustancia negra con patología tipo Lewy típica [Gilks et al., 2005]. En dos de ellos los cuerpos de Lewy se extendían a áreas límbicas. Ross y colaboradores estudiaron la presencia de la mutación G2019S del gen LRRK2 en 405 pacientes con enfermedad por cuerpos de Lewy, e identificaron 8 pacientes con la mutación [Ross et al., 2006 (a)]. Todos ellos habían sido diagnosticados en vida de EP. Giasson y colaboradores estudiaron 80 pacientes con un diagnóstico clínico-patológico de EP o demencia por cuerpos de Lewy (DCL) e identificaron 3 pacientes con la mutación G2019S [Giasson et al., 2006]. Dos de ellos presentaban patología tipo Lewy en la sustancia negra con extensión límbica y cortical, mientras que el tercer caso mostró marcada pérdida neuronal en sustancia negra y locus ceruleus pero sin la presencia de cuerpos de Lewy ni otras inclusiones distintivas. Los tres casos presentaron en vida un cuadro clínico compatible con una EP. Finalmente, Rajput y colaboradores han descrito un caso con parkinsonismo sin buena respuesta a la levodopa y portador de la mutación G2019S en el que el examen neuropatológico mostró una taupatía similar a la PSP, si bien la baja densidad de ovillos neurofibrillares en algunas áreas cerebrales y la ausencia de pérdida neuronal en el globus palidus y el núcleo subtalámico no permitían realizar el diagnóstico anatomopatológico de PSP [Rajput et al., 2006; Litvan et al., 1996 (b)].

Por lo tanto, la neuropatología del parkinsonismo asociado a mutaciones en el gen LRRK2 es heterogénea, incluso en casos con la misma mutación y pertenecientes a una misma familia. Esta neuropatología diversa y pleomórfica, que incluye los diversos fenotipos anatomopatológicos que se asocian a los parkinsonismos degenerativos, con casos que presentan patología tipo Lewy (sinucleinopatías), otros con depósitos de proteína tau (taupatías), o bien casos con inclusiones intracelulares ubiquitina positivas, o incluso casos de degeneración nigral sin inclusiones distintivas, sugieren que el gen LRRK2 podría tener un papel relevante en la fisiopatología de la EP y también en la de otros parkinsonismos degenerativos. Incluso algunos autores han sugerido que el gen LRRK2 podría ser la piedra "*roseta*" que permitiría comprender mejor los mecanismos etiopatogénicos implicados en este conjunto de enfermedades, pues el gen LRRK2 podría estar en la parte superior o inicial ("*upstream*") de una hipotética vía molecular o celular que podría ser común a los diversos parkinsonismos degenerativos [Taylor et al., 2006; Klein et al., 2007].

Un tema controvertido es la posibilidad que la proteína LRRK2 o dardarina sea un constituyente de los cuerpos de Lewy, tal y como sucede con la sinucleína, o que existan inclusiones positivas para LRRK2 en determinados casos con la mutación. Algunos autores han señalado que entre un 10 y un 15% de los cuerpos de Lewy de pacientes con una EP idiopática tiñen en la inmunohistoquímica con determinados anticuerpos para LRRK2 [Greggio et al. 2006; Zhu et al.,

2006(a); Zhu et al., 2006 (b)], mientras que otros grupos no han sido capaces de replicar estos resultados [Covy et al., 2006]. Por otro lado, en el caso con la mutación G2019S y pérdida neuronal en la sustancia negra sin cuerpos de Lewy ni otras inclusiones distintivas descrito por Giasson y colaboradores, se observó la presencia de neuritas distróficas en las neuronas de la sustancia negra al realizar una inmunohistoquímica con un anticuerpo anti-LRRK2 [Giasson et al., 2006].

Determinar en pacientes con EP de nuestro medio la frecuencia de las mutaciones en el gen LRRK2, en particular las mutaciones G2019S, la cuál es relativamente frecuente pacientes con EP de otras poblaciones occidentales, y la R1441G, muy frecuente en pacientes con EP del país vasco, así como definir mejor cuales son las características clínicas de los pacientes portadores de estas mutaciones, es especialmente relevante y pueden tener importantes repercusiones en la práctica clínica del futuro. Además, un mejor conocimiento del sustrato neuropatológico del parkinsonismo asociado a mutaciones en el gen LRRK2 puede ser de utilidad para conocer mejor cuales son los mecanismos fisiopatogénicos subadyacentes a esta forma genética de parkinsonismo, que a la vez podrían estar también implicados en las formas idiopáticas de EP y en la patogenia de otros parkinsonismos degenerativos.

2. HIPÓTESIS

Hipótesis de trabajo

1) En Catalunya las mutaciones G2019S y R1441G del gen LRRK2 son causa de parkinsonismo tanto familiar como esporádico.

2) Las manifestaciones clínicas motoras y no motoras del parkinsonismo asociado a mutaciones en el gen LRRK2 son heterogéneas.

3) El sustrato neuropatológico del parkinsonismo asociado a mutaciones en el gen LRRK2 es heterogéneo y determina el fenotipo clínico.

3. OBJETIVOS

Objetivos concretos

En relación a las hipótesis planteadas, los objetivos de este trabajo son:

1) Determinar la frecuencia de las mutaciones G2019S y R1441G del gen LRRK2 en pacientes con diagnóstico clínico de enfermedad de Parkinson en un hospital terciario de Barcelona.

2) Definir el fenotipo clínico de los pacientes con enfermedad de Parkinson portadores de una mutación en el gen LRRK2.

3) Determinar la presencia de las mutaciones G2019S y R1441G del gen LRRK2 en cerebros del Banco de tejidos Neurológicos del Hospital Clínic - Universitat de Barcelona diagnosticados de enfermedad de Parkinson, de parkinsonimo degenerativo, o de degeneración lobar frontotemporal, y estudiar las características clínicas y patológicas de los casos portadores de una mutación en este gen.

4. MÉTODOS

MÉTODOS

1) PACIENTES

1.A Estudio de mutaciones en el gen LRRK2 en pacientes con diagnóstico clínico de enfermedad de Parkinson

Han sido objeto de estudio 302 pacientes con un diagnostico clínico de EP probable o definida según los criterios de la United Kingdom PD Society Brain Bank [Hughes et al., 1992]. Estos pacientes se visitan en consultas externas de la Unidad de Parkinson del Servicio de Neurología del Hospital Clínic de Barcelona y se incluyeron en el estudio después de firmar un consentimiento informado. Se incluyeron de forma no consecutiva, tanto casos con antecedentes familiares de EP como casos esporádicos. Entre estos 302 pacientes no existía ningún vínculo familiar conocido. De cada paciente se recogieron datos clínicos básicos, así como los antecedentes familiares de EP o temblor. La presencia de una historia familiar positiva para EP se definió mediante el algoritmo diagnóstico propuesto por Marder y colaboradores [Marder et al., 2003], el cuál en base a una entrevista estructurada a los pacientes permite establecer los diferentes niveles de probabilidad o certeza sobre la presencia de una EP en los familiares del paciente que no pueden ser examinados. De cada paciente incluido en el estudio se obtuvo una muestra de sangre (20 ml. aproximadamente) para el análisis de mutaciones en el gen LRRK2.

1.B Estudio de mutaciones en el gen LRRK2 en tejido cerebral

Se han incluido en el estudio 110 cerebros con un diagnóstico patológico de enfermedad neurodegenerativa procedentes del Banco de Tejidos Neurológicos del Hospital Clínic - Universitat de Barcelona. Estos 110 casos incluían 33 casos de EP con cuerpos de Lewy en el examen neuropatológico, 25 casos de demencia por cuerpos de Lewy diagnosticada clínicamente i confirmada por la presencia de cuerpos de Lewy difusos en el examen neuropatológico, 35 pacientes con otros parkinsonismos degenerativos (8 con atrofia multisistémica (AMS), 21 con parálisis supranuclear progresiva (PSP), 3 con degeneración corticobasal (DCB) y 3 con parkinsonismo con degeneración nigral inespecífica), así como 17 casos con un diagnostico patológico de degeneración lobar frontotemporal (DLFT) (3 con enfermedad de Pick, 2 con una taupatía multisistémica, 9 con DLFT con inclusiones ubiquitina positivas (DLFT-U), y 3 con DLFT sin histología distintiva). El diagnostico clínico de estos casos se llevó a cabo por los médicos que atendían al paciente en vida, o en algunos casos, en base a los datos de la historia clínica y siempre utilizando los criterios de diagnóstico habituales [Hughes et al., 1992; Emre et al., 2007; McKeith et al., 2005; Gilman et al., 1999; Litvan et al., 1996 (a); Riley et al., 2000; McKhann et al., 2001]. El diagnostico patológico se realizó en el Banco de tejidos Neurológicos del Hospital Clínic - Universitat de Barcelona aplicando los criterios neuropatológicos establecidos [Hughes et al., 1992; McKeith et al., 1996; McKeith et al., 2005; Litvan et al., 1996 (b); Dickson et al., 2002; Gilman et al., 1999; McKhann et al., 2001; Shi et al., 2005] y siguiendo un protocolo estandarizado, utilizando secciones de diversas regiones cerebrales que fueron fijadas en formol y posteriormente parafinadas para ser procesadas y estudiadas con tinciones de hematoxilina-eosina y de Klüver Barrera (“luxol fast blue”), así como con inmunohistoquímica siguiendo el método de la avidina-biotina-peroxidada con anticuerpos específicos para amiloide BA4, tau fosforilada, alfa-sinucleína, ubiquitina y neurofilamentos. En cada caso incluido en el estudio se obtuvieron 25 mg de tejido cerebral (córtex frontal) para el análisis de las mutaciones en el gen LRRK2.

2) ANÁLISIS GENÉTICO: DETECCIÓN DE LAS MUTACIONES G2019S Y DEL CODON 1441 DEL GEN LRRK2

El DNA sanguíneo se aisló mediante el kit "DNA blood Mini Kit (250)" (Qiagen). El DNA procedente de tejido cerebral se extrajo usando el kit "DNeasy Tissue Mini Kit" (Qiagen).

La detección de las mutaciones del gen LRRK2 G2019S y las del codón 1441, incluyendo la R1441G, R1441C y R1441H, se realizó mediante amplificación y posterior digestión con enzimas de restricción (PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism). Para la detección de la mutación G2019S se utilizó la enzima de restricción SfcI, mientras que para la mutación R1441G (y otras mutaciones en este codón), se usó la enzima BstUI. Tras la digestión se llevó a cabo una electroforesis en un gel de acrilamida que fue teñido con bromuro de etidio. Las muestras con un patrón de electroforesis anormal fueron secuenciadas para confirmar la presencia de la mutación e identificar el cambio exacto de nucleótido.

El DNA genómico se amplificó mediante la técnica de reacción en cadena de la polimerasa (PCR) con el termociclador PTC-100 (MJ Research). Se utilizaron los "primers" previamente descritos [Paisán-Ruiz et al., 2004; Gilks et al., 2005] y manufacturados por la empresa Operon (Qiagen). La PCR se llevó a cabo en un volumen de 25 µL con 2,5 µL de "polymerase chain reaction buffer", 1,5mM de clorídeo de magnesio, 1,25mM de cada deoxinucleótido trifosfato (dNTP), 0,4µM de "Forward-primer", 0,4µM de "Reverse-primer", 2,5U de Taq DNA polimerasa y 50 ng de DNA genómico con las siguientes condiciones de tiempo y temperatura en cada paso del ciclo de la PCR: 5 minutos a 94℃, 30 ciclos de 30 segundos de desnaturalización a 94℃, 30 segundos de "annealing" a 58℃ y 90 segundos de extensión a 72° C con una extensión final de 5 minutos a 72℃. La secuenciación se realizó usando el Kit "Big Dye Terminator Cycle Sequencing Ready Reaction Kit" (Perkin Elmer) en el "ABI-prism automatic DNA sequencer" (Perkin Elmer).

3) CARACTERIZACIÓN CLÍNICA Y NEUROPATOLÓGICA DE LOS CASOS IDENTIFICADOS CON MUTACIÓN EN EL GEN LRRK2

Se obtuvo información sobre la edad de inicio, síntoma inicial, historia familiar (antecedentes de EP, temblor y origen geográfico de la familia), síntomas motores (características, distribución, gravedad), respuesta al tratamiento antiparkinsoniano, complicaciones motoras secundarias a la levodopaterapia (fluctuaciones motoras y discinesias) y síntomas no motores (depresión, ansiedad, alucinaciones, demencia) a lo largo de la enfermedad. En aquellos casos en que fue posible se contactó con los familiares del probando, estuvieran o no afectos de parkinsonismo. Tras ser informados y firmar un consentimiento informado, se procedió a una exploración neurológica y obtención de una muestra de sangre para el análisis genético.

En aquellos cerebros en los que se detectó una mutación en el gen LRRK2 se procedió a una revisión detallada de la historia clínica y las características neuropatológicas.

5. RESULTADOS

ARTÍCULO 1

Gaig C, Ezquerra M, Marti MJ, Muñoz E, Valldeoriola F, Tolosa E.

LRRK2 mutations in Spanish patients with Parkinson disease: frequency, clinical features, and incomplete penetrance.

Arch Neurol. 2006 Mar;63(3):377-82.

ORIGINAL CONTRIBUTION

LRRK2 Mutations in Spanish Patients With Parkinson Disease

Frequency, Clinical Features, and Incomplete Penetrance

Carles Gaig, MD; Mario Ezquerra, PhD; Maria Jose Marti, MD, PhD; Esteban Muñoz, MD, PhD; Francesc Valldeoriola, MD, PhD; Eduardo Tolosa, MD, FRCP

Background: Several pathogenic mutations in the *LRRK2* gene have been implicated in familial and sporadic cases of Parkinson disease (PD). The *R1441G* mutation is frequent in Spanish patients of Basque ethnicity with PD, and the *G2019S* mutation is a common mutation found in several populations worldwide.

Objectives: To determine the frequency of the *LRRK2 G2019S* and *R1441G* mutations in PD patients from the non-Basque northeast region of Spain (Catalonia), and to characterize their family history and clinical features.

Design: We screened patients for the presence of the *LRRK2 R1441G* and *G2019S* mutations. These *LRRK2* mutations were detected by restriction endonuclease digestion, and samples with an abnormal electrophoresis pattern were sequenced to identify the exact nucleotide change. The clinical features and family history of patients with *LRRK2* mutations were studied in detail.

Setting: The northeast region of Spain.

Patients: Three hundred two patients with PD.

Main Outcome Measures: Onset age, clinical features, and family history of PD and *LRRK2* mutations.

Results: The *R1441G* mutation was present in 0.7% of total PD cases. The *G2019S* mutation was found in 6.4% of familial and 3.4% of sporadic cases. Additionally, we found 1 patient with the *R1441C* mutation. Age at onset ranged from 33 to 78 years. Clinical features were not different from classic PD, except for 1 patient who presented with monosymptomatic leg rest tremor of 8 years' duration. In addition, a 91-year-old unaffected relative of a patient with the *G2019S* mutation was found to be a mutation carrier.

Conclusions: The *G2019S* mutation frequency in PD patients from northeast Spain is similar to that reported in other European regions. The *R1441G* mutation is very uncommon in Catalonia. The presence of an aged unaffected *G2019S* mutation carrier supports the previously described occurrence of incomplete penetrance in PD patients with *LRRK2* mutations.

Arch Neurol. 2006;63:377-382

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RECENTLY, PATHOGENIC MUTATIONS in the leucine-rich repeat kinase 2 (*LRRK2*) gene have been identified in patients with autosomal dominant forms of Parkinson disease (PD).[1,2] The *LRRK2* gene has 51 exons, encodes a large protein of 2527 amino acids (dardarin), and has an unknown function, although there are 5 predicted functional domains: a leucine-rich repeat domain, a Roc (Ras guanosine triphosphatase) domain, a C-terminal of Roc domain, a WD40 domain (β-transducin repeats), and a tyrosine kinase catalytic domain.[1,2] The clinical features of parkinsonism and the response to levodopa treatment in patients with *LRRK2* mutations seem to be indistinguishable from those of classic PD.[2-4] Features like dementia or atypical signs such as amyotrophy or supranuclear gaze palsy are rarely observed.[1,2,3,6]

Until now, several different missense mutations have been reported in the *LRRK2* gene.[1,2,4,5,7] One of them, the *G2019S* mutation, has been found in a significant proportion of patients with PD; it accounts for about 5% to 6% of familial and 1% to 2% of apparently sporadic cases.[8-10] The *R1441G* mutation has been described only in populations located in the Basque country and its neighboring region of northern Spain, Asturias. This mutation has been found in 8% of Basque PD patients[1] and in 2.7% of patients with late-onset PD in Asturias.[11] Two more mutations, located in position 1441 within the Roc domain, have been described in other populations: the *R1441C* and *R1441H* mutations, emphasizing the importance of this residue for the *LRRK2* pathogenic mechanism.[2,7]

The aims of this study were to determine the frequency of the *LRRK2 G2019S*

Table 1. Patients With PD With or Without *LRRK2* Mutations*

	LRRK2 Wild Type, No.	*LRRK2* Mutation			
		G2019S	*R1441G*	*R1441C*	Total†
Patients with PD (n = 302)	286	13 (4.3)	2 (0.7)	1 (0.3)	16 (5.3)
Familial PD (n = 94)	85	6 (6.4)	2 (2.1)	1 (1.1)	9 (9.6)
Onset ≥40 y (n = 77)	69	5 (6.5)	2 (2.6)	1 (1.3)	8 (10.4)
Onset <40 y (n = 17)	16	1 (5.9)	0	0	1 (5.9)
Sporadic PD (n = 208)	201	7 (3.4)	0	0	7 (3.4)
Onset ≥40 y (n = 177)	171	6 (3.4)	0	0	6 (3.4)
Onset <40 y (n = 31)	30	1 (3.2)	0	0	1 (3.2)

Abbreviation: PD, Parkinson disease.
*Data are presented as number (percentage) unless otherwise indicated.
†Combination of the 3 mutations.

and *R1441G* mutations in PD patients included in our PD DNA bank, composed of patients from the non-Basque northeast region of Spain (Catalonia), and to characterize in detail their family history and clinical features.

METHODS

PATIENTS

The *LRRK2* mutations were screened in 302 nonconsecutive and unrelated PD patients (mean ± SD age at onset, 53.8±13.3 years; range, 8-85 years; 170 [56.3%] males; 132 [43.7%] females). All subjects in this study resided in Catalonia and were recruited in the Neurology Service of the Hospital Clínic of Barcelona from January 1, 1997, to June 30, 2005. They all fulfilled commonly accepted diagnostic clinical criteria for PD.[12,13]

Patients included were observed longitudinally in our Movement Disorders Unit. Their family history of PD was periodically evaluated, and when positive, the conservative criteria of Marder et al[14] were used to assess its degree of certainty. Among the 302 PD patients, 94 had a family history (31.1%; mean ± SD age at onset, 53.1±14.0; range, 15-84 years), whereas 208 cases were sporadic (68.9%; mean age at onset, 54.2±13.0; range, 8-85 years). Written informed consent was obtained from all participants, and the local ethics authorities approved the project. The clinical data of patients with *LRRK2* mutations were reviewed. In some cases, it was possible to obtain blood samples from affected and unaffected relatives.

GENETIC ANALYSIS

The DNA from peripheral blood was isolated by using standard methods in all subjects. The *LRRK2 G2019S* mutation was detected by restriction endonuclease digestion with the *SfcI* enzyme as previously described,[8] running in an acrylamide gel electrophoresis and subsequently stained with ethidium bromide. The *R1441G*, *R1441C*, and *R1441H* mutations were screened by restriction endonuclease digestion with the *Bst*UI enzyme, and samples with an abnormal electrophoresis pattern were sequenced to identify the exact nucleotide change. Genomic DNA was amplified using polymerase chain reaction and done in 25 µL containing 2.5 µL of polymerase chain reaction buffer, 1.5mM magnesium chloride, 1.25mM concentration of each deoxynucleotide triphosphate (dNTP), 0.4µM forward primer, 0.4µM reverse primer, 2.5 U of *Taq* DNA polymerase, and 50 ng of genomic DNA. Cycle conditions were as follows: 5 minutes at 94°C, 30 cycles of 30 seconds' denaturation at 94°C, 30 seconds' annealing at 58°C, and 90 seconds' extension at 72°C, with a final extension of 5 minutes at 72°C. The samples were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Foster City, Calif) and run on an ABI-prism automatic DNA sequencer (Perkin Elmer).

