behaved in a sleep-like manner, and then lost her body tone. She slept for several tens of seconds, but continued to exhibit some automatic behaviors. She then woke up suddenly. These attacks were frequently seen after taking a meal. We concluded that these attacks were not epilepsy, because her EEG findings showed no epileptic discharge and the sleep attack disappeared after reducing the dose of levodopa and pergolide.

The report of a polysomnographic study showed that the REM latency was shorter in such patients than normal.[2] A report on MSLT documented its shorter latency. There have been some reports of a narcolepsy-like phenotype in patients with Parkinson's disease.[3-5] Her video images resembled narcolepsy clinically, although the sleep attack resolved after reducing her medication. Furthermore, no HLA-typing suggestive of narcolepsy was found.

The role of dopaminergic medications in sleep attack is not understood. Neither functional imaging of the dopamine transporter nor dopamine denervation has demonstrated any correlation between the dopamine system and sleepiness.[1] A weak but significant correlation was, however, found between the daily dose of levodopa, or a levodopa equivalent, and sedation.[1] The recently discovered neuropeptide hypocretin is important in maintaining wakefulness and its deficiency results in narcolepsy/cataplexy.[6] Some studies have found that the cerebrospinal fluid hypocretin-1 levels were normal in PD patients with daytime sleepiness,[7] but hypocretin neurotransmission is affected in PD.[8] 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice have increased amounts of REM sleep.[9] Hyperdopamine exposure induced electrophysiological REM activity in the hippocampal area of mice.[10] Furthermore, a D2 dopamine receptor agonist restored this REM activity.[10] Taken together, these data suggest that this sleep attack phenomenon could be a hypersensitivity or a hyperdose reaction to the D2 receptor.

In these patients with sleep attack, the ESS has been reported to be normal.[11,12] In addition, this patient did not notice her sleep attack and sleepiness. Thus, information from a patient's family is very important for diagnosing a sleep disturbance, particularly sleep attacks in PD patients.

REFERENCES

1. Arnulf I. Excessive daytime sleepiness in parkinsonism. Sleep Med Rev 2005;9:185-200.
2. Ulivelli M, Rossi S, Lombardi C, et al. Polysomnographic characterization of pergolide-induced sleep attacks in idiopathic PD. Neurology 2002;58:462-465.
3. Arnulf I, Bonnet AM, Damier P, et al. Hallucinations, REM sleep, and Parkinson's disease: a medical hypothesis. Neurology 2000; 55:281-288.
4. Moller JC, Stiasny K, Hargutt V, et al. Evaluation of sleep and driving performance in six patients with Parkinson's disease reporting sudden onset of sleep under dopaminergic medication: a pilot study. Mov Disord 2002;17:474-481.
5. Rye DB, Bliwise DL, Dihenia B, Gurecki P. FAST TRACK: daytime sleepiness in Parkinson's disease. J Sleep Res 2000;9:63-69.
6. Overeem S, Mignot E, van Dijk JG, Lammers GJ. Narcolepsy: clinical features, new pathophysiologic insights, and future perspectives. J Clin Neurophysiol 2001;18:78-105.
7. Overeem S, van Hilten JJ, Ripley B, Mignot E, Nishino S, Lammers GJ. Normal hypocretin-1 levels in Parkinson's disease patients with excessive daytime sleepiness. Neurology 2002;58:498-499.
8. Fronczek R, Overeem S, Lee SY, et al. Hypocretin (orexin) loss in Parkinson's disease. Brain 2007;130 (Part 6):1577-1585.
9. Monaca C, Laloux C, Jacquesson JM, et al. Vigilance states in a parkinsonian model, the MPTP mouse. Eur J Neurosci 2004;20: 2474-2478.
10. Dzirasa K, Ribeiro S, Costa R, et al. Dopaminergic control of sleep–wake states. J Neurosci 2006;26:10577-10589.
11. Korner Y, Meindorfner C, Moller JC, et al. Predictors of sudden onset of sleep in Parkinson's disease. Mov Disord 2004;19:1298-1305.
12. Tan EK, Lum SY, Fook-Chong SM, et al. Evaluation of somnolence in Parkinson's disease: comparison with age- and sex-matched controls. Neurology 2002;58:465-468.

