Movement Disorders
Vol. 25, No. 16, 2010, pp. 2854–2887

Brief Reports

Screening of Brazilian Families with Primary Dystonia Reveals a Novel *THAP1* Mutation and a De Novo *TOR1A* GAG Deletion

Patricia De Carvalho Aguiar, MD, PhD,[1,2]*
Tania Fuchs, PhD,[3] Vanderci Borges, MD, PhD,[1]
Kay-Marie Lamar, BS,[3]
Sonia Maria Azevedo Silva, MD, PhD,[1]
Henrique Ballalai Ferraz, MD, PhD,[1]
and Laurie Ozelius, PhD[3,4]

[1]*Department of Neurology and Neurosurgery, Universidade Federal de Sao Paulo, Sao Paulo, SP, Brazil;* [2]*Instituto Israelita de Ensino e Pesquisa Albert Einstein, Sao Paulo, SP, Brazil;* [3]*Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, New York, USA;* [4]*Department of Neurology, Mount Sinai School of Medicine, New York, New York, USA*

Abstract: **The *TOR1A* and *THAP1* genes were screened for mutations in a cohort of 21 Brazilian patients with Primary torsion dystonia (PTD). We identified a de novo delGAG mutation in the *TOR1A* gene in a patient with a typical DYT1 phenotype and a novel c.1A > G (p.Met1?) mutation in *THAP1* in a patient with early onset generalized dystonia with speech involvement. Mutations in these two known PTD genes, *TOR1A* and *THAP1*, are responsible for about 10% of the PTD cases in our Brazilian cohort suggesting genetic heterogeneity and supporting the role of other genes in PTD.** © 2010 Movement Disorder Society

Key words: dystonia; DYT1; TOR1A; DYT6; THAP1; de novo mutation

Primary torsion dystonia (PTD) is associated with mutations in two genes: *TOR1A* (*DYT1*)[1] and *THAP1* (*DYT6*).[2] A 3 bp (GAG) deletion in the coding region of the *TOR1A* gene is a major cause of early limb onset generalized dystonia in different ethnicities. Multiple mutations in the *THAP1* gene have been identified in families of European ancestry.[3,4] Clinical features can overlap with the DYT1 phenotype, but DYT6 families have a broader age of onset, from early childhood to adulthood, and are marked by prominent involvement of cranial and cervical muscles.[5] In this study, we screened Brazilian patients with primary dystonia for the GAG deletion in *TOR1A* and for mutations in *THAP1*.

PATIENTS AND METHODS

Twenty-one Brazilian probands (11 males and 10 females) with primary dystonia and available family members were recruited, after written informed consent, from the Movement Disorders Unit of Universidade Federal de Sao Paulo and examined by movement disorders specialists. All probands were classified as definite dystonia according to previously defined clinical criteria.[6] The study was approved by the institutional review boards.

Molecular Analysis

DNA was extracted from peripheral blood using the Purgene procedure (Gentra Systems, Minneapolis, MN). All patients were screened for the *TOR1A* GAG deletion as previously described.[1] Those who were negative for this mutation were further screened for *THAP1* mutations by direct sequencing as described.[2] European Caucasian DNA control samples (Sigma-Aldrich; n = 277) were sequenced for the identified *THAP1* mutation. To establish paternity, polymorphic markers spanning the *TOR1A* gene were tested including D9S159, D9S2160, D9S2161, D9S63, and D9S2162 using previously published PCR conditions[1] and analyzed on an ABI377 automated sequencer (PerkinElmer, Wellesley, MA).

RESULTS

The clinical characteristics of the patients are summarized in Table 1. More than half (62%) had generalized PTD with the remainder having segmental or focal PTD. We identified a delGAG mutation in the *TOR1A* gene in one patient (patient 7), in his affected sibling and in their unaffected mother but not in the

*Correspondence to: Dr. Patricia de Carvalho Aguiar, Department of Neurology and Neurosurgery-UNIFESP, Rua Botucatu,740, Sao Paulo, SP 04023-900, Brasil. E-mail: patriciamc@einstein.br

Potential conflict of interest: Nothing to report.

Received 30 July 2009; Revised 27 November 2009; Accepted 9 March 2010

Published online 5 October 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23133

TABLE 1. *Clinical characteristics of Brazilian primary dystonia patients*

Patient	Sex	Age of onset (yr)	Disease duration (yr)	Site of onset	Distribution	Classification	Dysphonia	Family history	Gene mutation
1	F	9	11	LA	G	G		N	
2	M	13	12	RA	G	G		Y	
3	F	4	19	RA	G	G	Y	P	
4	F	8	35	LL	G	G		N	
5	M	23	3	RL	G	G		N	
6	F	5	18	RL	G	G		N	
7	M	8	5	RA	G	G		Y	*TOR1A*
8	M	7	6	T	G	G		N	
9	M	8	5	RL	G	G		N	
10	F	7	15	LL	G	G		N	
11	F	7	35	RA	G	G	Y	N	
12	M	16	18	T	G	G		N	
13	F	19	1	C+T	C+T	S		N	
14	F	4	6	RL	G	G	Y	Y	*THAP1*
15	F	36	8	C	C	Fc		Y	
16	M	18	23	C+T	C+T+LA	S		N	
17	M	32	6	RA	RA+C	S		Y	
18	M	18	3	C	C	Fc		N	
19	F	13	41	C	C	Fc		P	
20	M	16	10	RA+LA	RA+LA+C	S		N	
21	M	48	1	RA	RA+C+LA	S		N	

F, female; LA, left arm; G, generalized; N, no; M, male; RA, right arm; Y, yes; LL, left leg; RL, right leg; T, trunk; C, cervical; Fc, focal; S, segmental; P, possible (other family members were reported as possibly having dystonia but were not available for examination).

mother's parents indicating a de novo mutation. Marker analysis revealed that the mother inherited a haplotype from each parent consistent with correct paternity (Fig. 1). *THAP1* was screened in the other 20 patients and we identified a novel c.1A > G (p.Met1?) mutation which cosegregated with the disease in affected family members but was also present in an 8-year-old brother and in the proband's 45-year-old father, both unaffected. This mutation was not present on 554 control chromosomes.

PATIENTS' DESCRIPTIONS

Patient 7

The proband (III.2 in Fig. 1) was a 13-year-old boy who developed right upper limb dystonia at the age of 8, which generalized over 3 years. There was no response to an initial levodopa trial and response to anticholinergic drugs was poor. On exam, his 14-year-old brother (III.1) showed typical writer's cramp in both arms with no change after 2 years. The 62-year-old maternal grandfather (I.2) complained of upper limbs tremor, which had begun 2 years previously. His past medical history was unremarkable. On exam, he presented upper limbs rest tremor, cogwheel rigidity, bradykinesia, and hypomimia. He was diagnosed with Parkinson's disease and started on Amantadine, with improvement of symptoms. The paternal uncle (II.1, 46 years old) reported upper left limb and mouth involuntary movements for 3 years. On exam, he showed orofacial dyskinesias and choreoathetoid movements of the upper left limb but denied previous exposure to neuroloeptics or other drugs otherwise, his past medical history was unremarkable. Brain image and laboratorial exams were normal. The probands's parents (II.2, II.3), 8-year-old sister (III.3), and paternal grandmother (I.1) did not show abnormalities on physical exam. Neither the grandfather nor the uncle had the delGAG mutation (Fig. 1).

Patient 14

A 12-year-old female presented to our clinic with a history of abnormal right inferior limb posture and twisting movements since the age of 4, which progressed to other body parts in 2 years. On physical exam, we observed severe generalized dystonia, the patient was wheelchair bound, all limbs, trunk, and cranial-cervical region were equally compromised, dysphonia was also present. She had a positive family history for dystonia, her 21-year-old brother had been affected with dystonia since the age of 6. In his case, dystonia was initially observed in the left upper limb

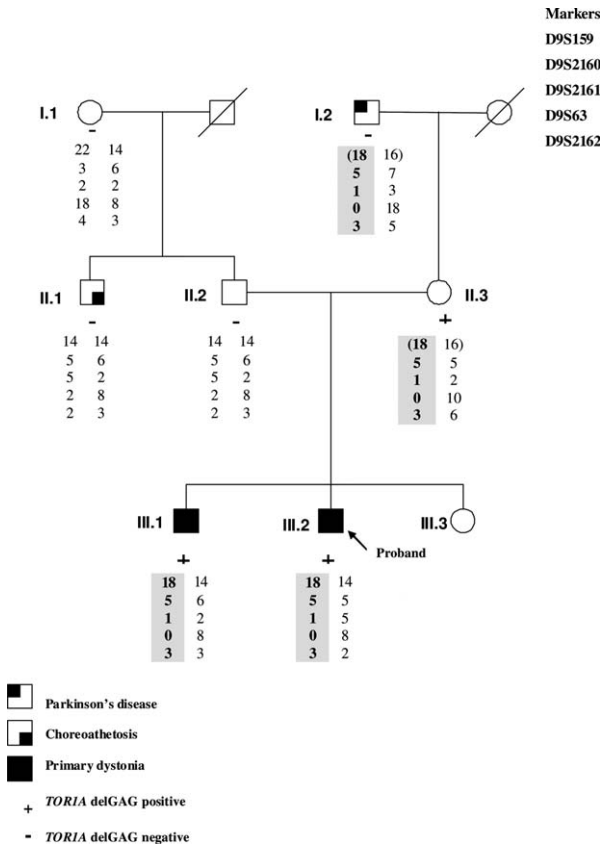


FIG. 1. De novo *TOR1A* delGAG mutation in proband's 7 family. Disease related haplotype is highlighted.

and then generalized, but remained asymmetrical with left side predominance but he was able to walk unassisted after a 15-year disease history. Dysphonia was present. An 8-year-old brother and both parents were unaffected. There was no consanguinity within the family. Past medical history, as well as laboratory and imaging exams were unremarkable.

DISCUSSION

We screened a cohort of Brazilian PTD cases for mutations in the two identified PTD genes and identified one case with a de novo mutation in *TOR1A* and one case with a novel mutation in the *THAP1* gene, both with a positive family history of dystonia.

Haplotype analysis of the family of proband 7 revealed a de novo delGAG mutation that arose in the probands' unaffected mother. His maternal grandfather has the disease haplotype, but not the mutation (Fig. 1). To our knowledge, this is the first report of a de novo *TOR1A* mutation in a Brazilian family and only the fourth de novo delGAG mutation reported.[7,8] This family is also interesting because of the spectrum of different movement disorders it presents. The proband has generalized dystonia and his brother has writers' cramp, both with mutations in the *TOR1A* gene. The maternal grandfather has Parkinson's disease and a paternal uncle has choreoathetoid movements of unknown etiology. It is possible that within this family, there are some genetic or environmental modifiers that lead to a higher risk of development of movement disorders in general. Association of more than one movement disorder in the same patient is not rare, but families with multiple movement disorders in different members are uncommon and of great interest to investigate susceptibility factors.[9,10]

THAP1 mutations have recently been identified in patients with PTD.[2,11,12] In these families, the age of onset varied from 5 to 49 years. The upper limb was the site of onset in the majority of the cases (58%), followed by the cranial region (29%), neck (17%), and inferior limb (10%). Speech was often involved. We identified a novel *THAP1* mutation, the first reported in a Brazilian family. The affected patients were within the described range for the disease age of onset and, consistent with the DYT6 phenotype, both had dysphonia. However, unlike the majority of the DYT6 cases, the proband had leg onset and rapid progression to the generalized form resulting in inability to walk, clinical features indistinguishable from typical DYT1 dystonia. This emphasizes the clinical overlap of these two forms of primary dystonia. In addition, other patients in our cohort had a positive family history and a similar phenotype (Table 1). However, no mutations were identified in either gene, which demonstrates the genetic heterogeneity of PTD. The fact that two family members are unaffected *THAP1* mutation carriers is consistent with the 60% penetrance previously reported,[5] but we cannot rule out the possibility that both mutation carriers will develop the disease in the future, since the age of onset spectrum is wide.

THAP1 is a member of a recently described family of atypical Zn finger DNA-binding proteins.[13] Most of the mutations described to date are localized in the THAP DNA-binding domain or remove the nuclear localization.[2,11,12] suggesting that aberrations in DNA binding and consequent dysregulation of transcription factor activity may play a role in the pathophysiology of dystonia. The novel c.1A>G *THAP1* mutation identified in the Brazilian family presumably results in elimination of the start codon. This could lead to the absence of the protein product with consequent haploinsuficiency or it is also possible that protein transla-

tion could start at one of the two in-frame downstream Methionines (M120 and M143), generating a truncated THAP1 protein missing the DNA-binding domain and therefore incapable of transcriptional regulation of its downstream targets. Further molecular studies are required to distinguish these hypotheses but unfortunately neither cells nor RNA are available from this family.

In the first comprehensive screening of PTD in a Brazilian cohort, we identified mutations in only 10% of cases consistent with genetic heterogeneity in this cohort.

Acknowledgments: Funding was provided by the Dystonia Medical Research Foundation (LJO), The Bachmann-Strauss Dystonia and Parkinson Foundation (LJO), and The National Institute of Neurological Disorders and Stroke (NS26636, LJO). We thank the patients and their families for participating in this study.

Author Roles: Patricia de Carvalho Aguiar was involved in research project conception, organization, execution, data analysis, and manuscript draft. Tania Fuchs was involved in research project organization, execution, data analysis, manuscript review, and critique. Vanderci Borges was involved in research project execution, manuscript review, and critique. Kay-Marie Lamar was involved in research project execution, manuscript review, and critique. Sonia Maria Azevedo Silva was involved in research project execution, manuscript review, and critique. Henrique Ballalai Ferraz was involved in research project conception, organization, manuscript review, and critique. Laurie Ozelius was involved in research project conception, organization, supervision, data analysis, manuscript review, and critique, funding obtaining.

Financial Disclosures: Patricia de Carvalho Aguiar received a doctoral stipend from Fundação CAPES, Brazil and also receives a post doctoral stipend from F. Hoffmann La-Roche, Switzerland. Henrique Ballalai Ferraz received a research grant from FAPESP, Brazil. Tania Fuchs, Vanderci Borges, Kay-Marie Lamar, Sonia Maria Azevedo Silva, and Laurie Ozelius report no disclosures.

REFERENCES

1. Ozelius LJ, Hewett JW, Page CE. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. Nat Genet 1997;17:40–48.
2. Fuchs T, Gavarini S, Saunders-Pullman R, et al. Mutations in the THAP1 gene are responsible for DYT6 primary torsion dystonia. Nat Genet 2009;41:286–288.
3. Bonetti M, Barzaghi C, Brancati F, et al. Mutation screening of the DYT6/THAP1 gene in Italy. Mov Disord 2009;24:2424–2427.
4. Paisán-Ruiz C, Ruiz-Martinez J, Ruibal M, et al. Identification of a novel THAP1 mutation at R29 amino-acid residue in sporadic patients with early-onset dystonia. Mov Disord 2009;24:2428–2429.
5. Saunders-Pullman R, Raymond D, Senthil G, et al. Narrowing the DYT6 dystonia region and evidence for locus heterogeneity in the Amish-Mennonites. Am J Med Genet A 2007;143:2098–2105.
6. Bressman SB, Sabatti C, Raymond D, et al. The DYT1 phenotype and guidelines for diagnostic testing. Neurology 2000;54:1746–1752.
7. Klein C, Brin MF, De Leon D, et al. De novo mutations (GAG deletion) in the DYT1 gene in two non-Jewish patients with early-onset dystonia. Hum Mol Genet 1998;7:1133–1136.
8. Hjermind LE, Werdelin LM, Sørensen SA. Inherited and de novo mutations in sporadic cases of DYT1-dystonia. Eur J Hum Genet 2002;10:213–216.
9. Edwards M, Russo N, Summers B, Morton J, Peake D, Bhatia K. An unusual family with multiple movement disorders. J Neurol 2003;250:793–796.
10. Müller J, Wenning GK, Wissel J, Poewe W. Intrafamilial heterogeneity of facial hyperkinesias: chance association of tics, cranial dystonia, and Huntington's disease? Mov Disord 2001;16:370–372.
11. Bressman SB, Raymond D, Fuchs T, Heiman GA, Ozelius LJ, Saunders-Pullman R. Mutations in THAP1 (DYT6) in early-onset dystonia: a genetic screening study. Lancet Neurol 2009;8:441–446.
12. Djarmati A, Schneider SA, Lohmann K, et al. Mutations in THAP1 (DYT6) and generalised dystonia with prominent spasmodic dysphonia: a genetic screening study. Lancet Neurol 2009;8:447–452.
13. Roussigne M, Roussigne M, Kossida S, et al. The THAP domain: a novel protein motif with similarity to the DNA-binding domain of P element transposase. Trends Biochem Sci 2003;28:66–69.

Grasping Premanifest Huntington's Disease – Shaping New Endpoints for New Trials

Ralf Reilmann, MD,[1]* Stefan Bohlen, MD,[1] Thomas Klopstock, MD,[2] Andreas Bender, MD,[2] Adolf Weindl, MD,[3] Philipp Saemann, MD,[4] Dorothee P. Auer, MD,[4] Erich B. Ringelstein, MD,[1] and Herwig W. Lange, MD[1]

[1]Department of Neurology, University Clinic Muenster (UKM), Westfaelische Wilhelms University of Muenster, Muenster, Germany; [2]Department of Neurology, Friedrich-Baur-Institute, Ludwig-Maximilians-University, Munich, Germany; [3]Department of Neurology, Technical University Munich, Germany; [4]Department of Radiology, Max Planck Institute of Psychiatry, Munich, Germany

Abstract: **Future clinical trials in subjects with premanifest Huntington's disease (preHD) may depend on the availability of biomarkers. It was previously shown in symptomatic HD that, the grip force variability coefficient-of-variation (GFV-C) in a grasping paradigm was correlated to the Unified-Huntington's-Disease-Rating-Scale-Total-Motor-Score (UHDRS-TMS) and increased in a 3 year follow-up study. To further elucidate its potential as a biomarker, we investigated whether GFV-C is able to detect a motor phenotype in preHD and is correlated to the genotype assessed by a disease-burden-score. The ability of preHD (n = 15) and symptomatic HD subjects (n = 20) to maintain stable grip forces, while holding an object (250 g and 500 g), was measured and compared with the controls (n = 19). GFV-C was increased in preHD at 500 g, in symptomatic subjects at both weights and was correlated to the disease-burden-score and UHDRS-TMS. GFV-C may be a useful objective and quantitative marker of motor dysfunction across genetically diagnosed premanifest and symptomatic HD subjects.** © 2010 Movement Disorder Society

Key words: huntington's disease; motor control; grip force; clinical physiology; biomarker

Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disease.[1] So far, no treatment for slowing disease progression is available.[2] Future disease modifying clinical trails may target subjects in premanifest stages to slow down or even prevent development of neurodegeneration.

Clinical trials in this setting will depend on reliable, objective, and quantitative biomarkers.[3] The phenotype of HD is complex, including cognitive, behavioral, psychiatric symptoms, involuntary movements, and deficits in voluntary motor control.[4] Motor symptoms are instrumental in establishing the diagnosis of manifest HD[5] and often serve as primary or coprimary endpoint in clinical trials in symptomatic HD.[6] Motor deficits are amenable to objective and quantitative analysis,[7] and subtle deficits of motor control were detected in premanifest gene-carriers.[3,8,9] Hence, it seems technically feasible to develop objective and quantitative measures of motor phenotype in HD to increase the sensitivity and power of future clinical trials using motor endpoints.

