BRIEF REPORTS

Direct Visualization of Parkinson's Disease by In Vivo Human Brain Imaging Using 7.0T Magnetic Resonance Imaging

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder resulting from progressive loss of dopaminergic neurons in the substantia nigra (SN) pars compacta. Therefore, imaging of the SN has been regarded to hold greatest potential for use in the diagnosis of PD. At the 7.0T magnetic resonance imaging (MRI), it is now possible to delineate clearly the shapes and boundaries of the SN. We scanned eight early and two advanced PD patients, along with nine age-matched control subjects, using a 7.0T MRI in an attempt to directly visualize the SN and quantify the differences in shape and boundaries of SN between PD subjects in comparison with the normal control subjects. In the normal controls, the boundaries between the SN and crus cerebri appear smooth, and clean "arch" shapes that stretch ventrally from posterior to anterior. In contrast, these smooth and clean arch-like boundaries were lost in PD subjects. The measured correlation analyses show that, in PD patients, there is age-dependent correlation and substantially stronger UPDRS motor score-dependent correlation. These results suggest that, by using 7.0T MRI, it appears possible to use these visible and distinctive changes in morphology as a diagnostic marker of PD.
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Key Words: Parkinson's disease; substantia nigra; 7.0T MRI; neurodegenerative disorder; brain imaging

In the majority of cases, the Parkinson's disease (PD) is idiopathic, and the diagnosis is dependent on constellation of symptoms. Thus, the definitive diagnosis is not available until postmortem histologic analysis, where degeneration of the substantia nigra para compacta (SNc) dopaminergic system is seen as depigmentation of SN. The neurodegeneration SNc result in the release of neuromelanin into the adjacent tissue, where it is phagocytosed and carried away by macrophages.[1] It is believed that >60% of dopaminergic neurons are lost before a patient begins to show clinical symptoms of PD, such as bradykinesia, cogwheel rigidity, and tremor.[2,3] Thus, in vivo direct observation of the SNc in the human brain has been one of the most sought-after goals in PD research, as it has the potential to lead to noninvasive premortem diagnosis of PD.[4–6]

In vivo observation of the region related to the SNc in the PD brain has been attempted using various imaging tools, including magnetic resonance imaging (MRI), positron emission tomography, single photon emission computed tomography (SPECT), and computed tomography.[7] Many studies have examined the MRI features of PD by using various MR techniques. For example, there has been use of an inversion recovery pulse sequence,[8–11] measuring the intensity or T_2 relaxation time,[12] diffusion-weighted images,[13] and phase-contrast images[14] or T_2-weighted imaging.[15] Despite this, none have been able to clearly show the shape and boundaries of the SN.

Recently, high-resolution phase-contrast MR images obtained with an ultra-high field system, such as 7.0T

Table 1. The information of patients in the early stages of PD (H&Y 1), patients in the advanced stages of PD (H&Y 3), and age-matched control subjects

Symptoms	ID	H&Y stage	Period	Age (yr)	Sex	UPDRS (right side at off medication)	UPDRS (left side at off medication)
Normal	C001	0	–	61	F	–	–
	C002	0	–	44	F	–	–
	C003	0	–	66	F	–	–
	C004	0	–	57	F	–	–
	C005	0	–	53	F	–	–
	C006	0	–	56	F	–	–
	C007	0	–	63	M	–	–
	C008	0	–	67	F	–	–
	C009	0	–	52	F	–	–
Early PD	P101	1	4	56	F	2	6.5
	P102	1	2	45	F	6	7
	P103	1	7	53	F	9.5	4
	P104	1	1	61	F	12.5	4
	P105	1	2	57	F	9	1
	P106	1	5	70	M	7	1
	P107	1	2	54	F	6	2
	P108	1	4	70	F	9	15.5
Advanced PD	P301	3	12	51	F	5	8
	P302	3	5	67	M	8.5	12.5

This table presents the information of selected age-matched normal controls, patients in the early stages of PD (H&Y 1), and patients in the advanced stages of PD (H&Y 3), respectively. This study includes nine age-matched normal controls and 10 patients in the early PD and advanced stages of PD. PDs were clinically diagnosed with H&Y stages 1–3. In the early stage of PD, the mean period of illness was 3.38 yr, whereas the mean period of illness in the advanced stage of PD was 8.5 yr. The mean age and sex of the subjects were closely matched in normal controls and PDs. The subject's condition, i.e., normal healthy control, Parkinson's H&Y stages 1 and 3 are indicated as C001–009, P101–108, and P301–302. The UPDRS motor scores are measured in the off medication state. Individual's other information such as their duration of illness, age, and sex are also shown in the table.

MRI, are beginning to demonstrate brain anatomy with exquisite detail, such as even the cortical layers, and provided us the tools for imaging of the midbrain areas.[16–20] Images obtained using 7.0T MRI also began to show deep brain areas, such as the hippocampus and the details of the structures within the hippocampus.[18,19] These findings suggest that 7.0T MRI could be used to observe the degeneration of the SN in PD patients.[18,19,21] Here, we demonstrate that there are visible and distinctive differences in morphology of SNc in PD patients when compared with normal controls and suggest that 7.0 T MRI may be a useful tool in diagnosis of PD.

Methods

Subjects

7.0T T2*-weighted MR images of the SN were obtained from patients with PD and age-matched control subjects (detailed information on the subjects is shown in Table 1). All subjects were informed of the purpose of the MR examinations and consented to enrollment in this study. The study protocol was approved by the Korean Food and Drug Administration and by the Institutional Medical Ethics Committees and Review Boards at Gachon University of Medicine and Science and Seoul National University Hospital.

The control group included nine subjects (one male and eight female) aged between 44 and 67 years (mean age, 57.7 ± 7.4 years) without known neurologic deficits or abnormal findings on conventional 1.5T MR images. The PD group included eight patients in the early stages of PD [one male and seven female, Hoehn and Yahr (H&Y) Stage 1] who were between the ages of 45 and 70 years (mean age, 58.3 ± 8.5 years; mean duration, 3.4 ± 2.6 years) and 2 patients in the advanced stages of PD (1 male and 1 female, H&Y Stage 3) who were between the ages of 51 and 67 years (mean age, 59 ± 11.3 years; mean duration, 8.5 ± 4.9 years).

MRI Examinations

The MRI used was a 7.0T research prototype MRI scanner (Magnetom 7T, Siemens). Axial images were all obtained from the control subjects and patients acquired using a 2D T2*-weighted gradient echo sequence aligned with AC-PC line. The specific MR imaging parameters used were as follows: repetition time = 750 ms; echo time = 17.8 ms; flip angle = 45°; total acquisition time = 12.50 min; bandwidth = 30; and matrix size = 1024 × 896. The in-plane resolution was 0.25 mm, and the slice thickness was 2 mm. All images were obtained without sedation. We developed a 7.0T optimized 8-channel SENSE coil designed specifically for use in this study. Axial images of the midbrain, which were aligned with the AC-PC line, including the SN and RN, were all obtained from the control subjects and patients.
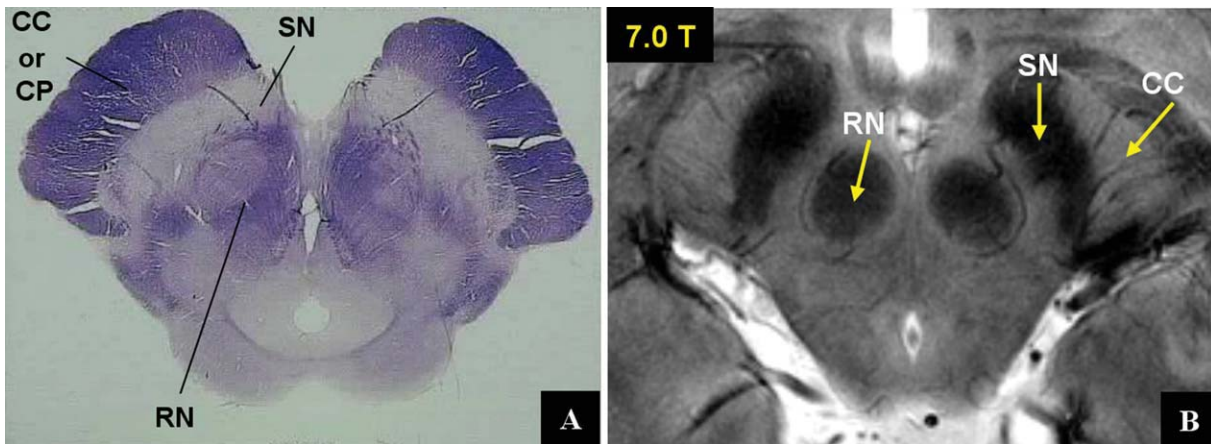
FIG. 1. Images of the SN. A cadaver image of a normal healthy subject showing the substantia nigra (SN), cerebral peduncle (CP) or crus cerebri (CC), red nucleus (RN), and other structures. Note that the smooth "arch" shape boundary between the SN and CP or CC. (B) MR images of the midbrain areas in a young normal healthy subject obtained using 7.0T MRI.