The G2019S *LRRK2* Mutation in Brazilian Patients with Parkinson's Disease: Phenotype in Monozygotic Twins

Renato P. Munhoz, MD,[1] Yosuke Wakutani, MD,[2] Connie Marras, MD,[3] Helio A. Teive, MD, PhD,[1] Salmo Raskin, MD, PhD,[4] Lineu C. Werneck, MD, PhD,[1] Danielle Moreno, BSc,[2] Christine Sato, BSc,[2] Anthony E. Lang, MD, FRCPC,[3,5] and Ekaterina Rogaeva, PhD[2,5]*

[1]Movement Disorders Unit, Neurology Service, Hospital de Clínicas, Federal University of Paraná, Curitiba, PR, Brazil; [2]Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario, Canada; [3]Movement Disorders Centre, Toronto Western Hospital, University of Toronto, Toronto, Ontario, Canada; [4]Genetika Laboratory, Curitiba, PR, Brazil; [5]Division of Neurology, Department of Medicine, University Health Network, Toronto, Ontario, Canada

Abstract: **Mutations in the Leucine-Rich Repeat Kinase 2 gene (*LRRK2*) are mainly responsible for idiopathic Parkinson's disease (PD) with either a dominant pattern of**

*Correspondence to: Ekaterina Rogaeva, Centre for Neurodegenerative Diseases, Department of Medicine, University of Toronto, 6 Queen's Park Crescent West, Toronto, Ontario, Canada M5S 3H2. E-mail: ekaterina.rogaeva@utoronto.ca

Received 22 March 2007; Revised 19 May 2007; Accepted 9 October 2007

Published online 12 November 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.21832

transmission or a sporadic occurrence due to the reduced penetrance. A majority of *LRRK2* kindreds demonstrate an extremely variable age-at-onset in affected members of the same family. The G2019S is the most common *LRRK2* mutation, which accounts for 1–5% PD patients in North America, and up to 40% of patients from an isolated Arab population. We assessed the frequency of the G2019S mutation in 83 Brazilian PD patients originally preselected for having an early age-at-onset (<50 years) and/or a positive family history. The mutation was detected in three probands (3.5%). Our clinical findings in these kindreds include the first description of the phenotype in identical twins discordant for handedness (a general phenomenon found in ~25% monozygotic twins). However, both twins developed right asymmetric PD. The clinical presentation of twins was strikingly similar including an identical PD onset at age 60. This observation may suggest that genetic factors predominantly determine age-at-onset. © 2007 Movement Disorder Society

Key words: mutation; Parkinson's disease; G2919S; *LRRK*; Brazil.

Parkinson disease (PD) is the most common neurodegenerative movement disorder, affecting ~3% of the population over 65 years of age.[1] Mutations in the novel PD gene (Leucine-Rich Repeat Kinase 2; *LRRK2*) have been described in both early- and late-onset idiopathic forms of the disease.[2,3] *LRRK2*-associated PD follows a dominant pattern of transmission, however PD may present in an apparently recessive or sporadic manner due to the reduced penetrance of *LRRK2* mutations. *LRRK2* is now recognized as the most common genetic cause of PD. There are at least 20 pathogenic *LRRK2* mutations, including the most frequent G2019S substitution, which accounts for ~5% of familial and ~1% sporadic PD patients in North American datasets.[4] Remarkably, the G2019S mutation is responsible for ~20% of Ashkenazi Jewish patients and up to 40% of PD patients from one isolated Arab population.[5,6] This mutation directly implicates the kinase activity of *LRRK2* in the pathogenic function since the glycine residue (G2019) is part of the D-Y-G motif of the kinase domain.

The prevalence of the G2019S mutation in Latin American PD patients remains unknown. Here we describe the assessment of the G2019S in a Brazilian PD dataset. Our clinical findings in three *LRRK2* families include the first description of the PD phenotype in monozygotic twins.

METHODS

Informed consent for research purposes was obtained from all individuals involved in the study approved by the research ethics board. Standard neurological examination was performed on all patients recruited from the Movement Disorders Unit, Neurology Service, Hospital de Clínicas, Federal University of Paraná, Brazil. All patients were of Brazilian origin and were diagnosed as having PD based on published criteria.[7] The sample characteristics are summarized in Table 1. The dataset is composed of 83 PD patients originally preselected for having an early age-at-onset (<50 years) (n = 59, 71%) and/or a positive family history (n = 39, 47%).