Guided by the clinical observation of the "milkmaid's grip,"[4] depicting the fluctuating strength of grip often seen in HD subjects, we previously explored the feasibility to use this clinical sign to objectively assess severity of motor dysfunction in symptomatic HD. A neurophysiological method using force transducers was applied to measure grip forces during grasping and holding an object.[10] In this cross-sectional study with subjects in different stages of HD, we showed that grip force variability expressed as coefficient-of-variation (GFV-C) was correlated to the severity of motor symptoms as assessed clinically on the Unified-Huntington's-Disease-Rating-Scale-Total-Motor-Score (UHDRS-TMS).[5] Based on these results, we hypothesized that GFV-C may be a measure of disease severity and motor phenotype progression in HD. In a consecutive 3 year follow-up study, we demonstrated that over time GFV-C increased in all participating subjects.[11] GFV-C was more sensitive to detect motor phenotype progression than the UHDRS-TMS. Likewise, in gait studies variability of motor performance was the most sensitive measure of abnormality in premanifest and manifest HD[12,13] suggesting that variability in motor output, that is, motor impersistency, maybe a more generalized feature of HD.

Based on these observation, the current study investigated whether changes in GFV-C during grasping (1) can be detected in premanifest HD and (2) are correlated to the genotype as assessed by a disease-burden-score (DBS) based on CAG-repeat length and age ([CAG-35.5] × age).[14]

PATIENTS AND METHODS

Subjects

About 20 symptomatic subjects with HD, 9 women and 11 men, mean age 43.9 ± 8.48 (SD) (range 27–

*Correspondence to: Ralf Reilmann, Department of Neurology, University Clinic Muenster (UKM), University of Muenster, Albert Schweitzer Strasse 33, Muenster 48129.
E-mail: r.reilmann@uni-muenster.de

Potential conflict of interest: Nothing to report.
Received 1 July 2009; Revised 15 March 2010; Accepted 17 May 2010
Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23300

61) years, 15 premanifest gene-carriers, 9 women and 6 men, 35.0 ± 7.0 (21–45) years, and 19 healthy and age matched control subjects, 12 women and 7 men, 41.7 ± 12.1 (20–63) years, participated in the study after giving their written informed consent in accordance with the declaration of Helsinki. The study was approved by the Institutional Review Board of the University of Muenster. Subjects were naïve regarding the aims of the study. CAG-expansion was known of all subjects in the premanifest and symptomatic HD group. Clinical assessment with the UHDRS-TMS[5] was performed by a physician experienced in HD and UHDRS-TMS rating. Only gene-carriers with a UHDRS-TMS $\leq$3 and UHDRS diagnostic confidence level <3 were allocated to the premanifest group.[5] The participants' cognitive status was assessed by the modified Mini Mental State Examination (mMMSE).[15] Four of the symptomatic subjects were taking small doses of neuroleptic medication, which, as was previously shown, have no systematic effect on the measures examined.[10] All premanifest subjects were free of medication. Exclusion criteria for subject recruitment were: (1) coexisting neurological diseases, (2) orthopedic disorders, (3) psychosis, (4) cognitive impairment on the mMMSE <23/30 points, or (5) other impairments that would interfere with the task. Neurological examination was normal in controls with no history of neurological, psychiatric disorders, or substance abuse.

EXPERIMENTAL SET-UP AND TASK

All subjects grasped and lifted a grip instrument with two force-torque sensors (Nano-40, ATI Industrial Automation, Apex, NC, USA), which measured the grip (normal) and load (tangential) force components (0.025 N resolution) of the thumb and index finger (Fig. 1). The object's weight could be modified to 250 g or 500 g. An electromagnetic sensor (Polhemus, VT) recorded the object's position (*x, y, z*, 0.75 mm resolution). The grip instrument was held adjacent to a marker 10 cm high for 35s. Subjects performed 5 practice trials and 13 consecutive recorded trials with each object weight (for a detailed description of the paradigm see Gordon et al.).[10]

Data was sampled at 400 Hz, stored and analyzed on a laboratory computer system (SC/ZOOM, University of Umeå, Sweden). Mean isometric grip forces and grip force variability in the static phase (expressed as coefficient-of-variation = SD/mean x 100) (GFV-C) were calculated during a 15s period starting 8s after the first cueing tone. As grip forces of the thumb and index finger in a static holding task are similar (opposite normal force vectors), grip force results presented in this study were restricted to forces of the thumb. Temporal measures assessed included: (1) contact phase – time between contact of the thumb and index finger, (2) preload phase – duration between contact and positive load force initiation, (3) load phase – time from load force onset to lift-off, and (4) transport phase – time from onset of lift to maximal grip force.[10]

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 16.0. Groups were compared using one-way ANOVA using Bonferroni corrections and Scheffés post-hoc tests; parametric Pearson tests were calculated to analyze correlations of measures with the DBS and UHDRS-TMS across all gene-carriers. Statistical significance was accepted at the $p \leq 0.05$ level (two-tailed). Results of the study were expressed in means ± standard deviation.

RESULTS

Between Group Comparisons

GFV-C was increased in the group of premanifest subjects compared to controls with the 500 g object (pHD: 6.26 ± 2.80; C: 4.77 ± 1.34; $p \leq 0.05$), while only a trend was seen with the lighter 250 g object (pHD: 7.16 ± 4.22; C: 5.59 ± 1.63; $p = 0.147$) (Fig. 1). GFV-C distinguished between symptomatic and control groups ($p \leq 0.001$ for both weights) and between premanifest and symptomatic groups ($p \leq 0.05$ for both weights).

Mean grip forces did not distinguish between groups. In addition, none of the temporal measures assessed (contact time, preload, load, and transport phase), showed changes in the premanifest stage (Table 1). Preload phases were prolonged in symptomatic subjects compared to controls for both weights and compared to premanifest subjects at the 500 g weight ($p \leq 0.05$ for all cases). In return, the load phase was shortened in symptomatic subjects compared to controls at the 500 g weight ($p \leq 0.05$); a similar trend was observed at the 250 g weight ($p = 0.066$).

Genotype and Phenotype Correlations

GFV-C exhibited significant correlations with DBS for both weights ($r = 0.516/p \leq 0.01$ at 250 g; $r = 0.608/p \leq 0.001$ at 500 g) (Fig. 1) and with UHDRS-TMS ($r = 0.430/p \leq 0.01$ at 250 g; $r = 0.520/p \leq 0.001$ at 500 g). Mean grip force and temporal meas-

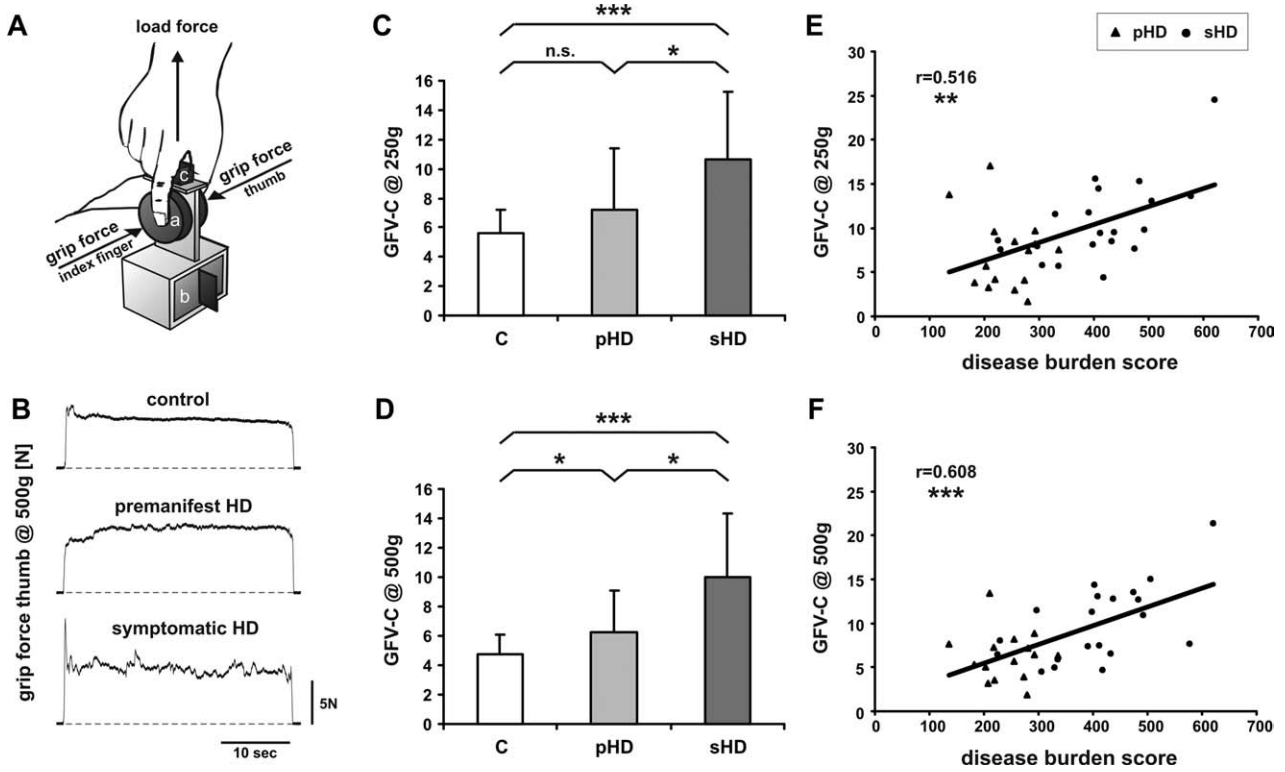


FIG. 1. Experimental set-up, sample recordings and results [A] Set-up of grip force device, showing (a) the force transducers for measuring the grip forces of the thumb and index finger, (b) the exchangeable weights (250 g or 500 g), and (c) the 3D position sensor; [B] representative sample curves of grip forces (thumb at 500 g), showing low variability in the static phase in the control subject, more variability in the premanifest subject, and highest variability in the symptomatic subject; [C] and [D] GFV-C (grip force variability coefficient-of-variation) as a measure of motor impersistency shown for the 250 g and 500 g object weights for controls (=C), premanifest HD (=pHD) and symptomatic HD (=sHD); [E] and [F] correlation of GFV-C with the disease-burden-score demonstrating a positive and fairly linear relationship between the quantitative motor measure and the genotype score across the pooled premanifest and symptomatic group. A transition from premanifest (triangles) to symptomatic subjects (circles) is seen with increasing GFV-C at both weights (bar diagrams show means ± SD / *=$p \leq 0.05$; **=$p \leq 0.01$; ***=$p \leq 0.001$).

ures were generally not correlated with either measure except for the load phase (500 g with DBS and UHDRS-TMS) and the contact phase (500 g with DBS) (see Table 1).

DISCUSSION

It was previously shown that variability of grip forces assessed by the coefficient-of-variation (GFV-C) is a measure correlated to the UHDRS-TMS[10] and progressing over time in symptomatic HD.[11] In this study, we showed for the first time that GFV-C is a measure sensitive in premanifest gene-carriers and correlated to the genotype of HD as expressed in the disease-burden-score (DBS).[14] GFV-C distinguished the group of premanifest subjects from controls and symptomatic HD patients at the 500 g object weight and a trend for increased GFV-C was also seen in the lighter object weight. Correlation of GFV-C with the UHDRS-TMS was confirmed as described before.[10]

We acknowledge several limitations of the study. (1) The sample size of 15 premanifest subjects is limited. However, the selection criteria applied were rigorous: a UHDRS-TMS score ≤ 3 was required to obtain a true premotor group. Commonly a diagnostic confidence level <4 is used in the UHDRS to recruit premanifest subjects, thus allowing subjects with motor signs with up to 98% specificity for HD to join this group.[16] These subjects may exhibit fairly high UHDRS-TMS scores and thus a relevant motor phenotype. (2) The measures used for correlation, that is, the DBS and the UHDRS-TMS both have limitations. For example, it is appreciated that CAG-repeat length only accounts for part of the variability seen in age-at-onset of HD.[17] The UHDRS-TMS may be affected by subjective error and limited sensitivity. Nevertheless, the observation of correlations between a neurophysiological motor measure and genotype as well as clinical phenotype scores, bearing considerable variability in themselves, seems noteworthy.

TABLE 1. *Group means, comparisons, and correlations*

	Weight [g]	C mean	pHD mean	sHD mean	pHD # C p	pHD # sHD p	sHD # C p	DBS (pHD + sHD pooled) r	DBS (pHD + sHD pooled) p	UHDRS-TMS (pHD + sHD pooled) r	UHDRS-TMS (pHD + sHD pooled) p
Grip force variability	250	5.59 ± 1.63	7.16 ± 4.22	10.61 ± 4.60 *(9.81 ± 3.47)*	0.147	0.030* *(0.066)*	≤0.001*** *(≤0.001***)*	0.516 *(0.317)*	0.002** *(0.082)*	0.430 *(0.327)*	0.010** *(0.073)*
(GFV-C)	500	4.77 ± 1.34	6.26 ± 2.80	9.99 ± 4.38 *(9.49 ± 3.80)*	0.048*	0.020* *(0.012*)*	≤0.001*** *(≤0.001***)*	0.608 *(0.581)*	≤0.001*** *(≤0.001***)*	0.520 *(0.467)*	≤0.001*** *(≤0.008**)*
Mean grip force	250	3.97 ± 1.67	4.86 ± 2.48	5.31 ± 2.95 *(5.53 ± 3.25)*	0.218	0.640 *(0.529)*	0.091 *(0.076)*	−0.028 *(0.018)*	0.872 *(0.925)*	0.144 *(0.187)*	0.410 *(0.314)*
[N]	500	5.38 ± 1.76	5.93 ± 2.79	7.05 ± 3.27 *(7.30 ± 3.56)*	0.492	0.485 *(0.246)*	0.150 *(0.047*)*	−0.016 *(0.077)*	0.927 *(0.682)*	0.131 *(0.176)*	0.453 *(0.344)*
Contact phase	250	64.0 ± 28.0	57.9 ± 17.4	64.0 ± 23.1 *(68.1 ± 22.5)*	0.465	0.369 *(0.169)*	0.999 *(0.637)*	0.144 *(0.240)*	0.408 *(0.194)*	0.114 *(0.182)*	0.513 *(0.328)*
[ms]	500	72.1 ± 25.3	62.3 ± 26.6	83.8 ± 33.3 *(87.3 ± 32.4)*	0.282	0.078 *(0.026*)*	0.321 *(0.128)*	0.337 *(0.376)*	0.048* *(0.037*)*	0.306 *(0.310)*	0.074 *(0.089)*
Preload phase	250	102.5 ± 60.6	140.9 ± 109.6	193.0 ± 155.4 *(204.8 ± 170.7)*	0.203	0.276 *(0.228)*	0.023* *(0.020*)*	0.007 *(0.015)*	0.966 *(0.936)*	−0.046 *(−0.029)*	0.794 *(0.877)*
[ms]	500	109.3 ± 68.2	115.1 ± 67.1	252.6 ± 219.2 *(267.6 ± 239.5)*	0.807	0.026* *(0.024*)*	0.011* *(0.009**)*	0.107 *(0.114)*	0.541 *(0.542)*	0.213 *(0.213)*	0.219 *(0.251)*
Load phase	250	244.8 ± 117.6	210.3 ± 210.0	173.0 ± 118.4 *(182.8 ± 129.7)*	0.549	0.509 *(0.661)*	0.066 *(0.148)*	−0.237 *(−0.206)*	0.171 *(0.267)*	−0.195 *(−0.163)*	0.261 *(0.381)*
[ms]	500	326.1 ± 178.9	292.8 ± 188.7	220.5 ± 127.7 *(237.1 ± 134.3)*	0.602	0.168 *(0.349)*	0.035* *(0.111)*	−0.387 *(−0.336)*	0.022* *(0.064)*	−0.335 *(−0.275)*	0.049* *(0.134)*
Transport phase	250	909.9 ± 291.8	1002.5 ± 513.6	1082.1 ± 328.5 *(1121.8 ± 354.7)*	0.512	0.580 *(0.456)*	0.092 *(0.061)*	−0.066 *(−0.059)*	0.705 *(0.751)*	0.052 *(0.082)*	0.768 *(0.661)*
[ms]	500	940.7 ± 308.0	992.3 ± 477.7	1141.0 ± 334.8 *(1200.0 ± 346.7)*	0.705	0.433 *(0.174)*	0.141 *(0.025*)*	−0.053 *(0.010)*	0.764 *(0.956)*	0.001 *(0.053)*	0.995 *(0.776)*

*= $p \leq 0.05$.
**= $p \leq 0.01$.
***= $p \leq 0.001$.
C = controls; DBS = disease-burden-score; GFV-C = grip force variability expressed as coefficient-of-variation; ms = milliseconds; N = Newton; pHD = premanifest HD; sHD = symptomatic HD; UHDRS-TMS = Unified-Huntington's-Disease-Rating-Scale-Total-Motor-Score / values in italics for sHD without neuroleptics.

With respect to the genotype and phenotype correlations and the between group differences all showing increasing GFV-C values with advancing disease stage, we hypothesize that GFV-C may be a measure capable of tracking motor phenotype progression across the different stages of HD. While, we acknowledge that cross-sectional results may not be used to precisely predict the longitudinal behavior of GFV-C, progression of GFV-C in symptomatic HD was shown previously.[11]

We conclude that GFV-C may be a useful objective and quantitative measure to assess motor phenotype in HD. It's validity and reliability as a biomarker of motor dysfunction should be further evaluated in prospective, blinded multicenter studies.

Acknowledgments: RR was supported by the fund "Innovative Medical Research" of the University of Münster Medical School # RE 12 02 25. We thank Thomas Merl, MD from the Department of Radiology, Max Planck Institute of Psychiatry, Munich, for help in scheduling subjects. IT support was provided by Jens Sommer, Ph.D. and Michael Deppe, Ph.D., University of Muenster. In addition, the support of all patients and their families is gratefully acknowledged.

Author Roles: Ralf Reilmann, Research project: Conception, Organization, Execution; Statistical Analysis: Design, Execution, Review and Critique; Manuscript: Writing of the first draft; Stefan Bohlen, Research project: Execution; Statistical Analysis: Design, Review and Critique; Manuscript: Writing of the first draft, Review and Critique; Thomas Klopstock, Research project: Organization, Execution; Statistical Analysis: Review and Critique; Manuscript: Review and Critique; Andreas Bender, Research project: Organization, Execution; Statistical Analysis: Review and Critique; Manuscript: Review and Critique; Adolf Weindl, Research project: Organization, Execution; Statistical Analysis: Review and Critique; Manuscript: Review and Critique; Philipp Saemann, Research project: Organization, Execution; Statistical Analysis: Review and Critique; Manuscript: Review and Critique; Dorothee P. Auer, Research project: Organization, Execution; Statistical Analysis: Review and Critique; Manuscript: Review and Critique; Erich B. Ringelstein, Research project: Organization; Statistical Analysis: Review and Critique; Manuscript: Review and Critique; Herwig W. Lange, Research project: Conception; Statistical Analysis: Review and Critique; Manuscript: Review and Critique.

Financial Disclosures: Dr. Reilmann is a consultant for Wyeth Pharma, Philadelphia, PA, USA, the Cure Huntington's Disease Initiative Inc., New York, NY, USA, received payments for clinical trial services from Neurosearch Inc., Denmark, and Medivation/Pfizer, USA, serves on the Advisory Board of the "Jacques and Gloria Gossweiler Foundation" and receives grant support from the High-Q-Foundation, the Cure Huntington's Disease Initiative Foundation, and the European Huntington's Disease Network (EHDN). Dr. Bohlen receives grant and salary support from the EHDN and from Neurosearch Inc., Denmark. Prof. Klopstock reports no disclosures. Dr. Bender reports no disclosures. Prof. Weindl reports no disclosures. Dr. Saemann reports no disclosures. Dr. Auer reports no disclosures. Prof. Ringelstein has received travel expenses and honorariums from Boehringer Ingelheim, Sygnis, Neurobiological Technologies, Novartis, Novo-Nordisc, Sanofi-Aventis, Solvay, Bayer Vital, M's Science, Sevier, UCB, Trommsdorf for serving as a member of Steering Committees, Safety Committees in clinical trials, and as a speaker and consultant. He has no ownership interest and does not own stocks of any pharmaceutical company. Dr. Lange receives salary support from the European Huntington's Disease Network and from Neurosearch, Denmark.