Data Analysis of the Lateral Boundaries of SN

For the quantitative analysis, we have segmented SN and then the center of mass was obtained to locate a center point. From this center point, we have defined the midline of SN along the direction of anterior-posterior (see white solid lines in Fig. 3A, D), which divides the lateral and the ventral aspects of SN. We select the lateral boundary of each subject and then these boundaries are normalized based on the length of midline of SN, which defined previously. After the normalization process, all dates have same length of midline; therefore, we could compare distance profile from the midline to the lateral boundary of SN.

To make the reference lateral boundary line between SN and CC, we have calculated mean boundary line of control group and used as a reference line. We then measured distance profiles by measuring the perpendicular distances from the midline to the lateral boundary of SN and calculated sum of absolute differences (SAD) for PD and normal control, i.e., SAD between individual data along the midline and reference. The SAD works by taking the absolute value of the difference between reference and individual line along the midline (see Fig. 3C, F). And then these differences are summed. We referred this SAD value as "undulation value." In Figure S1 (Supporting Information), the overall schematic diagram is shown.

Results

Ultra-High Field MRI of the SN

Figure 1A shows a photo of the midbrain area obtained from a cadaver. Figure 1B shows a typical MR image of the midbrain area obtained from a normal healthy subject using 7.0T MRI. The effects of magnetic susceptibility increase linearly with the strength of the main static magnetic field, especially when iron is deposited heavily in the SN. This strong T2*- or susceptibility weighted images are difficult to achieve with low field MRI.[15] Our results demonstrate that 7.0T MRI significantly increases the SNR and contrast, thus enabling substantially higher resolution and higher contrast images with increased sensitivity, which in turn enable us to detect the morphological changes of PD. As shown, with 7.0T, we are not only able to see the clear boundaries of between the SN and the CC and their surroundings but also allow us to quantitate directly the amount of undulation from the images obtained both from the normal and the patient.

Comparison of PD Patients and Healthy Normal Controls

Other representative samples of 7.0T images of the normal control and PD groups, namely two normal controls and PD patients with H&Y1 and the other with H&Y3 are selected and compared. First, two typical axial images of age-matched normal healthy controls obtained using 7.0T T2*-weighted imaging are shown in the left column of Figure 2. These two images clearly show the typical smooth boundaries between the SN and CC. However, in the right column of Figure 2, the boundaries of the two PD cases with H&Y1 and a H&Y2, respectively, are severely serrated in both PDs and clearly distinguishes the two groups, i.e., normal controls and PD patients. More specifically, the boundaries of PD patients are no longer "smooth-arch" shaped but rather appear serrated and distorted, suggesting probably due to the degradation of cells in the SN. These clear distinctions appear an important maker for the diagnosis of PD in vivo hither to unable to do with any other devices.
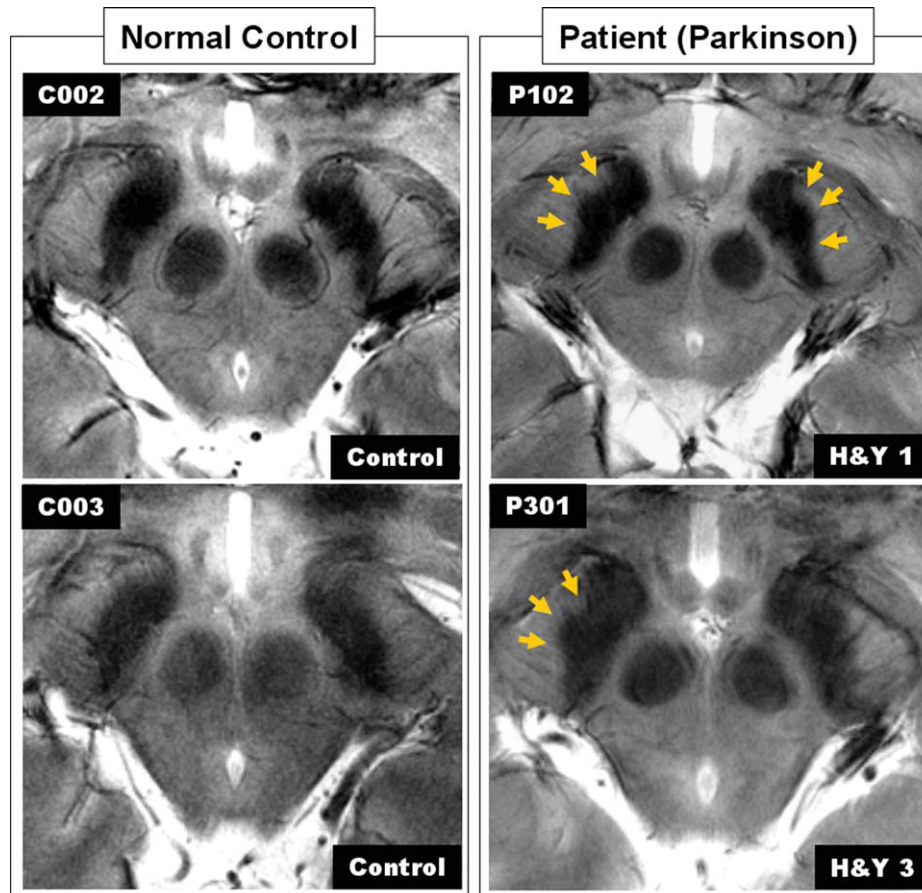
FIG. 2. The images of midbrain area from two typical normal controls and Parkinson's patients. These two Parkinson's cases (H&Y 1 and H&Y 3) show substantially serrated SN and their lateral boundaries compared with normal controls, suggesting that the SN of the PD patients mainly suffer degeneration of the SN.

Data Analysis of the Lateral Boundary of SN

Figure 3A–C and D–F are the results of analysis in typical PD patient and normal control, respectively. Representative images of a PD and a normal control obtained from 7.0T T2*-weighted SN images are shown in Figure 3A, D. In these images, white solid lines represent midline, which divides the lateral and the ventral aspects of SN, and white dotted lines represent distance from the midline to the lateral border of SN. Distance profiles of individual PD $[p(x)]$ for the lateral borderline and reference line $[m(x)]$ are shown in (B), and same process is applied for normal control $[c(x)]$ and the results is shown in (E). When we take the absolute differences between the reference and individual data, one obtains a typical data as shown in (C) and (F) for PD and normal control, respectively. As shown, the difference of PD patient $[|p(x) - m(x)|]$ is much larger than normal control $[|c(x) - m(x)|]$ (this example is the case of P102 patient and C002 normal control). Figure 3G shows a group difference, which is calculated by SAD (S_c and S_p, referred this as undulation value for control and PD, respectively) of the individuals divided by the number of control subjects and

patients who participated in the experiment. In case of the normal control, we averaged both (left and right) side, whereas in the case of the PD patient, we measured only the most affected side value. As it is seen, the values were significantly different between the two groups ($P = 0.0002$) and show much higher value in the PD patient than the normal control. Within each PD and control groups, correlation analysis show that there is substantial age-dependent correlation as well, especially for the patients (see Fig. 3H). As seen, the undulation value of PD group (S_p) is larger than normal control (S_c). When comparing the two correlation lines, the slope of the PD (0.314) is larger than that of normal control (0.117; see Fig. 3H).