Genomic DNA was isolated from blood samples using the QIAGEN DNA extraction kit. The presence of the G2019S mutation was evaluated using a restriction digest assay (SfcI) as described previously.[8] To confirm the monozygosity of the twins (no. 7368 and no. 7371) we genotyped six autosomal microsatellite markers: D2S2166, D2S319, D3S2748, D6S291, D12S1718, and D14S1056 (PCR conditions are available upon request).

RESULTS

Genetic Analysis

A mutation screening of *LRRK2* was performed on 83 independent PD patients of Brazilian origin. The heterozygous G2019S mutation was identified in three probands (no. 7008, no. 6714, and no. 7368) from the families BR-1, BR-2, and BR-3, respectively (see Fig. 1). Segregation analysis was possible only for the BR-3 family with four affected individuals available for study that demonstrated complete segregation of the G2019S with PD (Fig. 1c). The genotypes for the randomly selected highly polymorphic markers were identical for the patients no. 7368 and no. 7371 confirming the monozygosity of these twins from the family BR-3: D2S2166 (238/240 bp), D2S319 (126/128 bp), D3S2748 (105/105 bp), D6S291 (196/198 bp), D12S1718 (154/154 bp), and D14S1056 (117/117 bp).

Clinical Features

A summary of the clinical characteristics of all affected subjects with the G2019S mutation is shown in Table 2. Features generally considered atypical for most

TABLE 1. *General characteristics of the Brazilian dataset with Parkinson's disease (PD)*

Sample characteristics	PD probands (total n = 83)
Mean age-at-onset (range) ± SD	46.5 ± 12.7 (15–71 years)
Mean age of examination (range) ± SD	56.5 ± 13.2 (19–86 years)
Number of cases with early onset of PD (%)	59 (71%)
Number of cases with known family history (%)	39 (47%)
Female (%)	30 (36%)

SD, standard deviation.