REFERENCES

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993;72: 971–983.
2. Bonelli RM, Hofmann P. A systematic review of the treatment studies in Huntington's disease since 1990. Expert Opin Pharmacother 2007;8:141–153.
3. Paulsen JS, Langbehn DR, Stout JC, et al. Detection of Huntington's disease decades before diagnosis: the Predict-HD study. J Neurol Neurosurg Psychiatry 2008;79:874–880.
4. Walker FO. Huntington's disease. Lancet 2007;369:218–228.
5. Huntington Study Group. Unified Huntington's Disease Rating Scale: reliability and consistency. Mov Disord 1996;11:136–142.
6. Huntington Study Group. Randomized controlled trial of ethyl-eicosapentaenoic acid in Huntington disease: the TREND-HD study. Arch Neurol 2008;65:1582–1589.
7. Reilmann R. Motor deficits as biomarkers in Huntington's disease: perspectives for neuroacanthocytosis syndromes. In: Danek A, editor. Neuroacanthocytosis Syndromes, Berlin, Germany: Springer Verlag; 2004. p 233–242.
8. Siemers E, Foroud T, Bill DJ, et al. Motor changes in presymptomatic Huntington disease gene carriers. Arch Neurol 1996;53: 487–492.
9. Smith MA, Brandt J, Shadmehr R. Motor disorder in Huntington's disease begins as a dysfunction in error feedback control. Nature 2000;403:544–549.
10. Gordon AM, Quinn L, Reilmann R, et al. Coordination of Prehensile Forces during Precision Grip in Huntington's Disease. Exp Neurol 2000;163:136–148.
11. Reilmann R, Kirsten F, Quinn L, et al. Objective assessment of progression in Huntington's disease: a 3-year follow-up study. Neurology 2001;57:920–924.
12. Rao AK, Muratori L, Louis ED, et al. Spectrum of gait impairments in presymptomatic and symptomatic Huntington's disease. Mov Disord 2008;23:1100–1107.
13. Rao AK, Quinn L, Marder KS. Reliability of spatiotemporal gait outcome measures in Huntington's disease. Mov Disord 2005;20: 1033–1037.
14. Penney JBJ, Vonsattel JP, Macdonald ME, et al. CAG repeat number governs the development rate of pathology in Huntington's disease [see comments]. Ann Neurol 1997;41:689–692.
15. Stern Y, Sano M, Paulson J, Mayeux R. Modified mini-mental state examination: validity and reliability. Neurology 1987;37:179.
16. Hogarth P, Kayson E, Kieburtz K, et al. Interrater agreement in the assessment of motor manifestations of Huntington's disease. Mov Disord 2005;20:293–297.
17. Wexler NS, Lorimer J, Porter J, et al. Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. Proc Natl Acad Sci USA 2004;101:3498–3503.

Tolerability of Isradipine in Early Parkinson's Disease: A Pilot Dose Escalation Study

Tanya Simuni, MD,[1]* Emily Borushko, MPH,[1] Michael J. Avram, PhD,[2] Scott Miskevics, BS,[3] Audrey Martel, BS,[1] C. Zadikoff, MD,[1] Aleksandar Videnovic, MD,[1] Frances M. Weaver, PhD,[3] Karen Williams, BA,[1] and D. James Surmeier, PhD[4]

[1]*Department of Neurology, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA;* [2]*Department of Pharmacology, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA;* [3]*Hines VA Hospital, Hines, Illinois, USA;* [4]*Department of Physiology, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA*

Abstract: **Recent data suggests that isradipine, a dihydropyridine calcium channel blocker, is neuroprotective in preclinical models of parkinsonism. Isradipine has not been systematically studied in patients with Parkinson's disease (PD). The aim of this study was to evaluate safety and tolerability of isradipine controlled release (CR) in patients with early PD. Qualified subjects (n = 31) received isradipine CR, titrated from 5 to 20 mg daily dose over 8 weeks as tolerated. Eighty-one percent of subjects completed the study. Tolerability of isradipine CR was dose dependent: 94% for 5 mg dose; 87% for 10 mg; 68% for 15 mg; and 52% for 20 mg. Isradipine had no significant effect on blood pressure or PD motor disability. The two most common reasons for dose reduction were leg edema (7) and dizziness (3). There was no difference in isradipine tolerability between subjects with and without dopaminergic treatment, or with and without hypertension.** © 2010 Movement Disorder Society

Key words: Parkinson's disease; clinical trial; dihydropyridine calcium channel blockers

INTRODUCTION

Isradipine, a dihydropyridine (DHP) Ca^{2+} channel blocker (CCB) approved for treatment of hypertension, was shown to be neuroprotective in preclinical models of parkinsonism.[1] Two recent epidemiological studies demonstrated reduced risk of development of Parkinson's disease (PD) in subjects treated with CCBs compared to other antihypertensive agents.[2,3] To proceed with the pivotal studies of isradipine as a disease modifying agent in PD, safety and tolerability in the PD population has to be established, with emphasis on potential unwanted hypotensive effect.

PATIENTS AND METHODS

This was an open label dose escalation safety and tolerability trial performed at the Northwestern University (NU) Movement Disorders Center. The Institutional Review Board approved the protocol. All subjects signed an informed consent. Two groups of subjects (ages 30–75) were recruited: subjects with early PD either not requiring dopaminergic therapy (DT-) or on a stable regimen of a single dopaminergic agent (DT+), either levodopa (l-dopa) or a dopamine agonist. Use of MAO-B antagonists, amantadine, or anticholinergics was allowed in both groups. Initially, the protocol excluded subjects with hypertension. After the planned interim data analysis demonstrated no significant effect of isradipine on blood pressure in the first 20 subjects, inclusion criteria were revised to recruit subjects with hypertension on a stable regimen of one or two antihypertensive agents.

Inclusion Criteria

Idiopathic PD (based on UK brain bank diagnostic criteria) for $\leq$5 years, Hoehn and Yahr stage (H&Y) $<$2.5, stable regimen of PD medications (if treated) for $\geq$1 month before enrollment.

Exclusion Criteria

Atypical parkinsonian syndromes; baseline BP $<$90/60 mm Hg; orthostatic hypotension at screening [$>$20 mm Hg drop in systolic and 10 mm Hg in diastolic blood pressure (BP) after 2 min of standing]; other medical conditions that, in the opinion of the investigator, would preclude safe use of the drug, cognitive dysfunction [Mini Mental Status Examination (MMSE) score $<$24]; motor fluctuations.

Procedures

Consented subjects underwent screening procedures including a review of eligibility criteria, medical history and medications, Unified Parkinson's Disease Rating Scale (UPDRS) Part I- III,[4] H&Y,[5] Schwab and England Scale (S&E),[6] MMSE,[7] vital signs (including two sets of orthostatic BP), EKG, hematology, and serum chemistry. Subjects maintained twice daily BP logs during the study.

*Correspondence to: Tanya Simuni, Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, Illinois. E-mail: tsimuni@nmff.org

Potential conflict of interest: Nothing to report.

Received 16 March 2010; Revised 30 April 2010; Accepted 25 May 2010

Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23308

TABLE 1. *Baseline demographic and clinical characteristics*

Variable	Subjects N = 31	DT (+) N = 20	DT (−) N = 11	*P*-value
Gender male: female (N)	18:13	12:08	6:05	0.77[a]
Age (years)	58.87 (8.23)	58.45 (9.38)	59.64 (5.94)	0.71
Age at Dx (years)	56.77 (7.92)	56.05 (8.89)	58.09 (5.90)	0.50
PD duration (years)	2.26 (1.57)	2.50 (1.73)	1.82 (1.03)	0.25
History of HTN	6	4	2	
UPDRS I-III	13.55 (5.97)	14.63 (6.49)	11.59 (4.50)	0.18
H&Y	1.95 (0.20)	1.92 (0.24)	2.00 (0.0)	0.19
S&E	93.50 (5.75)	92.89 (6.31)	94.55 (4.72)	0.46
MMSE	29.45 (0.81)	29.32 (0.95)	29.70 (0.48)	0.16
Dopaminergic therapy (DT)				
Dopamine agonists (DA)		16		
Levodopa		4		
Amantadine	7	5	2	
MAO-B inhibitors	10	5	5	
Anticholinergics	1	1	0	
DT duration (for the treated subjects)		2.05 (1.73)		
Daily dose of PD medications (mg)		185 (232)[b]		

Values are mean (standard deviations) unless specified otherwise.
[a]P-value calculated using Chisquare.
[b]Expressed as levodopa equivalent dose.[8]
P, values calculated using Two-tailed *t* test unless specified otherwise; UPDRS, Unified Parkinson's Disease Rating Scale; H&Y, Hoehn and Yahr Stage; S&E, Schwab and England Activities of Daily Living Scale; MMSE Mini Mental State Examination; DT, Dopaminergic therapy (levodopa or dopamine agonist).

Subjects returned 7–10 days after screening and if they still qualified were started on isradipine CR 5 mg, to be taken every morning. Daily isradipine dose was increased by 5 mg every 2 weeks up to 20 mg, if tolerated and provided that BP remained stable (<15% change compared to baseline based on BP logs). In case of intolerability, the dose was reduced by 5 mg increments at any point during the study to the maximum tolerated dose. The subjects remained on that dose for the duration of the study. Drug exposure was 8 weeks for the initial cohort (20 subjects) and was increased to 12 weeks for the subsequent cohort to allow longer observation on a stable dose regimen. During the final visit "on" the drug or in case of early termination, all assessments were repeated and isradipine was tapered by 5 mg every 3 days. The final visit was performed 2 weeks later "off" study drug. UPDRS was performed at baseline, weeks 4, 8, 12 and at the final visit. Adverse events (AE) were recorded at every study visit and weekly phone contacts.

The "primary outcome measure" was the tolerability of isradipine defined as the proportion of subjects completing the study and the proportion of subjects tolerating each dose of isradipine. A dose was considered tolerable if the subject was able to either increase the dose to the next level or remain on a dose for the duration of the study. The dose was also considered tolerable in case early termination was due to nondrug related issues. The "Secondary outcome measures" included comparison of tolerability of isradipine between subjects treated or not treated with DT as well as with antihypertensive therapy; the effect of isradipine on PD motor disability. Safety outcomes included the frequency and type of AEs.

Statistical Analysis

De-identified data were entered into MS Access and converted into *SAS* v9.13 datasets for analysis. As this was a pilot study, we did not prespecify a tolerability threshold. Statistical significance was set at $P < 0.05$ and tested for tolerability between subjects in DT+ versus DT- groups as well as HTN+ versus HTN- subjects using Chi-square test. Changes in BP and heart rate were analyzed as the mean of all daily entries in BP diaries for each study visit and dose level. BP data were tested for statistical significance at each dose level, compared to subject's baseline values using two-tailed *t* test. Change in UPDRS, H&Y, and S&E scores was compared to baseline using two-tailed *t* test. Descriptive statistics were used to analyze safety outcomes.

RESULTS

Thirty-five subjects were screened and 31 were enrolled between January 2007 and March 2009. Subjects' baseline characteristics are presented in Table 1.

Tolerability of Isradipine CR

All subjects were included in the tolerability analysis. Twenty five subjects (81%) completed the study.

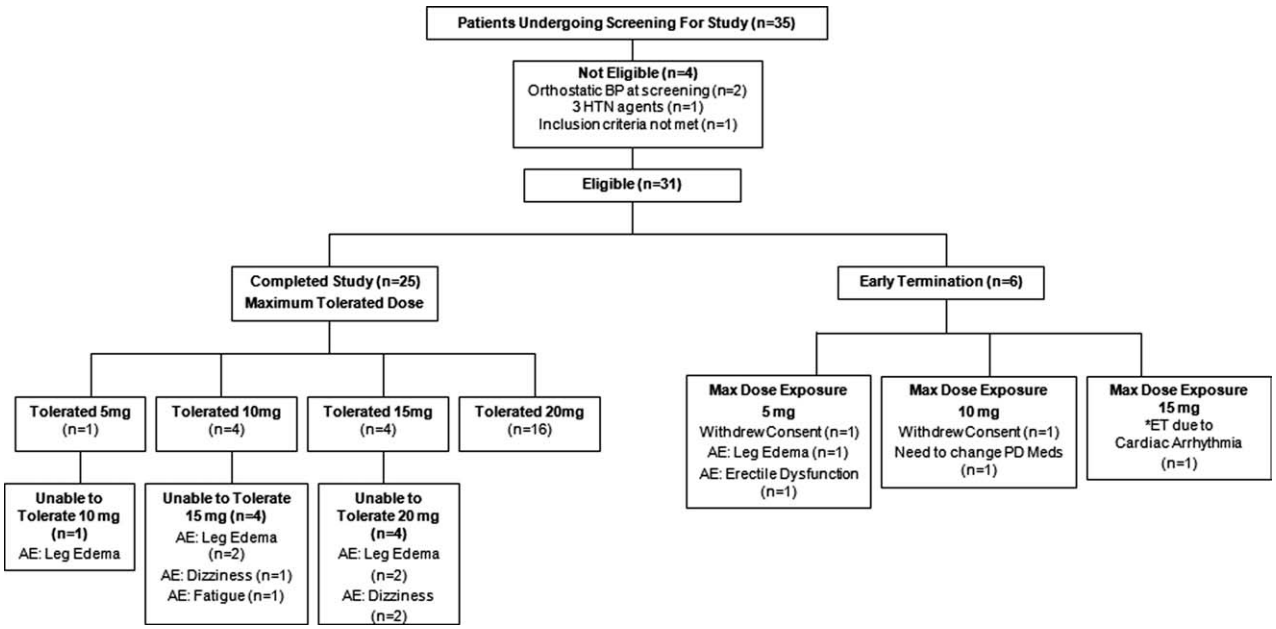


FIG. 1. Study flow chart.

There was no difference in the rate of completion between the DT- or DT+ groups (91% vs. 75%, $P = 0.28$). Concomitant hypertension did not affect the rate of study completion. Six subjects terminated the study early (Fig. 1). Two subjects terminated the study due to inability to tolerate the lowest dose of the drug (5 mg) due to leg edema (1) and erectile dysfunction (1). Both AEs resolved after discontinuation of the drug. Four subjects terminated the study due to issues unlikely related to the drug. The tolerability of isradipine CR was dose-dependent: 94% of subjects tolerated 5 mg; 87% tolerated 10 mg; 68% tolerated 15 mg; and 52% tolerated 20 mg daily dose.

There was no difference between the DT- and DT+ groups or between subjects with or without hypertension in tolerability or in the proportion of subjects reaching the highest dose of isradipine CR. Among the 25 subjects who completed the study, 16 (64%) ended on the 20 mg, 4 (16%) on the 15 mg, 4 (16%) on 10 mg, and 1 (4%) on 5 mg daily dose. Isradipine CR had no significant effect on blood pressure (supine, standing, or orthostatic) or heart rate at any dose. Exposure to DT and effect on BP were not correlated. There was no difference in the effect of isradipine on BP between HTN+ and HTN– subjects. Five subjects had single asymptomatic orthostatic BP recordings in the diaries. No subjects in the subgroup with hypertension required adjustment of their antihypertensive agent.

Isradipine CR Safety Data

There were no serious AEs. The three most common AEs were leg edema (17 subjects), dizziness (10), and fatigue (9). Most AEs were mild, dose dependent, and did not require dose adjustment. Eleven subjects, however, required a dose reduction (including two early terminations) for leg edema (7), dizziness (3), and fatigue (1). Symptoms occurred predominantly at daily doses above 10 mg (9 of 11).

There was no association between the incidence of leg edema and treatment with either dopamine agonists or amantadine. There was no correlation between dizziness and alterations in BP.

Isradipine CR had no effect on PD motor disability, as measured by the change in UPDRS, H&Y and S&E ratings over the course of the study. There was no change in PD motor disability after isradipine CR taper compared to the last observation on the drug.

DISCUSSION

This open- label dose- escalation study demonstrates dose-dependent tolerability of isradipine CR in patients with early PD. A major concern was the risk of isradipine induced hypotension in view of PD-related autonomic dysfunction that can be present even in early disease. Surprisingly, isradipine had no impact on

blood pressure in our cohort. Further study will be required to determine tolerability of isradipine in more advanced PD patients. The safety profile of isradipine in PD population was consistent with the isradipine package insert.[9] The most common AE was leg edema which is related to the potent vasodilatory effect of CCBs rather than fluid retention.[10]

There was no difference in tolerability of isradipine between subjects treated or not treated with dopaminergic medications. The rationale for including both groups was to determine the tolerability of isradipine in subjects who are typically recruited into studies of disease modifying agents, half of whom require initiation of DT during the course of the study.[11] Isradipine had no effect on PD motor disability in our cohort. This was expected given the short duration of treatment and the anticipated mode of action.

In conclusion, isradipine CR at doses up to 10 mg was well tolerated and safe in subjects with early PD. Tolerability of higher doses of isradipine needs to be investigated in a larger cohort of subjects. There was no evidence of an immediate symptomatic effect of isradipine on PD motor disability in this cohort. A randomized double blind study is underway to confirm these outcomes. These studies lay the foundation for the examination of the disease modifying efficacy of isradipine in PD.

Acknowledgments: We thank the staffs who participated in this study. We appreciate critical review of the draft and suggestions provided by Dr. George Bakris. This study was funded by Northwestern Foundation Dixon Translational Grant.

Financial Disclosures: Tanya Simuni, MD has served as a consultant and received honorarium from Novartis, Ibsen, General Electric, UCB Pharma, TEVA, Boehringer Ingelheim, GSK (terminated April 2009). Received research support from NIH, MJ Fox Foundation, TEVA, Takeda. Michael J. Avram, PhD is an editor of Anesthesiology and is on the editorial board of Clinical Pharmacology & Therapeutics. Dr. Avram has been a paid consultant for Alexza Pharmaceuticals, and Eisai Medical Research, Cindy Zadikoff, MD has served as a consultant and received honorarium from TEVA, Ibsen, Allergan. Aleksandar Videnovic, MD has research funding from the AAN Foundation and Parkinson's Disease Foundation. D. James Surmeier, PhD- has a pending use patent for the use of dihydropyridine Ca channel blocking agents in Parkinson's disease.

Author Roles: Tanya Simuni, MD: 1A, 1B, 1C, 2B, 2C, 3A. Emily Borushko, MPH: 1C, 3B. Michael J. Avram, PhD: 1A, 1B, 1C, 2C, 3B. Scott Miskevics, BS: 2A, 2B, 3B. Audrey Martel, BS: 1B, 1C. Cindy Zadikoff, MD: 1B, 1C, 2C, 3B. Aleksandar Videnovic, MD: 1B, 1C, 2C, 3B. Frances M. Weaver, PhD: 1A, 2C, 3B. Karen Williams, BA: 1B, 1C. D. James Surmeier, PhD: 1A, 2C, 3C.

REFERENCES

1. Chan CS, Guzman JN, Ilijic E, et al. 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. Nature 2007; 447:1081–1086.
2. Becker C, Jick SS, Meier CR. Use of antihypertensives and the risk of Parkinson disease. Neurology 2008;70 (Part 2): 1438–1444.
3. Ritz B, Rhodes SL, Qian L, et al. L-type calcium channel blockers and Parkinson disease in Denmark. Ann Neurol 2010;67: 600–606.
4. Goetz CG, Stebbins GT, Chmura TA, et al. Teaching tape for the motor section of the unified Parkinson's disease rating scale. Mov Disord 1995;10:263–266.
5. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology 1967;17:427–442.
6. Schwab RS, England A. Projection technique for evaluating surgery in Parkinson's disease. Gillingham FJ, DI, editors. Presented at the 3rd Symposium on Parkinson's Disease, Edinburgh, Scotland: E & S Livingstone; 1969. pp 152–157.
7. Folstein MF, Folstein SE, Mchugh PR. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–198.
8. Pahwa R, Wilkinson S, Smith D, Lyons K, Miyawaki E, Koller WC. High-frequency stimulation of the globus pallidus for the treatment of Parkinson's disease. Neurology 1997;49: 249–253.
9. Physician desk reference PDR: pdr.net/druginformation/isradipine. Accessed 12 June 2010.
10. Weir MR, Rosenberger C, Fink JC. Pilot study to evaluate a water displacement technique to compare effects of diuretics and ACE inhibitors to alleviate lower extremity edema due to dihydropyridine calcium antagonists. Am J Hypertens 2001;14 (Part 1): 963–968.
11. NINDS NET-PD Investigators. A randomized, double-blind, futility clinical trial of creatine and minocycline in early Parkinson disease. Neurology 2006;66: 664–671.