In addition to age-dependent correlation, we have also measured UPDRS motor score dependent correlation, and the result is shown in Fig. 3I. In this case, we measured both side of the undulation value of SN (S_p) and UPDRS motor scores in PD patients. The UPDRS motor scores are measured in the off medication state and detailed scores are shown in Table 1. As the UPDRS motor score increase, the undulation value also increased, and the slope of correlation line was 0.457. This
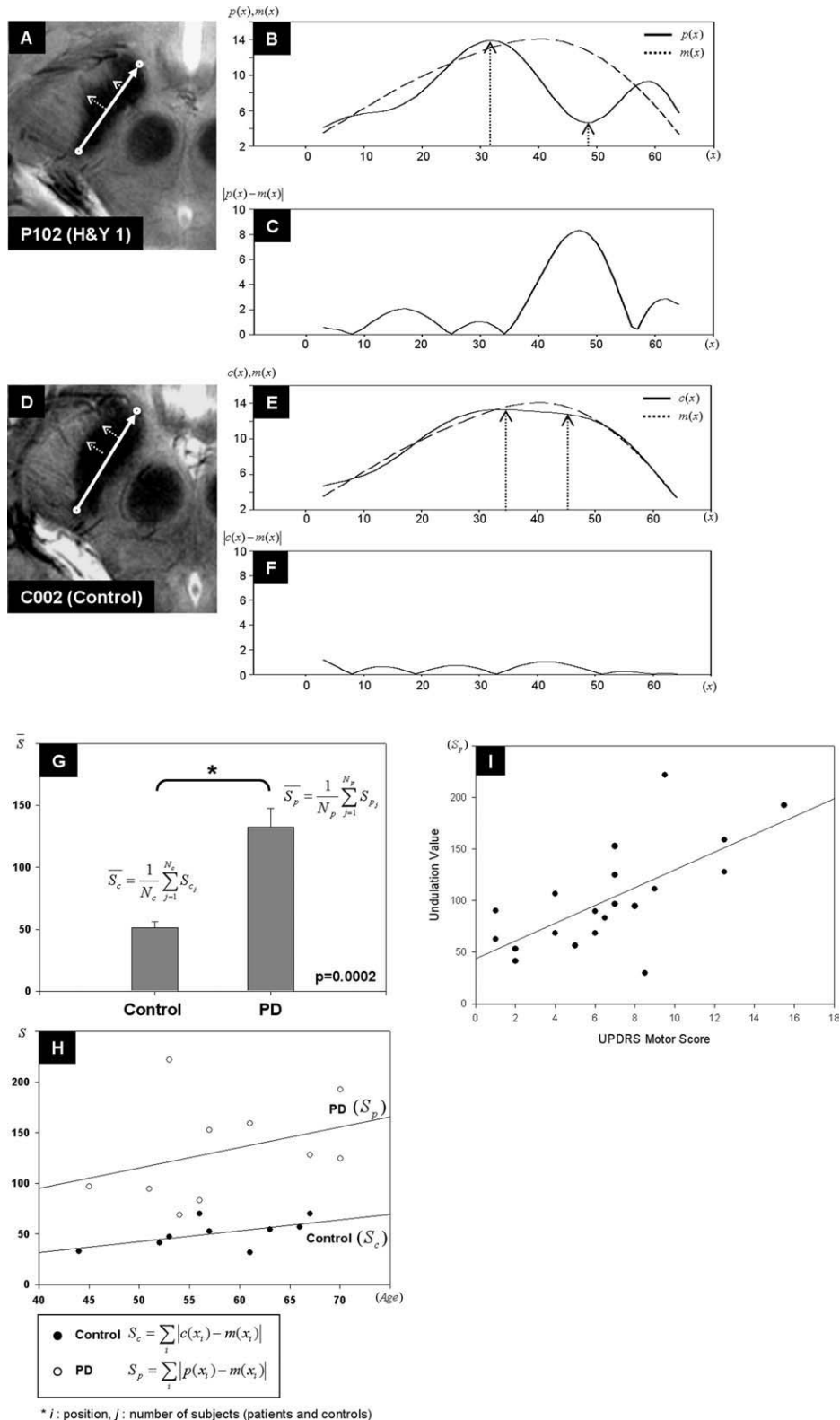
FIG. 3. Results of analysis in typical PD patient and normal control. (A) and (D) are the examples of the 7.0T T2*-weighted SN images of PD and control, respectively. As shown, for the quantification of PD, we have measured distance profiles of the individual PDs [$p(x)$] and normal controls [$c(x)$] along the white arrow. (B) and (E) are profiles measured along the white arrows shown in (A) and (D). The solid line is individual data while dotted line is reference data [$m(x)$] obtained by averaging of the nine normal healthy controls. (C) and (F) are the distance profiles of absolute differences measured between the individual (both PD and normal control) and reference data. (G) is the total mean value of differences of the control and PD. As shown significant differences between the two groups ($P = 0.0002$) are seen, i.e., normal healthy controls and the PDs. (H) "Undulation value" of PD (S_p) and normal control (S_c) group, respectively, are shown together with their correlation curves. As seen, the undulation values of PD group (S_p) are lager than normal control (S_c) suggesting potential value of the method in diagnosis of PDs. (I) Undulation value of both sides SN in PDs (S_p) are shown together with correlation line (slope of 0.457). As seen, the undulation values of PD group (S_p) found to have substantially stronger UPDRS motor score-dependent correlation.

correlation analysis shows that there is substantial UPDRS motor score-dependent correlation. These statistical results would obviously be useful in setting the criteria for diagnosis of PD patients in quantitative manner.

Discussion

The most interesting and important finding of these 7.0T MR imaging study appears to be the clear visualization and eventual quantitation of PDs and normal controls based on the difference in the gross anatomical shape and the quantitative undulation values between the controls and PDs. From the quantitative observation and quantitative analysis such as the undulation value, in vivo 7.0T T2*-weighted MR imaging could provide direct visualization of morphological deformation and quantitative estimation of the PDs from that of the normal controls in vivo. In this study, we have a relatively small number of PD patients and only two patients with more advanced PD. Therefore, the correlation analysis may be biased, particularly in undulation value of advanced PD. However, quantitative analysis with more patients may further improve the results of correlation analysis and is subject of future studies.

One of the major difficulties in the past for the in vivo diagnosis of PD has been the lack of clear image of SNc and surroundings such as CC; therefore, the difficulties in setting the quantitative diagnostic criteria. Most of the currently available diagnostic tools, such the lower field MRI, were simply insufficient for the diagnosis of PD. Some investigators have attempted to diagnose PD patients using conventional MRI, but the results were insufficient for practical use due to the limited resolution and contrast of the images.[11,13,22] In conclusion, this study has demonstrated that by using 7.0T MRI, one can visualize the pathologic features of PD within the SN. ∎

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Phenotype of the 202 Adenine Deletion in the *parkin* Gene: 40 Years of Follow-Up

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ABSTRACT

Background: We describe the four decades follow-up of 14 *parkin* patients belonging to two large eight-generation-long in-bred Muslim-Arab kindreds.

Results: All patients had a single base-pair of adenine deletion at nucleotide 202 of exon 2 (202A) of the *parkin* gene (all homozygous, one heterozygous). Parkinson's disease onset age was 17–68 years. Special features were intractable axial symptoms (low back pain, scoliosis, camptocormia, antecollis), postural tremor, and preserved cognition.

Conclusions: The 202A deletion of the *parkin* gene causes early-onset Parkinson's disease with marked levodopa/STN-DBS–resistant axial features. Postural tremor and preserved cognition, even after 40 years of disease, were also evident. © 2011 *Movement* Disorder Society

Key Words: Parkinson's disease; genetics; Parkin; PARK2; follow-up

Introduction

Mutations in the *parkin* gene (6q25.2-6q27, MIM 602544) are the most common cause of monogenic

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autosomal recessive Parkinson's disease (PD).[1,2] The phenotype includes early onset of classic PD symptoms, but may vary with respect to additional atypical features.[3–5] Exonic deletions or multiplications and truncating or missense mutations have been described.[1–6] No reports point to ethnic clusters of specific *parkin* mutations. We describe four-decades follow-up of 14 *parkin* patients belonging to two large in-bred Muslim-Arab kindreds.

Methods

PD patients of Arabic-origin with age of disease onset < 50 years were recruited from the Sheba Medical Center Movement Disorders Clinic. The Institutional Review Board approved the use of human subjects for this study. All patients and family members signed informed consent for participating in the study.

Participants were examined by a movement disorders specialist at 2–12 months intervals. Asymptomatic family members were examined once at the time of DNA collection. DNA was extracted from blood leukocytes. All exons of the *parkin* gene were screened for deletions, insertions, or point mutations by direct sequencing of the PCR products, sequenced on both strands as previously described.[4]

Results

Thirteen of 14 PD patients and 15 family members consented to genetic testing. Patient characteristics are summarized in Table 1 (10 men, 4 women; mean age 52 ± 10 years; range 35–73 years).