RESULTS

The *LRRK2* mutations were found in 16 patients, which represent 5.3% of all PD subjects studied in this cohort. Thirteen patients carrying the *G2019S* mutation, 2 patients with the *R1441G* mutation, and 1 patient with the *LRRK2 R1441C* mutation were identified (**Table 1**). The *R1441H* mutation was not present in any of our patients. An *R1441G* mutation was found in a familial PD patient originating from the northwest region of Spain, near Asturias, but the other *R1441G* mutation carrier had not known ancestors from this geographical area.

Nine patients (56.3%) had a family history of PD (**Figure**). Two other patients (patients 1 and 8; **Table 2**) reported tremor in 1 parent, but these families did not meet the conservative criteria of Marder and colleagues for familial PD,[14] and the cases were classified as sporadic. The pedigrees of patients with *LRRK2* mutations are shown in the Figure. The family of proband 4 showed incomplete penetrance. The proband's mother, who was a confirmed mutation carrier, did not have illness symptoms or signs of parkinsonism at age 91 years. Furthermore, a proband's half-sister, from the same mother but a different father, was affected by PD but was not a mutation carrier.

Two additional patients carrying the *G2019S* mutation (patients 17 and 18), relatives of probands 4 and 11, respectively, were identified, and their clinical features are also reported herein (Table 2 and Figure). The mean age at illness onset for the 18 patients with *LRRK2* mutations (mean±SD, 57.1±13.2 years; range, 33-78 years) did not differ significantly from PD patients without these mutations (mean±SD, 53.7±13.3 years; range, 8-85 years). Early onset (<40 years) was present in 2 patients, aged 33 and 35 years, respectively. One relative of patient 7 developed symptoms of PD at age 26 years, but extensive clinical information and DNA from this individual were not available.

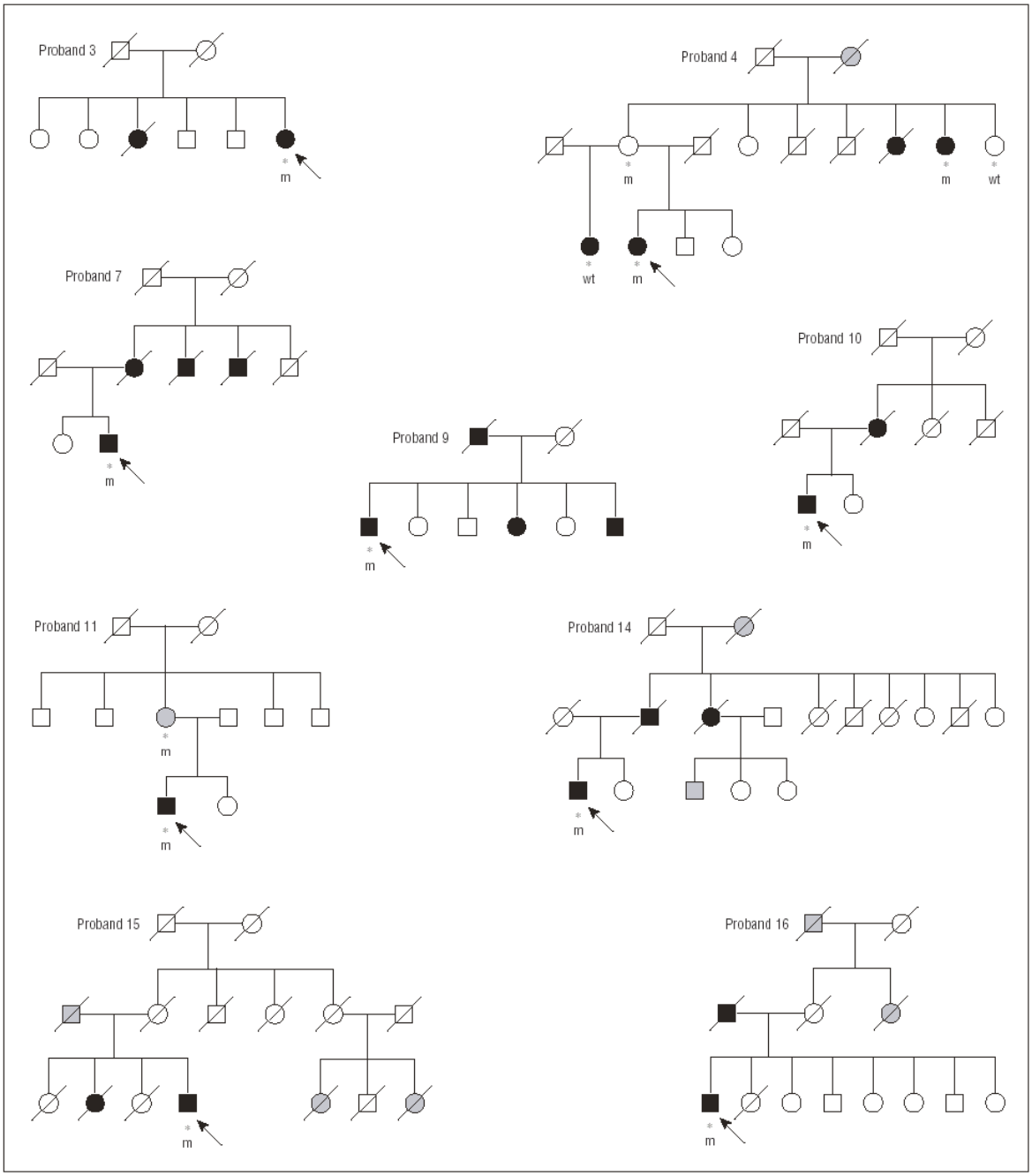

Figure. Pedigrees of familial patients with *LRRK2* mutations. Each proband is indicated by an arrow. Black symbols indicate affected family members; gray symbols, relatives who have only tremor; slash, deceased; circles, females; squares, males; asterisk, genotyped individual, with m for mutation carriers and wt for wild-type *LRRK2*.

The clinical features of patients with *LRRK2* mutations after a mean disease duration of 13 years are shown in Table 2 and **Table 3** (mean follow-up at our hospital, 6.3 years). The parkinsonism in patients with *LRRK2* mutations usually presented with unilateral rest tremor or motor slowness. During the course of the illness, the 3 cardinal parkinsonian signs of rest tremor, bradykinesia, and rigidity were present in most patients, with frequent persistence of the asymmetry of parkinsonian motor signs (Table 3). Only 1 case (patient 18; Table 2) differed from this clinical picture. At age 57 years this patient developed rest tremor of the left leg, which has remained mild and intermittent and been restricted to the left leg for 8 years, without the presence of other parkinsonian signs. Dementia was present in only 1 patient (patient

Table 2. Clinical Features of Patients With *LRRK2* Mutations*

Patient No./ Sex/Age at Onset, y	Disease Duration, y†	Family History of PD	Initial Symptom	Cardinal Signs‡	Asymmetry‡	Postural Instability	Freezing of Gait	Motor Fluctuations	Dyskinesias	Hoehn-Yahr Stage†	Psychiatric Symptoms
1/F/53	14	No§	Tremor, right limbs	T, R, B	Yes	Yes	+	++	+	V	D
2/M/48	18	No	Tremor, right arm	T, R, B	Yes	Yes	+	++	+	IV	A
3/F/62	9	Yes	Tremor, slowness, left arm	T, R, B	Yes	Yes	++	++	++	IV	–
4/F/66	4	Yes	Tremor, left leg	T, R, B	Yes	No	–	–	–	II	D,A
5/F/43	7	No	Slowness, right limbs	R, B	No	No	–	++	–	III	–
6/F/69	17	No	Tremor, right limbs	T, R, B	Yes	Yes	++	+	+	V	D,H
7/M/51	14	Yes	Slowness, right arm	R, B‖	Yes	No	++	+	++	III	–
8/F/60	25	No§	Tremor, right arm	T, R, B	No	Yes	++	++	++	V	D
9/M/60	16	Yes	Tremor, left leg	T, R, B	Yes	NA	+	+	NA	III	NA
10/M/47	17	Yes	Slowness, left limbs	R, B	Yes	Yes	++	+	+	IV	D
11/M/33	8	Yes	Slowness, right arm	T, R, B	Yes	No	+	++	+	III	–
12/F/35	25	No	Slowness, right leg	T, R, B	No	Yes	+	++	++	V	D,A,H
13/F/76	11	No	Generalized slowness	R, B‖	Yes	No	–	–¶	–¶	II	D
14/M/51	9	Yes	Slowness, left arm	T, R, B	Yes	No	++	++	+	III	–
15/M/75	10	Yes	Tremor, right limbs	T, R, B	Yes	Yes	++	+	–	V	–
16/F/65	19	Yes	Slowness, left limbs	T, R, B	Yes	Yes	+	++	+	V	DP
17/F/78#	6	Yes	Tremor, left limbs	T, B	No	Yes	–	–¶	–¶	III	–
18/F/57#	8	Yes	Tremor, left leg	T	Yes	No	–	–	–	I	–

Abbreviations: A, anxiety; B, bradykinesia; D, depression; DP, dopaminergic psychosis, delusions with visual hallucinations; H, transient hallucinations related to medication without psychosis after more than 10 years of illness; NA, data not available; PD, Parkinson disease; R, rigidity; T, rest tremor; –, not present; +, present but not disabling; ++, present and disabling.

*All patients had the *G2019S* mutation except for patients 14, 15, and 16, who had the *R1441G* mutation. All patients had a favorable response to levodopa except for patients 4 and 18, in whom levodopa treatment was not initiated.

†Data at last visit.

‡During illness course.

§One parent had tremor at old age but did not fulfill the conservative criteria of Marder et al[14] for familial PD.

‖Had minimal and infrequent rest tremor; Unified Parkinson's Disease Rating Scale III total score for rest tremor items = 1.

¶Two patients did not develop levodopa-induced motor complications after 6 (patient 13) and 2 (patient 17) years of treatment.

#Patient 17 is a relative (aunt) of patient 4; patient 18 is a relative (mother) of patient 11.

Table 3. Summary of Clinical Features in 18 Patients With *LRRK2* Mutations

Characteristic	No. (%)
Family history of PD	9 (56.3)*
Initial symptom	
Rest tremor	10 (55.5)
Clumsiness or motor slowness	9 (50.0)
Unilateral onset	17 (94.4)
Symptoms during illness course	
Rest tremor	14 (77.7)
Bradykinesia	17 (94.4)
Rigidity	16 (88.8)
Asymmetry	14 (77.7)
Postural instability	10 (58.8)†
Freezing of gait	13 (72.2)
Favorable response to levodopa	16 (100.0)‡
Motor fluctuations	14 (87.5)‡
Dyskinesias	11 (68.7)†‡
Psychiatric symptoms (depression or anxiety)	8 (47.0)†§
Dementia	1 (5.5)

Abbreviation: PD, Parkinson disease.

*Does not include 2 affected individuals who were relatives of a proband.

†Does not include a patient whose clinical data were not available.

‡Does not include 2 patients in whom levodopa treatment was not initiated.

§In all cases, psychiatric symptoms appeared after the onset of motor symptoms.

16; Table 2) with the *R1441G* mutation, who developed visual hallucinations and delusions with cognitive decline (Mini-Mental State Examination score, 12/30) and fluctuating attention after 15 years of illness. Atypical features of PD such as supranuclear gaze palsy or early dysautonomia were not observed in any of our patients.

Response to levodopa was positive in all cases. Treatment-induced motor complications appeared frequently after a mean illness duration of 7 years but in some cases appeared after 10 or as early as 2 years. All patients with treatment-induced motor complications had motor fluctuations, mainly wearing-off but also on-off phenomena (Table 3). Three patients (patients 2, 3, and 11; Table 2) were treated with bilateral subthalamic nuclei deep brain stimulation after 7 to 11 years of illness, with sustained control of motor PD symptoms and functional improvement in all cases.

COMMENT

We have found the *LRRK2 G2019S* mutation in 4.3% of our PD patients, accounting for 6.4% of familial and 3.4% of apparently sporadic cases. This is the first study that reports the frequency of the *LRRK2 G2019S* mutation in Spanish PD patients. The proportion of *LRRK2 G2019S* mutation carriers in both familial and isolated cases of PD is similar to or slightly higher than that reported previously in other studies performed in different populations, where this mutation accounts for about 2% to 6%

of familial and 0.6% to 2.0% of apparently sporadic cases.[7-10,15,16]

The *R1441G* mutation was present in 0.7% of our PD patients. This mutation was originally identified in 8% of Spanish families originating from the Basque region[1] and later in patients from a neighboring region, Asturias, with a lower frequency (2.7%).[11] This mutation has not been found in other world populations, including Portuguese PD patients in the Iberian peninsula,[16] suggesting that it is geographically restricted to northern Spain by a founder effect in the Basque people, a relatively homogeneous and historically isolated ethnic group. Haplotype analysis of this chromosomal region in PD patients supports this hypothesis.[1,11] We have found a lower *R1441G* mutation frequency in Catalonia when compared with Basque or Asturian regions, supporting the existence of a geographical gradient for this mutation as previously suggested.[11] In our population, 1 patient with an *R1441G* mutation originated from a region near Asturias, whereas the other patient carrier had not known Basque or Asturian ancestors. Therefore, the presence of this mutation in Catalonia could be explained by the existence of recent or ancient migration from the Basque population. Supporting the hypothesis of an ancient migration is the description of an ancestral gene flow between the Basque people and other populations of Europe, especially with the Catalan people, during the past few thousand years.[17]

As reported in previous studies,[2-6] the clinical features of parkinsonism in our patients with *LRRK2* mutations were similar to classic PD. Age at illness onset was variable, ranging from 33 to 78 years, although mean age at onset was similar to that of idiopathic PD. Onset was frequently asymmetrical, with rest tremor or motor slowness. A significant proportion of our patients had minimal or no rest tremor despite long disease duration. As noted in other studies, dementia was rare in patients with *LRRK2* mutations.[2-4] A clinical presentation different from classical PD occurred in 1 *G2019S* mutation carrier, who had monosymptomatic, mild, intermittent rest tremor in 1 leg for several years without developing additional parkinsonian symptoms or signs. This suggests that in some cases, the *LRRK2* mutation phenotype may have a benign course. All patients had a good response to levodopa but frequently developed treatment-induced motor complications, especially fluctuations and freezing of gait, that were disabling for many of them. Three of our patients were treated with chronic bilateral electrical stimulation of the subthalamic nuclei, with an excellent outcome.

Family history of PD was present only in 56.3% of our *LRRK2* mutation carriers. Negative family history for this subset of PD patients (43.7%) may be related to several factors, including incomplete penetrance,[10] undiagnosed PD in a relative, appearance of a mutation de novo, false paternity, and early death of family members before illness development. We found an unaffected 91-year-old relative who carries the *G2019S* mutation, supporting the existence of incomplete penetrance in this family. Previous studies have reported a penetrance between 70% and 100% in different families with *LRRK2* mutations.[1,18] In 1 study, the penetrance of the mutation was found to be age dependent, increasing from 17% at age 50 years to 85% at age 70 years.[3] However, the 91-year-old unaffected carrier reported herein, together with the previously reported 89-year-old unaffected subject carrying a similar mutation,[19] suggests that other factors besides age are important for *LRRK2* incomplete penetrance.

Identification of affected relatives of *G2019S* PD patients but who do not carry this mutation, as in 1 of our patients, has also been observed in other studies.[4,8] Nichols et al[8] reported the presence of such phenocopies in 5 of 19 affected sibships with the *G2019S* mutation. As suggested by Singleton,[20] these phenocopies may indicate that some risk factors might be present in other family members, independently of their *G2019S* status, and consequently increase the lifetime risk of PD.

In light of the high frequency of *LRRK2* mutations in PD, it seems likely that the identification of many patients and unaffected relatives with *LRRK2* mutations will be possible in the near future. This will facilitate the search of genetic and environmental factors that could condition disease susceptibility and age at disease onset. Furthermore, the detection of presymptomatic mutation carriers will allow researchers to perform studies of putative neuroprotective treatments.

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ARTÍCULO 2

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Screening for the LRRK2 G2019S and codon-1441 mutations in a pathological series of parkinsonian syndromes and frontotemporal lobar degeneration.

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Screening for the *LRRK2* G2019S and codon-1441 mutations in a pathological series of parkinsonian syndromes and frontotemporal lobar degeneration

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Abstract

Background: The neuropathology associated with *LRRK2* mutations is heterogeneous but Lewy body (LB) type pathology is the most common substrate encountered. While the prevalence of *LRRK2* mutations has been extensively studied in Parkinson's disease (PD), limited information is available on the frequency of *LRRK2* mutations in dementia with Lewy bodies (DLB) and in other pathological conditions associated with these mutations, such as non-specific nigral degeneration without LB, tau-immunopositive neurofibrillary tangle pathology, and ubiquitin-positive neuronal inclusions resembling those observed in a subtype of frontotemporal lobar degeneration (FTLD-U).
Objective: To further investigate the neuropathology associated with *LRRK2* mutations.
Methods: We have screened for the *LRRK2* G2019S and codon-1441 (R1441G/C/H) mutations in 110 cases from a Spanish Brain Bank, which include: 66 synucleinopathies (33 PD, 25 DLB and 8 multiple system atrophy cases), 29 tauopathies (21 progressive supranuclear palsy, 3 corticobasal degeneration and 5 tau-positive FTLD cases), 3 cases of non-specific nigral degeneration and 12 tau-negative FTLD (9 FTLD-U and 3 dementia lacking distinctive histology cases).
Results: The G2019S mutation was found in two cases: One case had a clinical and pathological diagnosis of PD and the other suffered from typical PD and on neuropathological examination had non-specific nigral degeneration without LB. A synonymous variant (R1441R; c.4323C>T) was detected in another PD case.
Conclusions: In this brain bank-based series, *LRRK2* G2019S mutation occurred in patients with parkinsonism associated with either typical brainstem LB pathology or non-specific nigral degeneration. *LRRK2* mutations were not encountered in other neurodegenerative disorders associated with synuclein and tau deposition.

Keywords: *LRRK2* G2019S mutation; *LRRK2* R1441G mutation; Parkinson's disease; Dementia with Lewy bodies; Frontotemporal lobar degeneration; Non-specific nigral degeneration; Neuropathology

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1. Introduction

Mutations in the leucine-rich repeat kinase 2 gene (*LRRK2*) have been found to cause a significant proportion of Parkinson's disease (PD) cases [1–3]. Two *LRRK2* mutations, the G2019S and the R1441G, are especially frequent in Spanish PD patients [1,4]. The motor features of *LRRK2* related PD are indistinguishable from classical PD [1–4], but the underlying neuropathology is heterogeneous [2]. Lewy-type pathology (synuclein positive Lewy bodies (LB) and neurites) is the most common reported neuropathological substrate associated with the *LRRK2* G2019S mutation [3,5,6]. Non-specific nigral degeneration without LB [6], tau-immunopositive neurofibrillary tangle pathology [7] and frontotemporal lobar degeneration with ubiquitin-immunoreactive neuronal inclusions (FTLD-U) [8], have also been described in some patients with the G2019S mutation. The underlying neuropathology of the R1441G mutation, to the best of our knowledge, has not yet been described.

Most studies analyzing the presence of *LRRK2* mutations have been conducted in PD patients in whom the diagnoses was exclusively clinical. Studies based on cases with a pathological diagnosis are sparse [3,5–8], and limited information is available on the frequency of *LRRK2* mutations in dementia with Lewy bodies (DLB), a disorder characterized by extensive central nervous system Lewy-type pathology, and in other pathological conditions associated with these mutations, such as non-specific nigral degeneration, tau-immunopositive neurofibrillary tangle pathology and ubiquitin-positive neuronal inclusions resembling those observed in FTLD-U [2]. In order to further investigate the neuropathology associated with *LRRK2* mutations, we screened for the G2019S and the codon-1441 mutations (R1441G/C/H) those cases from the Universitat-Hospital Clínic of Barcelona Brain Bank (UB-HC Brain Bank) with pathological diagnosis of PD or other degenerative parkinsonism, as well as those diagnosed as frontotemporal lobar degeneration (FTLD).

2. Subjects and methods

2.1. Subjects

One hundred and ten cases of several neurodegenerative disorders were studied (Table 1), which include: Thirty-three cases that had been diagnosed during lifetime of PD [9] and had brainstem LB pathology on neuropathological examination. Twenty-three of these 33 cases had PD with dementia (PDD) according to published criteria [10]. In addition, we studied twenty-five patients diagnosed clinically and pathologically as DLB according to established criteria [11]. We also studied 21 progressive supranuclear palsy (PSP), 3 corticobasal degeneration (CBD), 8 multiple system atrophy (MSA) and 3 cases of parkinsonism with non-specific nigral degeneration. Seventeen cases diagnosed as FTLD (3 Pick's disease, 2 multisystem tauopathy, 9 FTLD-U and 3 dementia lacking distinctive histology cases) were also included. Progranulin (PGRN) and tau (MAPT) gene mutations had been previously excluded in these FTLD cases [12].