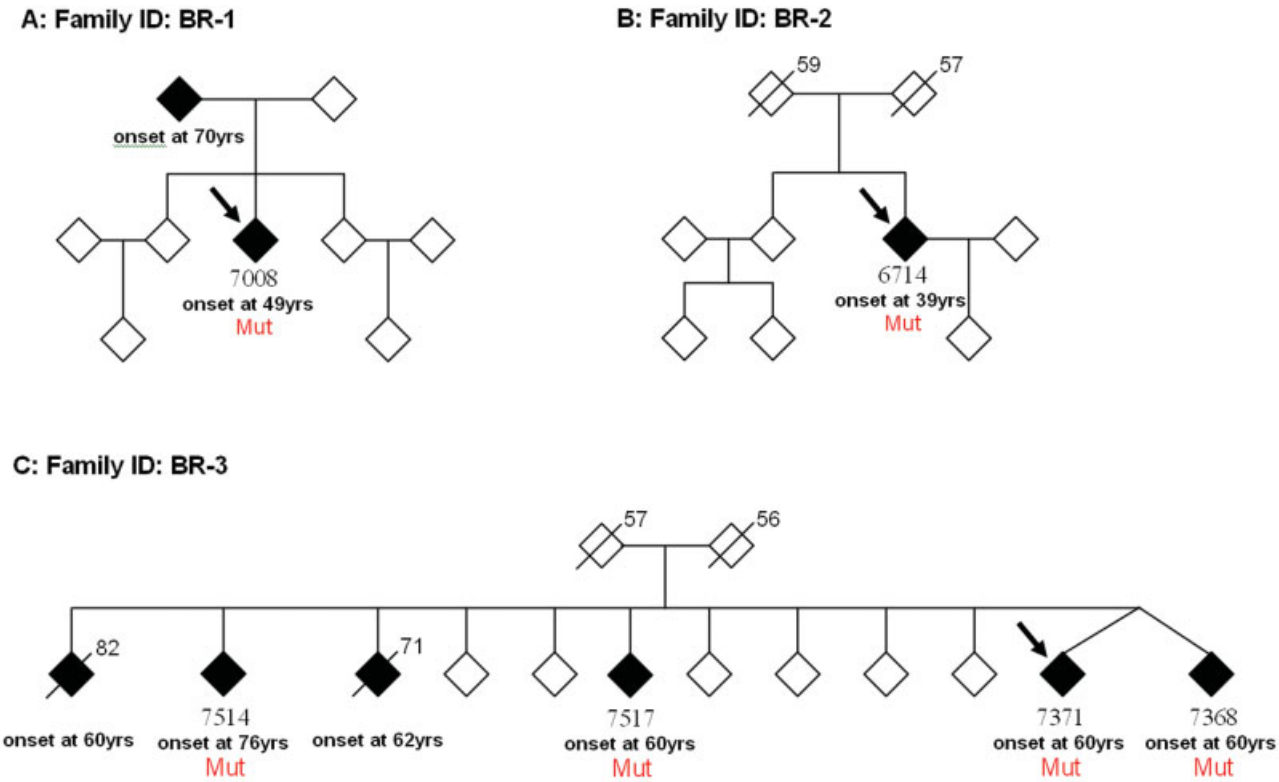

FIG. 1. The pedigree structure of the BR-1 (**a**), BR-2 (**b**), and BR-3 (**c**) families with the heterozygous G2019S mutation. Affected individuals are shown as filled symbols and the arrow points to the proband. The gender of the individuals has been masked to protect family confidentiality; Mut-heterozygous G2019S mutation. The age at death of deceased individuals is noted in the upper right corner of the symbols.

cases of PD (pyramidal signs, oculomotor disturbances, etc.) were absent. All subjects are of Brazilian origin. The ancestry for all patients was traced as far back as 3 to 4 generations and was also Brazilian except for the maternal great-grandmother of the proband from family BR-1, who was originally from Portugal.

Family BR-1 presented with autosomal dominant inheritance of PD (Fig. 1a). The proband (no. 7008) has an affected parent (not genotyped) who is currently 83-years-old with a 13-year history of left asymmetric resting tremor and bradykinesia. This subject reported good response to regular doses of levodopa (200 mg tid), peak-dose dyskinesias after about 6 years, wearing off phenomena after 9 years.

Family BR-2 presented with an apparently sporadic form of PD (Fig. 1b). The proband's (no. 6714) clinical features are described in Table 2.

Family BR-3 has 6 out of 12 siblings affected with PD (the mode of inheritance is inconclusive since the parents died in their mid 50's) (Fig. 1c). Mean age-at-onset for the six subjects was 63-years-old, ranging between 60 and 76 years of age (Fig. 1c). All four living affected subjects were formally assessed by one of the authors and were carriers of the G2019S mutation.

The proband (no. 7371) and its identical twin (no. 7368) are now 63-years-old, and share a strikingly similar clinical presentation of PD. Both twins developed right asymmetric PD, despite the fact that they were discordant for handedness: the patient no. 7371 is left-handed and patient no. 7368 is right-handed (a general phenomenon found in about one of every four monozygotic twins[9]). Both twins started with asymmetric resting tremor and rigidity concomitantly at the age of 60. On examination, cognition was normal, mild unilateral parkinsonism with good postural instability, and no atypical signs were detected. Both were initially treated with a dopamine agonist. The proband was started on pramipexole with good symptom control for about 1 year. At that point, pramipexole was stopped and L-dopa started at low doses due to lower limb edema and poor symptom control. At a recent follow-up cognition remains intact, with bilateral parkinsonism but still clearly right asymmetric. Pull test (UPDRS item no. 30) was scored as 1. L-dopa (125 mg tid) was started with good response and no motor or neuropsychiatric complications. The twin no. 7368 could not tolerate pramipexole at doses higher than 0.25 mg tid due to GI side effects. L-dopa was also added and this patient is now taking 125