In all 13 patients, the same *parkin* mutation was found: a single base-pair deletion of adenine at nucleotide 202 of exon 2 (202A), causing an out-frame mutation with an early-stop codon (12 homozygous, 1 heterozygous) and one patient was not genotyped. The mutant *parkin* lacks a part of the Ubl domain and the entire region of the RING box, suggesting loss of activity of E3.

Phenotype and Clinical Course

All patients belong to two large Muslim-Arab in-bred *hamulas* (kindreds). Each *hamula* can trace their ancestry to a few founders about eight generations ago. Family A traced back five generations and divided into three branches shown as Aa, Ab, and Ac Family B traced back eight generations.

Mean age ± SD at PD onset was 31 ± 15 years (range 17–68) and disease duration 21 ± 13 (median 19, range 1–41 years) (Table 1). The first patient (B-VII-22) was seen in our clinic in 1963, aged 27 years. She complained of "bent trunk" and slowing since the age of 23. Her first cousin (B-VII-25) was examined in 1989, aged 19 years due to scolisois and

Table 1. Demographic characteristics and motor features of Parkinson's disease (PD) patients with the *parkin* 202A deletion

Patient No.	*Parkin* 202A deletion	Gender	Age	Age of onset	PD duration	H&Y stage	Presenting sign	Rest tremor	Rig.	Asym	Brad.	Post. Inst.	Gait dist.	Post. tremor
Aa-II-3	HOM	M	58	17	41	3	Hand tremor	+	+	+	+	+	+	+
Aa-II-8	HOM	M	50	15	35	4	Leg dystonia	+	+	+	+	+	+	+
Aa-II-10	HOM	F	48	47	1	2	Leg tremor		+	+	+		+	
Aa-II-11	HOM	M	47	30	17	3	Leg tremor	+	+	+	+	+	+	+
Ab-IV-14	NG	M	73	68	5	3	Slow gait	+	+	+	+	+	+	
Ab-V-7	HOM	F	47	18	29	3	Hand tremor	+	+	+		+	+	+
Ac-IV-5	HOM	F	35	17	18	4	Hand tremor	+	+	+	+	+	+	+
B-VII-8	HOM	M	62	35	27	4	Leg tremor	+	+	+	+			+
B-VII-10	HOM	M	59	28	31	4	Hand tremor	+	+	+	+	+	+	+
B-VII-13	HOM	M	49	37	12	3	Leg tremor	+	+	+	+			+
B-VII-17	HOM	M	44	30	14	3	Hand tremor	+	+	+	+			+
B-VII-22	HOM	F	63	23	40	3	Camptocormia	+	+	+	+	+	+	+
B-VII-25	HOM	M	39	19	20	3	Hand tremor	+	+	+	+		+	+
Aa-II-6	HET	M	55	49	6	2	Hand tremor		+	+	+			
Mean±SD			52±10	31±15	21±13									

HET, heterozygous; HOM, homozygous; NG, not genotyped; Rig, rigidity; Asym, asymmetry; Brad, bradykinesia; Post Inst, postural instability; Dist, disturbance; Post, postural.

bradykinesia. The diagnosis of juvenile-onset PD was made in both.

The presenting symptom was hand tremor (n = 6), leg tremor (n = 4), foot dystonia (n = 1), camptocormia (n = 1), and gait disturbances (n = 1). Bradykinesia and rigidity were present in all patients and rest tremor in all but one. Eleven had postural hand tremor, three limb dystonia (two at PD onset) and three reported sleep benefit (Table 1).

Atypical motor features included prominent levodopa-resistant axial symptoms (n = 10): recurrent falls at onset (n = 1), gait disturbances at onset (n = 1), scoliosis (n = 1), camptocormia (progressive to fixed 90° trunk flexion, n = 2), antecollis (n = 1), lower back pain (LBP) (n = 8) (Table 2). Camptocormia and

antecollis 5 years after onset were observed in a heterozygous carrier with an intermediate PD phenotype (onset 49 years) and very slow disease progression.

Pain was a predominant symptom (painful dystonia = 2, LBP = 8). Two patients manifested autonomic dysfuntion with complaints of constipation (Table 1).

None of the patients developed significant cognitive impairment or dementia during follow-up of up to 40 years (median 19 years). Seven patients had depressive symptoms but none develeoped hallucinosis or psychosis.

Response to Treatment and Progression

Levodopa response was excellent for appendicular signs but only minor for axial signs. All patients devel-

Table 2. Nonmotor/atypical features and therapy-related features of Parkinson's disease (PD) patients with the *parkin* 202A deletion

Patient No.	Psych.	Cognitive decline	Sleep benefit	Autonomic features	Additional axial features	Response to levodopa	Wearing off	Levodopa induced dyskinesia	UPDRS III (on/off)	DBS (Yr after PD onset)
Aa-II-3		−				+	+	+	52	
Aa-II-8	DEP	−	+			+	+	+	20/27	+ (30)
Aa-II-10		−	+	constip	LBP	NA	NA	NA	17/NA	
Aa-II-11		−				+			46	
Ab-IV-14		−		constip		+	+		38/41	
Ab-V-7	DEP	−	+		LBP	+	+	+	14	
Ac-IV-5	DEP	−				+	+	+	48/61	+ (14)
B-VII-8	DEP	−			LBP	+	+	+	16/22	
B-VII-10	DEP	−			LBP	+	+	+	37/64	+ (26)
B-VII-13	DEP	−			LBP	+	+		4/20	
B-VII-17		−			LBP	+	+		20/30	
B-VII-22	DEP	−			Camptocormia, LBP	+	+	+	37/48	
B-VII-25		−			Scoliosis, LBP	+	+	+	16/28	
Aa-II-6		−			Antecollis, Camptocormia	NA	NA	NA	NA	

DEP, depression; constip, constipation; LBP, Lower back pain; NA, not applicable.

oped motor fluctuations. Despite consistently very slow disease progression, three patients developed severe motor fluctuations and underwent deep brain stimulation (STN-DBS) 14, 26, and 30 years after PD onset. Response to STN-DBS was modest with improvement in appendicular symptoms but no change in axial features, particularly postural instability, gait disorders, and LBP.

Genotype and Phenotype of Nonparkinsonian Family Members

Fifteen family members were genotyped. Five had no mutations and 10 were heterozygous, of whom nine were asymptomatic (age range 28–40 years). One family member, (70 year-old female) with postural tremor only (onset 60 years, B-VI-5), showed A-G heterozygosity for the 202A *parkin* deletion. Tremor remained her sole symptom during 10 years of follow-up and was levodopa unresponsive.

Discussion

The marked axial nature is the most striking feature of the *parkin* 202A deletion phenotype. This includes camptocormia, antecollis, scolisosis, and low back pain. Dystonia in PD manifests mainly in the limbs as equino-varus foot, striatal toe, ulnar deviation or hemidystonia and in the mid-line as blepharospasm or oromandibular dystonia.[7] Scoliosis, if considered a form of dystonia, is also seen. Camptocormia is a rare feature of PD and is attributed to dystonia or imbalance in muscle activity.[3,6–8] We postulate that LBP is part of the dystonic phenotype. In our patients, axial symptoms were painful and levodopa/STN-DBS unresponsive.

A second main characterisitic of the 202A *parkin* deletion phenotype was the preserved cognition after several decades since PD onset. Lack of dementia characterizes parkin disease.[9] To our knowledge long term follow-up up to 40 years has not yet been described. It has been suggested that the lack of dementia in parkin disease may be related to the absence of Lewy body pathology.[9] Hayashi et al. reported neuropathologic findings in a Japanese patient with a mutation in the *parkin* gene.[10] Loss of pigmented neurons and gliosis were most pronounced in the medial and ventrolateral regions of the SN pars compacta and in the locus ceruleus. There was no clinical evidence of dementia and no Lewy bodies were identified. Some neurofibrillary tangles and senile plaques were observed in the cerebral cortex. Another study also found diffuse tau pathology but no Lewy bodies.[11] Neuropsychiatric disturbances are reportedly common in carriers for *parkin* or *PINK1* mutations.[12,13] The 202A phenotype commonly included depression.