Table 1
Clinical and pathological diagnosis of cases screened for the *LRRK2* G2019S and codon-1441 mutations

Diagnosis (number of cases)	Number of cases with *LRRK2* mutation	
	G2019S mutation	Codon-1441 mutations
PD with LB type pathology ($n=33$)		
PD without dementia ($n=10$)	1	0
PDD ($n=23$)	0	1 [a]
DLB ($n=25$)	0	0
Parkinsonism with NSND ($n=3$)	1	0
Atypical parkinsonisms ($n=32$)		
PSP ($n=21$)	0	0
CBD ($n=3$)	0	0
MSA ($n=8$)	0	0
FTLD ($n=17$)		
PiD ($n=3$)	0	0
MST ($n=2$)	0	0
FTLD-U ($n=9$)	0	0
DLDH ($n=3$)	0	0

PDD: PD dementia; NSND: Parkinsonism with non-specific nigral degeneration; PiD: Pick's disease; MST: Multisystem tauopathy; DLDH: Dementia lacking distinctive histology.
[a] R1441R (c.4323C>T) variant was identified in one case.

Neuropathological diagnoses were made according to standard diagnostic criteria [13–17] and following a well-established protocol using formalin-fixed, paraffin-embedded sections of brain areas that were processed with haematoxylin–eosin and luxol fast blue-Klüver Barrera staining and immunohistochemistry following the avidin–biotin–peroxidase method with specific antibodies to BA4-amyloid, phosphorylated tau, ubiquitin, α-synuclein and neurofilaments. The clinical diagnoses and data were collected by a flow chart review process.

2.2. Genetic analysis

Genomic DNA was extracted from 25 mg of frozen brain tissue using the QIAamp DNA MiniKit (Qiagen). The *LRRK2* G2019S mutation was detected by restriction endonuclease digestion with the *SfcI* enzyme, running in an acrylamide gel electrophoresis and subsequently stained with ethidium bromide. The codon-1441 (R1441G/C/H) mutations were screened by restriction endonuclease digestion with the *Bst*UI enzyme. Samples with an abnormal electrophoresis pattern were sequenced in order to identify the exact nucleotide change as previously described [4].

RNA was isolated from 50 mg of frozen brain tissue (globus pallidus) of a case carrying a R1441R (c.4323C>T) variant, using the RNeasy lipid tissue mini kit DNase free (Qiagen). cDNA was obtained using TaqMan Reverse Transcription Reagents (Applied Biosystems). Specific primers were designed by using DNAstar (DNASTAR) in order to amplify

the adjacent exons to the c.4323C>T nucleotide change (29, 30, 31 and 32; Forward: GTTGGCATAGATGTGAAA GACT; Reverse: TACGCTCCGATAAAATGAT). Sequencing was performed by using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) with an automatic sequencer (ABI PRISM model 377).

2.3. Immunohistochemical studies

Immunohistochemical studies using three antibodies against different epitopes of LRRK2, the AP7099b (Abgent), the NB300-267 and NB300-268 (Novus Biologicals), were performed in the substantia nigra (SN) of those cases carrying *LRRK2* mutations and in additional sporadic PD case with LB pathology as described by others [6,18–20].

3. Results

Among the entire series of brains tested, a G2019S mutation was found in two cases (Table 1). One case had a clinical and pathological diagnosis of PD and the other suffered from typical PD and on neuropathological examination had non-specific nigral degeneration without LB. The clinical and neuropathological features of this latter case have been reported elsewhere [21]. Thus, the G2019S mutation was found with a frequency of 1 in 33 (~3%) among cases diagnosed as PD and 1 in 3 in cases diagnosed as parkinsonism with non-specific nigral degeneration. The G2019S mutation was not identified in any of the 25 DLB cases, nor in those cases with pathological diagnoses of PSP, CBD, MSA or FTLD. Similarly, the R1441G/C/H mutations were not identified in any of the cases studied. However, a synonymous genetic variant in the 1441-codon, located in the c.4323 position from the starting codon referenced with the NM_198578.2 NCBI number (R1441R; c.4323C>T), was detected in one PD case with LB pathology. This patient was a woman that presented clinically with sporadic parkinsonism at 55 years of age. She died at 71 years of age, after developing dementia and mild dysautonomia during the last 3 years of illness. We did not find evidence of abnormal brain alternative or splicing transcripts, and the *LRRK2* cDNA showed the normal sequence except for the c.4323C>T variant (NCBI database).

A review of the clinical history of the PD case in which a G2019S mutation was identified, revealed that the patient had presented to our Movement Disorders Clinic at 52 years of age with one-year history of rest tremor, bradykinesia and rigidity in the right limbs. Family history for PD or tremor was negative. She was diagnosed as PD and treatment with levodopa started with an excellent response. Three years later her motor symptoms were bilateral but more severe in the right side, and mild motor fluctuations of wearing off type had appeared. Her asymmetric parkinsonism progressed during the following years and postural instability developed at the ninth year of illness. At the twelfth year of illness severe choreiform peak dose dyskinesias, specially in the right limbs, and freezing of gait appeared. During the following years depression, anxiety and complex and disabling motor fluctuations developed. Left pallidotomy was performed at the nineteenth year of illness with moderate improvement of parkinsonian signs in the right limbs and levodopa induced motor complications. Cognitive decline or atypical signs for PD never developed during lifetime. She died at 78 years of age. Neuropathological examination showed a severe loss of pigmented neurons with the presence of α-synuclein positive LB and Lewy neurites in the SN, particularly in the pars compacta, as well as in other brainstem nuclei such as locus coeruleus, raphe nuclei and dorsal part of the nucleus of the hypoglossal and vagal nerves. LB and Lewy neurites were also observed in subthalamus, basal nucleus of Meynert and hypothalamus. LB in the cortical regions were infrequent and mostly observed in the amygdala, transentorhinal region and gyrus cinguli.

Immunohistochemistry using the antibodies to LRRK2 AP7099b, NB300-267 and NB300-268 did not show dystrophic neurites or stain LB or any other type of pathological inclusions in the SN of both cases carrying the G2019S mutation nor in another sporadic PD case with typical LB pathology additionally examined.

4. Discussion

Among 110 brains from the UB-HC Brain Bank diagnosed with various neurodegenerative disorders, we have found two cases with the G2019S mutation in the *LRRK2* gene. During lifetime these two individuals had been diagnosed as having PD, and on neuropathological examination one showed typical LB pathology while the other case presented non-specific nigral degeneration [21]. *LRRK2* G2019S and R1441G/C/H mutations were not found in any of the cases with DLB, PSP, CBD or MSA. A similar absence of *LRKK2* mutations in these disorders has been reported in other studies performed in brain tissue [5,6,22] as well as in clinical series [23–25]. In a recent study by Chen-Plotkin et al., however, the G2019S mutation was found in a case presenting as a CBD syndrome with no pathologic confirmation [26]. Although the number of cases screened for G2019S and R1441G mutation in our study is not so large, we believe that these results are relevant in light of the rather high frequency of such mutations found in our population as detected in clinical studies. The G2019S may account for 4.3 to 7.6% of Spanish PD patients [4,27], and the R1441G is found in 8% of Basque PD patients [1], and with a lower frequency, in PD cases from the neighboring regions of northern Spain, Catalonia (0.7%) and Asturias (2.7%) [4,28].

The *LRRK2* R1441G mutation was not found in any of our cases but the R1441R (c.4323C>T) variant, located in this critical codon of the gene where other mutations are found, was identified in one brain from a PD patient. This synonymous variant has not been identified in 302 clinical PD cases from our outpatient clinics studied previously [4]. In the brain tissue of this case with a R1441R variant,

LRRK2 alternative transcripts were not found, indicating that this synonymous variant is probably non-pathological. However, we cannot rule out a pathogenic effect of this variant by altering the mRNA stability or through a recently described mechanism, by which a synonymous mutation could change the function of the protein by altering the timing of cotranslational folding resulting in protein conformational changes [29].

With the inclusion of the case reported here, the neuropathology associated with the *LRRK2* G2019S mutation has been described in twenty cases with a clinical picture of progressive parkinsonism [[3,5–7,21,26]. In seventeen of these cases typical LB pathology was present, non-specific nigral degeneration occurred in two and tau-immunopositive neurofibrillary tangle pathology resembling PSP in one case. Thus, the most frequent histological findings encountered in parkinsonism with *LRRK2* G2019S mutation is neuronal loss is the SN with LB pathology.

We did not identify the G2019S mutation in any of our 25 pathologically proven DLB cases. Despite the evidence that G2019S mutation frequently causes LB pathology, the few studies that have been carried in DLB patients from Memory-Dementia Clinics or brain banks have failed to clearly identify mutations in this type of LB disorder [6,30,31]. One of these studies tested the presence of *LRRK2* G2019S mutation in 46 PD and 34 DLB cases from a Brain Bank series, and only found three PD cases carrying the mutation [6]. A second study screened for the G2019S mutation in 405 cases with PD or LB disease from another Brain Bank series, but the authors did not state how many of these cases were DLB. Eight of these 405 cases had this mutation and had been diagnosed as PD during lifetime [5]. A third study conducted in 85 LB disease cases from an autopsy series, without specifying how many of these cases were PD or DLB, failed to detect G2019S mutation [7]. A fourth study tested the presence of *LRRK2* mutations in 98 cases diagnosed as PD or DLB (78 of them pathologically proven but not stating how many of them were PD or DLB), and found the G2019S mutation in four of the cases with a diagnosis of PD [26]. Only two studies tested for G2019S and other *LRRK2* mutation patients with DLB diagnosed according to the consensus guidelines [11]. In each study 30 DLB patients were analyzed but *LRRK2* mutations were not found [30,31]. If confirmed in larger studies, the absence or a lower prevalence than in PD of *LRRK2* mutations in DLB cases, as described in the autopsy based series reported here, could provide clues to the mechanisms involved in the pathogenesis of the various types of LB diseases (PD and DLB).

The G2019S mutation has been reported in a 89-year old patient with a neuropathologically confirmed Alzheimer's disease (AD) [5] and in a 79-year old woman with a clinical picture of possible frontotemporal dementia without symptoms of parkinsonism but positive family history for tremor, whose neuropathological examination disclosed FTLD-U [8]. The G2019S mutation has been also identified in several subjects without any neurodegenerative disease [4,26], one of them with a normal neuropathological examination, indicating a reduced penetrance of the *LRRK2* G2019S mutation [5]. Thus, these two previously published cases of non-parkinsonian neurodegenerative disorders carrying the G2019S mutation may represent a coincidence, especially in the case with a pathological diagnosis of AD since *LRRK2* mutations have not been found in large series of AD patients [24,30]. However, few FTLD cases have been screened for *LRRK2* mutations [26], and pathology resembling FTLD-U has been observed in some cases with other *LRRK2* mutations [2]. Similarly, the patient with a clinical diagnosis of CBD syndrome carrying a G2019S mutation reported by Chen-Plotkin et al., could also represent a coincidental finding [26]. We did not find *LRRK2* mutations in any of our cases with FTLD-U or CBD, but more studies are needed to clarify whether the G2019S mutation can lead to FTLD-U or CBD phenotype.

Giasson and associates were able to demonstrate in a parkinsonian case with non-specific nigral degeneration and a G2019S mutation, the presence of dystrophic neurites in the SN intensely stained with the antibody to LRRK2 AP7099b [6]. In addition, antibodies to LRRK2 NB300-267 and NB300-268 have been reported to immunolabel a subset (10–15%) of LB from tissue sections of sporadic PD cases [18–20]. We were not able to detect pathological inclusions in the SN of our cases carrying the G2019S mutation or in additional sporadic PD case by using these three LRRK2 antibodies. Recent evidence suggest that the detection of some LB by these antibodies may be due to crossreactivity [32]. In addition, other studies indicate that LRRK2 is not a primary component of LB [33].

This study of *LRRK2* mutations in a brain bank series confirms that *LRRK2* G2019S mutation is relatively frequent among those cases of parkinsonism associated with either typical brainstem LB pathology or non-specific nigral degeneration without LB. *LRRK2* mutations were not encountered in other neurodegenerative disorders associated with synuclein and tau deposition. Larger studies analyzing the presence of *LRRK2* mutations in pathologically diagnosed LB and other neurodegenerative disorders, such as FTLD-U or CBD, are needed to further define the underlying neuropathology associated with these mutations. Clarification on how *LRRK2* mutations lead to different pathological phenotypes may provide important clues to the mechanisms involved in the pathogenesis of these disorders.

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ARTÍCULO 3

Gaig C, Martí MJ, Ezquerra M, Rey MJ, Cardozo A, Tolosa E.

G2019S LRRK2 mutation causing Parkinson's disease without Lewy bodies.

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SHORT REPORT

G2019S *LRRK2* mutation causing Parkinson's disease without Lewy bodies

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The G2019S leucine-rich repeat kinase 2 gene (*LRRK2*) mutation has been identified in a significant proportion of familial and sporadic cases of Parkinson's disease (PD). Until now, information on the neuropathological changes associated with the G2019S *LRRK2* mutation has been sparse. We report a 77-year-old patient who presented with a 14 year history of PD but, unexpectedly, histopathological examination disclosed mild neuronal loss in the substantia nigra without α-synuclein, tau or ubiquitin cytoplasmic inclusions. A G2019S *LRRK2* mutation was eventually detected. The present case confirms that clinical PD caused by G2019S mutations can be associated with non-specific nigral degeneration without Lewy bodies.

Recently, pathogenic mutations in a novel gene, the leucine-rich repeat kinase 2 gene (*LRRK2*), have been identified in a significant proportion of patients with autosomal dominant forms of Parkinson's disease (PD).[1 2] Several *LRRK2* mutations have been described, although until now only five, R1441C, R1441G, Y1699C, G2019S and I2020T, are considered definitely pathogenic.[3] G2019S is the most common and may account for approximately 5–6% of familial and 1–3% of sporadic PD cases.[4–6] The clinical features and response to levodopa treatment in patients with *LRRK2* mutations seem to be indistinguishable from classic PD.[3–6]

Although information is sparse, the neuropathological changes in patients with PD associated with *LRRK2* mutations seems to be heterogeneous.[2] In some cases, changes are consistent with classic PD, with neuronal loss in the substantia nigra (SN) and α-synuclein positive Lewy bodies (LB). However, in other cases, pathological findings vary and include the presence of non-specific neuronal loss with ubiquitin reactive cytoplasmic and nuclear inclusions, tau pathology reminiscent of progressive supranuclear palsy or pure nigral degeneration without specific α-synuclein, tau or ubiquitin inclusions.[2] We report a patient with a G2019S *LRRK2* mutation with a clinical picture compatible with PD, but histopathological examination disclosed non-specific nigral degeneration in the SN without α-synuclein, tau or ubiquitin cytoplasmic inclusions.

CASE REPORT

A 63-year-old woman presented with a 2 year history of rest tremor and slowness in her left limbs. She had a long history of diabetes mellitus type 2 and arterial hypertension. Although her family history for PD was negative, the patient explained that her mother and maternal uncle had suffered upper limb tremor at an older age. Neurological examination disclosed mild rest tremor, bradykinesia and rigidity in her left limbs. Her speech, face, ocular motility, gait and postural stability were normal. Tremor dominant PD, Hoehn and Yahr (H&Y) stage I, was diagnosed and levodopa treatment, 300 mg daily, was started with an excellent response.

Over the next 10 years, PD slightly progressed. Mild speech disturbance and facial hypomimia appeared. Rest tremor, bradykinesia and rigidity in the left limbs slowly worsened, and treatment with pramipexole 0.7 mg three times daily was added. Evident involvement of the right limbs occurred during the ninth year of her illness (H&Y stage II) but PD was still mild and asymmetric. After the 10th year of illness, shuffling and freezing of gait with postural instability developed (H&Y stage III). Levodopa was increased to 600 mg daily and moderate but bothersome coreiform dyskinesias in her left foot and abnormal posture in her trunk, which was bent to the right, appeared. Over the next 3 years her gait abnormalities worsened with the presence of frequent falls. Mild motor fluctuations of the wearing-off type also occurred. In the 13th year of illness, the patient needed assistance with walking (H&Y stage IV).

In the 14th year of her illness, the patient developed confusional syndrome with seizures related to heart failure–hypertensive cardiopathy and renal insufficiency–diabetic nephropathy. A brain MRI performed at that time disclosed severe periventricular leukoaraiosis with subcortical lacunar infarcts with mild rarefaction of the pons white matter. The confusional state and seizures were complicated by pneumonia, which led to death. Signs of cognitive impairment, hallucinations, delusions or atypical signs for PD never developed during the course of her illness.

Neuropathological examination was carried out following a well established protocol at the Universitat Barcelona-Clínic Brain Bank using haematoxylin-eosin and luxol fast blue–Klüver Barrera staining, and immunohistochemistry with specific antibodies to BA4 amyloid, phosphorylated tau, ubiquitin, α-synuclein, CD20 and CD3, neurofilaments and αβ-crystallin. The examination revealed mild depigmentation of the SN and multiple subcortical and basal ganglia lacunar infarcts. Neuronal loss in the SN was mild, involving both pars compacta and reticulata, with free extraneuronal melanin and prominent eosinophilic ubiquitin positive intanuclear inclusions compatible with Marinesco bodies (fig 1). Mild neuronal loss with abundant Marinesco bodies was also observed in the locus coeruleus. Neuronal loss was not detected in other brain structures. α-Synuclein immunohistochemistry did not disclose LB or Lewy neurites in the SN or other brain areas. Isolated tau positive neurofibrillary tangles and neuropil threads were observed in the hippocampus, transentorhinal cortex, locus coeruleus, nucleus basalis of Meynert and brainstem periaqueductal grey matter, but amyloid plaques were absent. A heterozygous G2019S *LRRK2* mutation was detected using the methods previously described from DNA obtained from frozen brain tissue.[6]

Abbreviations: H&Y, Hoehn and Yahr; LB, Lewy body; *LRRK2*, leucine-rich repeat kinase 2 gene; PD, Parkinson's disease; SN, substantia nigra

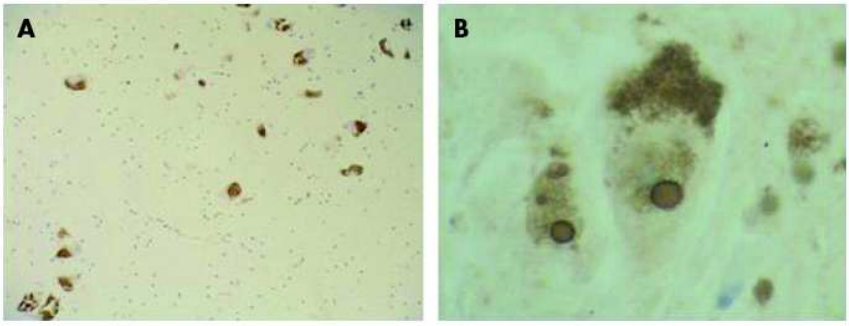

Figure 1 α-Synuclein immunohistochemistry (×100 objective) showing mild neuronal loss and gliosis of the substantia nigra without Lewy bodies or Lewy neurites (A). Ubiquitin immunohistochemistry (×1000 objective) showing prominent nuclear inclusions in neurons of the substantia nigra compatible with Marinesco bodies (B).