Carbidopa/Levodopa Pharmacy Errors in Parkinson's Disease

Nasim R. Khadem, BA, and
Melissa J. Nirenberg, MD, PhD*

Department of Neurology and Neuroscience, Weill Cornell Medical College, New York, New York, USA

Abstract: **Outpatient pharmacy errors are common, but little is known about their occurrence in Parkinson's disease (PD). We prospectively studied carbidopa/levodopa pharmacy errors in a cohort of PD outpatients. Over 1 year, pharmacy errors occurred in 8/73 (11%) subjects treated with this medication, producing adverse drug events (ADEs) in 7/8 (87.5%) and increased healthcare utilization in 6/8 (75%) cases. The most common errors were substitution of controlled-release for immediate-release carbidopa/levodopa 25/100 mg (5/8; 62.5%) or dispensation of the wrong carbidopa/levodopa dosage (2/8; 25%). All errors involved ongoing prescriptions, including three interpharmacy transfers. Three subjects (37.5%) questioned pharmacy staff about the change in appearance of the tablets, but the error was corrected in only 1/3 of these cases. Carbidopa/levodopa outpatient pharmacy errors are a common, preventable cause of morbidity and excessive healthcare utilization in PD. Education of healthcare providers, patients, and pharmacy staff is warranted to reduce these errors and associated ADEs.** © 2010 Movement Disorder Society

Key words: carbidopa/levodopa; healthcare utilization; Parkinson; outpatient; pharmacy error; prescription

INTRODUCTION

Medication errors cost the U.S. $177 billion annually and underlie the majority of adverse drug events (ADEs).[1–8] The most common causes of pharmacy errors include dispensation of the incorrect medication, strength, or dosage due to name confusion ("look-alike" or "sound-alike") errors and/or insufficient knowledge of the drug.[2,3,9–15] These errors not only cause morbidity and mortality, but also markedly increase healthcare utilization.[7,8] Thus, prevention of pharmacy errors has the potential to decrease the frequency of ADEs and reduce associated healthcare expenditures.

*Correspondence to: Dr. Melissa J. Nirenberg, Department of Neurology and Neuroscience, Weill Cornell Medical College, New York, New York, USA. E-mail: mjnirenb@med.cornell.edu

Potential conflict of interest: Nothing to report.

Received 7 March 2010; Revised 21 April 2010; Accepted 24 May 2010

Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23311

Levodopa (combined with a dopa decarboxylase inhibitor such as carbidopa) is the most potent and effective medication for Parkinson's disease (PD), and is eventually required for treatment of virtually all PD patients.[16] In the United States, levodopa is available in immediate-release (IR) and controlled-release (CR) carbidopa/levodopa formulations, each of which can be prescribed as either a brand-name drug (Sinemet®, Sinemet CR®) or in one of several generic forms. IR preparations have a shorter half-life, more rapid onset of benefit, and more predictable absorption pattern than CR formulations. The bioavailability of CR formulations is about two-thirds to three-quarters that of IR formulations, such that higher dosages are necessary to achieve the same clinical effect.[16,17] While there is considerable variability in the shape and size of the carbidopa/levodopa tablets supplied by different manufacturers, the color of each formulation is relatively consistent; IR 25/100 mg tablets, for example, are always yellow.[18]

After several patients in our practice experienced severe disability and excess healthcare utilization attributable to carbidopa/levodopa pharmacy errors, we decided to prospectively study this issue in PD. Our goal was to determine whether there were systematic changes that might reduce these errors and associated ADEs.

SUBJECTS AND METHODS

A cohort of nondemented outpatients with PD (n = 90) was recruited as previously described.[19] Inclusion criteria included PD by United Kingdom Brain Bank Criteria[20] and the ability to give informed consent and complete a battery of research questionnaires. Exclusion criteria included a modified Mini-Mental State Examination score <25, use of a dopamine receptor blocking agent, other neurodegenerative disease, or prior PD neurosurgery.

We prospectively recorded and characterized all identified carbidopa/levodopa pharmacy errors in this cohort over a 1-year period beginning on August 1, 2008. Data was obtained from subjects, caregivers, pharmacy staff, and chart reviews. All errors were confirmed by direct visualization of the tablets by the physician and/or pharmacist. Levodopa-equivalent daily doses were calculated as [IR levodopa + (0.70 x CR levodopa)]. Primary outcome measures included the type, duration, and cause of the pharmacy error, as well as associated ADEs and healthcare utilization. Written, informed consent was obtained from all subjects, in accordance with the Weill Cornell Institutional Review Board.

RESULTS

Over the course of 1 year, 73/90 (81.1%) of subjects in the cohort were treated with carbidopa/levodopa, of whom 8/73 (11%) experienced carbidopa/levodopa pharmacy errors (Table 1). Eight different pharmacies located in New York City (Manhattan, Queens, Bronx, Brooklyn), Massachusetts, and New Jersey were involved; local and chain pharmacies were equally represented. The prescriptions included four generated by a computerized physician order entry (CPOE) system (50%), three interpharmacy transfers (37.5%), and one telephone refill (12.5%). All were for generic rather than brand-name carbidopa/levodopa, as is prescribed for almost all patients in our practice.

Most errors (5/8; 62.5%) involved substitution of CR for IR carbidopa/levodopa 25/100 mg, resulting in underdosing. Other errors, each affecting 1/8 subjects (12.5%) included substitution of a higher (Subject A) or lower (Subject B) dosage than prescribed, and erroneous instructions to discontinue IR carbidopa/levodopa (Subject G). The mean duration of exposure to the incorrect dosage/formulation was 42 $\pm$ 35 days (range 0–90).

Of the eight subjects with carbidopa/levodopa pharmacy errors, 6 (75%) recognized a change in appearance of the tablets, and 3/6 (50%) questioned pharmacy staff whether there had been an error; however, the error was acknowledged and promptly corrected in only 1/3 (33.3%) of these cases. The other 2/3 subjects (66.7%) were misinformed that the change was due to differences in the appearance of generic carbidopa/levodopa tablets produced by various manufacturers.

All 7 (100%) subjects who took the incorrect formulation of carbidopa/levodopa experienced ADEs, including a single-car motor vehicle accident in which the wife of Subject C sustained a spinal compression fracture. ADEs were associated with psychological distress, inconvenience, and/or monetary losses in all cases, and increased healthcare utilization in 6/7 cases (85.7%). All subjects eventually returned to (or close to) baseline functional status after resuming the correct carbidopa/levodopa regimen.

All pharmacy errors were attributable to human error. In 7/8 cases (87.5%), this was entirely on the part of pharmacy staff. In the other case (subject G), the possibility of miscommunication between the pharmacist and the physician's staff could not be excluded. Most cases (7/8; 87.5%) involved the refill of a prescription that had previously been dispensed correctly. Only 1/8 (12.5%) involved a new prescription, and this was for the same formulation of carbidopa/levodopa as before (Subject A). In at least half of the cases, the labeling on the prescription bottle was deceptive (e.g., the label did not match the contents).

In one case (Subject H), a large pharmacy supply bottle of CR carbidopa/levodopa 25/100 mg had been placed on a shelf that was normally reserved for IR carbidopa/levodopa 25/100 mg, resulting in repeated medication dispensation errors not only to this subject, but presumably also to all other patients who filled prescriptions for IR carbidopa/levodopa 25/100 mg at this pharmacy for at least a 90-day period. Even after we reported the pharmacy error to the pharmacist, the underlying problem remained uncorrected; this was discovered a month later, when a second patient in our practice experienced the same error from this pharmacy, prompting further investigation.

DISCUSSION

In this study, we identified an alarmingly high rate of outpatient carbidopa/levodopa pharmacy errors, with an 11% incidence in subjects prescribed this medication during the one-year study period. This incidence was considerably higher than expected, falling within the upper range of the total annual estimated incidence of outpatient prescription pharmacy errors in the United States (5 to 13%).[4–6] ADEs were a common consequence, occurring in 7/73 subjects (9.6%) treated with carbidopa/levodopa, and 7/8 (87.5%) with a pharmacy error.

All identified carbidopa/levodopa errors occurred in patients on a stable medication dosage, presumably because patients familiar with a drug formulation were more likely to detect unexpected changes in its appearance or clinical effects. The actual incidence of pharmacy errors in the cohort was presumably higher, because additional errors likely remained undetected, particularly those without associated ADEs. Interpharmacy prescription transfers appeared to be high-risk events; although these occur infrequently, they accounted for 3/8 (37.5%) of the observed pharmacy errors.

The most common pharmacy error was the substitution of CR for IR carbidopa/levodopa, a striking example of a look-alike/sound-alike error. In this case the drug names and dosages are identical, and it is typically only the presence of two letters (e.g., "CR" or "ER") that specifies the controlled-release form, and the *absence* of those letters that denotes the immediate-release form. Changes in the way in which these prescriptions are written and supply bottles are labeled might potentially reduce the frequency of these errors.[11–15]

TABLE 1. *Type, duration, and outcome of pharmacy errors*

Subject	Rx type	Dosage on Rx (mg)	Dosage dispensed (mg)	LEDD prescribed (mg/day)	LEDD dispensed (mg/day)	Error type	Pharmacy type	How error identified	Patient asked pharmacy?	ADE	Duration (days)	Healthcare utilization
A 77/M	CPOE (new Rx, same dosage)	IR 25/100	IR 25/250	1000	2500	↑ ↑ ↑	Local	Color/size (by patient)	Y denied	Severe symptomatic OH, sleep disturbance	30	One call
B 85/M	Interpharmacy transfer	IR 25/250	IR 25/100	1000	400	↓ ↓ ↓	Local to chain (changed ownership)	Color (by wife)	N	Motor deterioration. Weak, slow, difficulty walking, impaired ADLs	60	Blood tests, local PCP visit, several outside neurology visits, one specialist visit, EMG/NCS, spine MRIs, bought a walker
C 79/M	Interpharmacy transfer	IR 25/100	CR 25/100	300	210	↓	Local to local (switched pharmacies)	Color (by MD)	N	Motor deterioration. Difficulty walking, fatigue, insomnia, MVA (spinal compression fx in wife)	30	One PCP visit, one specialist visit, blood & urine tests; ER visit (wife)
D 58/M	CPOE (refill)	IR 25/100	CR 25/100	550	385	↓	Chain	Color (by wife)	Y denied	Motor deterioration	30	One call
E 77/M	Interpharmacy transfer	IR 25/100	CR 25/100	450	315	↓	Chain to chain (switched pharmacies)	Color; hard to cut (by patient)	N	Motor deterioration; increased tremor	90	None
F 64/F	CPOE (refill)	IR 25/100	CR 25/100	400	280	↓	Local (consortium)	Color (by patient)	Y corrected	None (never taken)	0	None
G 62/M	Phone refill	IR 25/100	C/L discontinued	400	0	↓ ↓ ↓	Chain	Worsening symptoms	N	Motor deterioration. Felt "terrible"	2	One call
H 68/M	CPOE (refill)	IR 25/100	CR 25/100	800	560	↓	Local	Color (by patient)	N	Motor deterioration; FOG, insomnia, traumatic falls	90	Three calls, one page, one urgent neurology visit

↓↓↓, large reduction in dosage; ↑ ↑ ↑, large increase in dosage; ↓, small reduction in dosage; ADE, adverse drug event; ADLs, activities of daily living; CPOE, computerized physician order entry system; CR, controlled-release carbidopa/levodopa; EMG/NCS, electromyography and nerve conduction studies; ER, emergency room; FOG, freezing of gait; fx, fracture; IR, immediate-release carbidopa/levodopa; LEDD, levodopa-equivalent daily dosage; MD, medical doctor; MRI, magnetic resonance imaging scan; MVA, motor vehicle accident; OH, orthostatic hypotension; PCP, primary care provider; Rx, prescription.

While the CPOE may significantly decrease certain medication errors, it can also introduce new problems.[21–26] During our investigation of potential causes for carbidopa/levodopa pharmacy errors in our practice, we discovered that the Medi-Span® drug database (Indianapolis, IN) used by our EpicCare Ambulatory© electronic medical record (Verona, WI) would substitute the nontraditional abbreviation "OR" for the standard "PO" to denote oral administration. Thus, prescriptions for IR carbidopa/levodopa were printed as "carbidopa/levodopa 25/100 mg OR", which could be misinterpreted as carbidopa/levodopa 25/100 mg CR. This nontraditional abbreviation might potentially have contributed to up to 3/8 (37.5%) pharmacy errors in our cohort; the other 5/8 errors (62.5%) were clearly unrelated. The problem was corrected after we brought the issue to the attention of our Information Technology group.

Perhaps the most disturbing finding in this study was the frequency of missed secondary opportunities to correct the error and avoid or curtail ADEs. More often than not, when patients questioned pharmacy staff about the change in appearance of the medication tablets, they were falsely reassured. Even after we warned the pharmacist about the medication error that affected subject H, the underlying problem remained uncorrected for at least another month, such that many other patients were presumably also affected.

Given the frequency and serious consequences of carbidopa/levodopa pharmacy errors, we have instituted measures to increase patient education about their PD medications, in both routine patient care and a widely distributed patient-oriented webinar[26] and newsletter.[27] Since we implemented these changes, several additional patients in our practice reported carbidopa/levodopa pharmacy errors, each noting that they had averted adverse consequences because of their newly-acquired awareness of the issue. Thus, even when pharmacy errors occur, patient education can prevent ADEs and reduce unnecessary healthcare expenditures.

Study strengths include the prospective design, acquisition of data from multiple sources, and direct, real-time confirmation of all pharmacy errors. Limitations include the relatively small sample size, restriction to errors identified during clinical practice, and potential selection bias in favor of subjects with ADEs. The findings are also limited to a single academic practice, and therefore may not be applicable to other populations. While we restricted our study to carbidopa/levodopa, healthcare providers should also be cognizant of potential pharmacy errors related to other PD medications. An unintended reduction in the dosage of a dopamine agonist, for example, might potentially cause severe nonmotor symptoms due to dopamine agonist withdrawal syndrome.[19]

In summary, carbidopa/levodopa pharmacy errors are a common, preventable cause of morbidity and excess healthcare utilization in PD. Education of physicians, patients, and pharmacy staff is warranted to reduce these errors and minimize their adverse consequences.

Acknowledgments: We thank Malinka Velcheva and Bill Nikolov for technical assistance. This study was supported by the Parkinson's Disease Foundation.

Author Roles: Khadem was involved in acquisition, analysis, and interpretation of data, drafting and critical revision of the manuscript; Nirenberg was involved in conception and design, acquisition, analysis, and interpretation of data, drafting and critical revision of the manuscript, obtaining funding, and supervision.

Financial Disclosures: Khadem: Nothing to disclose. Nirenberg: Received research support from NIH/NINDS and the Parkinson's Disease Foundation; Consulting: Biovail, Editorial Work: AHC Media and Tarascon.

REFERENCES

1. Frey D, Rahman A. Medication management: an evidence-based model that decreases adverse events. Home Healthc Nurse 2003; 21:404–412.
2. Krahenbuhl-Melcher A, Schlienger R, Lampert M, Haschke M, Drewe J, Krahenbuhl S. Drug-related problems in hospitals: a review of the recent literature. Drug Saf 2007;30: 379–407.
3. Cina JL, Gandhi TK, Churchill W, et al. How many hospital pharmacy medication dispensing errors go undetected?. Comm J Qual Patient Saf 2006;32:73–80.
4. Balkrishnan R, Foss CE, Pawaskar M, Uhas AA, Feldman SR. Monitoring for medication errors in outpatient settings. J Dermatolog Treat 2009;20:229–232.
5. Hutchinson TA, Flegel KM, Kramer MS, Leduc DG, Kong HH. Frequency, severity and risk factors for adverse drug reactions in adult out-patients: a prospective study. J Chronic Dis 1986;39: 533–542.
6. Gandhi TK, Weingart SN, Seger AC, et al. Outpatient prescribing errors and the impact of computerized prescribing. J Gen Intern Med 2005;20:837–841.
7. Thomsen LA, Winterstein AG, Sondergaard B, Haugbolle LS, Melander A. Systematic review of the incidence and characteristics of preventable adverse drug events in ambulatory care. Ann Pharmacother 2007;41:1411–1426.
8. Gurwitz JH, Field TS, Harrold LR, et al. Incidence and preventability of adverse drug events among older persons in the ambulatory setting. JAMA 2003;289:1107–1116.
9. Bates DW, Cullen DJ, Laird N, et al.ADE Prevention Study Group. Incidence of adverse drug events and potential adverse drug events. Implications for prevention. JAMA 1995;274:29–34.
10. Winterstein AG, Sauer BC, Hepler CD, Poole C. Preventable drug-related hospital admissions. Ann Pharmacother 2002;36: 1238–1248.
11. Australian Council for Safety and Quality in Healthcare. Second national report on patient safety improving medication safety. Australian Council for Safety and Quality in Healthcare: 2002.

12. McCoy LK. Look-alike, sound-alike drugs review: include look-alike packaging as an additional safety check. Jt Comm J Qual Patient Saf 2005;31:47–53.
13. Walliser G, Grossberg R, Reed MD. Look-alike medications: a formula for possible morbidity and mortality in the long-term care facility. J Am Med Dir Assoc 2007;8:541–542.
14. Lambert BL, Lin SJ, Chang KY, Gandhi SK. Similarity as a risk factor in drug-name confusion errors: the look-alike (orthographic) and sound-alike (phonetic) model. Med Care 1999;37: 1214–1225.
15. Lambert BL. Predicting look-alike and sound-alike medication errors. Am J Health Syst Pharm 1997;54:1161–1171.
16. Nirenberg MJ, Fahn S. The Role of Levodopa and Catechol-O-Methyltransferase Inhibitors. In: Schapira A., Olanow C. W., editors. Principles of treatment in Parkinson's disease. Philadelphia: Butterworth Heinemann Elsevier; 2005. p 3–24.
17. Olanow CW, Stern MB, Sethi K. The scientific and clinical basis for the treatment of Parkinson disease. Neurology 2009;72 (21 Suppl 4):S1–136.
18. Ahlskog J. Initial symptomatic treatment of Parkinson's disease. In: Adler C. H., Ahlskog J. E., editors. Parkinson's disease and movement disorders: diagnosis and treatment guidelines for the practicing physician, First edition: Humana Press; 2000. 124–132.
19. Rabinak CA, Nirenberg MJ. Dopamine agonist withdrawal syndrome in Parkinson disease. Arch Neurol 2010;67:58–63.
20. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55: 181–184.
21. Shamliyan TA, Duval S, Du J, Kane RL. Just what the doctor ordered. Review of the evidence of the impact of computerized physician order entry system on medication errors. Health Serv Res 2008;43 (Part 1):32–53.
22. Ammenwerth E, Schnell-Inderst P, Machan C, Siebert U. The Effect of Electronic Prescribing on Medication Errors and Adverse Drug Events: A Systematic Review. J Am Med Inform Assoc 2008;15:585–600.
23. Rupp MT, Warholak TL. Evaluation of e-prescribing in chain community pharmacy: best-practice recommendations. J Am Pharm Assoc 2008;48:364–370.
24. Horsky J, Kuperman GJ, Patel VL. Comprehensive analysis of a medication dosing error related to CPOE. J Am Med Inform Assoc 2005;12:377–382.
25. Koppel R, Metlay JP, Cohen A, et al. Role of computerized physician order entry systems in facilitating medication errors. JAMA 2005;293:1197–1203.
26. Kaushal R, Kern LM, Barron Y, Quaresimo J, Abramson EL. Electronic prescribing improves medication safety in community-based office practices. J Gen Intern Med 2010.
27. Nirenberg, MJ. PD ExpertBriefing: Parkinson's Disease and its Treatment: Secrets, Myths and Misconceptions, 2009; Available at http://www.pdf.org/parkinson_briefing_treatments_myths.
28. Nirenberg MJ. Parkinson's disease secrets, myths and misconceptions; Parkinson's disease news & review. Winter: Parkinson's Disease Foundation; 2009, Available at http://www.pdf.org/pdf/NL_Winter_10.pdf.