There is controversy whether heterozygous carriers of *parkin* mutations are symptomatic and whether *parkin* is a susceptibility gene.[14,15] One of our heterozygous patients (onset 49 years) developed a mild phenotype but with antecollis and camptocormia. Another heterozygous family member presented solely with postural tremor (onset 60 years) with no other parkinsonian features during 10 years of follow-up. While homozygous mutations in the *parkin* gene result in early-onset PD, heterozygous mutations in the *parkin* gene are identified in patients with later-onset disease, raising the possibility that heterozygous mutations may have a role beyond that of loss of function and confer an increased susceptibility to the disease.[15]

The risk of developing PD in individuals with mutations in the *parkin* gene is age-dependent and penetrance remains unclear. Our asymptomatic carriers were aged 28–40 years and need to be further followed. A recent study identified mutations in the *parkin* gene in 10% of probands with disease onset before age 50.[16] They estimated the cumulative incidence of PD to age 65 years in relatives of mutation carriers to be 7%, compared to 1.7% in noncarrier relatives, and 1.1% in relatives of controls.

Periquet and coworkers analyzed *parkin* gene inheritance to discriminate between single founder effects and independent recurrent events by the use of intragenic and tightly flanking markers of the *parkin* gene region.[17] By studying a variety of *parkin* mutations in 48 European families they showed that the majority of exon rearrangements result from distinct mutational events, whereas point mutations may have arisen from a limited number of founders suggesting different mechanisms underlying these two groups of mutations. According to this hypothesis, the frequency of exon rearrangements would be expected, in the absence of selection, to increase with the passage of time because of new mutational events, whereas the frequency of point mutations as in our patients would be expected to remain stable, because new mutations would be rare. In this context, the 202A deletion, being a point mutation, could be related to a founder effect which persisted because of high rates of consanguinity in this population.

The fact that all patients in our two kindreds carry the same mutation suggests that it might be common among young-onset PD patients of Arabic descent. Indeed, the 202A deletion has also been observed among Jordanian patients.[17] However, the exact phenotype in this population has not been specified. While PD prevalence above the age of 65 years in Arab villages in Israel is similar to that of Western populations,[18] neither the prevalence of *parkin* mutations nor its impact on PD prevalence in young adults is known. In our studied population of Muslim Arab villages with large in-bred kindreds, carrier frequencies may be high.

Our phenotypic observations might be useful for raising the suspicion and recognition of carriers of the

parkin 202A deletion mutation among young-onset PD patients with Arabic ancestry. Postural tremor, prominent and/or painful axial features, slowness of progression of motor symptoms and preservation cognitive function are strong clues to *parkin* disease.

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LINGO1 Gene Analysis in Parkinson's Disease Phenotypes

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ABSTRACT

Background: Parkinson's disease (PD) and essential tremor (ET) may share some etiopathogenic factors. A genome-wide association study has shown that *LINGO1* gene variants are associated with increased risk of ET. We hypothesized that *LINGO1* variants could increase susceptibility to PD. Methods: A large series of PD subjects and healthy controls were genotyped for rs9652490 and rs11856808 *LINGO1* single nucleotide polymorphisms (SNPs). Results: We found an increased frequency of the rs11856808$^{T/T}$ genotype in PD compared with controls (odds ratio = 1.46; corrected *P* value = 0.02). A recessive genetic model was the best fit for rs11856808 influence on PD (recessive gene action test: corrected *P* value = 0.01). Stratification analysis showed that rs11856808$^{T/T}$ genotype frequency was higher in the tremor-dominant PD and the classical PD (C-PD) subgroups (recessive gene action test for the C-PD subgroup: corrected *P* value = 0.004). Discussion: Our results indicate that *LINGO1* variants could increase risk of PD, specifically those presenting the non-rigid-akinetic phenotypes, which suggests that *LINGO1* may have a role in the etiology of tremor in PD at least in the Spanish population. ©2011 *Movement* Disorder Society

Key Words: Parkinson's disease; *LINGO1*; essential tremor; genetics; association study

Parkinson's disease (PD) is one of the most frequent neurodegenerative diseases with a prevalence of 1–2% among individuals over the age of 60 years.[1] PD is due to the loss of dopaminergic neurons and presence of Lewy bodies in several brain areas, mainly in the substantia nigra pars compacta. The identification of genes associated with Mendelian inherited forms of PD supports the hypothesis that other genetic risk factors can also be associated with sporadic PD.[2]

Although essential tremor (ET) and PD are different neurological disorders, some evidence suggests that they may share common etiologic factors. In fact, relatives of subjects with tremoric PD have a fourfold increase in the prevalence of postural tremor compared with healthy controls.[3–5] Additionally, brainstem Lewy bodies have been described in some ET brains.[6,7]

Recently, a genome-wide association study (GWAS) showed that two intronic single nucleotide polymorphisms (SNPs), rs9652490 and rs11856808, of LINGO1 gene (Leucine-rich repeat and Ig domain containing Nogo receptor-interacting protein 1; OMIM ref no. 609791) were associated with ET.[8] LINGO-1 is a transmembrane protein that contains 12 leucine-rich repeat domains, an immunoglobulin domain and a short cytoplasmic tail encoded by a gene located on chromosome 15q24.[9] LINGO-1 expression is elevated in the substantia nigra of PD subjects compared with controls.[10] In addition, dopaminergic neurons of LINGO1 knockout mice are protected against degeneration.[10] Thus, LINGO1 seems to be a candidate gene for modifying PD risk. We genotyped two LINGO1 gene variants that have been previously associated with ET, to investigate whether LINGO1 variants could increase the risk of PD and its different subphenotypes in the Spanish population.

Subjects and Methods

Subjects were recruited from seven centers in Spain and included 721 PD individuals and 1,117 healthy controls. Recruitment data are reflected in the Supporting Information file 1. Selection criteria for PD included bradykinesia and at least one of the following: rigidity, resting tremor, postural instability, positive response to

dopaminergic therapy, and absence of atypical features or other causes of parkinsonism.[11] When information was available, PD subjects were divided into three different groups: tremor-dominant PD (TD-PD), classical PD phenotype (C-PD), and akinetic-rigid PD (AR-PD; Table 1).[12] The sample included 171 individuals with early-onset PD [EOPD; age at onset (AAO) < 50 years], and 550 subjects with late-onset PD (LOPD; AAO ≥ 51 years; Supporting Information Table 1). Given that the samples came from different regions of Spain to avoid influence of subpopulation differences on the results, we performed a reanalysis based on the geographical location of the individuals recruited, stratifying the sample into northern and southern Spain (Tables 2 and Supporting Information Table 5 and Fig. 1).

Two intronic polymorphisms of LINGO1 gene, rs9652490 and rs11856808, which had previously been associated with ET, were selected for genotyping.[8] Genotype success rate was over 96.7%. Genotyping methods are reflected in the Supporting Information file 1.

Pair-wise linkage disequilibrium (LD) D′ and r^2 measurements between rs9652490 and rs11856808 were calculated using Haploview software (http://www.broad institute.org/haploview/haploview).[13] Hardy–Weinberg equilibrium (HWE) analysis and "goodness-of-fit" test are described in the Supporting Information (Supporting Information file 1). Allelic and genotype frequency analysis and alternate/full model association tests were performed with PLINK v.1.07 software (Shaun Purcell; http://pngu.mgh.harvard.edu/purcell/plink/).[14] Multiple test correction was performed with Westfall and Young's step-down max(T) permutation procedure implemented in PLINK v.1.07 by running 100,000 permutations within each group.[14] Level of statistical significance was considered at corrected P values ≤ 0.05. Haplotype analysis and influence of LINGO1 variants on the AAO are detailed in the Supporting Information file 1.

To replicate the association found by our group in additional populations, we performed a meta-analysis based on genotype data of the two LINGO1 SNPs from two GWAS in PD and controls[15,16] using the Meta-Disc v. 1.1.1 program (http://www.hrc.es/investigacion/metadisc.html; Biostatistics Unit, Hospital Ramón y Cajal, Madrid, Spain).