DISCUSSION

Our patient presented with a progressive PD syndrome which started unilaterally with rest tremor, and showed a good response to levodopa with development of classic treatment induced motor complications without the presence of atypical features for PD. The patient fulfilled the accepted criteria used for a clinical diagnosis of PD but, unexpectedly, the histopathology studies disclosed only mild neuronal loss in the SN without α-synuclein positive LB or Lewy neurites.[7]

Cases of parkinsonism associated with *LRRK2* mutations with histopathological findings similar to those reported here led us to search for a mutation in this gene. Pure nigral degeneration without α-synuclein positive LB and Lewy neurites was described as the underlying neuropathological substrate in the first PD family linked to the PARK8 locus, the Sagamihara kindred, that later was found to carry the missense mutation I2020T in the *LRRK2* gene.[8 9] Non-specific nigral degeneration has also been reported in patients with other *LRRK2* mutations. In one subject (family D; Western Nebraska; mutation R1441C), the SN showed marked neuronal loss without α-synuclein inclusions but ubiquitin positive inclusions were observed whereas two other individuals of this kindred presented with LB disease and another had tau positive inclusions.[2] In three subjects carrying the mutation Y1699C, non-specific SN degeneration with ubiquitin positive neuronal inclusions was observed in two cases whereas LB formation was present in one case.[2 10]

To date, the neuropathology associated with the commonest *LRRK2* mutation, G2019S, has been examined in only 14 cases.[5 11 12] In 13 cases, LB and Lewy neurites were found and, therefore, some authors have suggested that *LRRK2* G2019S may be an α-synucleinopathy.[5 11 12] Our patient, together with another reported case, confirms that the histopathology in patients with the G2019S mutation can be non-specific nigral degeneration without α-synuclein positive LB, and indicates that there is no correlation between the type of *LRRK2* mutation and the underlying neuropathological changes.[12] In addition, tau immunopositive neurofibrillary tangle pathology has been observed recently in a case of parkinsonism with the G2019S mutation.[13] In light of this pleomorphic neuropathology associated with different *LRRK2* mutations, some authors have hypothesised that the underlying pathogenic mechanism may be an upstream pathway of other proteins implicated in the pathogenesis of neurodegeneration.[2]

The G2019S mutation has also been observed in a control subject without a personal or family history of neurological or neurodegenerative disease who died at age 68 years. His neuropathological examination did not disclose any neurodegenerative abnormalities.[11] The G2019S mutation was also found in a 89-year-old patient with neuropathologically confirmed Alzheimer's disease.[11] Both cases may reflect the incomplete penetrance of the *LRRK2* mutations, which is probably influenced by age and other genetic and environmental factors.

Parkinsonism in the patient reported here showed a benign course for the first 10 years. The rapid worsening of the illness over the last 4 years, with major gait disturbances, could be explained by the development of ischaemic lesions involving the subcortical white matter and basal ganglia. Her initial benign parkinsonism may have been related to the final mild SN neuronal loss found at the pathological examination. Another explanation for this benign course is the absence of α-synuclein positive LB pathology. Patients with other genetic causes of PD syndrome, such as those with *Parkin* gene mutations, may also present with nigral degeneration without α-synuclein inclusions and frequently have less progressive parkinsonism compared with idiopathic PD patients.[14 15] Therefore, it is reasonable to hypothesise that the absence of α-synuclein positive LB pathology may be a marker of a less aggressive pathogenic process with a slower rate of neuronal loss in the SN.

The presence of Marinesco bodies found in our patient has been described in neurons from other *LRRK2* patients.[2] Marinesco bodies are nuclear ubiquitin positive inclusions found in pigmented neurons of the SN nigra that increase in frequency with advancing age. Although no pathological associations have been clearly established, it has recently been suggested that these nuclear inclusions may not represent a benign phenomenon as they are associated with a significant decline in striatal dopaminergic markers.[16] Furthermore, pigmented neurons of the SN containing LB more likely present Marinesco bodies than those pigmented neurons without LB, and proteasome dysfunction can lead to similar abnormalities in cultured cells.[16] Although their pathological role is still unclear, Marinesco bodies may represent the accumulation or aggregation of ubiquitinated proteins induced by dysfunction of the ubiquitin–proteosome pathway and, consequently, it is possible that they may be involved directly in dopaminergic cell death or, in contrast, may represent an epiphenomenon in response to the risk of neuronal cell death.

Although LB disease is the most frequent autopsy finding in the cases reported in the literature, the present case confirms that clinical PD caused by G2019S mutations can be associated with non-specific nigral degeneration, without LB, α-synuclein or any other distinctive inclusion. More extensive series, autopsy and brain bank based, are necessary to clearly define the underlying neuropathology associated with each *LRRK2* mutation.

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6. DISCUSIÓN

6.1 Prevalencia de mutaciones en el gen LRRK2 en la enfermedad de Parkinson

De entre 302 pacientes con EP visitados en la Unidad de Parkinson del Hospital Clínic de Barcelona hemos detectado una mutación en el gen LRRK2 en 16 casos. Estos resultados representan una frecuencia de mutaciones en el gen LRRK2 del 5,3%. La frecuencia de las mutaciones en los pacientes con historia familiar de EP ha sido del 9,6%, siendo del 10,4% en los casos con una EP familiar y una edad de inicio de la enfermedad tardía ($\geq$ 40 años), y del 5,9% en los pacientes con una EP familiar pero con una edad de inicio temprana (< 40 años). La frecuencia en los casos esporádicos ha sido del 3,4%, siendo similar en los casos con un inicio tardío (3,4%) o temprano (3,2%).

6.1.1 La mutación G2019S

La mutación más frecuente en el gen LRRK2 ha sido la G2019S, presente en 13 pacientes, lo que significa el 4,3% de los pacientes estudiados: un 6,4% en los casos con antecedentes familiares de EP y un 3,4% en los casos esporádicos. Esta frecuencia de la mutación G2019S en pacientes con EP es muy parecida a la observada en estudios realizados en otras regiones de España y Portugal [Infante et al., 2006; Mata et al., 2006; Bras et al., 2006; Ferreira et al., 2007; González-Fernández et al., 2007; Gorostidi et al., 2009; Gao et al., 2009], y es superior a la reportada en otras poblaciones europeas, norte-americanas, sur-americanas o asiáticas (Tabla 5). En el Norte de África y entre los judíos asquenazíes es donde la frecuencia de la mutación G2019S es más alta, estando presente en el 39% de los pacientes con EP norte-africanos y en un 18% de los pacientes con EP de origen judío asquenazí [Lesage et al., 2006; Ozelius et al., 2006].

Diversos estudios han demostrado que los pacientes con la mutación G2019S del gen LRRK2 y pertenecientes a poblaciones tan dispares de Europa, América o el Norte de África, así como los judíos asquenazíes, comparten todos ellos un mismo haplotipo ancestral común en la región del cromosoma 12 donde se halla el gen LRRK2. La presencia de este haplotipo común en los portadores de la mutación G2019S sugiere que esta mutación se originó a partir de un único ancestro fundador, quien probablemente vivió hace unos 1000 o 2000 años en el Norte de África u Oriente Medio [Kachergus et al., 2005; Lesage et al., 2005 (a); Lesage et al., 2005 (b); Goldwurm et al., 2005; Zabetian et al., 2006 (a); Lesage et al., 2006; Ozelius et al., 2006; Orr-Urtreger et al., 2007]. El posterior e histórico flujo migratorio des del Norte de África a Europa a través de la Península Ibérica, explicaría el gradiente geográfico en la frecuencia de la mutación G2019S que existe entre el Norte de África, la Península Ibérica, y el resto de poblaciones europeas y occidentales (Tabla 5). Esto también explicaría que esta mutación sea tan infrecuente en países asiáticos como la India, Taiwán o Japón [Tan et al., 2005; Zabetián et al., 2006 (b); Punia et al., 2006]. Sin embargo, no todos los pacientes con la mutación G2019S presentan el mismo haplotipo en el cromosoma 12. Un segundo haplotipo, diferente al supuestamente originado en el Norte de África u Oriente Medio, ha sido detectado también en algunos pocos pacientes de origen europeo [Zabetian et al., 2006 (a)], mientras que un

Tabla 5. Frecuencia de la mutación G2019S en el gen LRRK2 en diferentes poblaciones

Población [Referencia]	Pacientes con EP		Casos familiares de EP		Casos esporádicos de EP		Controles	
	Nº casos	Nº G2019S	Nº casos	Nº G2019S	Nº casos	Nº G2019S	Nº casos	Nº G2019S
ESPAÑA								
Catalunya [Gaig et al., 2006]	**302**	**13 (4,3%)**	**94**	**6 (6,4%)**	**208**	**7 (3,4%)**	**NA**	**NA**
Cantabria [Infante et al., 2006]	98	8 (8,2%)	16	3 (18,7%)	82	5 (6,1%)	310	0
Asturias [Mata et al., 2006]	225	5 (2,2%)	50	0	175	5 (2,9%)	100	0
País Vasco [González-Fernández et al., 2007]	67	6 (8,9%)	50	5 (10%)	17	1 (5,8%)	NA	NA
País Vasco [Gorostidi et al., 2009]	418	16 (3,8%)	181	10 (5,5%)	237	6 (2,5%)	1	138 (0,7%)
Andalucia [Gao et al., 2009]	187	3 (1.6%)	15	0	172	3 (1.7%)	287	1 (0.3%)
PORTUGAL								
Portugal [Bras et al., 2005]	124	7 (5,6%)	22	2 (9,1%)	102	5 (4,9%)	126	0
Portugal [Ferreira et al., 2007]	138	9 (6,5%)	31	5 (16,1%)	107	4 (3,7%)	101	0
NORTE ÁFRICA								
Marruecos, Túnez y Algeria [Lesage et al., 2006]	76	30 (39%)	27	10 (37%)	49	20 (41%)	151	2 (1,3%)
JUDIOS ASQUENAZÍES								
Norte América [Ozelius et al., 2006]	120	22 (18,3%)	37	11 (29,7%)	83	11 (13,3%)	317	4 (1,3%)
Israel [Orr-Urtreger et al., 2007]	342	51 (14,8%)	96	25 (26%)	246	26 (10,6%)	841	17 (2%)
EUROPA								
Italia [Goldwurm et al., 2006]	1092	19 (1,7%)	236	10 (4,2%)	856	9 (1%)	440	0
Grecia [Xiromerisiou et al., 2007]	290	1 (0,3%)	55	0	235	1 (0,4%)	235	0
Alemania [Schlitter et al., 2006]	120	2 (1,6%)	41	1 (2,4%)	79	1 (1,2%)	336	0
Reino Unido [Williams-Gray et al., 2006]	538	2 (0,4%)	75	1 (1,3%)	463	1 (0,2%)	909	0
Noruega [Asly et al, 2005]	433	7 (1,6%)	65	6 (9,2%)	368	1 (0,2%)	519	0
Rusia [Pchelina et al., 2006]	208	3 (1,4%)	51	2 (3,9%)	157	1 (0,6%)	161	0
NORTE AMERICA								
Estados Unidos [Kay et al., 2006]	1425	18 (1,3%)	329	10 (3%)	1096	8 (0,7%)	1647	1 (0,06%)
SUD AMERICA								
Chile [Pérez-Pastene et al., 2007]	166	5 (3%)	29	1 (3,4%)	137	4 (2,9%)	153	0
ASIA								
Taiwán [Tan et al., 2005]	675	0	58	0	617	0	325	0
Japón [Zabetián et al (b)., 2006]	586	2 (0,3%)	32	0	554	2 (0,3%)	317	0
India [Punia et al., 2006]	778	1 (0,1%)	60	0	718	1 (0,1%)	212	0

NA:No aplicable.

tercer haplotipo ha sido descrito en pacientes japoneses [Zabetian et al., 2006 (b)]. La presencia de al menos tres haplotipos diferentes asociados a la mutación G2019S sugiere, o bien un fundador común muy antiguo, o más probablemente, la presencia de un "*hot spot*" mutacional en este codón del gen LRRK2.

6.1.2 La mutación R1441G

La mutación R1441G se ha detectado en tan sólo dos de nuestros pacientes (0,7% de los pacientes). Esta mutación fue descrita inicialmente en familias con EP en el País Vasco y se detectó en un 8% de los pacientes de esta región (20% en los casos familiares y 4,6% en los casos esporádicos) [Paisán-Ruiz et al., 2004]. Un estudio posterior detectó una frecuencia de la mutación R1441G menor, del 2,2% en pacientes con EP esporádica de Asturias [Mata et al., 2005 (b)], sugiriendo la presencia de un gradiente geográfico en cuanto a la frecuencia de esta mutación en el norte de España (Tabla 6) [González-Fernández et al., 2007; Gorostidi et al., 2009]. Más recientemenete la mutación R1441G del gen LRRK2 ha sido detectada en algunos pacientes con EP del sur de España [Gao et al., 2009], pero no se ha identificado en otros estudios realizados en poblaciones de España y Portugal, sugiriendo que esta es una mutación limitada geográficamente al País Vasco y algunas regiones, sobretodo del norte, de la peninsula Ibérica [Bras et al., 2005; Infante et al., 2006; Ferreira et al., 2007].

El análisis de polimorfismos genéticos realizados en pacientes con la mutación R1441G ha demostrado que todos ellos presentan un mismo haplotipo en el cromosoma 12q12. Esto sugiere un efecto fundador, probablemente en el País Vasco unos 300-400 años atrás, en el siglo XVII, lugar desde donde posteriormente la mutación se extendió a las regiones vecinas del norte de España [Paisán-Ruiz et al., 2004; Mata et al., 2005 (b); Simón-Sánchez et al., 2006 (b); González-Fernández et al., 2007; Mata et al., 2009 (a)].

La frecuencia de la mutación R1441G en nuestro medio, a juzgar por los resultados obtenidos en nuestro estudio, es muy inferior a la observada en el País Vasco y Asturias (Tabla 6), confirmando la presencia de un gradiente geográfico en la frecuencia de esta mutación en las regiones del norte de España. Ninguno de los dos pacientes en los que encontramos la mutación R1441G su familia era de origen vasco. En uno, la familia era originaria del norte de España, en concreto de León, mientras que el otro paciente su familia provenía de Valencia. La presencia de la mutación R1441G en pacientes de Catalunya se puede explicar por la existencia de una migración relativamente reciente de personas originarias del País Vasco, o bien por una migración más antigua, pues existen estudios de genética poblacional que señalan la existencia de un flujo genético del País Vasco a otras poblaciones europeas, y en especial a la catalana, durante los últimos milenios [Hurles et al., 1999].

Hasta hace muy poco no se habían detectado pacientes con la mutación R1441G fuera de España [Paisán-Ruiz et al., 2004; Berg et al., 2005; Di Fonzo et al., 2006; Pérez-Pastene et al., 2007]. Sin embargo recientemente se han identificado dos pacientes con esta mutación, uno en Estados

Unidos y otro en Uruguay [Deng et al., 2006 (a); Mata et al., 2009 (b)]. La paciente norte-americana con EP y la mutación R1441G es de origen hispano y aparentemente no tiene ancestros españoles [Deng et al., 2006 (a)]. En el paciente uruguayo con la mutación R1441G, sus ancestros parecen ser italianos, pero este paciente presenta un haplotipo en la región del gen LRRK2 diferente al descrito en los pacientes vascos con la mutación R1441G, sugiriendo la posibilidad de que la mutación en este paciente uruguayo pudiera provenir de un fundador diferente [Mata et al., 2009 (b)]. El hecho de que la mutación R1441G pudiera estar presente en otras poblaciones sin tener un origen ancestral vasco, tan solo hace remarcar más la idea de que el codón 1441 del gen LRRK2 es un codón predispuesto a mutar ("*mutational hot spot*"), tal y como también lo demuestra el hecho que en este codón se han descrito otras mutaciones, la R1441C y la R1441H [Zimprich et al., 2004 (a); Zabetian et al., 2005], las cuales también parecen derivar de múltiples fundadores diferentes [Haugarvoll et al., 2008; Ross et al., 2009].

Tabla 6. Frecuencia de la mutación R1441G en el gen LRRK2 en diferentes poblaciones de la Península Ibérica

Población [Referencia]	Pacientes con EP		Casos familiares de EP		Casos esporádicos de EP		Controles	
	Nº casos	Nº R1441G	Nº casos	Nº R1441G	Nº casos	Nº R1441G	Nº casos	Nº R1441G
ESPAÑA								
Catalunya [Gaig et al., 2006]	**302**	**2 (0,7%)**	**94**	**2 (2,6%)**	**208**	**0**	**NA**	**NA**
País Vasco [Paisán-Ruiz et al., 2004]	137	11 (8%)	30	6 (20%)	107	5 (4,6%)	80	0
País Vasco [González Fernández et al., 2007]	67	10 (14,9%)	50	10 (20%)	17	0	NA	NA
Asturias [Mata et al., 2005 (b)]	225	5 (2,2%)	NA	NA	225	5 (2,2%)	100	0
País Vasco [Gorostidi et al., 2009]	418	55 (13,1%)	181	49 (27,1%)	237	6 (2,5%)	1	0
Andalucia [Gao et al., 2009]	187	3 (1.6%)	15	0	172	3 (1.7%)	287	1 (0.3%)
Cantabria [Infante et al., 2006]	98	0	16	0	82	0	310	0
PORTUGAL								
Portugal [Bras et al., 2005]	124	0	22	0	102	0	126	0
Portugal [Ferreira et al., 2007]	138	0	31	0	107	0	101	0

NA:No-aplicable

6.1.3 La mutación R1441C

La mutación R1441C del gen LRRK2 la hemos detectado en uno de nuestros pacientes (0,3%). En un estudio internacional se incluyeron los 33 pacientes con la mutación R1441C que se habían identificado en el mundo hasta ese momento [Haugarvoll et al., 2008, Apéndice 1]. Estos 33 pacientes pertenecían a 20 familias de diversos países como Italia, Alemania, Bélgica, Irlanda, los Estados Unidos o Singapur, e incluía el paciente que hemos identificado con la mutación R1441C. En

este estudio se analizaron los polimorfismos alrededor del gen LRRK2 en el cromosoma 12 y se identificaron diversos haplotipos. Un haplotipo era común en los pacientes italianos, alemanes, norte-americanos y en nuestro paciente de origen español [Di Fonzo et al., 2006; Haugarvoll et al., 2008]. Un segundo haplotipo se identificó en los paciente belgas y en la familia D, la familia norte-americana en la que inicialmente se identificó la mutación R1441C [Zimprich et al., 2004 (a); Haugarvoll et al., 2008; Nuytemans et al., 2008]. Un paciente alemán y otro irlandés compartían un tercer haplotipo, que no pudo ser bien definido y que podría ser diferente a los otros dos haplotipos identificados. Finalmente el paciente de Singapur con la mutación R1441C presentaba unos alelos que no podían ser asignados a ninguno de los otros haplotipos. Así pues, existen como mínimo dos haplotipos diferentes asociados a la mutación R1441C del gen LRRK2, lo que indicarían la presencia de al menos dos fundadores independientes de esta mutación [Haugarvoll et al., 2008].

6.2 Mutaciones en el gen LRRK2: Historia familiar, casos esporádicos y penetrancia reducida

Solo 9 (56,3%) de los 16 pacientes con mutaciones en el gen LRRK2 que hemos identificado presentaban antecedentes familiares de EP. La ausencia de historia familiar en los 7 (43,7%) de los casos restantes se puede explicar de diversas formas. Una posible explicación para estos casos aparentemente esporádicos es el no diagnóstico de la EP en los familiares del paciente, cuando probablemente estos habían padecido la enfermedad en vida al haber desarrollado un temblor en la vejez. Otras posibilidad es que los padres, abuelos u otros familiares portadores de la mutación hubieran fallecido tempranamente, sin haber tenido suficiente tiempo para desarrollar la enfermedad [Kachergus et al., 2005]. Otras posibles explicaciones para los casos aparentemente esporádicos podrían ser la falsa paternidad, la penetrancia incompleta [Gilks et al., 2005], o bien que la mutación hubiera aparecido de “novo”. Esta última posibilidad, que la mutación hubiera aparecido de “novo” en un sujeto, es posible como lo indica la presencia de diferentes haplotipos asociados a una misma mutación del gen LRRK2 que sugieren la presencia de múltiples fundadores comunes no relacionados entre si [Zabetian et al., 2006 (a); Zabetian et al., 2006 (b); Haugarvoll et al., 2008; Ross et al., 2008].

Un ejemplo de penetrancia reducida o incompleta es la madre de una de nuestras pacientes con la mutación G2019S del gen LRRK2. Esta madre tenía 91 años edad, y si bien no mostraba ningún síntoma ni signo de parkinsonismo, se confirmó que era portadora de la mutación. Otras personas de edad avanzada y sin enfermedad neurológica pero portadoras de mutaciones en el gen LRRK2 han sido también descritas [Kay et al., 2005], e incluso en alguna de ellas se ha podido realizar el estudio postmortem del tejido cerebral sin hallarse pérdida neuronal en la sustancia negra ni otras alteraciones indicativas de neurodegeneración incidental, como podrían ser la presencia de cuerpos de Lewy u otras inclusiones de sinucleína [Ross et al., 2006 (a)].

Aún no se conoce con exactitud cual es la penetrancia de las mutaciones del gen LRRK2, pero parece ser que la probabilidad de que una persona portadora de una mutación en este gen desarrolle la enfermedad a lo largo de la vida esta entre el 30 y el 85% [Kachergus et al., 2005;

Goldwrum et al., 2007; Haugarvoll et al., 2008]. Un trabajo estudió la penetrancia de la mutación G2019S en 13 familias con esta mutación [Kachergus et al., 2005]. En total analizaba a 20 pacientes con EP, incluyendo los probandos de las familias, y 15 sujetos asintomáticos, todos ellos portadores de la mutación G2019S. En este trabajo se observó que la penetrancia era dependiente de la edad, pues se incrementaba del 17% a la edad de 50 años al 85% a los 70 años [Kachergus et al., 2005]. Lesage y colaboradores al estudiar 13 familias francesas y del Norte de África con EP autosómica dominante y la mutación G2019S, observaron una penetrancia del 33% a los 50 años, y del 100% a los 75 [Lesage et al., 2005 (a)].