Clinical Course of the First Asian Family with Parkinsonism Related to SNCA Triplication

Takeshi Sekine, MD,[1] Hajime Kagaya, MD,[2] Manabu Funayama, PhD,[1,3] Yuanzhe Li, PhD,[3] Hiroyo Yoshino, BS,[3] Hiroyuki Tomiyama, MD, PhD,[1] and Nobutaka Hattori, MD, PhD[1,3]*

[1]Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan; [2]Department of Neurology, Nakadori General Hospital, Akita, Japan; [3]Research Institute for Diseases of Old Age, Graduate School of Medicine, Juntendo University, Tokyo, Japan

Abstract: **Triplication of *SNCA* is a rare cause of familial Parkinson's disease compared with duplication. Its clinical course is believed to be more robust than duplication, though it is uncertain. Marked as the first among the Asian population, we identified a Japanese family (paternal grandfather, father, and son) with *SNCA* triplication based on genetic and clinical analyses. The proband had a completely triplicated region including *SNCA*. This allele did not share any common haplotypes with those of previously reported Japanese families with *SNCA* duplication. Clinical analysis indicated early onset, rapidly progressive parkinsonism with mild levodopa response. Further studies are needed to clarify the gene dose effect of *SNCA*.** © 2010 Movement Disorder Society

Key words: SNCA; triplication; duplication; familial Parkinson's disease

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer disease. About 5% of patients with PD have the familial form, which is caused by a single gene disorder of *SNCA, LRRK2, UCH-L1, PRKN, DJ-1, PINK1*, or *ATP13A2*. Duplication of the *SNCA* gene is relatively frequent in autosomal dominant PD, and is also seen in sporadic PD due to its low penetrance.[1–5] However, triplication of *SNCA* is rare and to our knowledge, only three families have been described so far.[6–8] The clinical course of triplication is believed to be more robust than duplication due to its gene dose effect,

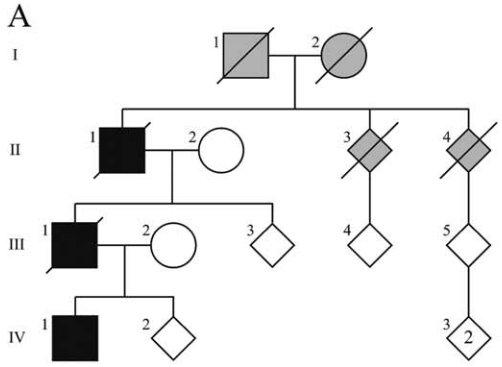
*Correspondence to: Nobutaka Hattori, Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. E-mail: nhattori@juntendo.ac.jp

Potential conflict of interest: Nothing to report.

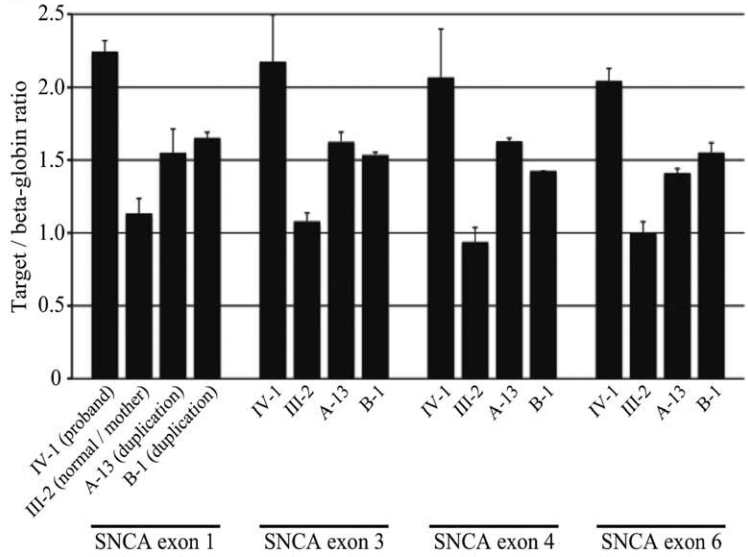
Received 16 November 2009; Revised 9 April 2010; Accepted 24 May 2010

Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23313

A



B



C

	IV-1/control	III-2/control
SNCA Exon 1	2.1	1.0
SNCA Exon 3	2.3	1.1
SNCA Exon 4	1.9	1.2
SNCA Exon 5	1.7	1.0
SNCA Exon 6	2.0	1.1

Gene dosage ratio.

D

	Triplication				Reported Japanese duplication families																			
Microsatellite	IV-1				Family A			Family F			Family B			Family C			Family D			Family G			Family E		
D4S3475	223	219	219	219	221	219	219	219	219	219	219	221	221	221	221	221	221	221	221	219	221	221	221	219	219
D4S3477	261	263	263	263	261	265	265	265	265	265	263	261	261	265	261	261	261	261	261	263	261	261	263	263	263
D4S3480	227	219	219	219	227	236	236	219	236	236	219	228	228	236	228	228	228	228	228	219	228	228	228	219	219
D4S3479	176	178	178	178	176	172	172	178	172	172	178	174	176	172	174	176	176	174	174	178	174	174	174	178	178
D4S3474	211	211	211	211	215	209	209	205	209	209	205	205	213	203	205	213	213	205	205	211	205	205	205	205	205

FIG. 1. Pedigree of SNCA triplication family (**A**). Black symbols: affected patients, gray symbols: unaffected individuals. Gene dosage analyses using real-time PCR (**B**) and MLPA (**C**). Haplotype analysis (**D**). The disease haplotypes are highlighted in gray.

but the precise picture remains to be defined as there is insufficient information about triplication.

Here we report the first Asian family with *SNCA* triplication presented with early-onset and severe clinical features of parkinsonism.

METHODS

Subjects

The pedigree studied is shown in Figure 1A. The DNA was extracted from peripheral blood samples obtained from the proband (IV-1) and his mother (III-2). Approval for the study was obtained from the ethics review committee of Juntendo University. To compare the gene dosages and haplotype of *SNCA* and its flanking region, we also examined patients from Japanese families with duplication of the *SNCA* gene.[1,4]

Genetic Analyses for SNCA

Mutation screening was performed as described previously.[1] Semiquantitative multiplex polymerase chain reaction (PCR) of genomic DNA samples was performed using real-time PCR to detect the dosage of *SNCA* (7500 Fast Real-Time PCR system, Applied Biosystems, Foster City, CA). In the first step, we targeted exon 4 of *SNCA* to screen the gene dosage. The "beta-globin" gene was amplified as an endogenous reference, and patients with *SNCA* duplication, who were confirmed by fluorescence in situ hybridization, were used as positive control (Patients A-13 and B-1).[1] The primer and probe sequences and the methods were described previously.[1] In the second step, we performed semiquantitative analysis on *SNCA* exons 1, 3, 6 and flanking genes (*LOC345278, MMRN1*, and *KIAA1680*) for the patients found in the first step to carry multiplication of this gene.

To confirm the gene dosage, we also performed multiplex ligation-dependent probe amplification (MLPA). Employing the SALSA MLPA P051-C1 Parkinson-1 probe mix (MRC Holland, Amsterdam, The Netherlands) using the DNA detection/quantification protocol provided by the manufacturer, products were quantified by the ABI 3130 Genetic Analyzer and Gene Mapper

v3.7 (Applied Biosystems, Foster City, CA). The MLPA data were analyzed as described previously.[9]

To determine whether the same haplotype was shared between our probands with *SNCA* multiplication, we performed haplotype analysis of the proband, mother, and samples of previously described families,[1,4] using five microsatellite including D4S3475, D4S3477, D4S3480, D4S3479, and D4S3474.[4]

RESULTS

Gene Dosage Analysis

Gene dosage analysis, both real-time PCR method and MLPA, revealed that the proband (IV-1) had four *SNCA* alleles (Fig. 1B,C), whereas the mother (III-2) had two normal *SNCA* alleles (Fig. 1B,C). These findings indicated that the proband had triplication of *SNCA*. The range of triplication includes whole exons of *SNCA, MMRN1* and exon 1 of *KIAA1680* according to the result of real-time PCR (data not shown).

Haplotype Analysis

Haplotype analysis indicated that the haplotype of the proband was different from that of Japanese families with *SNCA* duplication, as reported by our group previously (Fig. 1D).[4]

Case Records

II–1

This is the grandfather of the proband. At 49 years of age, he developed bradykinesia, slowness of speech, and sialorrhea. Hyposmia was not obvious. At age 50, he noted resting tremor in the left hand and foot. In addition, he became akinetic and depressed. At age 51, he was diagnosed with PD and treated with 600 mg/day levodopa (L-dopa). The treatment alleviated parkinsonism, but at age 52, he developed frequent drop attacks due to severe orthostatic hypotension. Another autonomic nervous system-related symptom was severe constipation. After several drop attacks, he suffered head contusion, which was followed by progressive deterioration of cognitive function until age 54. The parkinsonism was Hoehn & Yahr stage V at age 55. He died during an attack of pneumonia at age 57.

III–1

This is the father of the proband. At age 33 years, he developed masked face, resting tremor, bradykinesia, and antecollis. However, he did not complain of hyposmia. He was diagnosed the same year with PD and treated with L-dopa, which resulted in alleviation of symptoms. At age 34, he became apathic and developed dizziness due to orthostatic hypotension. At age 35, he became a pathological gambler and suffered from insomnia. At age 36, he was arrested for stealing. Subsequently, he was admitted to the hospital for further evaluation and treatment. On examination, the clinical symptoms included bradykinesia, masked face, muscle rigidity, antecollis and disturbed postural reflex. However, the gait showed no remarkable disturbance. He also had orthostatic hypotension, impotence, and constipation. In addition to the motor and autonomic symptoms, he had neurosis, insomnia, and suicide intent. This symptom complex was collectively diagnosed as depression. The parkinsonism gradually worsened after discharge from the hospital and was staged as Hoehn & Yahr stage V at age 37. He died at age 40.

IV–1

The proband was a 31-year-old man with familial parkinsonism of three generation. At age 28 years, he developed tremor and rigidity in the left hand and foot. No complaint of hyposmia was reported. One year later, he developed bradykinesia, orthostatic hypotension, and mild decline of intellectual activity. At age 30, he was admitted to the hospital and diagnosed with Parkinson disease, Hoehn & Yahr stage III. He had resting tremor, muscle rigidity, bradykinesia (masked face and gait disturbance), and disturbed postural reflex. Remarkable orthostatic hypotension was noted (systolic blood pressure in supine position 120 mm Hg, falling to 80 mm Hg on standing). Constipation was not prominent. No pyramidal signs were noted. As for intellectual activity, the WAIS-R score was 75 (verbal IQ 78 and motor IQ 76), although the mini mental state examination score was 29/30. There was no history of hallucination, nightmare, or REM sleep behavior disorder. Magnetic resonance imaging of the brain did not showed any remarkable findings. Single photon emission computed tomography of cerebral blood flow showed reduced blood flow in the parieto-occipital area. Myocardial scintigraphy of ^{123}I-metaiodobenzylguanidine showed markedly low heart-to-mediastinum ratio of 1.27 in the early stage and 1.23 in the late stage.

Treatment of parkinsonism consisted of 150 mg/day of L-dopa to alleviate symptoms. The dose was later increased to 300 mg/day of L-dopa, and combined with pramipexol (3 mg/day), selegiline (2.5 mg/day), and droxydopa (800 mg/day). Doubling the selegirin dose resulted in a transient episode of drug hypersensitivity,

but the symptoms were almost relieved and the Hoehn & Yahr stage improved to III.

DISCUSSION

This is the first case report of parkinsonism with *SNCA* triplication in an Asian family. The paternal grandfather, father and son of one family developed parkinsonism with ages at onset (AAO) under 50, suggesting autosomal dominant hereditary pattern and high penetrance. Based on the quantitative PCR study, we confirmed that this family have triplicated genetic region, which includes the *SNCA* gene.

To clarify the mechanism of copy number variation of the *SNCA* gene, we performed haplotype analysis of this triplication family and other unrelated Japanese family members with *SNCA* duplication. If the triplicated allele were generated from a duplicated allele, the proband of triplication might share a common haplotype with other families. However, the result of the analysis indicated that the triplication family had a different allele. Thus, the mechanism of *SNCA* triplication, whether it is self-duplicated by two-step or generated from recombination process, remains an important issue to be analyzed in future studies.

Limited to the three consecutive generations (proband, farther, and paternal grandfather), the penetrance is high. However, only three individuals were affected among 15 members of the family, and incomplete penetrance is the more favorable explanation. On the other hand, the AAO of the three affected members were under 50 years, which is younger than AAO of patients with *SNCA* duplication (mean $\pm$ SD: 48.5 $\pm$ 11.2 years)[4].

The AAO was markedly different between the generations. The paternal grandfather (II-1) developed parkinsonism at about 50 years of age, which is almost similar to *SNCA* duplication. On the other hand, both the proband and his father developed parkinsonism around 30 years of age. This phenomenon might imply anticipation between II-1 and III-1. In the case of a Swedish family, the ancestors of the family members with triplication were considered to have carried duplication.[8,10] Alternatively, it could reflect insufficient information about parkinsonism in the grandfather due to diagnostic difficulties in the past.

The clinical features of this family included early-onset parkinsonism with cognitive or mental disorders, and an aggressive disease course within a period of about 10 years. Treatment with L-dopa was effective at least in the early stage, though it did not improve the outcome. The two features; early AAO and cognitive or mental disturbances, are explainable by the dosage effect of α-synuclein; a higher production of the protein is associated with earlier AAO. Furthermore, a larger amount of the product results in a wider pathological picture. The clinical course of PD is mainly influenced by the AAO in general, but the mechanism of early ineffectiveness of L-dopa remains elusive. A more generalized pathology, which commonly involves the autonomic nervous system, manifested by orthostatic hypotension, is one possible explanation of the poor prognosis.

Finally, *SNCA* triplication is a rare event compared to the frequency of duplication. The clinical phenotype of triplication is severe among the Japanese population. In other words, the copy number of *SNCA* could determine the severity of the PD phenotype similar to the age at onset in the Asian population.[1,4] Further research on *SNCA* triplication is needed including its mechanism and differences in the clinical features between duplication and triplication.

Acknowledgments: We are grateful to the patients and their families, and all participants. This work was supported by High-Tech Research Center Project, Grant-in-Aid for Scientific Research (to N.H., 17390256, and to H.T., 21591098), Grant-in-Aid for Scientific Research on Priority Areas (to N.H., 08071510), and Grant-in-Aid for Young Scientists (to M.F., 20790625) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Financial Disclosures: M.F.: Grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology and Grant-in-Aid for Young Scientists (20790625). H.T.: Grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Grant-in-Aid for Scientific Research (21591098) from the Japanese Ministry of Health, Labor and Welfare, and Grant-in-Aid from the Research Committee of Muro disease (Kii ALS/PDC) (21210301). N.H.: Grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Grant-in-Aid for Scientific Research (09005213), and Grant-in-Aid for Scientific Research on Priority Areas (08071510), and Health and Labor Sciences Research Grants from the Japanese Ministry of Health, Labor and Welfare (H19-021 and H20-015).

Author Roles: Sekine—Research project: Execution; Statistical Analysis: Design, Execution, Review and Critique; Manuscript: Writing of the first draft. Kagaya—Research project: Execution; Statistical Analysis: Execution, Review and Critique; Manuscript: Review and Critique. Funayama—Research project: Organization, Execution; Statistical Analysis: Design, Execution, Review and Critique; Manuscript: Review and Critique. Li—Research project: Execution; Statistical Analysis: Execution, Review and Critique; Manuscript: Review and Critique. Yoshino—Research project: Execution; Statistical Analysis: Execution, Review and Critique; Manuscript: Review and Critique. Tomiyama—Research project: Organization; Statistical Analysis: Review and Critique; Manuscript: Review and Critique. Hattori—

Research project: Conception, Organization; Manuscript: Review and Critique.

REFERENCES

1. Nishioka K, Hayashi S, Farrer MJ, et al. Clinical heterogeneity of alpha-synuclein gene duplication in Parkinson's disease. Ann Neurol 2006;59:298–309.
2. Ibáñez P, Bonnet AM, Débarges B, et al. Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. Lancet 2004;364:1169–1171.
3. Chartier-Harlin MC, Kachergus J, Roumier C, et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 2004;364:1167–1169.
4. Nishioka K, Ross OA, Ishii K, et al. Expanding the clinical phenotype of SNCA duplication carriers. Mov Disord 2009;24: 1811–1819.
5. Ahn TB, Kim SY, Kim JY, et al. Alpha-Synuclein gene duplication is present in sporadic Parkinson disease. Neurology 2008; 70:43–49.
6. Singleton AB, Farrer M, Johnson J, et al. Alpha-Synuclein locus triplication causes Parkinson's disease. Science 2003;302:841.
7. Ibáñez P, Lesage S, Janin S, et al. Alpha-synuclein gene rearrangements in dominantly inherited parkinsonism: frequency, phenotype, and mechanisms. Arch Neurol 2009;66: 102–108.
8. Fuchs J, Nilsson C, Kachergus J, et al. Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication. Neurology 2007;68:916–922.
9. Keyser RJ, Lombard D, Veikondis R, et al. Analysis of exon dosage using MLPA in south African Parkinson's disease patients. Neurogenetics 2009 Dec 15. [Epub ahead of print].
10. Ross OA, Braithwaite AT, Skipper LM, et al. Genomic investigation of alpha-synuclein multiplication and parkinsonism. Ann Neurol 2008;63:743–750.

Spinocerebellar Ataxia Type 10: Frequency of Epilepsy in a Large Sample of Brazilian Patients

Hélio A.G. Teive,[1]* Renato P. Munhoz,[1] Salmo Raskin,[1] Walter O. Arruda,[1] Luciano de Paola,[1] Lineu C. Werneck,[1] and Tetsuo Ashizawa[2]

[1]Department of Internal Medicine, Movement Disorders Unit, Neurology Service, Hospital de Clínicas, Federal University of Paraná, Curitiba, Paraná, Brazil; [2]Department of Neurology, University of Florida, Gainesville, Florida, USA

Abstract: **Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant disorder caused by an ATTCT repeat intronic expansion in the SCA10 gene. SCA 10 has been reported in Mexican, Brazilian, Argentinean and Venezuelan families. Its phenotype is overall characterized by cerebellar ataxia and epilepsy. Interestingly, Brazilian patients reported so far showed pure cerebellar ataxia, without epilepsy. Here, authors provide a systematic analysis of the presence, frequency and electroencephalographic presentation of epilepsy among 80 SCA10 patients from 10 Brazilian families. Overall, the frequency of epilepsy was considered rare, been found in 3.75 % of the cases while this finding in populations from other geographic areas reaches 60% of SCA10 cases.** © 2010 Movement Disorder Society

Key words: spinocerebellar ataxia type 10; SCA; autosomal dominant cerebellar ataxia; epilepsy

Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant disorder caused by a large expansion of a pentanucleotide (ATTCT) repeat in the intron 9 of the SCA10 gene on chromosome 22.[1–5] SCA10 is the only neurodegenerative disease caused by an expansion of a pentanucleotide repeat. Pathogenic alleles range from 800 to 4500 ATTCTs (normal 10 to 29).[1,3] SCA10 has previously been reported in Mexican families, in which the disease presented with a unique combination of pure cerebellar ataxia, epilepsy and, at times, polyneuropathy, pyramidal signs, and cognitive dysfunction.[1–5] In 2004, we described the clinical phenotype of five Brazilian families with SCA10 presenting with pure cerebellar ataxia but no associated epilepsy.[6]

*Correspondence to: Dr. Hélio A.G. Teive, Rua General Carneiro 1103/102, Centro, Curitiba, Paraná 80060-150, Brazil.
E-mail: hagteive@mps.com.br

Potential conflict of interest: Nothing to report.