Results

No deviation from HWE was found for rs9652490 in PD or control group. However, a significant departure from HWE was observed among PD individuals for rs11856808 ($n = 721$; P value = 0.03, Pearson), but not in control group. Goodness-of-fit test, considering a disease prevalence of 0.01, showed the highest nonsignificant χ^2 for rs11856808 under a recessive genetic model ($\chi^2 = 2.48$; P value = 0.29 with 2 df) suggesting that the

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Table 1. Demographic data of the sample

Group	Controls ($n = 1{,}117$)	PD				
		Entire PD ($n = 721$)	TD-PD ($n = 30$)	C-PD ($n = 361$)	AR-PD ($n = 104$)	Unclassifiable PD[a] ($n = 226$)
Age (yr), mean (SD)	63.59 (14.55)	66.96 (10.59)	69,50 (8.18)	67.06 (10.68)	66.00 (10.79)	67.41 (11.99)
Age range (yr)	17–104	22–95	49–87	22–95	39–87	41–88
AAO (y), mean (SD)	NA	59.53 (12.51)	62.95 (8.24)	57.55 (11.58)	56.81 (11.59)	63.47 (13.72)
AAO range (y)	NA	17–90	46–76	17–84	34–84	18–90
Female (%)	49.4	42.0	40	36.8	41.3	50.9

[a]PD subjects with no information on motor features available.
PD, Parkinson's disease; TD-PD, tremor-dominant PD; C-PD, classical PD phenotype; AR-PD, akinetic-rigid PD; AAO, age at onset; SD, standard deviation; NA, data not available.

rs11856808 HWE departure in PD subjects was a consequence of a real genotype association.[17] Analysis of genotype frequency distribution showed that rs11856808[T/T] frequency was higher in PD subjects than in controls [odds ratio (OR) = 1.46; 95% confidence interval (CI) = 1.06–2.02; corrected P value = 0.02; Table 2)] Alternate/full model association tests confirmed that the rs11856808 association with PD was explained by a recessive genetic model (corrected P value = 0.01; Supporting Information Table 3).

Stratification analysis of PD sample according to subphenotypes showed that rs11856808[T/T] genotype frequency was only significant in the C-PD phenotype group (OR = 1.74; 95% CI = 1.19–2.56; corrected P value = 0.01; Table 2), which followed a recessive model (corrected P value = 0.004; Supporting Information Table 3). Interestingly, although nonsignificant, the highest rs11856808[T/T] genotype frequency was observed among the TD-PD subgroup (16.7%) suggesting that we cannot exclude an effect of rs11856808[T/T] on this PD subtype owing to the small sample size ($n = 30$). We found no significant allelic association between *LINGO1* SNPs and the entire PD sample compared with controls (Table 2).

As previous reports suggested that subjects with TD-PD and C-PD phenotype may have an earlier AAO than the AR-PD group,[18] we aimed to analyze whether the overrepresentation of rs11856808[T/T] genotype in the C-PD subgroup was influenced by subjects with an earlier AAO within this group. We performed Kaplan–Meier survival analysis considering recessive, dominant, and additive models, which showed no influence of *LINGO1* variants on the AAO (data not shown). Additionally, stratification analysis according to the AAO showed a trend for the rs11856808[T/T] genotype in both the EOPD and the LOPD groups compared with controls (OR = 1.31; 95% CI = 0.77–2.22; corrected P value = 0.06 and OR = 1.51; 95% CI = 1.07–2.15; corrected P value = 0.06, respectively; Supporting Information Table 4). However, after considering only the individuals with the C-PD phenotype in both groups, EOPD and

LOPD, we observed an overrepresentation of rs11856808[T] allele and rs11856808[T/T] genotype frequencies in the LOPD C-PD group compared with controls (corrected $P = 0.02$; for both, allelic and genotype tests) but found no allelic or genotype association for the EOPD C-PD group.

Given the different geographical origin of the samples across Spain, we wanted to investigate whether there were any regional differences. As the Spanish population is largely homogeneous,[19] we arbitrarily stratified our population into a northern and southern Spanish population (Supporting Information Fig. 1).

Breslow–Day test[14] was used to investigate between cluster heterogeneity and the test of the homogeneity of OR tests based on partitioning the χ^2 statistic, which was used to analyze the heterogeneity of the association. Both tests are complemented in *PLINK*.[14]

Breslow–Day test for homogeneity showed no significant differences in the allelic ORs of rs9652490 and rs11856808 between the northern and southern Spanish population (data not shown; P values> 0.2), suggesting the absence of population substructure. However, when both subpopulations were analyzed separately by a χ^2 test, significant differences were observed in rs11856808 allelic association in the southern subpopulation (OR = 1.39; corrected P value = 0.04) (Supporting Information Table 5). Interestingly, the recessive gene action test for the rs11856808[T/T] genotype was also significant in the southern Spanish population (corrected P value = 0.004; data not shown) but not in the northern Spanish population. As rs9652490 and rs11856808 were in high LD ($D' = 0.992$ and $r^2 = 0.455$), we performed a haplotype analysis that showed no differences between the PD group and controls (Supporting Information Table 6).

Rs9652490 and rs11856808 meta-analysis of two PD GWAS[15,16] and our study, accounting for 2,564 PD subjects and 5,018 controls, showed no differences in allelic or genotype distribution frequencies between cases and controls (Supporting Information Fig. 2).

Table 2. Allelic and genotype frequency distribution of *LINGO1* variants

Group	Controls (n = 1,117)				Entire PD sample (n = 721)				TD-PD (n = 30)				C-PD (n = 361)				AR-PD (n = 104)				UNK-PD (n = 226)			
rs9652490 allele[a]/genotypes	G	AA	AG	GG	G	AA	AG	GG	G	AA	AG	GG	G	AA	AG	GG	G	AA	AG	GG	G	AA	AG	GG
Freq./count (freq.)	0.171	750 (0.682)	324 (0.295)	26 (0.024)	0.192	461 (0.655)	216 (0.307)	27 (0.038)	0.183	20 (0.667)	9 (0.300)	1 (0.033)	0.199	229 (0.638)	117 (0.326)	13 (0.036)	0.158	72 (0.713)	26 (0.257)	3 (0.030)	0.196	140 (0.654)	64 (0.299)	10 (0.047)
OR [95% CI]					1.15 [0.97–1.37]	Ref	1.09 [0.88–1.34]	1.69 [0.97–2.93]	1.09 [0.56–2.11]	Ref	1.04 [0.47–2.31]	1.44 [0.19–11.16]	1.21 [0.97–1.50]	Ref	1.18 [0.91–1.53]	1.64 [0.83–3.24]	0.91 [0.62–1.35]	Ref	0.84 [0.52–1.33]	1.20 [0.35–4.07]	1.18 [0.91–1.54]	Ref	1.06 [0.77–1.46]	2.06 [0.98–4.37]
P value[b]					0.11		0.15		0.80		0.94		0.09		0.20		0.65		0.70		0.21		0.16	
P value[c]					0.18		0.25		0.96		0.91		0.14		0.31		0.85		0.88		0.32		0.22	
rs11856808 allele[a]/genotypes	T	CC	CT	TT	T	CC	CT	TT	T	CC	CT	TT	T	CC	CT	TT	T	CC	CT	TT	T	CC	CT	TT
Freq./count (freq.)	0.312	498 (0.463)	484 (0.450)	94 (0.087)	0.332	326 (0.464)	286 (0.407)	90 (0.128)	0.367	13 (0.433)	12 (0.400)	5 (0.167)	0.353	158 (0.439)	150 (0.417)	52 (0.144)	0.291	53 (0.515)	40 (0.388)	10 (0.097)	0.311	102 (0.488)	84 (0.402)	23 (0.11)
OR [95% CI]					1.09 [0.95–1.26]	Ref	0.90 [0.74–1.11]	1.46 [1.06–2.02]	1.28 [0.75–2.17]	Ref	0.95 [0.43–2.10]	2.04 [0.71–5.85]	1.20 [1.00–1.43]	Ref	0.98 [0.76–1.26]	1.74 [1.19–2.56]	0.91 [0.66–1.24]	Ref	0.78 [0.50–1.19]	0.99 [0.49–2.03]	0.99 [0.79–1.25]	Ref	0.84 [0.62–1.16]	1.20 [0.72–1.98]
P value[b]					0.22		**0.01**		0.38		0.32		**0.04**		**0.008**		0.53		0.48		0.96		0.34	
P value[c]					0.34		**0.02**		0.56		0.39		0.07		**0.01**		0.73		0.65		0.99		0.46	

PD, Parkinson's disease; TD-PD, tremor-dominant PD; C-PD, classical PD phenotype; AR-PD, Akinetic-rigid PD; UNK-PD, Unknown PD phenotype; Freq., Frequency; CI, confidence interval; OR, odds ratio.

[a]Minor allele frequency.

[b]Uncorrected P values for genotype test.

[c]Corrected P values after Westfall–Young max(T) step-down procedure (100,000 permutations). Corrected P values $\leq$ 0.05 are highlighted in bold.