Sin embargo en otro estudio, realizado en 19 familias con la mutación G2019S, se observó una menor penetrancia, del 15% a los 50 años y del 32% a los 80 años [Goldwrum et al., 2007]. En este estudio se incluyeron 51 personas con la mutación, 10 de ellas afectas por la enfermedad. Con el objetivo de evitar un sesgo de selección, se excluyeron del análisis de la penetrancia a los probandos con EP de cada una de las 19 familias, lo que explicaría la menor penetrancia observada en este estudio en comparación con los trabajos previos [Goldwrum et al., 2007]. Esta estimación más baja se aproxima más a la penetrancia de la mutación G2019S calculada en los judíos Asquenazíes portadores de la mutación G2019S, que es del 32-35% a lo largo de la vida [Ozelius et al., 2006]. En otro estudio realizado en 29 familias con EP y la mutación G2019S se estimó una penetrancia del 10% entre los 50-54 años de edad, y del 67% entre los 90-94 años [Latourelle et al., 2008]. En este estudio solo se incluyeron en el análisis de la penetrancia a los padres de los probandos.

Un estudio más reciente, en el que participó nuestro centro, ha estimado una penetrancia intermedia entre los estudios previamente comentados [Healy et al., 2008; Apéndice 2]. En este estudio se incluyeron 1045 personas pertenecientes a 133 familias con la mutación G2019S del gen LRRK2. De estas 1045 personas, 327 eran pacientes con EP (incluyendo probandos y casos no probandos de cada familia) y 718 eran familiares no afectos. Se estimó mediante un análisis de supervivencia de Kaplan-Meier un riesgo de padecer una EP del 28% a los 59 años, del 51% a los 69 años y del 74% a los 79. En este estudio también se analizaron casos con mutaciones en el gen LRRK2 diferentes a la G2019S y se estimó una penetrancia para ellas del 40% a los 59 años de edad, del 64% a los 69 años, y del 84% a los 79 [Healy et al., 2008].

Los estudios de casos y controles o de cohortes de familias para determinar la penetrancia de una mutación pueden conllevar un sesgo de selección. Los estudios basados en poblaciones seleccionadas aleatoriamente y que analizan los casos incidentales pueden evitar este sesgo de selección. En este sentido, otro estudio reciente realizado por Hulihan y colaboradores analizó la presencia de la mutación G2019S del gen LRRK2 en 238 pacientes con EP esporádica y 371 controles reclutados aleatoriamente entre la población berebere de Túnez [Hulihan et al., 2008]. Se identificaron 72 (30%) pacientes y 7 (2%) controles con la mutación, y a partir de estos 79 sujetos, y sin incluir a sus familiares, se analizó la penetrancia de la mutación G2019S, que se estimó en menos del 20% antes de los 50 años y en más del 80% en los sujetos mayores de 70 años.

La penetrancia podría ser diferente en función del tipo de mutación en el gen LRRK2. En un trabajo en el que colaboramos y que analizaba la penetrancia de la mutación R1441C, se observó que menos del 20% de los portadores de la mutación tenían síntomas de la enfermedad antes de los 50 años, mientras que a los 75 años el 90% de ellos la habían desarrollado [Haugarvoll et al., 2008]. En la familia japonesa (*"Sagamihara kindred"*) en la que se identificó el locus cromosómico donde se halla el gen LRRK2 y que es portadora de la mutación I2020T, los análisis de segregación enfermedad-haplotipo asociado, han estimado una penetrancia del 65% [Funayama et al., 2002]. En cambio en las familias vascas con la mutación R1441G se ha sugerido una penetrancia casi del 100% a los 80 años [Paisán-Ruiz et al., 2004]. En un estudio mas reciente, en el que se incluyeron 68 con EP y portadores de la mutación R1441G y 239 familiares asintomáticos, se ha estimado una penetrancia de la enfermedad del 12,5% a los 65 años y del 83,4% a los 80 para la mutación R1441G [Ruiz-Martínez et al., 2010]

Por lo tanto, la penetrancia de las mutaciones del gen LRRK2 es incompleta y dependiente de la edad, lo que explicaría en parte la presencia de casos sin antecedentes familiares de la enfermedad. La penetrancia reducida también explicaría por que en algunas familias la enfermedad puede presentar un patrón de herencia aparentemente autosómico recesivo (pseudorecesivo), como podría ser la afectación de 2 hermanos sin la presencia de antecedentes de EP en los padres o en otras generaciones de la familia [Lesage et al., 2007], o bien la detección de mutaciones en el gen LRRK2 en personas de edad avanzada sin enfermedad neurológica [Kay et al., 2005].

La penetrancia incompleta y la edad de inicio de la enfermedad variable sugieren que otros factores genéticos diferentes a las mutaciones en el gen LRRK2, o factores ambientales, o bien una combinación de ambos, podrían tener un papel relevante en el desarrollo de la enfermedad. La descripción en familias con mutaciones en el gen LRRK2 de fenocopias, es decir familiares afectos de EP pero que no son portadores de la mutación, si bien pueden representar una mera coincidencia de una enfermedad frecuente como la EP idiopática en una familia con la mutación, también sugiere que en algunas de estas familias otros factores genéticos o ambientales podrían influenciar el desarrollo de la enfermedad [Kachergus et al., 2005; Nichols et al., 2005; Hernández et al., 2005 (a); Latourelle et al., 2008]. En una de nuestras pacientes con la mutación G2019S, su hermana presentaba una EP pero no era portadora de la mutación. Nichols y colaboradores identificaron 5 fenocopias en 19 parejas de hermanos con EP y la mutación G2019S en uno de ellos [Nichols et al., 2005], mientras que Latourelle y colaboradores detectaron 9 fenocopias en 29 familias con esta mutación [Latourelle et al., 2008]. Se han identificados familias en las que coexisten mutaciones en el gen LRRK2 y en otros genes que causan formas monogénicas o mendelianas de parkinsonismo, como el gen de la parkina [Dächsel et al., 2006; Ferreira et al., 2007; Marras et al., 2010] o el gen GIGYF2 (o TNRC15; PARK11) [Lautier et al., 2008], o bien con mutaciones en genes como el de la glucocerebrosidasa, cuya homocigosis causa la enfermedad de Gaucher y que en estado de heterocigoto podría incrementar el riesgo de padecer la EP [Gan-Or et al., 2008], o bien con el numero de repeticiones CAG/CAA de la ataxia espinocerebelosa tipo 2 (SCA2) [Charles et al., 2007] o del haplotipo H1 del

gen tau [Golub et al., 2008], que también se han asociado a un mayor riesgo para desarrollar una EP. Sin embargo aun no se conoce ningún factor genético o ambiental que modifique la expresión (edad de inicio, gravedad de la enfermedad) del parkinsonismo asociado a mutaciones en el gen LRRK2.

6.3 Características clínicas de los pacientes con mutaciones en el gen LRRK2

6.3.1 Edad de inicio de la enfermedad

En nuestro trabajo la edad media de inicio de la enfermedad en los pacientes portadores de mutaciones en el gen LRRK2 fue de 57,1 años, siendo similar a la edad media de inicio de los pacientes con EP en los que no detectamos mutaciones en este gen, que era de 53,7 años. Dos casos con la mutación G2019S presentaban una edad de inicio temprana, antes de los 40 años, concretamente a los 33 y 35 años de edad, mientras que tres casos presentaron un inicio posterior a los 70 años, el más tardío de ellos a los 78 años. La media y el rango de edades de inicio de la enfermedad es similar a la observada en otros estudios con pacientes con parkinsonismo y mutaciones en el gen LRRK2 [Kachergus et al., 2005; Gilks et al., 2005; Ferreira et al., 2007; González-Fernández et al., 2007; Healy et al., 2008; Haugarvoll et al., 2008].

En el estudio internacional en el que colaboramos y en el cual se incluyeron 356 pacientes con EP y mutaciones en el gen LRRK2 (327 de ellos tenían la mutación G2019S), la edad media de inicio de la enfermedad fue de 58,1 años [Healy et al., 2008]. Cuando se comparó la edad media de inicio entre los pacientes con la mutación G2019S y los pacientes con otras mutaciones en el gen LRRK2, se observó que estas eran similares, siendo del 57,5 años para la mutación G2019S y de 59,9 años para las otras mutaciones en este gen. En el estudio internacional sobre la mutación R1441C en el que también colaboramos, se observó una edad media de inicio de la enfermedad de 60 años para los pacientes portadores de esta mutación [Haugarvoll et al., 2008].

La mayoría de pacientes con mutaciones en el gen LRRK2 presentan una edad al inicio de la enfermedad superior a los 50 años. Un 8% de los pacientes desarrollan la enfermedad antes de los 40 años, y menos de un 3% después de los 80 [Healy et al., 2008]. Estos datos concuerdan con el incremento de la penetrancia en las diferentes mutaciones en el gen LRRK2 a partir de los 50 años de edad [Kachergus et al., 2005; Goldwrum et al., 2007; Haugarvoll et al., 2008].

6.3.2 Síntomas motores

En la mayoría de nuestros pacientes con parkinsonismo y mutaciones en el gen LRRK2, así como en los reportados en la literatura, los síntomas motores parkinsonianos presentan habitualmente un inicio unilateral. El temblor de reposo, seguido de la lentitud motora y la combinación de ambos síntomas son las alteraciones motoras más frecuentes al comienzo. Habitualmente afectan a la extremidad superior, en algunas ocasiones tanto al brazo como a la pierna, y más raramente afectan a la extremidad inferior de forma aislada [Di Fonzo et al., 2005; Gilks et al., 2005; Ferreira et al., 2007; Haugarvoll et al., 2008].

A lo largo de la enfermedad, en la mayoría de pacientes la asimetría inicial persiste, siendo más grave en el hemicuerpo donde se inicio la enfermedad, tal y como sucede en la EP idiopática. En nuestro estudio hasta el 22,3% de los pacientes no presentaron temblor de reposo a pesar de una duración considerable de la enfermedad. Habitualmente, a medida que la enfermedad progresa los pacientes presentan con frecuencia inestabilidad postural o trastornos de la marcha con bloqueos o caídas, lo cual también ocurre en la EP idiopática [Paisán-Ruiz et al., 2005 (a); Di Fonzo et al., 2005; Nichols 2005 et al., Gilks et al., 2005; Aasly et al., 2005; Hernandez et al., 2005 (a); Ferreira et al., 2007; Healy et al., 2008].

La respuesta al tratamiento con levodopa en nuestros pacientes con mutaciones en el gen LRRK2, así como en la gran mayoría de pacientes reportados en la literatura [Aasly et al., 2005; Hernandez et al., 2005 (a); Healy et al., 2008], es excelente o buena. Muchos de ellos desarrollan complicaciones motoras como fluctuaciones y discinesias inducidas por la levodopaterapia, que en ocasiones son invalidantes y requieren tratamientos quirúrgicos como la palidotomía o la estimulación cerebral profunda a nivel del núcleo subtalámico [Di Fonzo et al., 2005; Nichols 2005 et al., Aasly et al., 2005; Ferreira et al., 2007; Healy et al., 2008]. Tres de nuestros pacientes con la mutación G2019S fueron tratados con estimulación cerebral profunda subtalámica bilateral, consiguiéndose controlar con éxito las complicaciones motores secundarias al tratamiento con levodopa que padecían. Diversos estudios sugieren que los pacientes con mutaciones en el gen LRRK2 pueden ser tan buen candidatos al tratamiento con estimulación cerebral profunda como los pacientes con EP idiopática [Schüpbach et al., 2007; Healy et al., 2008].

Las características clínicas no parecen diferir entre pacientes con diferentes mutaciones del gen LRRK2 [Ferreira et al., 2007; Healy et al., 2008; Haugarvoll et al., 2008]. Tampoco se han observado diferencias en cuanto a las características clínicas entre pacientes homocigotos y heterocigotos para la mutación G2019S del gen LRRK2 [Ishihara et al., 2008], lo que indicaría la ausencia de un efecto de sobredosificación génica, que si ocurriría en otras formas mendelianas de EP, como por ejemplo en las multiplicaciones del gen de la sinucleína [Singleton et al., 2003; Ibañez et al., 2004; Farrer et al., 2004].

En ninguno de nuestros pacientes con parkinsonismo y mutaciones en el gen LRRK2 se observaron signos atípicos para la EP como oftalmoparesia supranuclear o la presencia temprana de disfunción autonómica severa o demencia. Estos signos atípicos son en general infrecuentes en pacientes con mutaciones en el gen LRRK2. En diversos estudios no se han detectado la presencia mutaciones del gen LRRK2 en pacientes con un diagnóstico clínico de parkinsonismo atípico, como DCL, PSP, DCB o AMS [Hernandez et al., 2005 (b); Tan et al., 2006; Ozelius et al., 2007]. Tampoco se han detectado mutaciones del gen LRRK2 en otros estudios que incluían pacientes con clínica de temblor esencial [Deng et al., 2006 (b)], demencia tipo Alzheimer [Hernandez et al., 2005 (b); Toft et al., 2005 (b); Lee et al., 2006; Zabetian et al., 2007] u otras enfermedades neurodegenerativas como la esclerosis lateral amiotrófica [Whittle et al., 2007].

En base a lo expuesto, puede concluirse que las características del síndrome motor en el parkinsonismo asociado a mutaciones en el gen LRRK2, tanto al inicio como en las fases más avanzadas de la enfermedad, así como la respuesta al tratamiento con levodopa y las complicaciones derivadas, son en la gran mayoría de casos similares a los observados en la EP clásica. Sin embargo pueden haber excepciones, con pacientes que presenten un enfermedad más benigna, como una de nuestras pacientes con la mutación G2019S quien presentaba un fenotipo clínico raramente observado en pacientes con EP idiopática y caracterizado por la presencia de un temblor de reposo aislado en una pierna durante más de 8 años sin síntomas motores adicionales ("*Monosymptomatic rest tremor- like*"). Algún estudio reciente, como el de Healy y colaboradores, sugieren que los pacientes con mutaciones en el gen LRRK2 podrían tener una enfermedad más benigna, con una progresión algo más lenta que los pacientes con EP sin mutaciones en este gen [Healy et al., 2008].

6.3.3 Síntomas no motores: demencia y trastornos psiquiátricos

En general los síntomas no motores en los pacientes con EP y mutaciones en el gen LRRK2 parecen ocurrir con una frecuencia similar a la EP idiopática [Healy et al., 2008]. Los síntomas disautonómicos como la disfunción urinaria, el estreñimiento o la disfunción eréctil, así como los trastornos del sueño como el insomnio o el trastorno de conducta de sueño REM, ocurrirían con frecuencia en los pacientes con mutaciones en el gen LRRK2 [Goldwrum et al., 2006; Ferreira et al., 2007; Healy et al., 2008]. Los trastornos psiquiátricos como la depresión o la ansiedad también son frecuentes en pacientes con mutaciones en el gen LRRK2. Hasta el 47% de nuestros pacientes con mutación en este gen presentaban o habían presentado depresión, ansiedad o ambos trastornos psiquiátricos.

Ciertos síntomas no motores podrían ser menos frecuentes en pacientes con mutaciones en el gen LRRK2 que en pacientes con EP sin estas mutaciones. Se ha sugerido por ejemplo, un menor riesgo de desarrollar demencia en estos pacientes [Aasly et al., 2005; Hernández et al., 2005 (a); Healy et al., 2008]. Tan solo uno de nuestros pacientes, con la mutación R1441G, presentó una demencia a los 15 años de enfermedad. La demencia en este paciente presentaba unas características muy similares a la demencia asociada a la EP idiopática, con alucinaciones visuales, ideas delirantes y fluctuaciones en el estado cognitivo.

También se ha sugerido que los pacientes con mutaciones en el gen LRRK2 podrían tener una frecuencia menor de hiposmia [Healy et al., 2008]. Si bien algún estudio ha demostrado una pérdida del sentido del olfato similar entre pacientes con la mutación G2019S y pacientes sin esta mutación [Silveira-Moriyama et al., 2008], otros estudios indican que tan sólo el 43-51% de los pacientes con la mutación G2019S del gen LRRK2 presentarían hiposmia [Healy et al., 2008; Silveira-Moriyama et al., 2010], lo que contrasta con la frecuencia de hiposmia habitualmente reportada en pacientes con EP que es superior al 70% [Katzenschlager et al., 2004].

6.4 Fenotipo neuropatológico de los pacientes con mutaciones en el gen LRRK2

De entre los 110 casos con un diagnóstico clínico-patológico de diversas enfermedades neurodegenerativas que hemos estudiado, hemos encontrado mutaciones en el gen LRRK2 en 2 casos. Ambos pacientes presentaban la mutación G2019S y en vida habían sido diagnosticados de EP. En el examen neuropatológico ambos presentaban pérdida neuronal en la sustancia negra, pero tan solo uno de ellos esta pérdida neuronal se acompañaba de cuerpos y neuritas de Lewy. El otro caso no mostró patología de Lewy ni ningún otro tipo de inclusión distintiva.

En un caso con un diagnóstico clínico de EP y presencia de patología tipo Lewy en el estudio postmortem identificamos la variante sinónima R1441R, localizada en el mismo codón donde se hallan otras mutaciones patogénicas del gen LRRK2 (R1441C/R1441G y R1441H). Sin embargo, no creemos que esta variante genética sea patogénica, pues no produce ningún cambio de significado en la codificación de aminoácidos ni la hemos detectado en otros pacientes con EP que hemos estudiado. Esta variante sinónima tampoco produce una alteración en el "splicing" del gen LRRK2, al no asociarse con la presencia de transcritos alternativos al esperado.

En ninguno de nuestros casos con un diagnóstico clínico-patológico de EP u otra enfermedad neurodegenerativa detectamos la mutación en el gen LRRK2 de origen vasco, la R1441G. Recientemente se ha descrito el examen neuropatológico de un paciente con EP y la mutación R1441G, mostrando la presencia de pérdida neuronal en la sustancia negra sin cuerpos de Lewy ni otras inclusiones específicas [Martí- Massó et al., 2009].

6.4.1 Neuropatología subadyacente asociada a la mutación G2019S del gen LRRK2

Con los dos casos que hemos aportado, se han descrito en la literatura el examen histopatológico de un total de 21 pacientes con parkinsonismo asociado a la mutación G2019S del gen LRRK2 (Tabla 7) [Gilks et al., 2005; Ross et al., 2006 (a); Giasson et al., 2006; Rajput et al., 2006; Chen-Plotkin et al., 2007; Silveira-Moriyama et al., 2008]. Dieciocho de estos 21 casos mostraron patología por cuerpos de Lewy, tal y como ocurre en la EP clásica, mientras que dos casos presentaban pérdida neuronal en la sustancia negra sin cuerpos de Lewy ni otras inclusiones distintivas. Finalmente un caso presentaba una taupatía con ovillos neurofibrilares similares a los observados en la PSP (Tabla 7). Así pues, se puede decir que el hallazgo neuropatológico más frecuente en el parkinsonismo secundario a la mutación G2019S es la pérdida neuronal en la sustancia negra asociado a patología tipo Lewy.

6.4.2 Parkinsonismo, degeneración nigral inespecífica y mutaciones en el gen LRRK2

En la literatura se han descrito diversos pacientes con parkinsonismo asociado a mutaciones en el gen LRRK2 en los que el examen postmortem mostraba pérdida neuronal en la sustancia negra sin cuerpos de Lewy ni otras inclusiones distintivas (Tabla 7) [Hasegawa et al., 1997; Wszolek et al., 2004 ; Zimprich et al., 2004 (a); Khan et al., 2005; Giasson et al., 2006]. Algunos de estos pacientes

con degeneración nigral inespecífica presentaban inclusiones intranucleares ubiquitina positivas, mientras que algunos otros casos presentaban inclusiones intracitoplasmáticas parecidas a las observadas en la DLFT con inclusiones ubiquitina positivas (DLFT-U) (Tabla 7). Tan solo uno de estos casos con degeneración nigral inespecífica y mutación en el gen LRRK2 era portador de la mutación G2019S [Giasson et al., 2006]. El paciente que hemos identificado es el segundo caso descrito con degeneración nigral inespecífica y la mutación G2019S, y confirma que esta mutación del gen LRRK2 puede presentar este tipo de cambios histopatológicos, sin la presencia de cuerpos de Lewy o de otras inclusiones distintivas. A diferencia de nuestro paciente, el otro caso descrito con degeneración nigral inespecífica y mutación G2019S, no presentaba inclusiones intranucleares (ni intracitoplasmáticas) ubiquitina positivas [Giasson et al., 2006].