Received 3 March 2010; Revised 4 May 2010; Accepted 1 June 2010

Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23324

TABLE 1. *Clinical and genetic aspects of Brazilian, Mexican, Argentinian, and Venezuelan patients with SCA 10*

	Brazilian patients	Mexican patients	Argentinean patients	Venezuelan patients
Number of patients	80	19	5	5
Age of onset (yr)	35.5 (22–46)	26.7 (14–44)	35	14 (case report)
Number of ATTCT repeats	1,820 (20)	2,838	1,100	4,400
Correlation between size of ATTCT repeats and age of onset	Inverse correlation	Inverse correlation	–	–
Cerebellar ataxia	100%	100%	100%	100%
Pyramidal signs	6 (mild hyperreflexia), 3 (mild spasticity)	6 ("soft" pyramidal signs), 2 (pyramidal signs)	2	–
Epilepsy	3.75%	72.2%	100%	80%
Peripheral Neuropathy	0%	66%	–	–
Ethnical origin (by history)	Indian ancestry 75%	Indian ancestry 100%	Mixed Spanish and Amerindian	Unknown

The objective of our study is to analyze the frequency and characteristics of epilepsy in a large sample of Brazilian patients with SCA10.

METHODS

We studied 80 patients from 10 unrelated families with SCA10, selected out of 180 Brazilian genetically proven SCA families followed at the Hospital de Clínicas, Federal University of Paraná in Curitiba, Brazil, from 1990 to 2009. This cohort includes all cases of SCA 10 diagnosed so far in our service. Signed informed consent was obtained based on a protocol approved by the local Ethics Committee. Five of these ten families have already been published by the authors elsewhere.[6] All patients were evaluated by 3 neurologists (HT, WOA, and RPM) and a medical geneticist (SR). History, physical examination, and routine laboratory tests, including complete blood count, blood urea nitrogen, creatinine, electrolytes, glucose, liver, and thyroid function tests, and venereal disease research laboratory (VDRL), were performed. The diagnosis of epilepsy was ascertained via clinical history. Detailed family history of each patient was obtained and the information was double-checked with close relatives. The following studies were also performed in all patients: brain computed tomography and magnetic resonance imaging (MRI), electroencephalography (EEG), and routine cerebral spinal fluid (CSF) analysis. Molecular analysis of the ATTCT repeat expansion in the SCA10 gene was performed by polymerase chain reaction (PCR) amplification using primers attct-L (5′-AGAAAA CAGATGGCAGAATGA-3′) and attct-R (5′-GCCTGGGC AACATAGAGAGA-3′), as described previously. Patient deoxyribonucleic acid samples that showed a single normal SCA10 allele by PCR underwent Southern blot analysis to assess large expansions.

RESULTS

From the total 80 patients examined, 40 (50%) were male with mean age of onset of 35.5 years, and mean disease duration of 15.3 years. Among the 10 families, number of affected members studied varied from 1 (Ref. 7) to 21 subjects (mean 8 per family). All patients presented with cerebellar syndrome (predominantly gait ataxia, with dysarthria and nystagmus). Six (7.5%) patients had mild lower limbs hyperreflexia with spasticity in three. Three (3.75 %) of the 80 patients had a history compatible with seizures, including generalized tonic-clonic seizures in two cases and a combination of myoclonic, complex partial, and generalized tonic-clonic seizures, with occasional status epilepticus in the third patient. The later case has been previously published as a 28-year-old woman with progressive cerebellar ataxia starting at childhood, followed by seizures/epilepsy (at age 23 years) and progressive cognitive dysfunction (at age 24 years), and definite dementia (at age 27 years).[7] Both cases with tonic-clonic seizures belonged to the same family. This family is the largest of our cohort with 21 affected members studied so far. Molecular genetic testing of this patient showed an expanded allele of 850 ATTCT repeats. The other 2 patients with SCA 10 and epilepsy had expanded alleles with 1250 (35-year-old female patient) and 1500 repeats (55-year-old male patient).

Brain MRI of all index cases (n: 10) showed cerebellar atrophy. Brainstem atrophy was found in 3 cases and brain atrophy in 1 case. Interictal EEG of these 3 cases was abnormal in only 1 patient, showing diffuse disorganization but no clear cut epileptiform activity (patient published previously).[7] Patients with SCA10 with epilepsy did not differ from molecular and demographic standpoints in regards to those with pure cerebellar ataxia.

The comparison between Brazilian, Mexican (published by Rasmussen et al.),[4] Argentinean, and Venezuelan patients, with SCA10 is showed in Table 1.

DISCUSSION

SCA10 is an autosomal dominant neurodegenerative disease initially described only in Mexican families.[1–6] In 2002, Matsuura et al.[8] studied the presence of SCA in several non-Mexican populations, including White American, French-Canadian, Italian, Japanese, and Spanish patients, in whom no pathogenic ATTCT expansion repeat was detected. Later, Teive et al.[6] reported on 28 SCA 10 patients from five new Brazilian families with a new phenotype: pure cerebellar ataxia, without epilepsy. This study also showed that SCA10 is the second most common autosomal dominant cerebellar ataxia in Brazil (after SCA type 3), as had already been shown for the Mexican population where SCA type 2 is the most common form. In both countries, all SCA10 families reported Amerindian ancestry.[4,6,7,9] Two additional reports on non-Brazilian South American populations diagnosed with SCA 10 were published more recently. Gatto et al.[10] reported on two SCA 10 Argentinean patients presenting with cerebellar ataxia and epilepsy, associated with additional motor signs (dystonia in 1 case and parkinsonism on the other). Gallardo and Soto[11] described a patient from Venezuela, also genetically confirmed with SCA 10, in whom cerebellar ataxia and cognitive dysfunction coexist with epilepsy. Almeida et al.[12] studied the ancestral origin of the ATTCT repeat expansion in SCA10 concluding that there may be a common ancestral for SCA10 in Latin America, probably with Amerindian origin, who later on spread into the mixed populations of Mexico and Brazil.

Here, we report a large series of Brazilian patients with the SCA10 mutation, showing that epilepsy, one of the particular aspects of this disorder in Mexico, Argentina, and Venezuela, is very uncommon, leaving the presentation of a pure cerebellar ataxia. The incidence of epilepsy in developed countries is reportedly between 0.04 and 0.07%/year. In resource-poor countries, these figures are higher, around 0.12%/year with prevalence rates between 0.6 and 1%.[13] These figures were recently confirmed in a descriptive study of epilepsy epidemiology, with the caveat that regional environmental exposures and socioeconomic status may have biased the statistics.[14] Specifically, data regarding to the epidemiology of epilepsy in Brazil is somewhat scattered. A recent project by Li et al.,[15] part of a WHO/ILAE/IBE Global Campaign, disclosed a prevalence of 0.92%. Thus, the 3.75% rate of epilepsy in our sample seems to rest above the expected frequency in the general population but significantly below the 60% reported in Mexican families with SCA 10.

These data demonstrated that the phenotypic expression of the SCA10 mutation in Brazilian families, with predominantly pure cerebellar ataxia, is rather different from Mexican, as well as from Argentinean and Venezuelan cases, where cerebellar ataxia and epilepsy represent the most common phenotype (up to 60% in the Mexican patients). Our 3 patients with epilepsy presented with generalized tonic-clonic seizures in 2 cases, and in only 1 case, previously published, we found myoclonic seizures, complex partial seizures and generalized tonic-clonic seizures. This patient had a progressive cerebellar ataxia, with epilepsy and dementia. Brain MRI of these cases showed predominantly cerebellar atrophy, and the EEG tracings did not reveal specific abnormalities.

Based on our cohort, the differing phenotype of Brazilian and Mexican patients cannot be explained based on the ATTCT repeat expansion size, mostly because the repeat size of these two SCA 10 populations overlapped. Of importance, in the Mexican SCA 10 patients with epilepsy there was a wide range of the repeat expansion sizes, suggesting that this molecular variable is probably not independently related with epilepsy.[6] Other alternative explanations, such as somatic and germline instability of the ATTCT repeat in SCA 10 and the effect of interruptions in the expanded ATTCT repeats, may contribute to this phenotypic variation and should be studied in future investigations.[16–18]

Acknowledgments: This work was supported by NIH NS041547 (TA).

Financial Disclosures: Dr. H.A.G. Teive: Stock Ownership in medically-related fields none; Intellectual Property Rights none; Consultancies none; Expert Testimony none; Advisory Boards none; Employment Federal University of Parana; Partnerships none; Contracts none; Honoraria none; Royalties none; Grants none; Other none. Dr. R.P. Munhoz: Stock Ownership in medically-related fields: none; Intellectual Property Rights: none; Consultancies none; Expert Testimony: none; Advisory Boards: none; Employment Pontifical Catholic University of Parana, Brazil; Partnerships: none; Contracts: none; Honoraria: none; Royalties: none; Grants: none; Other: none. Dr. S. Raskin: Stock Ownership in medically-related fields: none; Intellectual Property Rights: none; Consultancies: none; Expert Testimony: none; Advisory Boards: none; Employment Pontifical Catholic University of Parana, Brazil; Partnerships: none; Contracts: none; Honoraria: none; Royalties: none; Grants: none; Other: none. W.O. Arruda: Stock Ownership in medically-related fields none; Intellectual Property Rights none; Consultancies none; Expert Testimony none; Advisory Boards none; Employment Federal

University of Parana, Brazil; Partnerships none; Contracts none; Honoraria none; Royalties none; Grants none; Other Support to attend scientific meetings from Bayer-Schering, Biogen-Idec, Merck-Serono and Teva. L. De Paola: Stock Ownership in medically-related fields none; Intellectual Property Rights none; Consultancies none; Expert Testimony none; Advisory Boards none; Employment Federal University of Parana, Brazil; Partnerships none; Contracts none; Honoraria none; Royalties none; Grants none; Other none. L.C. Werneck: Stock Ownership in medically-related fields none; Intellectual Property Rights none; Consultancies none; Expert Testimony none; Advisory Boards none; Employment Federal University of Parana, Brazil, Partnerships none; Contracts none; Honoraria none; Royalties none; Grants none; Other none. T. Ashizawa: Stock Ownership in medically-related fields US Patents #6,855,497 and #6,048,529; Intellectual Property Rights none; Consultancies none; Expert Testimony none; Advisory Boards National Ataxia Foundation Medical and Research Advisory Board, Myotonic Dystrophy Foundation Scientific Advisory Board; Employment The University of Florida Medical Branch, and University of Florida; Partnerships none; Contracts none; Honoraria none; Royalties from Baylor College of Medicine for US Patent 6,855,497; Grants NIH RC1NS068897, R01NS41547, Muscular Dystrophy Association, National Ataxia Foundation; Other none.

Author Roles: Hélio A. G. Teive: Conception, Organization, Execution of Research project; Design, Execution, Review and Critique of Statistical Analysis; Writing of the first draft of Manuscript. Renato P. Munhoz: Organization, Execution of Research project; Design, Execution of Statistical Analysis; Writing of the first draft, Review and Critique of Manuscript. Salmo Raskin: Execution of Research project; Review and Critique of Statistical Analysis; Review and Critique of Manuscript. Walter O. Arruda: Execution of Research project; Review and Critique of Manuscript. Luciano de Paola: Execution of Research project; Writing of the first draft of Manuscript. Lineu C. Werneck: Organization of Research project; Review and Critique of Statistical Analysis; Review and Critique of Manuscript. Tetsuo Ashizawa: Conception, Execution of Research project; Review and Critique of Statistical Analysis; Review and Critique of Manuscript.

REFERENCES

1. Matsuura T, Achari M, Khajavi M, Bachinski LL, Zoghbi HY, Ashizawa T. Mapping of a gene for a novel spinocerebellar ataxia with pure cerebellar signs and epilepsy. Ann Neurol 1999;45:407–411.
2. Zu L, Figueroa KP, Grewal R, Pulst S-M. Mapping of a new autosomal dominant spinocerebellar ataxia to chromosome 22. Am J Hum Genet 1999;64:594–599.
3. Matsuura T, Yamagata T, Burgess DL, et al. Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. Nat Genet 2000;26:191–194.
4. Rasmussen A, Matsuura T, Ruano L, et al. Clinical and genetic analysis of four Mexican families with spinocerebellar ataxia type 10. Ann Neurol 2001;50:234–239.
5. Grewal RP, Achari M, Matsuura T, et al. Clinical features and ATTCT repeat expansion in spinocerebellar ataxia type 10. Arch Neurol 2002;59:1285–1290.
6. Teive HA, Roa BB, Raskin S, et al. Clinical phenotype of Brazilian families with spinocerebellar ataxia 10. Neurology 2004;63:1509–1512.
7. Raskin S, Ashizawa T, Teive HAG, et al. Reduced penetrance in a Brazilian family with spinocerebellar ataxia type 10. Arch Neurol 2007;64:591–594.
8. Matsuura T, Ranum LPW, Volpini V, et al. Spinocerebellar ataxia type 10 is rare in populations other than Mexicans. Neurology 2002;58:983–984.
9. Teive HAG, Arruda WO, Raskin S, Ashizawa T, Werneck LC. The history of spinocerebellar ataxia type 10 in Brazil: travels of a gene. Arq Neuropsiquiatr 2007;65:965–968.
10. Gatto EM, Gao R, White MC, et al. Ethnic origin and extrapyramidal signs in an Argentinean spinocerebellar ataxia type 10 family. Neurology 2007;69:216–218.
11. Gallardo M, Soto A. Clinical characterization of a Venezuelan family with spinocerebellar ataxia type 10. Mov Disord 2009; 24(suppl 1):S12 (Poster).
12. Almeida T, Alonso I, Martins S, et al. Ancestral origin of the ATTCT repeat expansion in Spinocerebellar ataxia type 10 (SCA10). Plos One 2009;4:e4553.
13. de Boer HM, Mula M, Sander JW. The global burden and stigma of epilepsy. Epilepsy Behav 2008;12:540–546.
14. Banerjee PN, Filippi D, Hauser WA. The descriptive epidemiology of epilepsy—a review. Epilepsy Res 2009;85: 31–45.
15. Li LM, Fernandes PT, Noronha ALA, et al. Demonstration project on epilepsy in Brazil. Arq Neuropsiquiatr 2007;65 (suppl 1): 5–13.
16. Matsuura T, Fang P, Lin X, et al. Somatic and germline instability of the ATTCT repeat in spinocerebellar ataxia type 10. Am J Hum Genet 2004;74:1216–1224.
17. Hagerman KA, Ruan H, Edamura KN, Matsuura T, Pearson CE, Wang YH. The ATTCT repeats of spinocerebellar ataxia type 10 display strong nucleosome assembly which is enhanced by repeat interruptions. Gene 2008;434:29–34.
18. Matsuura T, Fang P, Pearson CE, et al. Interruptions in the expanded ATTCT repeat of spinocerebellar ataxia type 10: repeat purity as a disease modifier? Am J Hum Genet 2006; 78:125–129.

Olfactory Heterogeneity in *LRRK2* Related Parkinsonism

Laura Silveira-Moriyama, MD, PhD,[1] Renato Pupi Munhoz, MD,[2] Margarete de J. Carvalho, MD,[3] Salmo Raskin, MD,[2] Ekaterina Rogaeva, PhD,[4] Patricia de C. Aguiar, MD, PhD,[5] Rodrigo A. Bressan, MD, PhD,[5,6] Andre C. Felicio, MD,[5,6,7] Orlando G.P. Barsottini, MD,[7] Luiz Augusto F. Andrade, MD,[5] Hsin F. Chien, MD,[3] Vincenzo Bonifati, MD, PhD,[8] Egberto R. Barbosa, MD, PhD,[3] Helio A. Teive, MD,[2] and Andrew J. Lees, MD[1]*

[1]Reta Lila Weston Institute of Neurological Studies, UCL Institute of Neurology, London, United Kingdom; [2]Movement Disorders Unit, Neurology Service, Internal Medicine Department Hospital de Clínicas, Federal University of Paraná, Curitiba-PR, Brazil; [3]Department of Neurology, University of Sao Paulo School of Medicine, Sao Paulo-SP, Brazil; [4]Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario, Canada; [5]Instituto do Cérebro - Instituto Israelita de Ensino e Pesquisa Albert Einstein, Hospital Israelita Albert Einstein, São Paulo-SP, Brazil; [6]Laboratório Interdisciplinar de Neurosciencias Clínicas - LiNC, Universidade Federal de Sao Paulo, UNIFESP, São Paulo, Brazil; [7]Department of Neurology and Neurosurgery, Universidade Federal de São Paulo, SP, Brazil; [8]Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands

***Abstract:* *LRRK2* mutations can cause familial and sporadic Parkinson's disease (PD) with Lewy-body pathology at post-mortem. Studies of olfaction in *LRRK2* are sparse and incongruent. We applied a previously validated translation of the 16 item smell identification test from Sniffin' Sticks (SS-16) to 14 parkinsonian carriers of heterozygous G2019S *LRRK2* mutation and compared with 106 PD patients and 118 healthy controls. The mean SS-16 score in *LRRK2* was higher than in PD ($p < 0.001$, 95% CI for $\beta = -4.7$ to -1.7) and lower than in controls ($p = 0.007$, 95% CI for $\beta = +0.6$ to $+3.6$). In the *LRRK2* group, subjects with low scores had significantly more dyskinesia. They also had younger age of onset, longer disease duration, and reported less frequently a family history of PD, but none of these other differences reached significance. Odor identification is diminished in *LRRK2* parkinsonism but not to the same extent as in idiopathic PD.** © 2010 Movement Disorder Society

Key words: olfaction; smell; *LRRK2*; PARK8; parkinsonism

A number of mutations in 12 different loci can cause familial parkinsonism. Of these, mutations in the α-synuclein and *LRRK2* gene have been associated at post-mortem with Lewy bodies (LB) in the substantia nigra, which has been traditionally considered the hallmark of idiopathic Parkinson's disease (PD).[1] Hyposmia is as common as rest tremor in PD (about 85% of patients),[2] and it is likely to be caused by the pathological alterations found in the olfactory bulb and primary olfactory cortex,[3,4] which are believed to be invariable sites of pathology of LB disease.[5] Concordantly, LB have also been found in the rhinencephalon in four *LRRK2* cases examined post-mortem.[6]

Previous reports of smell tests in *LRRK2* mutation carriers are sparse, have varied methodology and showed mixed results (see Table 1, which contains references 7–12); only one performed statistical comparison with sporadic PD and controls adjusted for age and gender.[6] We have analyzed the sense of smell in a series of *LRRK2* carriers with levodopa (L-dopa)-responsive parkinsonism and compared to sporadic PD and control subjects.

PATIENTS AND METHODS

Genetic Testing

The *LRRK2* mutation carriers were identified in previous screening studies as described by Munhoz et al,[13] Aguiar et al,[14] and Di Fonzo et al.[15]

Smell Testing

A previously validated Brazilian-Portuguese translation of the 16 item smell identification test from Sniffin' Sticks (SS-16)[16] was used.

Subjects

Smell testing was performed in 14 parkinsonian carriers of heterozygous G2019S *LRRK2* mutation. Eleven (78.6%) were female and two (14.3%) were smokers. Clinical data of some of these patients is published elsewere,[13,14] Ten patients had at least one relative with PD, and seven had at least one affected first degree relative. All patients had bradykinesia, 12 had rest tremor, 12 had rigidity, but only 6 had gait impairment or postural instability. Twelve reported significant improvement with

Additional Supporting Information may be found in the online version of this article.

*Correspondence to: Prof. Andrew Lees, Reta Lila Weston Institute of Neurological Studies, UCL Institute of Neurology, 1 Wakefield St, London, WC1N 1PJ, United Kingdom. E-mail: alees@ion.ucl.ac.uk

Potential conflict of interest: The authors do not have any conflict of interest.