TABLE 2. *Clinical characteristics of patients with the G2019S LRRK2 mutation*

Family ID	BR-1	BR-2	BR-3	BR-3	BR-3	BR-3
Subject ID	7008	6714	7514	7517	7368	7371
Age at examination	51	50	79	75	63	63
Age of onset	49	39	76	60	60	60
Disease duration	2	11	3	15	3	3
Most affected side	Right	Right	Right	Left	Right	Right
Resting tremor	+	+	+	+	+	+
Rigidity	+	+	+	+	+	+
Bradykinesia	+	+	+	+	+	+
Postural instability	−	+	−	+	+	+
Asymmetry at onset	+	+	+	+	+	+
L-dopa response (mg/day)	+ (200)	+ (1,000)	+ (200)	+ (300)	+ (375)	+ (375)
Dopamine agonist–pramipexole mg/day	3	Not tried	Not tried	0.25	0.75	−
Peak dose dyskinesia (years from onset)	−	+ (5)	−	+ (11)	−	−
Off-dystonia	−	+	−	−	−	−
Motor fluctuations (years from onset)	−	+ (5)	−	+ (12)	−	−
Surgery (pallidotomy)	−	+	−	−	−	−
Sleep benefit	−	−	−	−	−	−
Psychosis (years from onset)	−	+ (6)	−	−	−	−
Dementia	−	−	−	−	−	−
Anxiety/depression	−	+	−	+	+	−
Orthostatic hypotension	−	−	−	−	−	−
Urinary urgensy	−	−	+	−	−	−
Urinary incontinence	−	−	−	−	−	−
REM sleep behaviour disorder	−	+	−	−	−	−
Restless legs syndrome	−	−	+	−	−	−
Hoehn and Yahr scale score	1.5	3	2	3	3	3
Atypical signs	−	−	−	−	−	−

mg tid. The progression of symptoms and clinical parameters of the twins are very similar, including the now bilateral disease with mild postural instability. Additionally, patient no. 7368 has mild depression and anxiety, well controlled with low dose amitriptyline.

Clinical features of the remaining genotyped affected family members are described in Table 2. The two deceased subjects had a formal diagnosis of PD during life. The older sibling started with a left asymmetric resting tremor and rigidity in its early 60's, and died from a myocardial infarction at age 82. Data on the second deceased affected subject was available from medical records only. Right asymmetric parkinsonism started by the age of 62 with resting tremor, bradykinesia, rigidity, and postural instability with repeated falls. A good initial response to L-dopa was described but the patient eventually died from pulmonary complications after a right hip fracture at age 71.

DISCUSSION

The current study is the first to analyze the frequency of the G2019S mutation in a Latin-American country, which demonstrated that this mutation is not a common cause of PD in Brazilian patients with early-onset and/or family history of PD. The G2019S was found in three probands (3.5%), representing 1.7% of our sporadic early-onset patients and 5.1% of familial patients. A similar Portuguese study analyzing 124 late- and early-onset PD patients found the G2019S mutation in 6% of cases.[10] The mutation frequency in our dataset is comparable to that reported in three other recent studies of PD patients in North America (1.6%), Germany (1.3%), and Italy (3%).[11–13] These studies also showed that there are no differences in the frequencies of the G2019S mutation in probands with early-onset versus late-onset PD, therefore age-at-onset may not be a distinguishing feature for this specific genetic subtype of PD. In regard to familial PD, our results are in the lower range of those found across Europe, and are comparable to the frequencies found in North America and North European countries.[11,14–16]

Since we evaluated only the most common known mutation, a reliable estimate of the role of *LRRK2* mutations in the Brazilian PD population requires sequencing of the entire *LRRK2* gene in all the patients. Ongoing sequencing study may reveal specific *LRRK2* mutation responsible for PD in our patients, however, Brazilian population is very heterogeneous as result of five centuries of interethnic marriages between individuals of European, African, and Amerindian origin. Analysis of population-specific alleles indicates that almost one third of those with European ancestry are Portuguese in origin. The patients studied here were collected from the south of Brazil where admixture studies showed that 82% of

the population is of European, 7% African, and 11% Amerindian ancestries.[17]

The clinical presentation of our cases with the G2019S mutation was similar to that of idiopathic PD, which is in keeping with the literature.[18] Additionally, two of our patients were notable for depression and anxiety and one had restless legs syndrome. Two cases had a relatively mild phenotype with slow disease progression (duration >10 years) and only mild treatment-related complications. As in the patients presented here, the majority of families with *LRRK2* mutations demonstrate an extremely variable age-at-onset in affected members of the same family.[3] Notably, our study includes the first report on monozygotic twins with the *LRRK2* mutation. There is only one report describing the finding that the identical twin of a G2019S mutation carrier remains unaffected 24 years after the onset in his brother.[10] However unpublished follow-up data revealed that these siblings were in fact dizygotic twins (e-mail communication with Dr. John Hardy; 1/5/2007). Of importance, monozygosity of the twins reported here was confirmed by the identical genotypes of polymorphic markers. Evaluation of the clinical presentation in these Brazilian twins revealed a very similar phenotype including an identical onset at age 60. Our finding may suggest that age-at-onset is largely genetically determined, although it is impossible to draw firm conclusions on the basis of one twin pair.