Tabla 7. Hallazgos neuropatológicos en casos con parkinsonismo y mutaciones en el gen LRRK2 reportados hasta finales del año 2009

Mutación en el gen LRRK2	Número de casos reportados [Referencias]	Neuropatología			
		Patología tipo LB	Taupatía (PSP "*like*")	DLFT-U	Degeneración nigral inespecífica
G2019S	21 [Gilks et al., 2006 ; Giasson et al., 2006 ; Ross et al., 2006 (a) ; Rajput et al., 2006 ; Silveira-Moriyama et al., 2008 ; Chen-Plotkin et al., 2009]	18	1	-	2
I2020T	7* [Hasegawa et al.,1997 ; Funayama et al., 2002; Hasegawa et al., 2008]	1	-	-	6
R1441C	4 [Wszolek et al., 2004]	2	1	1	-
R1441G	1 [Martí-Massó et al., 2009]	-	-	-	1
Y1699C	3 [Wszolek et al., 2004; Khan et al., 2005]	1	-	2	-

Se incluyen los dos casos que hemos identificado. LB: Lewy bodies; PSP: Parálisis supranuclear progresiva; DLFT-U: Degeneración lobar frontotemporal con inclusiones ubiquitina positivas

* Los siete casos con la mutación I2020T corresponden todos ellos a la familia japonesa *"Sagamihara kindred"*. Un sujeto de esta familia no ha sido incluido en la tabla. Este sujeto es portador de la mutación y en vida padeció un parkinsonismo compatible con una atrofia multisistémica (AMS) que el examen postmortem confirmó. En este caso con AMS, si bien la mutación I2020T podría haber causado la enfermedad, otra posibilidad sugerida por los autores es que pudiera tratarse de la coincidencia de una AMS en un sujeto portador de esta mutación en el gen LRRK2.

En nuestra paciente con degeneración nigral inespecífica, la pérdida neuronal en la sustancia negra era leve y en vida presentó un cuadro clínico compatible con la EP, con un inicio unilateral con temblor de reposo y buena respuesta a la levodopa. El curso de la enfermedad fue lentamente

progresivo, en especial durante los primeros diez años. Posteriormente, durante los últimos 4 años de enfermedad la paciente empeoró de forma más rápida, con un importante trastorno de la marcha. Este parkinsonismo, relativamente benigno al menos durante los primeros años de la enfermedad, podría correlacionarse con la pérdida neuronal leve observada en la sustancia negra de la paciente. Otra posible explicación al parkinsonismo relativamente benigno que presentó esta paciente podría ser la ausencia de patología tipo Lewy. Los pacientes con parkinsonismo por mutaciones en el gen de la parkina presentan habitualmente degeneración nigral inespecífica, sin inclusiones de sinucleína, y su parkinsonismo frecuentemente presenta un curso más lentamente progresivo y benigno que la EP idiopática [Mori et al., 1998; Hayashi et al., 2000; Khan et al., 2003]. Es posible que la ausencia de agregados de sinucleína en forma de cuerpos de Lewy pudiera ser un marcador de un proceso degenerativo menos agresivo, con una pérdida neuronal en la sustancia negra más lenta que en aquellos casos que si presentan patología tipo Lewy.

La presencia de inclusiones intranucleares ubiquitina positivas en forma de cuerpos de Marinesco en las neuronas de la sustancia negra de nuestra paciente también se ha observado en otros pacientes con mutaciones en el gen LRRK2 [Zimprich et al., 2004 (a)]. El significado patológico de los cuerpos de Marinesco es desconocido, pues estas inclusiones intranucleares ubiquitina positivas se observan con frecuencia en sujetos de edad avanzada sin enfermedad neurológica. Algún estudio, sin embargo sugiere, que los cuerpos de Marinesco podrían tener un significado patológico [Beach et al., 2004]. Estos cuerpos intranucleares se han asociado con una reducción de los marcadores dopaminérgicos estriatales. Además se ha observado que las neuronas dopaminérgicas con cuerpos de Lewy presentan con más frecuencia cuerpos de Marinesco que aquellas neuronas dopaminérgicas sin cuerpos de Lewy. Finalmente, en cultivos celulares se ha observado que la disfunción de sistema ubiquitina-proteosoma genera inclusiones intranucleares similares a los cuerpos de Marinesco [Beach et al., 2004]. Todos estos datos indican que los cuerpos de Marinesco podrían representar la agregación de proteínas por la disfunción del sistema ubiquitina-proteosoma y jugar quizás, un papel directo o indirecto en la degeneración de las neuronas dopaminérgicas.

6.4.3 Ausencia de mutaciones en el gen LRRK2 en enfermedades neurodegenerativas diferentes a la enfermedad de Parkinson

No hemos detectado la mutación G2019S ni ninguna de las mutaciones en el codón 1441 (R1441G/C/H) del gen LRRK2 en ningún caso con un diagnóstico clínico-patológico de DCL, PSP, DCB, AMS o DLFT. La mayoría de estudios realizados en pacientes con un diagnóstico clínico o patológico de estas enfermedades neurodegenerativas tampoco han sido capaces de detectar mutaciones en el gen LRRK2 [Giasson et al., 2006; Ross et al., 2006 (a); Ross et al., 2006 (b); Hernandez et al 2005 (b); Tan et al., 2006; Madzar et al., 2009]. Tan solo recientemente se ha detectado la presencia de la mutación G2019S en el gen LRRK2 en una paciente de 79 años de edad que en vida presentó un cuadro clínico de demencia pero sin parkinsonismo, y en quien el estudio postmortem mostró la presencia de una DLFT-U [Dächsel et al., 2007]. Esta paciente presentaba antecedentes familiares de temblor. En otro estudio más reciente, la mutación G2019S se detectó en

un paciente con un síndrome clínico de DCB pero sin confirmación neuropatológica [Chen-Plotkin et al., 2008]. La mutación G2019S también se ha detectado en otro sujeto de 89 años sin parkinsonismo pero con una enfermedad de Alzheimer neuropatológicamente confirmada [Ross et al., 2006 (a)].

La descripción de estos casos aislados de mutaciones en el gen LRRK2 en pacientes con enfermedades degenerativas como la enfermedad de Alzheimer o la DCB podrían representar una simple coincidencia. Por ejemplo, en el caso de la enfermedad de Alzheimer no se han detectado mutaciones en el gen LRRK2 en series largas de pacientes con esta enfermedad neurodegenerativa tan frecuente [Hernandez et al., 2005 (b); Toft et al., 2005 (b)]. Además, las mutaciones en el gen LRRK2 presentan una penetrancia reducida [Kachergus et al., 2005; Goldwrum et al., 2007] y no siempre causan enfermedad neurológica en vida, como lo demuestra la descripción de sujetos de edad avanzada sin enfermedad neurológica y portadores de una mutación en este gen [Kay et al., 2005], siendo en alguno de ellos el examen neuropatológico normal [Ross et al., 2006 (a)]. El caso de la paciente con una DLFT-U y mutación G2019S es sin embargo más controvertido [Dächsel et al., 2007], pues algunos pacientes con parkinsonismo y mutaciones en el gen LRRK2 presentan un fenotipo neuropatológico con pérdida neuronal e inclusiones intracitoplasmáticas ubiquitina positivas en la sustancia negra, muy parecidas a las observadas en este subtipo de DLFT [Zimprich et al., 2004 (a)].

Un hecho posiblemente relevante es que no hemos detectado en ningún caso con un diagnóstico clínico-patológico de DCL las mutaciones G2019S o del codón 1441 del gen LRRK2. Otros estudios tampoco han detectado estas u otras mutaciones en el gen LRRK2 en pacientes con un diagnóstico clínico o clínico-patológico de DCL [Toft et al., 2005 (b); Ross et al., 2006 (a); Giasson et al., 2006; Rajput et al., 2006; Haubenberger et al., 2007; Chen-Plotkin et al., 2008]. Dado que el sustrato neuropatológico más frecuentemente asociado a la mutación G2019S es la patología tipo Lewy (Tabla 7), es destacable el hecho de que no se hallan descrito casos de DCL, definida según los criterios actuales (es decir, la demencia aparece en el primer año de la enfermedad [McKeith et al., 2005]), que sean portadores de esta u otras mutaciones en el gen LRRK2. La ausencia de mutaciones en el gen LRRK2 en casos de DCL concordaría con los estudios que sugieren que los pacientes con EP y mutaciones en el gen LRRK2 parecen tener un menor riesgo de desarrollar demencia [Aasly et al., 2005; Hernández et al., 2005 (a); Healy et al., 2008]. Algunos autores consideran que la EP y la DCL, dadas sus similitudes clínicas y su sustrato neuropatológico indistinguible, representarían expresiones clínicas diferentes de una misma enfermedad [Lippa et al., 2007]. De confirmarse la ausencia o la rareza de las mutaciones en el gen LRRK2 en pacientes con DCL, el mejor conocimiento de los mecanismos fisiopatogénicos a través del cual las mutaciones en este gen causan neurodegeneración, nos podría ayudar a esclarecer que mecanismos biológicos conllevan a la diferente presentación clínica de las enfermedades por cuerpos de Lewy (EP, EP con demencia y DCL).

6.4.4 ¿Es la proteína LRRK2 un componente de los cuerpos de Lewy?

Un tema que sigue siendo controvertido es la posibilidad de que la proteína LRRK2 sea un constituyente de los cuerpos de Lewy o que existan inclusiones positivas para LRRK2 en determinados casos con mutaciones en este gen [Greggio et al. 2006; Zhu et al., 2006(a); Zhu et al., 2006 (b); Covy et al., 2006; Giasson et al., 2006]. Nosotros no fuimos capaces de detectar inclusiones patológicas en la sustancia negra ni de teñir los cuerpos de Lewy mediante anticuerpos anti-LRRK2 en los dos casos con la mutación G2019S en el gen LRRK2 identificados, ni en un caso adicional estudiado con EP esporádica. La posibilidad de que la proteína LRRK2 sea un constituyente de los cuerpos de Lewy sigue siendo un tema de discusión, con diversos trabajos en la literatura apoyando esta posibilidad [Alegre-Abarrategui et al., 2008; Perry et al., 2008], y otros señalando lo contrario y sugiriendo que los resultados positivos son probablemente debidos a la falta de especificidad de los anticuerpos anti-LRRK2 disponibles en la actualidad, los cuales reaccionarían de forma cruzada con otras proteínas presentes en los cuerpos de Lewy [Covy et al., 2006; Higashi et al., 2007].

7. CONCLUSIONES

CONCLUSIONES

1) La frecuencia de mutaciones en el gen LRRK2 en pacientes con enfermedad de Parkinson que atienden a una consulta especializada en un hospital terciario de Barcelona es del 5,3%, siendo la mutación G2019S la más frecuente (el 4,3% de los pacientes son portadores). La mutación en el gen LRRK2 "de origen vasco," la R1441G, es más infrecuente (0,7%), y la mutación R1441C es muy infrecuente (0,3%).

2) Las mutaciones en el gen LRRK2 son más frecuentes en casos familiares de enfermedad de Parkinson (un 9,6% de los casos son portadores) que en casos esporádicos (un 3,4% de los pacientes son portadores). Hasta en un 43,7% de los pacientes con mutaciones en el gen LRRK2 no existen antecedentes familiares de parkinsonismo.

3) Las características clínicas de los síntomas motores y no motores del parkinsonismo asociado a mutaciones en el gen LRRK2 es indistinguible de los observados en la enfermedad de Parkinson clásica. Tanto los trastornos psiquiátricos, como la depresión o la ansiedad, así como un cuadro de demencia, se pueden observar en el parkinsonismo asociado a mutaciones en el gen LRRK2.

4) Las alteraciones neuropatológicas del parkinsonismo asociado a la mutación G2019S del gen LRRK2 son heterogéneas. Si bien el sustrato neuropatológico más frecuente es la patología tipo Lewy, describimos un caso que confirma que uno de los fenotipos neuropatológicos asociados a la mutación G2019S es la degeneración nigral inespecífica sin inclusiones distintivas.

5) No hemos detectado mutaciones en el gen LRRK2 en cerebros asociados a otro tipo de enfermedad neurodegenerativa diferente a la enfermedad de Parkinson, como la demencia por cuerpos de Lewy, la parálisis supranuclear progresiva o la atrofia multisistémica. Su existencia habría que considerarla probablemente excepcional o coincidental.

8. Apéndices

Apéndice 1

Haugarvoll K, Rademakers R, Kachergus JM, Nuytemans K, Ross OA, Gibson JM, Tan EK, Gaig C, Tolosa E, Goldwurm S, Guidi M, Riboldazzi G, Brown L, Walter U, Benecke R, Berg D, Gasser T, Theuns J, Pals P, Cras P, De Deyn PP, Engelborghs S, Pickut B, Uitti RJ, Foroud T, Nichols WC, Hagenah J, Klein C, Samii A, Zabetian CP, Bonifati V, Van Broeckhoven C, Farrer MJ, Wszolek ZK.

Lrrk2 R1441C parkinsonism is clinically similar to sporadic Parkinson disease.

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Lrrk2 R1441C parkinsonism is clinically similar to sporadic Parkinson disease

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ABSTRACT

Objective: Leucine-rich repeat kinase 2 (*LRRK2*) mutations are the most common cause of Parkinson disease (PD). Several dominantly inherited pathogenic substitutions have been identified in different domains of the Lrrk2 protein. Herein, we characterize the clinical and genetic features associated with Lrrk2 p.R1441C.

Methods: We identified 33 affected and 15 unaffected *LRRK2* c.4321C>T (p.R1441C) mutation carriers through an international consortium originating from three continents. The age-specific cumulative incidence of PD was calculated by Kaplan-Meier analysis.

Results: The clinical presentation of Lrrk2 p.R1441C carriers was similar to sporadic PD and Lrrk2 p.G2019S parkinsonism. The mean age at onset for parkinsonism was 60 years, range 30–79 years; fewer than 20% of the patients had symptoms before the age 50 years, while by 75 years >90% of them had developed symptoms. Haplotype analysis suggests four independent founders for the p.R1441C mutation.

Conclusions: The distribution in age at onset and clinical features in Lrrk2 p.R1441C patients are similar to idiopathic and Lrrk2 p.G2019S parkinsonism. Several independent founders of the p.R1441C substitution suggest this site is prone to recurrent mutagenesis.

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GLOSSARY

COR = C-terminal of Roc; **GTPase** = guanosine triphosphatase; **LBD** = Lewy body disease; **PD** = Parkinson disease; **SNPs** = single nucleotide polymorphisms.

The recent discovery of mutations in the leucine-rich repeat kinase 2 (*LRRK2* [MIM *609007]) gene in clinically typical, late-onset Parkinson disease (PD) highlights the role of genetics in this disorder.[1,2] To date, pathogenicity has been demonstrated for five Lrrk2 protein substitutions (p.R1441C, p.R1441G, p.Y1699C, p.G2019S, and p.I2020T).

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Table Clinical and pathologic features in *LRRK2* c.4321C>T (p.R1441C) mutation carriers

Features	Values
Mean ± SD age in affected carriers, y; range (n)*	68 ± 10; 50–85 (33)
Mean ± SD age in unaffected carriers, y; range (n)†	62 ± 14; 33–84 (15)
Mean ± SD age at onset, y; range	60 ± 13; 30–79
Mean ± SD Unified Parkinson Disease Rating Scale III; range	19 ± 11; 6.5–42
Mean ± SD Hoehn & Yahr staging; range	2.5 ± 0.8; 1.5–5
Asymmetry (n)‡	Present (17)
Levodopa response (n)§	Favorable (25)
Familial/sporadic Parkinson disease, n	29/4
Additional symptoms (n)	Depression (9), fluctuations (10), anxiety (6), mild cognitive impairment (4), dementia (1), hallucinations (2)
Pathologic features (n)	Nigral degeneration in all (4), Lewy bodies in brainstem (1), widespread Lewy body disease(1), tau deposits (1)

Data from the last neurologic examination; however, the presence of asymmetry was recorded at the initial examination. Pathology findings are presented in references 2 and 22.
*Includes living mutation carriers.
†Includes deceased individuals and three obligate mutation carriers.
‡No asymmetry was found in three patients and no data were available in 13 patients.
§Two had no response to levodopa; data are not available on six patients.

They are located within the guanosine triphosphatase (GTPase), the C-terminal of Roc (COR), and the kinase domains of the protein. Furthermore, the p.G2385R substitution in the WD40 motif of Lrrk2 is consistently associated with increased risk for PD in the Asian population.[3-7]

The Lrrk2 p.R1441 amino acid residue in the GTPase domain is the second most common location of pathogenic Lrrk2 substitutions, after p.G2019S. The p.R1441 residue may be prone to mutagenesis as the two pathogenic substitutions (p.R1441C; c.4321C>T and p.R1441G; c.4321C>G) and one putatively pathogenic substitution (p.R1441H, c.4322G>A) affect the very same residue. An additional putative pathogenic substitution (p.A1442P; c.4324G>C) was recently reported in the adjacent amino acid.[8]

Further clinical and genetic investigation may translate neurogenetic discoveries into improved care for patients. These studies provide a framework for advancing our understanding of the molecular pathology of parkinsonism and may guide future functional research. The present collaboration represents a worldwide initiative to collect mutation carriers and characterize clinical and genetic features of the pathogenic Lrrk2 p.R1441C substitution.

METHODS

Study population. This international consortium was formed to identify all reported Lrrk2 p.R1441C substitution carriers. A Medline search was conducted using the terms *LRRK2* and *R1441C*, including publications up to June 2007.[2,9-16] Additionally, unreported mutation carriers who had come to the attention of any of the participating centers were included. We identified 33 affected and 15 unaffected p.R1441C substitution carriers.

PD was diagnosed by movement disorder neurologists according to published criteria and all participating neurologists filled out a clinical evaluation form designed for this study (Z.K.W.).[17] If available for genetic and clinical evaluation, family members of the index case were also examined. Study instruments included the Unified PD Rating Scale and Hoehn and Yahr staging.[18,19] A summary of the clinical characteristics of the affected mutation carriers is provided in the table. The institutional review boards at each participating institution approved the study.

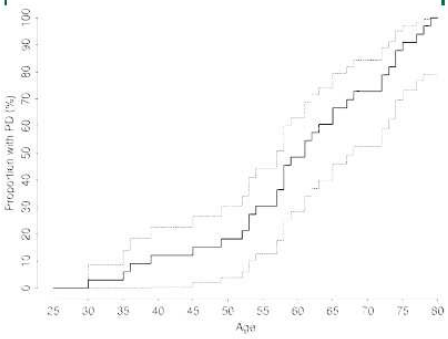
Genomic DNA were extracted from peripheral blood using standard protocols. Direct sequencing of exon 31 was used to verify *LRRK2* c.4321C>T (p.R1441C) mutation carrier status identified through screening procedures and to determine genotypes for adjacent single nucleotide polymorphisms (SNPs). Sequencing was performed utilizing the Applied Biosystems Big-Dye Terminator v3.1 Cycle Sequencing kit. Data were analyzed with SeqScape software version 2.1.1 (Applied Biosystems). Microsatellites were amplified by PCR using fluorescently labeled primers, run on an ABI 3730 genetic analyzer and analyzed using GeneMapper 4.0 Software (Applied Biosystems). Microsatellite allele sizes were normalized using CEPH-control DNA (1331-01 and 1331-02). Twenty-seven markers (9 SNPs and 18 microsatellites) spanning 16 Mb across the *LRRK2* locus were systematically selected to determine whether subjects carrying the *LRRK2* c.4321C>T (p.R1441C) mutation shared a common haplotype. Seventy-four mutation carriers and noncarriers from 20 families were genotyped to determine the disease-carrying p.R1441C haplotypes as shown in figure e-1 on the *Neurology*® Web site at www.neurology.org. In each family, all available individuals were genotyped to establish gametic phase. In smaller families where phase could not be established allele sharing was used to assign the most likely haplotype.

Statistical analysis. PHASE v.2.1.1 was used to estimate the frequency of the *LRRK2* c.4321C>T haplotypes in 80 Caucasian US controls previously genotyped for microsatellite markers within the *LRRK2* locus.[20,21] Haplotype frequencies were only estimated when phase could be established. The age-specific cumulative incidence of PD in affected p.R1441C substitution carriers was estimated using the Kaplan-Meier method, considering age at onset as the time variable.