Received 24 February 2010; Revised 2 May 2010; Accepted 1 June 2010

Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23325

Movement Disorders, Vol. 25, No. 16, 2010

TABLE 1. *Summary of literature data regarding olfaction in LRRK2*

Article	Mutations	Number of cases	Test used	Method of analysis	Finding
Lohmann et al., 2009[7]	G2019S	5 affected, 12 nonaffected carriers, and 8 noncarriers (family F-030)	UPSIT	Classification of subjects into categories of smell loss according to percentile of age and gender matched controls (raw score not provided)	3 of 5 affected carriers, 8 of the 10 unaffected carriers and 5 of 8 noncarriers who were examined for olfaction had moderate to severe anosmia
Healy et al., 2008[8]	Various	43 affected carriers	UPSIT	Not disclosed	Hyposmia detected in "only 51% of patients with mutations in *LRRK2*"
Lin et al., 2008[9]	R1441H	1 affected member and one nonaffected carrier (family TA)	UPSIT	Raw score	Score 29/40 for the affected member and 27/40 (aged 32) for the nonaffected member
	G2385R	2 affected carriers (family TB)	UPSIT	Raw score	Scores 15/40 and 15/40
Silveira-Moriyama et al., 2008[6]	G2019S	19 affected carriers (sporadic and familial PD from various different families)	UPSIT	Multiple linear regression comparing *LRRK2* with control and PD groups when adjusting for age and gender.	*LRRK2* patients scored lower than controls and not different from PD
Berg et al., 2005[10]	R793M	1 affected carrier	8 item Sniffin Sticks	Raw score	Score 2/8
	S1096C	1 affected carrier	UPSIT	Raw score	Score 35/40
	S1228T	2 affected carrier	8 item Sniffin Sticks	Raw score	Scores 5/8 and 7/8
	I2020T	1 affected carrier	8 item Sniffin Sticks	Raw score	Scores 7/8
Khan et al., 2005[11]	Y1699C	4 affected carriers from Lincolnshire kindred	UPSIT	Raw score	Scores 34/40, 24/40, 25/40, and 36/40
Markopoulou et al., 1997[12]	R1441C	3 affected carriers	UPSIT	Raw score	Scores 25/40, 28/40, and 34/40
		3 nonaffected carriers	UPSIT	Raw score	Scores 39/40, 32/40, and 31/40

UPSIT; University of Pennsylvania Smell Identification Test.
Some of the mutations in the *LRRK2* gene have not yet been proven to be pathogenic.

L-dopa; of these six developed dyskinesias. Six had visual hallucinations but none were demented. Mean age was 66 years [range 42–84 years, standard deviation (SD), 14.6 years], mean age of onset was 55.6 years (range 32–80 years, SD 14.7 years), and mean disease duration was 10.4 years (range 3–32 years, SD 7.9 years).

For the comparison groups, we used data from 106 PD patients and 118 control subjects tested for a previous study and published elsewhere.[16] In the PD group, mean age was 61.3 years (range 33–83 years, SD 11.0 years), 35 (33%) subjects were female and four (3.8%) were smokers. Mean age of onset was 49.8 years (range 25–76 years, SD 11.4 years) and mean disease duration was 11.4 years (range 1–28 years, SD 6.0 years). In the control group mean age was 63.0 years (range 33–89 years, SD 9.8 years), 15 (12.7%) subjects were female and 22 (18.6%) were smokers.

Consent was obtained from all participants and the protocol was approved by the local ethics committees. Participants with active upper respiratory tract infection or previous history of head trauma leading to loss-of-consciousness were excluded.

Statistical Analysis

To compare the SS-16 score between subject groups (*LRRK2*, PD, controls) we used a multiple linear regression analysis for the SS-16 as dependent variable and as covariates gender, age, smoking status, and group (two indicator variables to compare *LRRK2* with the other groups). Assumptions underlying the regression analyses were checked by a study of the residuals. We applied to the SS-16 results an arbitrary cut-off ($\leq$9 for subjects aged $<$60 and $\leq$8 for those aged $\geq$60) previously recommended to differentiate PD subjects from controls in Brazil,[16] and calculated the difference in proportions of subjects with normal or abnormal SS-16 between the groups using Chi-Squared tests. Clinical features in the *LRRK2* patients with and without low SS-16 were compared using t-tests and Chi-Squared statistics. A significance level of 0.05 was used throughout.

RESULTS

The mean SS-16 score in *LRRK2* was higher than in PD ($p < 0.001$, 95% CI for $\beta = -4.7$ to -1.7) and lower than in controls ($p = 0.007$, 95% CI for $\beta = +0.6$ to $+3.6$) when adjusting for the covariates age ($p < 0.001$, 95% CI for $\beta = -0.1$ to -0.0), gender ($p = 0.07$, 95% CI for β -1.5 to $+0.1$) and smoking ($p = 0.8$, 95% CI for $\beta = -0.9$ to $+1.2$). Figure 1 shows the box plot of SS-16 results.

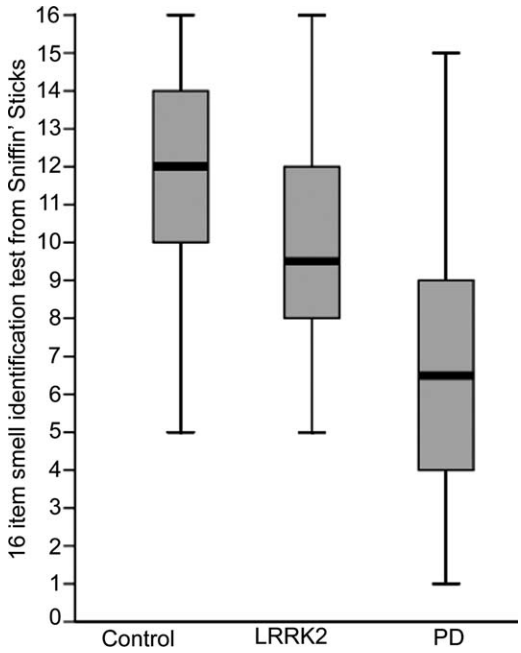


FIG. 1. Box plot of scores in the 16 item smell identification test from Sniffin' Sticks in the three patient groups. The median (the horizontal line) is within the box containing the central 50% of the observations and the extremes of the "whiskers" contain the central 95% of the ordered observations. PD = Parkinson's disease. SS-16 = 16 item smell identification test from Sniffin' Sticks.

The number of patients with abnormal SS-16 scores in *LRRK2* was higher than in controls (Fisher's exact test $p = 0.006$) and lower than in PD ($p = 0.004$) (see pie chart in Supporting Information Figure S1). In the *LRRK2* group, those with low SS-16 scores had significantly more dyskinesia. They also had younger age of onset, longer disease duration, and less family members affected, but these differences did not reach statistical significance (see details Supporting Information Table S1).

DISCUSSION

Our study provides independent confirmation of the smell deficit found in *LRRK2* parkinsonian patients, as none of the patients in the current study were included in any of the previous reports listed in Table 1. Hyposmia was less frequent in *LRRK2* than in idiopathic PD. LB disease is the most commonly reported pathological signature in G2019S cases[17,18] but other pathological substrates have also been reported in patients with *LRRK2* mutations, including tau-positive neurofibrillary tangles,[19] mixed tau and LB, TDP-43 positive inclusions,[20] ubiquitin only inclusions,[21] and also no characteristic pathological deposition of protein was found, but only cell loss and gliosis of the substantia

nigra.[22,23] It is possible that in *LRRK2* LB pathology is associated with hyposmia, whereas other substrates are not, but direct evidence of a link between pathological hallmark and olfactory function in *LRRK2* is scant. The rhinencephalon was examined in only six *LRRK2* cases (five cases of the G2019S[6,18] and one of the Y1699C mutation[11]) and in all LB were found in olfactory structures available; one of the G2019S patients had been smell tested and had severe hyposmia while alive.[6] The Y1699C mutation can present with accumulation of α-synuclein, concomitant tau pathology, or cell loss with no protein accumulation; smell testing of four subjects showed mixed results,[11] but these patients were not examined post-mortem. The R1441C mutation can present with accumulation of α-synuclein, tau, or no protein accumulation; smell testing of six members revealed mixed results,[12,24] In six out of eight cases of the I2020T mutation examined no α-synuclein accumulation was detected at post-mortem[23] and one carrier of this mutation had a normal UPSIT-40 score of 35/40.[10] Assuming that hyposmia is a marker for LB disease and given that the majority of G2019S patients are found to have LBs at autopsy, we would expect more than half of G2019S patients to be hyposmic. This is not the case in our study.

It is difficult to know what lies between the data presented here and that presented in 2008,[6] which failed to show a significant difference in smell test scores between PD and *LRRK2* subjects. It is possible this difference only reflects lack of power in the first study. The basic demographics of the all European subjects included in the 2008 study were similar to the Brazilian subjects in terms of age (mean [SD] in years 58.8 [5.96] for Europeans, 66 [14.6] for Brazilians) and disease duration (11.4 [7.9] for Europeans, 10.4 [7.9] for Brazilians), and the smell tests used (UPSIT for the European study, Sniffin' Sticks for the Brazilians) are of similar sensitivity and specificity, despite the UPSIT having a slightly higher repeatability.[2,16,25] Our current data is more in line with the findings of Healy et al, 2008,[8] who despite lacking comparisons with controls and PD, described that only half the *LRRK2* subjects presented with smell deficit. No previous study has tried to associate smell with clinical features in *LRRK2*. The only feature significantly associated with smell loss in the *LRRK2* group was the presence of dyskinesia, an association not yet investigated in PD patients, although hyposmia is independent of the overall severity of motor features of PD.[26–32]

The clinical and pathological similarities between *LRRK2* related parkinsonism and idiopathic PD indicate that monogenetic *LRRK2* parkinsonism may be a paradigm for the development of LB disease, and a careful look at discrepancies between these two conditions may provide insight into the pathogenesis of PD. Direct cell-to-cell transmission of α-synuclein has been demonstrated using both cell culture and animal models[33] providing a plausible mechanism to explain the findings of LB in embryonic graft cells that had been transplanted into the basal ganglia of PD patients[34,35] and leading to theories that an as yet unidentified external agent entering the nervous system through the nose and olfactory nerve causes PD. This would explain the fact that lesions of the olfactory areas are found in virtually all PD patients, as well as subjects with incidental Lewy type pathology.[36] That hyposmia is found in patients who present with a genetic form of parkinsonism goes against this notion, especially because *LRRK2* subjects also present with Lewy body pathology, and this can be found in the rhinencephalon.[6,11] Nevertheless, the variable penetrance of *LRRK2*[8] suggests factors other than the genetic mutations play a role in the pathogenesis of *LRRK2* related parkinsonism.

Acknowledgments: This work was funded by the Reta Lila Weston Trust for Medical Research and Foundation Philantropique Edmond Safra. Dr Laura Silveira-Moriyama is beneficiary of a Reta Lila Weston Fellowship.

Financial Disclosures: L Silveira-Moriyama: Honoraria: Britania Pharm.; Employment: Reta Lila Weston Institute of Neurological Studies. RP Munhoz: Employment: Pontificia Universidade Catolica. M de J Carvalho: Employment: Faculdade de Medicina da Fundação do ABC. E Rogaeva: Employment: Centre for Research in Neurodegenerative Diseases, University of Toronto, Canada. P de C Aguiar: Grants: Edmund J Safra Philantropic Foundation (Switzerland), Conselho Nacional de Desenvolvimento Científico e Tecnológico- CNPq (Brasil); Employment: Instituto Israelita de Ensino e Pesquisa Albert Einstein (São Paulo-SP, Brazil); Other: post doctoral stipend from F. Hoffmann La-Roche (Switzerland) in 2009. RA Bressan: Advisory Boards: Novartis, Lilly, Janssen, Astra Zeneca; Honoraria: Novartis, Lilly, Janssen, Astra Zeneca; Grants: FAPESP, Safra Foundation, CNPq; Employment: Federal University of Sao Paulo – UNIFESP. AC Felicio: Employment: Federal University of Sao Paulo, Brazil. OGPBarsottini: Employment: Instituto Israelita de Ensino e Pesquisa- Instituto de Cérebro (HIAE)/ Universidade Federal de São Paulo. LAF Andrade: Employment: Instituto do Cérebro - Instituto Israelita de Ensino e Pesquisa. Hospital I.Albert Einstein–São Paulo. HF Chien: Employment: self-employed. V Bonifati: Grants: Netherlands Organization for Scientific Research (NWO, VIDI grant); the Erasmus MC Rotterdam (Erasmus Fellowship); and the "Internationaal Parkinson Fonds" (The Netherlands); Employment: Erasmus MC, Rotterdam, The Netherlands. ER Barbosa: Honoraria: Boehringer-Ingelheim and Hoffmann-LaRoche; Employment: Hospital das Clínicas da Fac Med Univ São Paulo. HA Teive: Employment: Federal University of Paraná,

Curitiba, Brazil. AJ Lees: Consultancies: Genus; Advisory Boards: Novartis, Teva, Meda, Boehringer Ingelheim, GSK, Ipsen, Lundbeck, Allergan, Orion; Honoraria: Novartis, Teva, Meda, Boehringer Ingelheim, GSK, Ipsen, Lundbeck, Allergan, Orion; Grants: PSP Association, Weston Trust–The Reta Lila Howard Foundation; Employment: UCL/UCLH.

Author Roles: 1. Research project: A. Conception, B. Organization, C. Execution; 2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 3. Manuscript: A. Writing of the first draft, B. Review and Critique; L Silveira-Moriyama: 1A, B, C; 2A, B; 3A. R.P Munhoz: 1B, C; 2C, 3C. M de J Carvalho: 1C; 2C, 3C. S Raskin: 1C; 2C, 3C. E Rogaeva: 1C; 2C, 3C. P de C Aguiar: 1C; 2C, 3C. RA Bressan: 1C; 2C, 3C. AC Felicio: 1C; 2C, 3C. OGPBarsottini: 1C; 2C, 3C. LAF Andrade: 1C; 2C, 3C. HF Chien: 1C; 2C, 3C. V Bonifati: 1C; 2C, 3C. ER Barbosa: 1B,C; 2C, 3C. HA Teive: 1B,C; 2C, 3C. AJ Lees: 1A, B,C; 2C, 3C.

REFERENCES

1. Lees AJ, Hardy J, Revesz T. Parkinson's disease. Lancet 2009; 373:2055–2066.
2. Silveira-Moriyama L, Petrie A, Williams DR, et al. The use of a color coded probability scale to interpret smell tests in suspected parkinsonism. Mov Disord 2009;24:1144–1153.
3. Silveira-Moriyama L, Holton JL, Kingsbury A, et al. Regional differences in the severity of Lewy body pathology across the olfactory cortex. Neurosci Lett 2009;453:77–80.
4. Pearce RK, Hawkes CH, Daniel SE. The anterior olfactory nucleus in Parkinson's disease. Mov Disord 1995;10:283–287.
5. Braak H, Del TK. Invited Article: nervous system pathology in sporadic Parkinson disease. Neurology 2008;70:1916–1925.
6. Silveira-Moriyama L, Guedes LC, Kingsbury A, et al. Hyposmia in G2019S LRRK2-related parkinsonism: clinical and pathologic data. Neurology 2008;71:1021–1026.
7. Lohmann E, Leclere L, De AF, et al. A clinical, neuropsychological and olfactory evaluation of a large family with LRRK2 mutations. Parkinsonism Relat Disord 2009;15:273–276.
8. Healy DG, Falchi M, O'Sullivan SS, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol 2008;7:583–590.
9. Lin CH, Tzen KY, Yu CY, Tai CH, Farrer MJ, Wu RM. LRRK2 mutation in familial Parkinson's disease in a Taiwanese population: clinical, PET, and functional studies. J Biomed Sci 2008;15:661–667.
10. Berg D, Schweitzer K, Leitner P, et al. Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease*. Brain 2005;128:3000–3011.
11. 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.
12. Markopoulou K, Larsen KW, Wszolek EK, et al. Olfactory dysfunction in familial parkinsonism. Neurology 1997;49:1262–1267.
13. Munhoz RP, Wakutani Y, Marras C, et al. The G2019S LRRK2 mutation in Brazilian patients with Parkinson's disease: phenotype in monozygotic twins. Mov Disord 2008;23:290–294.
14. Aguiar PC, Lessa PS, Godeiro C, Jr., et al. Genetic and environmental findings in early-onset Parkinson's disease Brazilian patients. Mov Disord 2008;23:1228–1233.
15. Di Fonzo A, Rohe CF, Ferreira J, et al. A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. Lancet 2005;365:412–415.
16. Silveira-Moriyama L, Carvalho MJ, Katzenschlager R, et al. The use of smell identification tests in the diagnosis of Parkinson's disease in Brazil. Mov Disord 2008;23:2328–2334.
17. Giasson BI, Covy JP, Bonini NM, et al. Biochemical and pathological characterization of Lrrk2. Ann Neurol 2006;59:315–322.
18. Ross OA, Toft M, Whittle AJ, et al. Lrrk2 and Lewy body disease. Ann Neurol 2006;59:388–393.
19. Rajput A, Dickson DW, Robinson CA, et al. Parkinsonism, Lrrk2 G2019S, and tau neuropathology. Neurology 2006;67: 1506–1508.
20. Covy JP, Yuan W, Waxman EA, Hurtig HI, Van DV, Giasson BI. Clinical and pathological characteristics of patients with leucine-rich repeat kinase-2 mutations. Mov Disord 2009;24:32–39.
21. Dachsel JC, Ross OA, Mata IF, et al. Lrrk2 G2019S substitution in frontotemporal lobar degeneration with ubiquitin-immunoreactive neuronal inclusions. Acta Neuropathol (Berl) 2007;113:601–606.
22. Gaig C, Marti MJ, Ezquerra M, Rey MJ, Cardozo A, Tolosa E. G2019S LRRK2 mutation causing Parkinson's disease without Lewy bodies. J Neurol Neurosurg Psychiatry 2007;78:626–628.
23. Hasegawa K, Stoessl AJ, Yokoyama T, Kowa H, Wszolek ZK, Yagishita S. Familial parkinsonism: study of original Sagamihara PARK8 (I2020T) kindred with variable clinicopathologic outcomes. Parkinsonism Relat Disord 2009;15:300–306.
24. Zimprich A, Biskup S, Leitner P, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 2004;44:601–607.
25. Doty RL, McKeown DA, Lee WW, Shaman P. A study of the test-retest reliability of ten olfactory tests. Chem Senses 1995;20:645–656.
26. Doty RL, Deems DA, Stellar S. Olfactory dysfunction in parkinsonism: a general deficit unrelated to neurologic signs, disease stage, or disease duration. Neurology 1988;38:1237–1244.
27. Hawkes CH, Shephard BC. Olfactory evoked responses and identification tests in neurological disease. Ann N Y Acad Sci 1998; 855:608–615.
28. Stern MB, Doty RL, Dotti M, et al. Olfactory function in Parkinson's disease subtypes. Neurology 1994;44:266–268.
29. Doty RL, Stern MB, Pfeiffer C, Gollomp SM, Hurtig HI. Bilateral olfactory dysfunction in early stage treated and untreated idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1992; 55:138–142.
30. Lehrner J, Brucke T, Kryspin-Exner I, Asenbaum S, Podreka I. Impaired olfactory function in Parkinson's disease. Lancet 1995; 345:1054–1055.
31. Barz S, Hummel T, Pauli E, Majer M, Lang CJ, Kobal G. Chemosensory event-related potentials in response to trigeminal and olfactory stimulation in idiopathic Parkinson's disease. Neurology 1997;49:1424–1431.
32. Double KL, Rowe DB, Hayes M, et al. Identifying the pattern of olfactory deficits in Parkinson disease using the brief smell identification test. Arch Neurol 2003;60:545–549.
33. Desplats P, Lee HJ, Bae EJ, et al. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. Proc Natl Acad Sci USA 2009;106:13010–13015.
34. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat Med 2008;14:504–506.
35. Li JY, Englund E, Holton JL, et al. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med 2008;14:501–503.
36. Hawkes CH, Del TK, Braak H. Parkinson's disease: a dual-hit hypothesis. Neuropathol Appl Neurobiol 2007;33:599–614.