Acknowledgments: This work was supported by grants from the Parkinson Society of Canada, Canadian Institutes of Health Research, Japan-Canada and Canadian Institutes of Health Research Joint Health Research Program, W. Garfield Weston Fellows (ER), Center of Excellence grant (CM), and National Parkinson Foundation (AEL).

REFERENCES

1. Lang AE, Lozano AM. Parkinson's disease. First of two parts. N Engl J Med 1998;339:1044–1053.
2. Zimprich A, Biskup S, Leitner P, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 2004;44:601–607.
3. Paisan-Ruiz C, Jain S, Evans EW, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 2004;44:595–600.
4. Hedrich K, Klein C, Rogaeva E, et al. Role of heterozygous mutations in Parkinson's disease genes. Lancet Neurol 2007;69: 140–147.
5. Lesage S, Durr A, Tazir M, et al. LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. N Engl J Med 2006; 354:422–423.
6. Ozelius LJ, Senthil G, Saunders-Pullman R, et al. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. N Engl J Med 2006;354:424–425.
7. Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease. Neurology 2001;57: 1497–1499.
8. Paisán-Ruíz C, Lang AE, Kawarai T, et al. LRRK2 gene in Parkinson's Disease: mutation analysis and case control association study. Neurology 2005;65:696–700.
9. Sommer IE, Ramsey NF, Mandl RC, Kahn RS. Language lateralization in monozygotic twin pairs concordant and discordant for handedness. Brain 2002;125:2710–2718.
10. Bras JM, Guerreiro RJ, Ribeiro MH, et al. G2019S dardarin substitution is a common cause of Parkinson's disease in a Portuguese cohort. Mov Disord 2005;20:1653–1655.
11. Kay DM, Zabetian CP, Factor SA, et al. Parkinson's disease and LRRK2: frequency of a common mutation in U.S. movement disorder clinics. Mov Disord 2006;21:519–523.
12. Hedrich K, Winkler S, Hagenah J, et al. Recurrent LRRK2 (Park8) mutations in early-onset Parkinson's disease. Mov Disord 2006; 21:1506–1510.
13. Goldwurm S, Di Fonzo A, Simons EJ, et al. The G6055A (G2019S) mutation in LRRK2 is frequent in both early and late onset Parkinson's disease and originates from a common ancestor. J Med Genet 2005;42:e65.
14. Lesage S, Ibanez P, Lohmann E, et al. G2019S LRRK2 mutation in French and North African families with Parkinson's disease. Ann Neurol 2005;58:784–787.
15. Zabetian CP, Samii A, Mosley AD, et al. A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations. Neurology 2005;65:741–744.
16. Nichols WC, Pankratz N, Hernandez D, et al. Genetic screening for a single common LRRK2 mutation in familial Parkinson's disease. Lancet 2005;365:410–412.
17. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci USA 2003;100:177–182.
18. Khan NL, Jain S, Lynch JM, et al. Mutations in the gene LRRK2 encoding dardarin (PARK8) cause familial Parkinson's disease: clinical, pathological, olfactory and functional imaging and genetic data. Brain 2005;128:2786–2796.

Survival in Multiple System Atrophy

Anette Schrag, MD, PhD,[1] Gregor K. Wenning, MD, PhD,[2] Niall Quinn, MD,[3] and Yoav Ben-Shlomo, MD, PhD[4]*

[1]Department of Clinical Neurosciences, Royal Free & University College Medical School, London, United Kingdom; [2]Clinical Department of Neurology, Innsbruck Medical University, Innsbruck, Austria; [3]Institute of Neurology, University College London, Queen Square, London, United Kingdom; [4]Department of Social Medicine, University of Bristol, Bristol, United Kingdom

Abstract: **We here report survival in patients with multiple system atrophy (MSA) in a large, prospectively studied group of patients with MSA. Eighty-five of 100 patients**

*Correspondence to: Y. Ben-Shlomo, Department of Social Medicine, University of Bristol, Canynge Hall, Whiteladies Road, Bristol BS8 2PR, United Kingdom. E-mail: y.ben-shlomo@bristol.ac.uk

Received 22 February 2007; revised 10 October 2007; accepted 11 October 2007

Published online 28 November 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.21839