RESULTS

We identified 33 affected and 15 unaffected Lrrk2 p.R1441C carriers from 20 families, including four patients with no family history of parkinsonism. Clinical features were comparable

Figure Cumulative incidence of PD in affected carriers



Kaplan-Meier curve of age-specific cumulative incidence of Parkinson disease (PD) calculated from 33 affected Lrrk2 p.R1441C substitution carriers, including the 95% CIs (dotted).

to typical, late-onset sporadic PD including all four cardinal signs of PD (tremor, bradykinesia, rigidity, and postural instability). The age-specific cumulative incidence was calculated only from affected carriers (figure). The clinical spectrum of disease includes asymmetry at symptom onset and a favorable response to levodopa therapy. Nonmotor symptoms included hallucinations, depression, anxiety, cognitive impairment, and pain, as frequently reported in sporadic PD (table). The most common initial symptom in Lrrk2 p.R1441C patients was rest tremor (57%), followed by bradykinesia (18%) and mixed motor symptoms (18%). One patient was reported to have dementia and four additional patients had mild cognitive impairment; there were no data on the cognitive status in 6 of the 33 affected Lrrk2 p.R1441C carriers. There were insufficient data on Lrrk2 p.R1441C carriers who did not have evidence for parkinsonism to comment on possible nonmotor complications.

Pathology findings have previously been reported for four Lrrk2 p.R1441C carriers from Family D (Western Nebraska).[22] Lewy body disease (LBD) was found in two patients but the pathologic spectrum also included pure dopaminergic cell loss in the substantia nigra (n = 1) without distinctive pathology and tau pathology (n = 1) (table).[2]

Genotyping of Lrrk2 p.R1441C carriers from 20 families revealed two major haplotypes for which gametic phase could be established, of four classes in total (figure e-1). The first haplotype class was identified in all Italian patients, as well as in German, Spanish, and American patients. The second haplotype was present in all Belgian families and the American Family D (Western Nebraska). This indicates a minimum of two independent founders.[9,14] A German and an Irish patient shared a third haplotype for which phase could not be determined. In addition, the proband from Singapore carried alleles that could not be assigned to any of the other haplotype classes. Genetic analysis employing PHASE v2.1.1 estimates that the two haplotypes most commonly found in *LRRK2* c.4321C>T (p.R1441C) mutation carriers each have a frequency of <1% in 80 control subjects. The allele frequencies in US control subjects were comparable with frequencies obtained from 300 Belgian control subjects (data not shown). The small number of carriers did not allow mutation age to be estimated from the size and genetic variability within each haplotype class.[4]

DISCUSSION The present study examines clinical and genetic features of Lrrk2 p.R1441C substitution carriers from three continents. The clinical spectrum of Lrrk2 p.R1441C substitution carriers includes all four cardinal signs of PD (table). Furthermore, asymmetry at disease onset and a favorable response to levodopa therapy were frequently present as seen in sporadic PD.[23] A family history of parkinsonism was observed in most cases, and may be the only clinical feature that differentiates Lrrk2 p.R1441C substitution carriers from sporadic PD. Indeed, all cases examined herein would fulfill published criteria for a diagnosis of idiopathic PD.[17]

The age-specific cumulative incidence of PD in carriers of the Lrrk2 R1441C mutation is shown in the figure. Fewer than 20% of the mutation carriers showed PD symptoms before the age 50 years, while at age 75 years >90% of them had developed symptoms. Accurate penetrance estimates are crucial for proper genetic counseling. Our figures on the age-specific cumulative incidence of disease must be interpreted with caution as they may overestimate the risk for PD in p.R1441C mutation carriers. This is due to ascertainment bias as most carriers were identified in studies targeting series of large, multicase PD families (thereby biased toward high penetrance). The penetrance estimate for a given mutation may be lower if measured among carriers from unselected, consecutive series of patients (including familial and sporadic PD), even more so, in studies from population-based cohorts. The rarity of Lrrk2 p.R1441C makes prospective population studies difficult, although less biased penetrance estimates on Lrrk2 p.G2019S carriers,

which are especially frequent in Ashkenazi and Berber-Arab peoples, may be feasible.[24,25]

Our genetic investigation of p.R1441C mutation carriers reveals evidence of several founders originating from different parts of the world (figure e-1).[20] Lrrk2 p.R1441C is evidently a hotspot for mutation events. Indeed, this peptide region appears especially susceptible as two other putatively pathogenic amino acid substitutions, p.R1441G and p.R1441H, affect the same residue, and a third, p.A1442P, is adjacent. These residues are normally highly conserved across species and even between the ancestral Lrk1 within invertebrates and Lrrk1 and Lrrk2 in vertebrate radiations.[26]

The present study highlights the clinical overlap between p.R1441C substitution carriers, p.G2019S carriers, and idiopathic PD, indicating that the effect of mutations in different domains of the Lrrk2 protein lead to similar phenotypes. It is hypothesized that *LRRK2* mutations cause PD through a dominant gain of function effect.[20] Lrrk2 p.G2019S appears to increase kinase activity in several studies but results are conflicting regarding the impact of other substitutions on Lrrk2 kinase activity, including p.R1441C located in the GTPase domain.[27] However, GTP binding to the Roc domain may be critical for Lrrk2 phosphorylation and subsequent kinase activation.[28-30] Lrrk2 mutations may exert their effects by interfering with the cellular and stoichiometric interaction of Lrrk2 with its binding partners or alter Lrrk2 cellular stability and localization.[27]

As for the Lrrk2 p.G2019S substitution, LBD may be the most frequent pathologic finding in p.R1441C carriers particularly in cases where no atypical signs are present (e.g., supranuclear palsy). The pathologic spectrum can also include pure dopaminergic cell loss in the substantia nigra without distinctive pathology and tau pathology.[2] These findings highlight that the clinical syndrome referred to as PD may be present in the absence of LBD.

The discovery of *LRRK2* mutations in clinically typical, late-onset PD provides an unprecedented opportunity to advance our knowledge regarding the molecular mechanisms defining the disease and to develop better animal models for initial compound screening. Prospective studies may further elucidate the clinical course of Lrrk2 parkinsonism. Genetic testing may not be warranted in routine clinical practice; however, it is crucial for further research. Asymptomatic mutation carriers will provide insight into the preclinical disease course, facilitate biomarker development, and may be the first to benefit from neuroprotective treatment aimed at halting disease progression.

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Apéndice 2

Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, Brice A, Aasly J, Zabetian CP, Goldwurm S, Ferreira JJ, Tolosa E, Kay DM, Klein C, Williams DR, Marras C, Lang AE, Wszolek ZK, Berciano J, Schapira AH, Lynch T, Bhatia KP, Gasser T, Lees AJ, Wood NW; International LRRK2 Consortium.

Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study.

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Phenotype, genotype, and worldwide genetic penetrance of *LRRK2*-associated Parkinson's disease: a case-control study

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Summary

Background Mutations in *LRRK2*, the gene that encodes leucine-rich repeat kinase 2, are a cause of Parkinson's disease (PD). The International *LRRK2* Consortium was established to answer three key clinical questions: can *LRRK2*-associated PD be distinguished from idiopathic PD; which mutations in *LRRK2* are pathogenic; and what is the age-specific cumulative risk of PD for individuals who inherit or are at risk of inheriting a deleterious mutation in *LRRK2*?

Methods Researchers from 21 centres across the world collaborated on this study. The frequency of the common LRRK2 Gly2019Ser mutation was estimated on the basis of data from 24 populations worldwide, and the penetrance of the mutation was defined in 1045 people with mutations in *LRRK2* from 133 families. The *LRRK2* phenotype was defined on the basis of 59 motor and non-motor symptoms in 356 patients with *LRRK2*-associated PD and compared with the symptoms of 543 patients with pathologically proven idiopathic PD.

Findings Six mutations met the consortium's criteria for being proven pathogenic. The frequency of the common LRRK2 Gly2019Ser mutation was 1% of patients with sporadic PD and 4% of patients with hereditary PD; the frequency was highest in the middle east and higher in southern Europe than in northern Europe. The risk of PD for a person who inherits the LRRK2 Gly2019Ser mutation was 28% at age 59 years, 51% at 69 years, and 74% at 79 years. The motor symptoms (eg, disease severity, rate of progression, occurrence of falls, and dyskinesia) and non-motor symptoms (eg, cognition and olfaction) of *LRRK2*-associated PD were more benign than those of idiopathic PD.

Interpretation Mutations in *LRRK2* are a clinically relevant cause of PD that merit testing in patients with hereditary PD and in subgroups of patients with PD. However, this knowledge should be applied with caution in the diagnosis and counselling of patients.

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Introduction

Parkinson's disease (PD) affects 1–2% of people older than 65 years. Respective mutations in five genes—*SNCA* (α-synuclein), *PARK2* (parkin), *PARK7* (DJ-1), *PINK1*, and *LRRK2*—can cause parkinsonism that resembles idiopathic PD.[1] *LRRK2* encodes the 51-exon, multidomain protein, leucine-rich repeat kinase 2 (LRRK2); mutations in *LRRK2* cause an autosomal dominant PD that, in most patients, produces a α-synuclein-type neuropathology.[2–4]

Although individual *LRRK2* genotype–phenotype correlations have been reported,[5–7] these have been reported for a mean of only five mutation carriers per study, and the results can not be easily compared because of the use of different clinical approaches, sample sizes, diagnostic criteria, and genotyping techniques.

The International *LRRK2* Consortium was established to pool worldwide data to answer important clinical questions through collective experience and research. Three questions have been considered by the consortium: can *LRRK2*-associated PD be distinguished from idiopathic PD; which mutations in *LRRK2* are pathogenic; and what is the age-specific cumulative risk of PD for individuals who inherit or are at risk of inheriting a deleterious mutation in *LRRK2*?

Methods

Patients and procedures

Researchers at 21 primary centres collaborated on this study. The clinical and genetic data were collected and analysed at The National Hospital for Neurology and Neurosurgery, Queen Square, London, UK. The study was approved by the joint Research Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery, London, UK. Informed consent was obtained from each patient by the local ethics committees.

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Clinical data were collected at each participating centre on a standardised, two-part pro forma questionnaire. Each patient was assessed by a neurologist who was a specialist in movement disorders, and a separate pro forma was completed for each patient. The average time to complete the pro forma was 21 mins per patient. Most of the clinical data were collected prospectively and not specifically for this project. The first part of the pro forma consisted of 13 sections that focused on population demographics and genotyping techniques for the patients and controls. Each centre provided details on the number of patients with PD who were screened for mutations in *LRRK2*, their ethnic backgrounds, and the number of controls used, to confirm the pathogenicity of novel mutations. The second part of the pro forma was a 59-section clinical questionnaire on motor and non-motor symptoms. The pro formas were completed by the neurologist.

Validated scales were used to assess olfaction (University of Pennsylvania smell identification test [UPSIT]),[8] cognition (the mini-mental state examination),[9] and to stage the disease and its progression (the Hoehn and Yahr scale[10]). We attempted to assess the severity of other symptoms, such as anxiety, depression, sleep disturbance, dysautonomia, and dystonia, with unified standardised criteria; however, this was difficult to standardise across centres, and instead we chose a simplified "symptom present", "symptom absent", or "symptom not recorded" scoring method because it was more reproducible.

The rate of disease progression was measured by the time taken to reach each stage on the Hoehn and Yahr scale and the percentage of patients at each stage of the scale with PD for longer than 10 years, starting from symptom onset rather than time of diagnosis.

The clinical features of mutations in *LRRK2*-associated PD were compared with a series of 543 clinically documented, pathologically proven cases of idiopathic PD from the Queen Square Brain Bank (QSBB). All QSBB patients were screened for the LRRK2 Gly2019Ser mutation, and carriers were excluded. The same phenotyping methods were used for patients with mutations in *LRRK2* as were used in the QSBB group.

Mutation-screening methods were not standardised; however, most centres used direct DNA sequencing, and we relied on the quality controls at each participating centre. Mutations were arbitrarily designated as 'proven' pathogenic if they were found in three or more unrelated participants, or if the mutation segregated within a large family with many members who had PD and was not found in more than 1000 ethnically matched controls. The consortium acknowledges that many reported mutations do not fulfil these criteria but are likely to be pathogenic.

Statistical analysis

The penetrance of all mutations in *LRRK2* (age-specific cumulative risk of PD) was estimated with the Kaplan-Meier life table survival method and maximum-likelihood estimates calculated with the pedigree analysis package for Java (jPAP Version 1.5.0).[11,12] Kaplan-Meier survival function was calculated for mutation carriers and individuals whose probability of carrying a mutation was greater than 90% (as inferred from jPAP) in affected and unaffected individuals.[13] No minimum size was imposed on families (ie, the analysis was not restricted to large families with many affected [penetrating] members). To further minimise any ascertainment bias, the index case of each pedigree was excluded. Similarly, sporadic (singleton) cases were excluded when data were not available on at least both parents. A Kaplan-Meier curve to estimate the time to onset of PD was plotted using the R project for statistical computing,[14] and the curve was inverted to give an empirical, cumulative-risk step function.

Maximum-likelihood estimates of age-specific penetrance were calculated by maximising the log likelihood of seeing the variant genotypes while restricting affection probabilities to age-specific incidence intervals. PD incidence rates were based on previous estimates in 588 patients.[15] The frequency of the disease allele was set to 0·001. The model assumed that an earlier disease onset corresponded to a higher liability. The incidences determine a series of points that are ordered inversely on the liability scale. The genotype-specific probability of an individual developing PD at a particular age was evaluated within the corresponding liability interval. For an unaffected individual, the age was right censored at the age of last follow-up or death, and the genotype-specific affection probability evaluated from the upper limit of that liability interval to infinity. The sample had ascertainment correction as per the method of Cannings and Thompson.[16]

The variant effect was characterised by dominance and displacement. If the disease allele is denoted as A, the dominance is calculated by the rate of differences between the mean probabilities of the heterozygous and homozygous carriers of the disease-variant ([μAa–μaa]/[μAA–μaa]) and the wild-type homozygotes. The dominance was set to equal 1 in this analysis, to investigate the variant effect with a dominant contribution towards the phenotype. The displacement is the difference between the mean liabilities of the homozygotes relative to the total standard deviation within genotypes ($\mu_{AA}-\mu_{aa}$)/□. The total mean liability and the variance (□²) were restricted to equal 0 and 1, respectively. Ascertainment correction was done by maximising the conditional likelihood of the pedigree, given the phenotypic and genotypic information of the index case. A likelihood-ratio test was used to assess the heterogeneity of risk by sex, ethnic group (white or not white), and mutation. Owing to the high frequency of LRRK2 Gly2019Ser carriers in our sample, all the other pathogenic mutations were analysed together.

Role of the funding source

The study sponsors had no role in the study design, the conduct of the study, data collection, data analysis, data interpretation, nor writing of the report. The investigators

had full responsibility for submitting the manuscript for publication. The corresponding author had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the analysis.

Results

19 376 unrelated patients with PD were genotyped for *LRRK2* mutations. *LRRK2* 6055G→A, the mutation that causes LRRK2 Gly2019Ser, was found in 201 families and in 179 patients with apparently sporadic PD. Table 1 summarises the frequency of LRRK2 Gly2019Ser in 24 populations; there were insufficient data to estimate the population frequencies of mutations other than LRRK2 Gly2019Ser; therefore, clinical data are reported as all mutations in *LRRK2*, the combination of mutations in *LRRK2* other than *LRRK2* 6055G→A, and *LRRK2* 6055G→A (LRRK2 Gly2019Ser) only.

The worldwide frequency of LRRK2 Gly2019Ser was 1% of patients with sporadic PD and 4% of patients with hereditary PD (table 1). The highest frequency of LRRK2 Gly2019Ser was seen in north African Arabs (hereditary 36%, sporadic 39%) and Ashkenazi Jews (hereditary 28%, sporadic 10%). The frequency was higher in southern European countries than in northern European countries. LRRK2 Gly2019Ser was rarely seen in Asians (Chinese, Japanese, Korean, and Indian); it was found in only four of 4172 patients (<0·1%). Completed pro formas were available for 356 patients with PD (hereditary or sporadic) who had any mutation in *LRRK2*, 313 of whom had LRRK2 Gly2019Ser. The mean disease duration was shorter for patients with any mutation in *LRRK2* than for patients with idiopathic PD (10·9 years [SD 7·8 years] *vs* 15·1 years [SD 7·1 years], mean difference 4·2 years, 95% CI 3·2–5·2; p<0·0001).

The mean age of PD onset for all *LRRK2* mutation carriers was 58·1 years (14·0 years). This did not differ significantly by sex, and was similar in patients with LRRK2 Gly2019Ser (57·5 years [13·9 years] and carriers of all LRRK2 non-Gly2019Ser mutations (59·9 years [13·8 years]). Patients in the QSBB series developed PD at a slightly older age than *LRRK2* mutation carriers (61·0 years [10·9 years], mean difference 2·9 years, 95% CI 1·3–4·5; p<0·0001). 15 patients (3%) with LRRK2 Gly2019Ser developed PD after the age of 80 years, including five patients who developed PD after 90 years.

Mutations in *PARK2* (parkin) are the second most common genetic cause of parkinsonism after mutations in *LRRK2*.[17,18] Across the consortium, we identified 184 homozygous carriers of *PARK2* mutations who had a mean age of PD onset of 29·2 years (10·4 years). Figure 1 shows the age of onset versus the cumulative percent of patients with mutations in *LRRK2*, *PARK2*, or in the QSBB series. *LRRK2*-associated PD develops at a younger age than the age of onset in the QSBB group; however, although the onset of *LRRK2*-associated PD occurs at a slightly younger age than idiopathic PD does, it is of little clinical relevance. More clinically useful is the difference in the age of PD onset between the patients with *LRRK2* mutations and those in the QSBB series, and patients who are homozygous for mutations in *PARK2*. Only 29 *LRRK2* mutation carriers (8%) or 25 patients with idiopathic PD (4%) developed symptoms of PD before the age of 40 years; by contrast, 155 (84%) of the patients

	Patients with sporadic PD		Patients with hereditary PD		Controls	
	N	Patients with mutations (%)	N	Patients with mutations (%)	N	Patients with mutations (%)
North African Arabs	56	22 (39%)	143	51 (36%)	739	4 (<1%)
Ashkenazi Jews	259	25 (10%)	78	22 (28%)	410	4 (1%)
Portuguese	317	13 (4%)	85	12 (14%)	100	0 (0%)
Chilean*	137	4 (3%)	29	1 (3%)	153	0 (0%)
Spanish	806	22 (3%)	283	14 (4%)	544	0 (0%)
Swedish*	200	4 (2%)	127	0 (0%)	200	0 (0%)
French	300	5 (2%)	174	5 (3%)	348	0 (0%)
Italian and Sardinian	2516	37 (2%)	633	26 (4%)	1040	1 (<1%)
North American (white)	2606	26 (1%)	1450	45 (3%)	4934	1 (<1%)
British	1145	9 (1%)	192	4 (2%)	1786	0 (0%)
Norwegian	371	3 (1%)	64	6 (1%)	572	0 (0%)
Russian*	157	1 (1%)	10	0 (0%)	126	0 (0%)
Irish	236	1 (<1%)	35	1 (3%)	212	0 (0%)
Greek*	235	1 (<1%)	0	0 (0%)	0	0 (0%)
German and Austrian	803	2 (<1%)	231	3 (1%)	436	0 (0%)
Australian*	578	2 (<1%)	252	6 (2%)	0	0 (0%)
Japanese*	526	1 (<1%)	60	1 (2%)	372	1 (<1%)
Indian*	718	1 (<1%)	82	0 (0%)	1200	0 (0%)
Serbian*	47	0 (0%)	51	2 (4%)	161	0 (0%)
Cretan	174	0 (0%)	92	1 (1%)	0	0 (0%)
Chinese*	1360	0 (0%)	973	1 (<1%)	938	0 (0%)
Basque	117	0 (0%)	41	0 (0%)	425	0 (0%)
Korean*	436	0 (0%)	17	0 (0%)	0	0 (0%)
Polish*	153	0 (0%)	21	0 (0%)	190	0 (0%)
Total white	10714	126 (1%)	3690	123 (3%)	10913	2 (<1%)
Total worldwide	14253	179 (1%)	5123	201 (4%)	14886	11 (<1%)

PD was subdivided into those with an affected first-degree relative (hereditary) and those without a family history of PD (sporadic/singleton). *No clinical data were received from these populations; estimates are based on published data.