Disappearance of Essential Tremor After Stroke

Michel J.-M. Dupuis,[1]* Frédéric L.A. Evrard,[1] Philippe G. Jacquerye,[1] Gaëtane R. Picard[1] and Oliver G. Lermen[2]

[1]*Neurology Department, Pierre, Ottignies, Belgium;*
[2]*Neurosurgery Department, Pierre, Ottignies, Belgium*

Video 

Abstract: **Improvement of a patient's essential tremor (ET) after a stroke has rarely been reported. In such patients, cerebral imaging could help to identify structures involved in the maintenance of ET and improves the knowledge of its physiopathology. This article reports the disappearance of ET, after a stroke in 4 patients and reviews similar previously published cases. These cases suggest that the interruption of cerebellar loops during a stroke could be responsible for the disappearance of ET.** © 2010 Movement Disorder Society

Key words: physiopathology; case study; anatomical structures

In the past 22 years, 12 cases of disappearance of essential tremor (ET) after a stroke have been published. In this article, we report 4 new cases. These strokes create an interruption of loops between the cerebellum and the cerebral cortex.

CASE REPORT

In 1989, we reported a patient[1] suffering from a cerebellar stroke interrupting homolateral ET. Since then, 4 cases were collected in a general hospital treating around 250 ischemic strokes and 30 hematomas per year from a population of about 200.000 people.

Case 1

This right-handed man born in 1925 was admitted in January 2000 for the abrupt onset of a right motor deficit including the face, associated to right dysmetria, dyschronometria, and dysarthria. Medical history included myocardial infarction and coronary stenting, diabetes, hypertension, hypercholesterolemia, and past smoking habits. His idiopathic epilepsy had been treated by valproate (1300 mg/day), since 1980. The patient's father died at the age of 96 years and during his last 2 years had suffered from a tremor of both hands. In the last 10 years before stroke, the patient complained of a worsening tremor of both hands and he did not dare to eat soup with a spoon. There was neither intentional tremor or impairment of gait. His tremor hadn't responded to primidone. On admission, the MRI showed a left pontine paramedian stroke (Fig. 1a). He recovered after a few weeks, with only residual right intermediate plantar response and brisker osteotendinous reflexes. An MRI showed only small hyperintense Flair T2 signals in the pons, 10 months later (Fig. 1b).

Since the day of the stroke, the tremor on the right side has totally disappeared (video). The patient can now use a screw pull or his cutlery.

Case 2

This right-handed woman born in 1930 noted left lower limb paresis and upper limb paresthesia, when she awakened after her nap in May 2008. Five hours later, clinical examination showed left hemiparesis, face included, predominant on the lower limb, intermediate plantar response, numerous mistakes for position of toe and index, and possible index to nose dysmetria. There was a history of untreated hypertension, hypercholesterolemia, and for the last 10 years ET in both hands, without dysmetria. MRI imaging revealed a 6×12 mm stroke of the posterior limb of the right internal capsule (Fig. 1c). She was treated with acetylsalicylic acid, amlodipine, and statin. She was transferred to the rehabilitation unit with a gradual recovery of autonomy, went back home after 40 days, and continued to improve. After the stroke, the patient noticed that the tremor disappeared totally on the left side.

Case 3

This right-handed man was born in 1948. For more than 10 years, he had had bilateral postural hand tremor partially improved by alcohol ingestion and treated intermittently with propranolol 40 mg. In September 2008, he was admitted for left fluctuating clumsiness and sensory disturbances of the upper and lower limbs. There was a left-sided slow fall in the Mingazzini sign, intermediate plantar response, tactile hypoesthesia mainly of the hand, and mild dysmetria with closed eyes without adiadoccokynesia. Stroke workup showed a cortico-subcortical rolandic and prerolandic infarct on MRI (Fig. 1d). He was treated with acetylsalicylic acid and statin. In 3 days, he recovered from his neurologic defi-

*Correspondence to: Dr Michel Dupuis, Avenue Reine Fabiola 9, B-1340 Ottignies, Belgium. E-mail: mi.dupuis@clinique-saint-pierre.be

Potential conflict of interest: The authors report no conflicts of interest.

Received 31 December 2009; Revised 31 March 2010; Accepted 1 June 2010

Published online 10 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23328

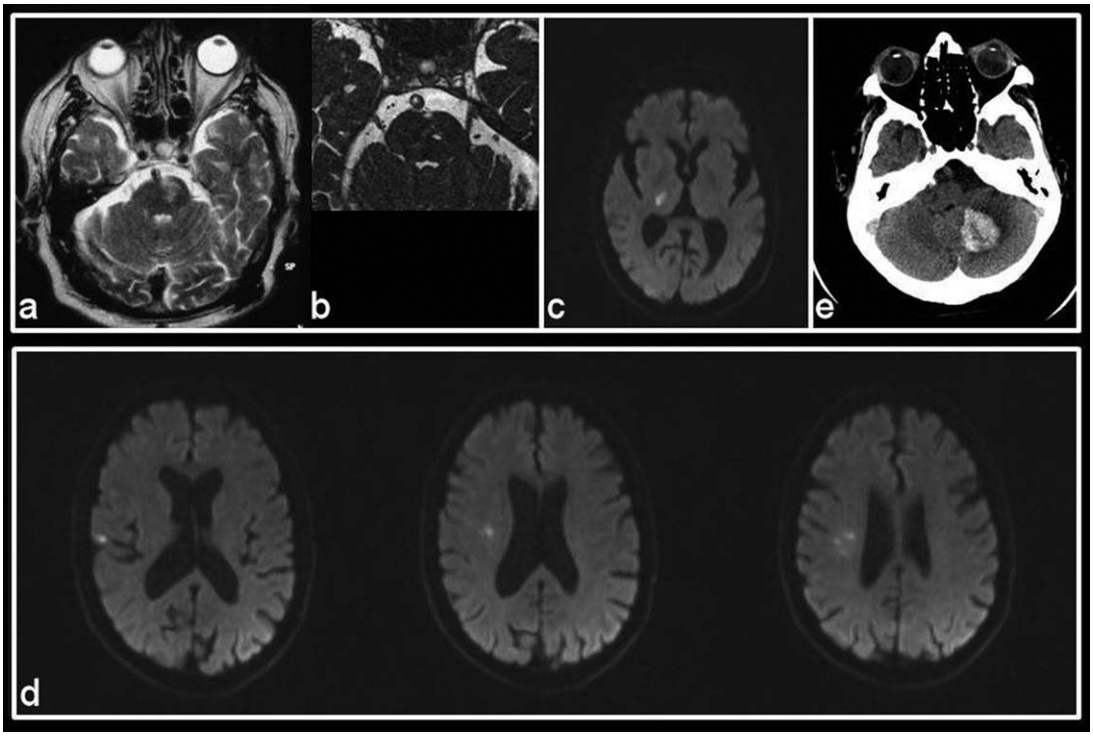


FIG. 1. Patient 1: T2-MRI acute stage (a) and 10 months later (b); Patient 2 (c): diffusion-MRI; Patient 3 (d): diffusion-MRI (all the positive diffusion images are shown); Patient 4 (e): CT-scan.

cit. After the stroke, he was pleased to see the disappearance of the tremor on the left side. Unfortunately, the tremor reappeared 3 months later and was equal in both hands in February 2009.

Case 4

This right-handed woman born in 1933 was admitted in September 2009 for unsteadiness, vomiting, and left cerebellar hematoma (Fig. 1e). Two days later, she developed generalized convulsive seizures. There was a left dysmetria, adiadoccokynesia, and normal eye movements. An MRI EPI T2 showed multiple small (<10 mm) subcortical small hemorrhages, sparing rolandic areas, suggesting amyloïdosis. Valproate was administered for epilepsy attributed to a supratentorial hemorrage. She had noted for the last 10 years a mild tremor when holding an object. After the stroke, her tremor disappeared on the left side.

DISCUSSION

Each of our 4 patients had suffered from bilateral postural hand tremors for years preceding their strokes; 2 of these patients have a family history. Whereas in all the cases, the ET totally disappeared on the side clinically involved with the stroke, the improvement in Case 3 lasted only 3 months.

Three previous cases of disappearance of ET following cortico-subcortical hemispheric cerebral lesions have been reported (Fig. 2; Table 1). Constantino and Louis[3] reported a man who suffered from a first left hemiparesis resulting from right cerebral infarction at the age of 61 years. About 2 years later, he suffered once more from left paresis of the upper and lower limbs and noticed disappearance of ET since then. The CT scan showed only an old extensive frontal stroke, which does not exclude a small lacunar stroke as in Case 1.

Le Pira et al[4] reported the observation of a patient with a disabling familial ET. After a transitory paresis, tremor of the right hand but not of the foot disappeared during 6 months, and reappeared as a discrete tremor within 2 years of follow-up. An MRI showed a small lesion involving the left corona radiata adjacent to the precentral gyrus.

Kim JS et al[5] reported a case with familial ET, where the patient suffered from a right hand sensory-motor deficit resulting from a small frontal precentral stroke demonstrated by an MRI. The right-hand tremor totally disappeared after the stroke, although there prevailed a mild weakness and sensory deficit. These last two observations and Case 3 show that a limited rolandic or prerolandic cortico-subcortical stroke can be responsible for disappearance of ET, although only transitory in all cases, maybe because of cortical plasticity.

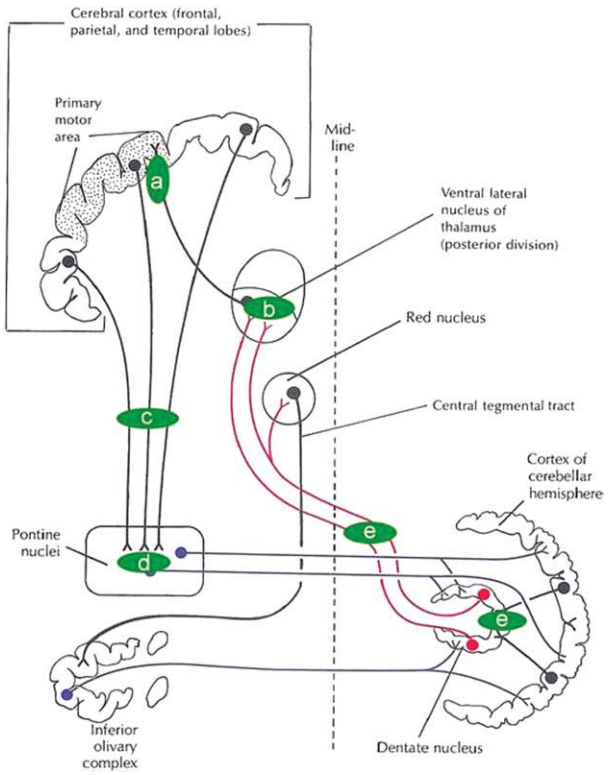


FIG. 2. Connections between the cerebellum and the cerebral cortex, with permission, from Barr's Human Nervous System.[2] Locations of strokes are indicated as follows: a: Cortico-subcortical hemispheric cerebral lesions; b: Thalamic lesions; c: Posterior limb of internal capsule lesions; d: Pontine lesions; e: Cerebellar lesions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In contrast, 12 cases of persisting disappearance of ET after a deep stroke have been reported (Fig. 2; Table 1). Three thalamic lesions[6–8] were reported, including a thalamic hemorrhage, a thalamic lacune and another thalamic ischemia involving VIM.

Two cases of "capsulothalamic" strokes have been published. In one case,[9] a right paresis with Babinski sign was noted, and the CT showed a stroke of the posterior limb of the internal capsule. The other case is a poster abstract stating left capsulothalamic stroke documented by an MRI[10]. Case 2 is similar to these cases, with sensory-motor deficit and cerebellar signs linked to a stroke of the posterior limb of the internal capsule. Typically, this region is vascularized by the anterior choroidal artery.[11] In 10% of the population, there is an anatomical variance, where the anterior choroidal artery irrigates a small thalamic territory.[12] Furthermore, the anterior choroidal artery is not typically reported in the four main thalamic vascular supplies.[13] Therefore, lesions of the posterior limb structures have to be considered in patients with disappearance of ET.

Nagaratnam et al[14] reported a case of a pontine stroke documented by a CT scan and contralateral disappearance of ET, similar to Case 1. Rajput et al[15] reported a left cerebellar hemorrhage involving the deep cerebellar nuclei documented by a CT with homolateral disappearance of familial ET. Rottschy et al[16] reported in a poster a case of progressive disappearance of ET, because of a cerebellar tumor. Our previously published Case 1 concerns an ischemic cerebellar stroke including dentate nucleus and superior cerebellar peduncle with homolateral disappearance of ET that lasted for 10 months until death. Case 4 concerns a cerebellar hematoma involving all the deep cerebellar nuclei, with a very short follow-up period.

We interpret (Fig. 2) the four cerebellar lesions and the three thalamic lesions as involving an efferent cerebellar pathway. The three capsular and two pontine strokes involve an interruption of the afferent cerebellar pathway (fronto-ponto-cerebellar tract). Both tracts are necessary for ET maintenance. Another explanation for capsular lesions would be the interruption of sensory radiations. The three corticosubcortical hemispheric cerebral lesions, close to the primary sensorimotor area, are probably also linked to the interruption of the efferent cerebellar pathways between the thalamus and the cortex. Involvement of the afferent cerebellar pathways seems less probable as they come from a very wide area of the cerebral cortex.

In summary, all of these "curative strokes" can be interpreted as interrupting the connecting pathways between primary sensory-motor area and cerebellum (Fig. 2), and semeiologically we would interpret future cases of disappearance of ET after a stroke as having such a location. No curative strokes have been reported until now concerning other structures like the inferior olivar complex.

In view of the number of cases in our practice, we believe that there is a general under reporting of disappearance of ET after a stroke. Our findings support a more systematic reporting associated with the use of MRI diffusion to prove recent small ischemic strokes.

This method of collecting data is also used for other movement disorders and is similar to classical clinico-anatomical findings. This data could usefully be correlated to information gathered from neurosurgery of movement disorders, neuropathology,[17] MRI,[18] and functional neuroimaging of ET for a better understanding of its physiopathology.[19]

LEGENDS TO THE VIDEO

Case 1: 9 years and 10 months after pontine stroke: disappearance of controlateral essential tremor on the right hand side.

TABLE 1. *Locations of the strokes interrupting the tremor and corresponding references*

Author	Year	Symptoms	Lesions	Imaging	Follow-up
(a) Cortico subcortical hemispheric cerebral lesions					
Constantino and Louis	2003	Left paresis	Right frontal	CT	12 yr
Le Pira et al.	2004	Right paresis	Corona radiata	MRI	2 yr
Kim et al.	2006	Sensory motor stroke	Frontal precentral	MRI	5 wk
Dupuis	This study; Case 3	Left sensorimotor	Rolandic and prerolandic	MRI	6 mo
(b) Thalamic lesions					
Im et al.	1996	Unknown	Thalamic hematoma	CT	Unknown
Nakamura et al.	1999	Unknown	Thalamic infarction	CT	Unknown
Barbaud et al.	2001	Right numbness	Left V.I.M (thalamus)	MRI	6 mo
(c) Posterior limb of internal capsule lesions					
Duncan et al.	1988	Right paresis and Babinski	Capsular posterior limb	CT scan	6 mo
Duval et al.	1997	Unknown	Left capsulo thalamic	MRI	1 yr
Dupuis	This study; Case 2	Left sensorimotor and dysmetria	Right posterior limb capsular	MRI	10 mo
(d) Pontine lesions					
Nagaratnam and Kalasabail	1997	Right paresis and Babinski	Left pontine	CT	10 mo
Dupuis	This study; Case 1	Right paresis and dysmetria	Left pontine lacune	MRI	9 yr
(e) Cerebellar lesions					
Dupuis et al.	1989	Right dysmetria	Right cerebellar	M.R.I.	10 mo
Rajput et al.	2008	Left ataxia	Left cerebellar hematoma	CT	6 yr
Dupuis	This study; Case 4	Left dysmetria	Left cerebellar hematoma	CT + MRI	1 mo
Rottschy et al.	2009	Bilateral ataxia	Right cerebellar tumor	CT	Unknown

Letters "a" to "e" indicates the locations of strokes labelled in Fig. 2.

Financial Disclosures: The authors have no financial disclosures.

Author Roles: Michel J-M Dupuis: Conception of Research project; Writing of the first draft and review and critique of the manuscript. Frédéric LA Evrard: Writing of the first draft and review and critique of the manuscript. Philippe G Jacquerye: Writing of the first draft and review and critique of the manuscript. Gaëtane R Picard: Writing of the first draft and review and critique of the manuscript. Oliver G Lermen: Writing of the first draft and review and critique of the manuscript.

REFERENCES

1. Dupuis MJ, Delwaide PJ, Boucquey D, Gonsette RE. Homolateral disappearance of essential tremor after cerebellar stroke. Mov Disord 1989;4:183–187.
2. Kiernan JA. Barr's The Human Nervous System: An Anatomical Viewpoint. Ninth ed. Philadelphia: Lippincott Williams & Wilkins; 2009, figure 10–15, p169.
3. Constantino AEA, Louis ED. Unilateral disappearance of essential tremor after cerebral hemispheric infarct. J Neurol 2003;250: 354–355.
4. Le Pira F, Giuffrida S, Panetta MR, Lo Bartolo ML, Politi G. Selective disappearance of essential tremor after ischaemic stroke. Eur J Neurol 2004;11:422–423.
5. Kim J, Park J, Kim W, Kim H, Kim Y, Lee K. Disappearance of essential tremor after frontal cortical infarct. Mov Disord 2006; 21:1284–1285.
6. Im JH, Kim JS, Lee MC. Disappearance of essential tremor after small thalamic hemorrhage. Clin Neurol Neurosurg 1996;98:40–42.
7. Nakamura Y, Miura K, Yamada I, Takada K. Disappearance of essential tremor after thalamic infarction. Rinsho Shinkeigaku 1999;39:340–342.
8. Barbaud A, Hadjout K, Blard JM, Pagès M. Improvement in essential tremor after pure sensory stroke due to thalamic infarction. Eur Neurol 2001;46:57–59.
9. Duncan R, Bone I, Melville ID. Essential tremor cured by infarction adjacent to the thalamus. J Neurol Neurosurg Psychiatry 1988;51:591–592.
10. Duval L, Felten D, Tailia H, Renard JL, Bequet D. Essential tremor: Controlateral disappearance after stroke (Poster P670). J Neurol 1997:S241.
11. Vuadens P, Bogousslavsky J. Stroke Syndromes. In: Bogousslavsky J, Caplan LR, editors. Anterior choroïdal artery territory infarcts, Second ed. Cambridge UK: Cambridge University Press; 2001. p451–460.
12. Barth A, Bogousslavsky J, Caplan LR. Thalamic infarcts and hemorrhages, Second ed. In: Bogousslavsky J, Caplan LR, editors. Cambridge UK: Cambridge University Press; 2001. p 461–468.
13. Fujii K, Lenkey C, Rhoton A. Microsurgical anatomy of the choroidal arteries: Lateral and third ventricles. J Neurosurg 1980;52:165–188.
14. Nagaratnam N, Kalasabail G. Contralateral abolition of essential tremor following a pontine stroke. J Neurol Sci 1997;149:195–196.
15. Rajput AH, Maxood K, Rajput A. Classic essential tremor changes following cerebellar hemorrhage. Neurology 2008;71:1739–1740.
16. Rottschy C, Kronenbuerger M, Csontos V, Montagne J, Eickhoff SB, Schulz JB. Gradual disappearance of essential tremor due to a cerebellar Tumor (Poster We367), Mov Disord 2009;24suppl 1: S513.
17. Louis E. Essential tremor with ubiquitinated Purkinje cell intranuclear inclusions, Acta Neuropathol 2010;119:375–377.
18. Nicoletti G, Manners M, Novellino F, et al. Diffusion tensor MRI changes in cerebellar structures of patients with familial essential tremor. Neurology 2010;74:988–994.
19. Elble RJ, Deuschl G, An update on essential tremor. Curr Neurol Neurosci Rep 2009;9:273–277.