Discussion

We analyzed two *LINGO1* variants that have been previously associated with increased ET risk in subjects with PD from a Spanish population. Since the first association of *LINGO1* gene with ET was described,[8] the analysis of subsequent independent ET series replicated the association in some of them.[8,20–23] Given that PD and ET could potentially share some etiologic factors, we investigated the role of *LINGO1* variants in PD risk. Although we found no significant allelic association, we observed an increased frequency of rs11856808[T/T] carriers among PD subjects compared with controls. rs11856808[T/T] frequency was significantly higher after comparing the C-PD phenotype subgroup with controls, but differences in rs11856808 genotype frequency distribution were not statistically significant after considering other PD subgroups' comparisons with controls (Table 2). The observed rs11856808[T/T] genotype frequency in our sample that led to a departure from HWE in the PD group but not in controls was explained by a recessive model of heritability for rs11856808[T] allele.[17] This fact supports the notion that the increased rs11856808[T/T] frequency in PD could be a result of an association between PD and rs11856808 rather than caused by the population structure or by possible genotyping errors. Moreover, a recessive model as best fit for rs11856808 variant was confirmed with recessive gene action analysis of *PLINK*, when comparing the entire PD group or the C-PD subgroup versus controls (Supporting Information Table 3). Although the first study of *LINGO1* variants suggested that a multiplicative model explains better rs11856808 association with ET,[8] our findings are consistent with more recent series in which rs9652490 association with ET followed a recessive model.[21–23] The direction of effect of the PD risk rs11856808[T] allele seen in our series is also consistent with that seen in the later studies where similar ORs were observed.[8,21] Our findings suggest that *LINGO1* variability may influence both ET and PD. We hypothesize that the high proportion of PD patients with predominant tremor in our series could be responsible for the recessive genetic model observed in our series. We found no difference in genotype and allelic frequencies between EOPD and LOPD, but the association with *LINGO1* was observed in the LOPD C-PD group and not in the EOPD C-PD group, suggesting that *LINGO1* influence could be dependent upon age and PD phenotype.

A previous study described a significant association between rs9652490[A] allele and PD in two different series of PD from North America and Norway.[24] However, the results of the latter study were based on an analysis of a pooled sample set in which the control group showed a strong HWE departure (*P* value = 0.007, Pearson); hence, the assumption of association can be argued. Haubenberger et al.[25] failed to replicate the rs9652490[A] allele association with PD in the Austrian population. However, as observed in our PD sample, rs9652490[G/G] genotype frequency was higher among PD subjects than in controls. Additionally, the Austrian study followed different selection criteria when dividing PD patients into motor subgroups.[25] Indeed, they included PD individuals within their non-TD-PD group that we would have considered as having C-PD or AR-PD. Subsequently, they could have missed a potential rs9652490[G/G] effect in the PD patients with mild to moderate rest tremor. Recently, another study showed that rs9652490[G] allele was not associated with PD in the Polish population, but their results should be interpreted with caution, as their sample size was smaller than ours.[26]

Reanalysis of our series according to their geographical origin showed no population stratification in the series, although only the southern population showed significant differences in allelic and genotype frequencies between PD and controls (Supporting Information Table 5). However, we are aware that initial diagnostic errors between PD and ET can be a possible source of confusion in our study. It is not uncommon that a PD patient is diagnosed as having ET and vice versa in the initial and even in later stages of disease.[27–29] In fact, a study showed that ~33% of subjects with ET had a wrong initial diagnosis, being PD the most frequent source of misdiagnosis throughout the follow-up.[29] Moreover, it has been described that ET subjects can show certain degree of bradykinesia.[30]

Our results support the hypothesis that *LINGO1* gene variants could have a role in the risk of developing PD in the Spanish population, especially among those with non-rigid-akinetic PD phenotypes. The meta-analysis of this study, which also included previously published data, suggested that *LINGO1* is not a major risk factor for PD in Caucasian population (Table 7 and Fig. 2 of Supporting Information). However, *LINGO1* gene variants may still behave as risk factors for PD and/or PD subtypes with predominant tremor in specific populations, like that of Spain. However, we should be cautious about our results, which need to be replicated in other well-characterized PD series. ∎

APPENDIX

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Instability of Syllable Repetition in Parkinson's Disease—influence of Levodopa and Deep Brain Stimulation

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ABSTRACT

The aim of this study was to test the hypothesis of a fundamental impairment of vocal pace performance in Parkinson's disease (PD) based on a syllable repetition paradigm and the influence of levodopa and deep brain stimulation of the subthalamic nucleus (STN-DBS). Twenty-two PD patients under stable dopaminergic medication, 14 patients with STN-DBS, and 30 controls were tested. Participants had to repeat the syllable /pa/ in a steady pace. Percental coefficient of variance (COV) of interval length was measured for the description of pace stability. Patients were tested in the OFF state and again in the ON state after levodopa administration or ongoing STN-DBS. COV was elevated in both PD subgroups. COV was not influenced by levodopa administration but showed a further deterioration under STN-DBS. The impaired syllable repetition capacity shows similarities to the patterns of more complex speech rhythm abnormalities in PD and therefore might share the same pathophysiology. ©2011 *Movement* Disorder Society

Key Words: Parkinson's disease; syllable repetition; motor speech performance; subthalamic nucleus deep brain stimulation; levodopa

Hypokinetic dysarthria in Parkinson's disease (PD) implies alterations of speech rate that have been attributed to a reduced range of articulatory movements[1] as a consequence of manifestation of hypokinesia on the speech production system. With regard to the aspect of speech rate and rhythm, Parkinsonian patients feature an articulatory acceleration in the course of reading and further irregularities as abnormally placed pauses and difficulties initiating articulation which could result from the inability to maintain a speech motor program.[2,3] The characteristic basal ganglia dysfunction in PD is thought to induce an instability of motor sequences that normally are performed in an automatic fashion and which require little attentional resources.[4,5]

From the therapeutic point of view, the effect of dopaminergic stimulation on speech rhythm in PD finally remains inconclusive, whereas most of the studies found no significant changes after short-term levodopa administration or under long-term dopaminergic medication.[6,7] On the other hand, studies on the effect of deep brain stimulation of the subthalamic nucleus (STN-DBS) have shown a variable response of speech to stimulation.[8,9] There is some evidence for a crucial role of contact side and amplitude of stimulation on speech intelligibility, whereas in some patients, the progression of speech difficulties was not modifiable by adjusting the medication or stimulation.[10,11]

The aim of this study was to test the hypothesis of a fundamental impairment of speech motor performance in PD based on a syllable repetition paradigm.[5] Furthermore, the influence of short-time levodopa administration and the effect of STN-DBS were tested by comparison of motor speech performance in defined OFF and ON states. If hypokinesia of the voice apparatus was the major cause for impaired syllable repetition, one might expect an amelioration of speech rhythm performance in the medicamentous ON state and with STN-DBS ON as well. On the other hand, if there was no influence of levodopa on steadiness of syllable repetition or a diverse effect of levodopa treatment and STN-DBS, unsteadiness of syllable repetition should rather mirror a nondopaminergic deficit and might hint to a general impairment to maintain a speech motor program in PD.

Patients and Methods

Our study had been approved by the local Ethics Committee of the Ruhr University Bochum. Written informed consent was obtained from each participant.

From 2005 to 2008, 36 consecutive patients with PD were recruited for this study. Fourteen patients of this series (DBS group) had undergone stereotactic surgery for bilateral deep brain stimulation (DBS) of the subthalamic nucleus 10 to 24 months prior to this investigation (mean 14.27 months) and were on stable stimulation and dopaminergic medication for at least 2 months prior to this investigation. Stimulation amplitudes ranged from 2.4 to 3.9 V (mean 3.14 V) with pulse duration of 60 microseconds and a frequency of 130 Hz. The other subgroup included 22 patients

Table 1. Participants' characteristics and comparison of COV between PD and control group and between OFF and ON state

	PD_medical treatment (PD_mt; 9 men, 13 women)			PD_DBS (9 men, 5 women)			Control group (15 men, 15 women)	
	Mean	SD		Mean	SD		Mean	SD
Age (yr)	63.82	10.31		65.64	6.81		65.97	7.59
Duration (yr)	4.18	4.63		12.07	5.21			
Hoehn and Yahr_OFF	2.41	0.17		3.11	0.53			

	OFF (mean/SD)	ON (mean/SD)	Comp. OFF vs. ON	OFF (mean/SD)	ON (mean/SD)	Comp. OFF vs. ON		
UPDRS III	32.10/13.06	18.77/8.06	$P < 0.01$	34.36/8.47	13.36/9.09	$P < 0.01$		
VKT (ms)	11,791/5,751	13,341/5,198	n.s.	14,429/7,511	13,293/5,242	n.s.	11,405/5,814	
COV_{5-20}	2.00/1.04	1.91/0.87	n.s.	2.22/1.32	2.90/1.27	$P = 0.035$	1.08/0.26	

The levels of significance refer to the comparison of the control group with the accordant PD group (ANOVA).
*$P < 0.01$.