Table 1: **Frequency of LRRK2 Gly2019Ser in patients with PD and controls across 24 world populations**

	Time of progression to H and Y stage		Proportion of patients at each H and Y stage with PD for 10 years or longer	
	All mutations (n=321)	Gly2019Ser (n=291)	All mutations (n= 257)	Gly2019Ser (n=226)
H and Y stage 1	4·0 years	4·1 years	6%	6%
H and Y stage 2	7·2 years	7·4 years	27%	31%
H and Y stage 3	9·4 years	9·1 years	45%	44%
H and Y stage 4	12·6 years	12·9 years	57%	61%
H and Y stage 5	15·6 years	16·2 years	79%	82%

Average number of years it took each patient to reach each stage of the H and Y scale and the percentage of patients at each stage who had symptoms for 10 years or longer. H and Y=Hoehn and Yahr scale

Table 2: **Rate of disease progression in patients with all *LRRK2* mutations and LRRK2 Gly2019Ser**

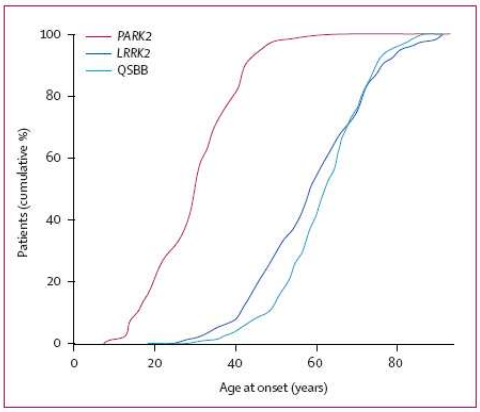

Figure 1: Age of PD onset plotted against the cumulative percentage of patients in the *PARK2*, *LRRK2*, or QSBB series
QSBB=Queen Square brain bank.

who were homozygous for *PARK2* mutations had presented with symptoms of PD by the age of 40 years.

The patients with mutations in *LRRK2* had a mean duration from the onset of PD to Hoehn and Yahr scale stage 2 (bilateral symptoms, no difficulty walking) of 7·2 years (SD 4·1 years) and a similar duration was seen between LRRK2 Gly2019Ser and LRRK2 non-Gly2019Ser carriers; however, the numbers in the latter group were small and did not include many different mutations. There was no difference when adjusted for age or sex. The rate of disease progression in *LRRK2* mutation carriers measured by the time to progression through each point on the Hoehn and Yahr scale and the percentage of those patients at each stage of the Hoehn and Yahr scale that had symptoms of PD for more than 10 years are summarised in table 2.

For clinicians, the occurrence of falls is a more practical measure of the severity of disease than disease rating scales. The mean time to first fall in patients with a mutation in *LRRK2* was longer than in the QSBB series (12·6 years [SD 7·9 years] *vs* 9·3 years [SD 5·9 years]; difference 3·3 years, 95% CI 2 ·4–4·2 years; p<0·0001).

At some point during the course of the disease, tremor, bradykinesia, and rigidity were seen in 93% (331) of patients with mutations in *LRRK2* and 94% (510) of patients in the QSBB series. Tremor was the most common presenting symptom in both groups (63% [224] of patients with mutations in *LRRK2* and 52% [282] in the QSBB series), then bradykinesia (27% [92] and 36% [198], respectively), and rigidity (10% [36] and 12% [65], respectively). The higher incidence of tremor in the patients with mutations in *LRRK2* than the QSBB was significant (odds ratio [OR] 1·49, 95% CI 1·1–2·0; p<0·003). Of the 271 patients (76%) with mutations in *LRRK2* who had descriptive accounts of their tremor, the tremors were characterised as 'rest' tremor in 73%, and 'leg' tremor—described by four independent centres as an abduction–adduction leg movement—was a first symptom in 9% of patients, compared with only three of 193 patients (2%) in the QSBB series who had descriptive accounts of their tremor.

Any form of dystonia was seen in 126 of 301 patients (42%) with mutations in *LRRK2* compared with 121 of 487 patients (25%) with idiopathic PD; in most patients this was a painful "off period" foot dystonia. Dystonia occurred during the first 2 years of the disease in 22 of 126 patients (18%) with mutations in *LRRK2* compared with 5 of 21 (4%) of patients with idiopathic PD (OR 4·5, 95% CI 2·4–8·4; p<0·0001). Only one patient with a mutation in *LRRK2* had dystonia before dopamine-replacement treatment. Atypical examples of dystonia that affected the arm (n=2), neck (n=2), tongue (n=1), and that caused blepharospasm (n=4) were also reported in patients with mutations in *LRRK2*.

Dopamine-replacement regimens varied across centres, but the clinical assessment of the responses were good or excellent in 88% (313 of 356) of patients, modest in 9% (32), and poor in 3% (10). The clinical responses were similar to those in patients with idiopathic PD (good or excellent in 83% [450 of 543], modest in 12% [65], and poor in 5% [28]). Patients with idiopathic PD needed treatment earlier than patients with mutations in *LRRK2*: the mean time from the onset of PD to the start of dopamine-replacement treatment was 4·01 years (SD 2·50 years) for patients with mutations in *LRRK2* and 3·03 years (2·90 years) for patients with idiopathic PD (difference 0·98 years, 95% CI 0·61–1·35 years; p<0·0001). 66 (19%) patients with mutations in *LRRK2* compared with 38 (7%) patients with idiopathic PD (p=0·003) were not on dopamine-replacement treatment 5 years after disease onset.

Drug-induced, interdose dyskinesia was reported by 206 (58%) patients with mutations in *LRRK2* and by 293 (54%) patients in the QSBB group. Although the incidence of dyskinesia was similar in both groups, the time to onset was longer in patients with mutations in *LRRK2* than in patients with idiopathic PD (8·4 years [SD 4·6 years] *vs* 5·6 years [SD 3·7 years], difference 2·8 years, 95% CI 2·3–3·3 years; p<0·0001). Only 39 (11%) patients with mutations in *LRRK2* were dyskinetic after 5 years of treatment, and only 114 (32%) were dyskinetic after 10 years of treatment. By comparison, 136 (25%) patients with idiopathic PD were dyskinetic after 5 years of treatment and 223 (41%) patients were dyskinetic after 10 years of treatment.

Stereotactic functional neurosurgery was done on 22 patients with mutations in *LRRK2*: 18 had unilateral or bilateral subthalamic nucleus stimulation, three had pallidotomy, and one had thalamotomy. The mean time from PD onset to surgery was 11·4 years (SD 6·2), and the indications were usually either motor fluctuation or dyskinesia. Of the 12 patients who had detailed measurements of clinical outcome, eight were good or excellent, two moderate, and two poor.

The sample sizes for each non-motor symptom were reduced owing to missing data or not using validated or self-reported diagnostic scales in routine practice. Formal mini-mental state examination data on cognition were obtained on 162 patients with mutations in *LRRK2*. These results and those for other neuropsychiatric symptoms are shown in figure 2. 37 patients (23%) with mutations in *LRRK2* had evidence of cognitive impairment (mini-mental state examination score ≤24) compared with 340 patients (70%) with idiopathic PD. The mean disease duration of patients with cognitive impairment was 15·2 years (SD 5·9 years) for those with mutations in *LRRK2* and 14·4 years (SD 5·9 years) for those with idiopathic PD. However, this must be considered in light of a mean 4·2 years (3·2–5·2 years) longer duration for the entire QSBB series. A more comparable measure is the proportion of patients who develop cognitive impairment within 2 years of symptom onset: 6 patients (3·4%) with mutations in *LRRK2* compared with 48 patients (9·8%) with idiopathic PD (p=0·0016).

The response rate for olfaction was low (n=43) because only UPSIT data were accepted. Abnormal olfaction was found in 22 patients (51%) with LRRK2 Gly2019Ser after a mean disease duration of 5·6 years (SD 4·3).

Urinary symptoms affected 58 of 204 (28%) of the patients with LRRK2 Gly2019Ser, but these did not vary significantly when stratified by disease duration or by mutation. The most common symptoms were frequency and urge incontinence. 48% (93 of 194) of patients had constipation. 11% of men with LRRK2 Gly2019Ser reported erectile dysfunction, all of whom were older than 60 years.

69% (186 of 268) of patients with LRRK2 Gly2019Ser had sleep disturbances; however, there was no significant difference from the controls when this symptom was stratified by disease duration or by mutation. Insomnia and sleep fragmentation were the most common symptoms. Formal sleep studies identified 13 patients with rapid eye movement (REM) sleep behaviour disorder and five patients with restless legs syndrome. However we do not have accurate frequency estimates for these two symptoms because most patients did not have formal sleep studies.

9% (30 of 342) of patients with *LRRK2*-associated PD had diabetes mellitus, which was unexpectedly high and might be due to the effect of multiple-comparison testing. The mean age of these diabetic patients was 54 years (range 42–66 years) but we could not establish the age of diabetes onset. The prevalences of other common medical conditions were similar to those reported for the general population.

There were no apparent differences between the patients with mutations in *LRRK2* and those with idiopathic PD groups with respect to employment history, exposure to toxins, or educational status; however, with the exception of smoking history (n=306), these sections of the pro forma were poorly completed. Smoking (past or present) was seen in 28% of patients with LRRK2 Gly2019Ser compared with 39% of the QSBB series. There was no correlation between smoking history and age of symptom onset.

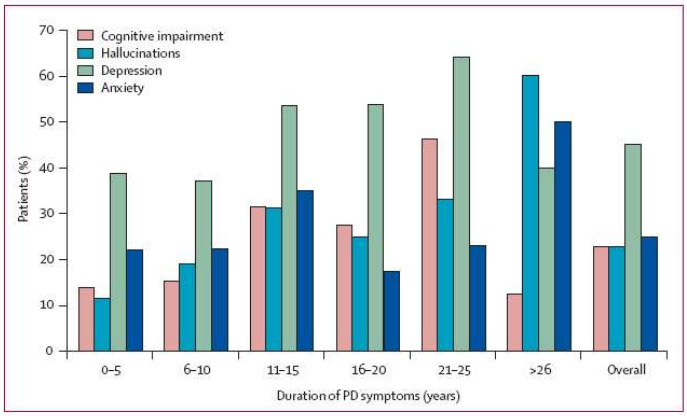

Figure 2: **Point prevalence of neuropsychiatric symptoms in patients with mutations in *LRRK2* after various durations of the symptoms of PD**

In this study, six mutations in *LRRK2* met the consortium's criteria for being 'proven pathogenic': Gly2019Ser (n=391), Arg1441Gly (n=33), Arg1441Cys (n=9), Arg1441His (n=5), Ile2020Thr (n=5), and Tyr1699Cys (n=2), where n is the number of unrelated individuals or families that carry the mutation. Conclusions made about mutations of low frequency are likely to be less robust than those drawn from mutations of higher frequency.

Penetrance estimates were calculated for LRRK2 Gly2019Ser on the basis of results from 1045 patients in 133 families. These comprised 327 affected patients (index and non-index cases) and 718 unaffected participants, with a mean of 8 individuals per family (range 3–45). Sporadic (singleton) cases were included when there were data on age and affection status from at least both parents (n=67). Similar calculations were made for all mutations in *LRRK2* combined, on the basis of results for 1387 patients in 152 families and 94 singletons.

Survival analysis was done on a subset of carriers after exclusion of all index cases (152). Kaplan-Meier analysis estimated the cumulative risk of PD as 36% at 59 years, 59% at 69 years, and 80% at 79 years. In the maximum-likelihood estimation, there were no significant differences in displacement by sex or ethnic group; however, there were significant displacements for patients with LRRK2 Gly2019Ser (mean displacement 1·66, SE 0·06; p< 0·02) and patients with the other mutations in *LRRK2* combined (mean displacement 2·00, SE 0·13; p<0·01). When this difference was taken into account, the cumulative risk for carriers of LRRK2 Gly2019Ser was 28% at 59 years, 51% at 69 years, and 74% at 79 years; the cumulative risk for carriers of LRRK2 non-Gly2019Ser mutations combined was 40% at 59 years, 64% at 69 years, and 84% at 79 years. Figure 3 shows the penetrance estimates calculated with the Kaplan-Meier and the maximum-likelihood methods.

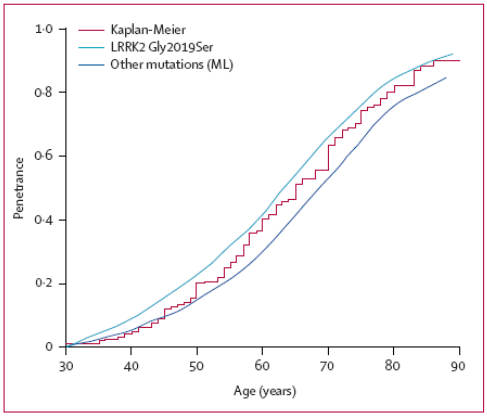

Figure 3: **Age-specific risk of PD**
Risk is estimated with the Kaplan-Meier method for the whole sample and with the maximum-likelihood estimation (ML) for all patients with mutations in *LRRK2* combined.

Discussion

Our prevalence data for *LRRK2*-associated PD imply that most clinicians who treat movement disorders will treat patients with this disorder at a clinically relevant frequency. For example, table 1 shows that 1% of white patients with sporadic PD and 3% with hereditary PD have the LRRK2 Gly2019Ser mutation, which means there is a prevalence of 3 cases per 100 000 people (estimate based on a PD prevalence of 200 cases per 100 000, and 15% of cases of PD are hereditary).[19] This prevalence is similar to those for other neurological disorders, such as multiple system atrophy (4 per 100 000), progressive supranuclear palsy (6 per 100 000), motor neuron disease (6 per 100 000), and common single-gene disorders, such as Huntington's disease (2 per 100 000) and haemophilia A (5 per 100 000).[20–23] Moreover, 3 cases per 100 000 is an underestimate of the overall prevalence of mutations in *LRRK2* because it does not include mutations other than LRRK2 Gly2019Ser or the higher frequency of LRRK2 Gly2019Ser in particular populations (table 1).

The core feature of the 356 patients with LRRK2 Gly2019Ser-associated PD was asymmetrical, tremor-predominant parkinsonism with bradykinesia and rigidity that responded to dopamine replacement and functional neurosurgery. With respect to these symptoms, patients with LRRK2 Gly2019Ser-associated PD are indistinguishable from patients with idiopathic PD. Tremor was more common in patients with LRRK2 Gly2019Ser-associated PD, and abduction–adduction leg tremor should be regarded as a useful diagnostic clue.

See Online for webtable

For **more on alternative genetic testing sites** see http://www.geneclinics.org

Non-motor symptoms generally occurred at similar frequencies in patients with LRRK2 Gly2019Ser to those reported for patients with idiopathic PD; however, patients with LRRK2 Gly2019Ser had a lower risk of cognitive impairment and hyposmia than did patients with idiopathic PD. The patients who had normal olfaction are an interesting subgroup that might have had their olfactory pathways spared. Owing to insufficient reliable olfactory data in the QSBB series, we were unable to make comparisons; however, to detect anosmia in only 51% of patients with mutations in *LRRK2* is unexpectedly low when olfactory dysfunction is reported in 80–100% of patients with idiopathic PD.[8,24]

Several factors imply that LRRK2 Gly2019Ser-associated PD is less severe than idiopathic PD. A more benign progression was seen: 6% of patients at Hoehn and Yahr scale stage 1 and 31% of patients at stage 2 had had PD for longer than 10 years. Another factor is a longer time to the first fall. Furthermore, patients with idiopathic PD needed dopamine-replacement treatment earlier than patients with LRRK2 Gly2019Ser did and were more prone to drug-induced dyskinesia.

Patients with LRRK2 Gly2019Ser-associated PD had a greater propensity to dystonia than did patients with idiopathic PD. Although this dystonia was nearly three times more probable in the first 2 years of the disease, it was seen only rarely in patients with LRRK2 Gly2019Ser-associated PD before dopamine-replacement treatment. This distinguishes LRRK2 Gly2019Ser-associated PD from PD due to recessive genes, in which pretreatment dystonia can be prominent.[25–27]

LRRK2 Gly2019Ser-associated PD can be easily distinguished from *PARK2*-associated PD on the basis of an older age of onset; the symptoms of PD were rare before 40 years in patients with LRRK2 Gly2019Ser-associated PD, whereas symptom onset after 40 years was rare in patients who were homozygous for mutations in *PARK2*. This rule of thumb has clinical uses, particularly in the genetic counselling of patients with sporadic PD and their affected siblings, in whom the mode of inheritance is often unclear.

A limitation of this study is that despite careful efforts to standardise data collection, the data are derived from international tertiary centres rather than from one community-based population, which introduces the possibility of bias.[28]

Until now, six mutations in *LRRK2* satisfy the requirements for proven pathogenicity as defined by the consortium. Until new data are reported, clinicians should be cautious about any interpretation and advice they give to patients with regard to other mutations in *LRRK2*, particularly because many of the non-synonymous mutations that were originally reported as pathogenic have been proven not to be. The webtable lists the consortium members who offer diagnostic (non-research) testing of *LRRK2*. A list of alternative testing sites is also available. Because exon 31 (Arg1441Cys, Arg1441Gly, Arg1441His) and exon 41 (Gly2019Ser, Ile2020Thr) are mutational hotspots, many institutions restrict testing to these exons.

Previous estimates of *LRRK2* penetrance were based on smaller sample sizes than reported here, and whether probands were included is not clear in some studies.[5,7,29,30,31]

The selection bias was minimised in this study by excluding the proband for all families and only including the families of sporadic (singleton) cases if there were data available on at least both parents. The authors of previous studies reported that the cumulative risk of PD by the age of 59 years ranges from 13–45%,[29,31] and from 21–85% at 69 years.[30,31] The range was widest at 79 years, with estimates from 32%[32] to 100%.[5,31] Figure 3 shows the intermediate values from the consortium data; for example, a person who inherits LRRK2 Gly2019Ser has a 28% risk of PD at 59 years, 51% at 69 years, and 74% at 79 years. This reduced penetrance explains the high prevalence of mutations in patients with sporadic PD and the occasional occurrence of mutations in controls. There was no difference in penetrance by sex or ethnic group; however, there was a suggestion that carriers of LRRK2 Gly2019Ser had lower penetrance than did carriers of other *LRRK2* mutations. Because the carriers of the other mutations are under represented in our sample, more investigations need to be done to assess the specific effects of these mutations.

The high concordance between the Kaplan-Meier and the maximum-likelihood estimates corroborate the reliability of these results and highlight the need for the careful selection of patients to obtain unbiased estimates with the Kaplan-Meier method. Indeed, the inclusion of putative carriers who were not validated by the segregation analysis, sporadic cases without family data, or index cases in the survival analysis resulted in, on average, a 10% increase in the penetrance estimates at any age range.

Hospital-based and volunteer patient proband series have been criticised for overestimating mutation penetrance because they can over-represent multiplex families (families with many affected individuals), and the data from these might not be appropriate to use when counselling patients with sporadic PD.[33] Multiplex families are also likely to share the combined influences of susceptibility genes and epigenetic and environmental factors, which increase risk but were not taken into account in the ascertainment adjustment. In this regard, our estimates are most appropriately used for the estimation of the age-specific cumulative risks of PD in patients with a known family history, either in those who have a mutation or in those who are at risk of having a mutation. Although the provision of penetrance data to the relatives of patients with sporadic PD who are carriers of mutations in *LRRK2* might be a challenge, if these relatives also carry the mutation, their risk of developing PD is at least no greater than the estimates in figure 3, and probably lower.

In general, we only recommend testing for the common LRRK2 Gly2019Ser mutation and only within the framework of pretest and post-test counselling. Without reliable penetrance estimates, we recommend that testing for other pathologically proven mutations is considered on a case-by-case basis.

There are two additional caveats. First, patients should be counselled that the frequency of mutations is greatly determined by ethnic group; for example, we showed that the common LRRK2 Gly2019Ser mutation is found in 10% of Ashkenazi Jews with sporadic PD and in 4% of Portuguese patients with sporadic PD but in only 1% of white North Americans. This means that it is probably only appropriate to test patients with sporadic PD who are in high-risk groups. Second, patients should be aware that the main benefit of testing is to improve diagnostic accuracy. The presence of a pathogenic mutation does not influence treatment choices, a point that also applies to the presymptomatic testing of individuals with a family history of PD, at least until such time as neuroprotective drugs are discovered.

Contributors

The study was thought of and designed by DGH, AHVS, AJL, and NWW. The data were acquired by all the authors, and were analysed by DGH, MJF, and SSO'S. DGH and AJL drafted the article, and all the authors reviewed the manuscript. DGH and MJF did the statistical analysis. Funding was obtained by DJH and NWW. The study was supervised by NWW, AJL, and DGH.

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Conflicts of interest

ZKW, MJF, and the Mayo Clinic have a financial interest in two technologies associated with this research. Both technologies have been patented and licensed to commercial entities. The Mayo Clinic has received royalties that exceed the federal threshold for considerable financial interest from the licensing of these technologies. ZKW and MJF have received no personal royalties for these technologies.

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