(medical treatment group) on stable dopaminergic therapy consisting of levodopa and different dopamine agonists. As preparation for the testing, dopamine agonists had been stopped for at least 3 days, and the last levodopa dosage was given in the antecedent evening, at least 12 hours prior to the examination. The medical treatment group underwent a standardized levodopa challenge with administration of 200 mg of a soluble levodopa preparation. Patients were tested in the drug-naive defined OFF state and again in the best ON state 30 to 45 minutes after levodopa admission. The DBS group was tested under ongoing STN stimulation (Stim$_{ON}$/Med$_{OFF}$) and again 30 minutes after turning off the stimulation (Stim$_{OFF}$/Med$_{OFF}$).We tested 30 age-matched healthy persons as control group.

For the speech test, each participant was asked to repeat the syllables /pa/ at least 25 times in a "comfortable" self-chosen steady (isochronous) pace without accelerating or slowing articulatory velocity. Based on the oscillographic sound pressure signal of the digitally recorded audio material, the period from onset of one vocalization until the following vocalization was defined as "interval." Stability of pace of the utterances was defined as relative coefficient of variation (COV_{5-20}) calculated for the intervals 5 to 20 in relation to the average interval length of the first four utterances (avIntDur$_{1-4}$) following the formula: $COV_{5-20} = SD_{5-20}/[(avIntDur_{1-4})/\sqrt{16}] \times 100$. As a parameter for speech breathing, each participant was asked to keep the vowel /a/ as long as possible on one breath, defined as vowel keeping time (VKT). Detailed information about the performance and analysis of the speech rhythm task is published elsewhere.[5]

Statistics

Winstat was used for statistical analyses. ANOVA with post hoc Bonferroni adjustment was performed to assess differences of motor performance and speech variables for group and condition. t-Test for dependent samples was used for intragroup comparisons in both PD subgroups as the variables were widely normally distributed (Kolmogorov–Smirnov test). Pearson's correlation was used to test for significant correlations. The level of significance was set at $P = 0.05$.

Results

Detailed results are summarized in Table 1 and Figure 1.

Comparison Between Control Group and the PD Subgroups

As compared with the control group, COV_{5-20} was significantly elevated in both PD groups in the OFF and ON state.

Comparison Between OFF and ON State in the PD Subgroups

In the PD_medical treatment group, COV_{5-20} in the OFF and ON state showed no significant differences. In the PD_DBS group, COV_{5-20} in the ON state was significantly higher than in the OFF state. No correlation was seen between VKT and COV_{5-20}.

Discussion

In our study, both groups of PD patients (PD_medical treatment and PD_DBS) showed significant difficulties to keep an isochronous vocal rhythm both in the OFF and ON states.

In the PD_medical treatment group, COV_{5-20} showed no significant changes after levodopa administration. The lack of efficacy of levodopa on vocal pace stability might be an evidence for the incapability to maintain a once chosen speech motor program caused by dysfunctional basal ganglia circuits, which do not respond to dopaminergic stimulation. This hypothesis is corroborated by previous findings of instability of syllable repetition in a large sample of Parkinsonian patients being on stable long-term dopaminergic medication.[5] Provided that basic syllable repetition tasks and much more complex speech
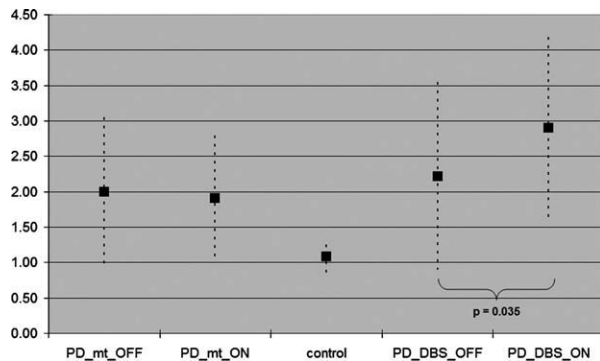
FIG. 1. COV in PD patients in ON and OFF state in comparison to the control group; x-axis: groups/y-axis: COV; PD_mt, PD medical treatment group; PD_DBS, PD deep brain stimulation group.

tasks share the same underlying mechanisms, our results are in line with the finding that speech rate and pause ratios of Parkinsonian speakers based on reading and monolog tasks do not respond to dopaminergic therapy.[6,7,12] A recent positrone emission tomography (PET)-based investigation on motor timing in Parkinsonian patients based on a finger tapping paradigm revealed differential patterns of activation depending on the availability of dopaminergic stimulation with a lack of adequate frontal activation caused by an excessive inhibitory pallidal outflow, which could be normalized by apomorphine administration.[13] As the accuracy of repetitive timed movements showed no significant difference in the OFF and ON state, the Parkinsonian patients obviously switched to alternative neural pathways, e.g., the cerebellum, to maintain the motor program.[13] Related to the aspect of acoustical rhythm perception, the basal ganglia have been shown to be part of a system involved in detecting and generating an internal beat, and that this capacity seems to be compromised in PD.[14]

In the PD_DBS group, the already elevated COV_{5-20} in the OFF state showed a further deterioration under stimulation, which bears some analogy to patterns of dysfunctional articulator movements under high voltage STN-DBS with subsequent deterioration of overall intelligibility.[10,15] Several hypotheses could explain the worsening effect of STN-DBS on the stability of syllable repetition and overall speech performance in PD. On the one hand, STN could have a different somatotopy for speech and body motor control. As a consequence, the site of STN stimulation which leads to the best effect on limb movement might provoke a worsening of dysarthria as it has been reported for contacts placed dorsomedial to the STN.[16] On the other hand, deterioration of speech under STN-DBS might be induced by the spread of current to adjacent areas such as the corticobulbar tract or pallidofugal and cerebellothalamic pathways.[17] However, if our findings of further vocal pace deterioration under STN-DBS were caused by the spread of current to corticobulbar pathways for laryngeal motor control, one would expect a significant effect on sustained phonation (as measured by VKT) that is not observed in our series.

Summarized, our finding of pace instability of syllable repetition in PD confirms previous findings and seems to corroborate the paradigm of the crucial role of basal ganglia in the performance and maintainance of automatic motor sequences.[4,5] In PD, the maladaptive neuronal motor speech networks apparently are not reconstituted by short-term dopaminergic stimulation and may be even deteriorated by STN-DBS. ∎

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Serum Insulin-Like System Alterations in Patients with Spinocerebellar Ataxia Type 3

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ABSTRACT

Spinocerebellar ataxias (SCAs) constitute a group of autosomal dominant neurodegenerative disorders with no current treatment. The insulin/insulin-like growth factor 1 (IGF-1) system (IIS) has been shown to play a role in the neurological dysfunction of SCAs and other polyglutamine disorders. We aimed to study the biomarker profile of serum IIS components in SCA3. We performed a case–control study with 46 SCA3 patients and 42 healthy individuals evaluating the peripheral IIS profile (insulin, IGF-1, IGFBP1 and 3) and the correlation with clinical, molecular, and neuroimaging findings. SCA3 patients presented lower insulin and IGFBP3 levels and higher insulin sensitivity (HOMA2), free IGF-I, and IGFBP1 levels when compared with controls. IGFBP-1 levels were directly associated with CAG expanded repeat length; IGF-1 was associated with the volumetries of specific brainstem regions on magnetic resonance imaging (MRI). Insulin levels and sensitivity were related to age at onset of symptoms. Our findings indicate an involvement of IIS components in SCA3 neurobiology and IGFBP-1 as a potential biomarker of the disease. © 2011 Movement Disorder Society

Key Words: spinocerebellar ataxia; polyglutamine disorders; IGF-1; insulin; IGFBP; Machado-Joseph disease

Spinocerebellar ataxias (SCAs) constitute a group of autosomal dominant neurodegenerative disorders with no current treatment.[1,2] The most prevalent SCAs are those caused by CAG glutamine trinucleotide repeat expansions,[1] being part of the so-called group of polyglutamine (polyQ) disorders.[2]

The insulin/insulin-like growth factor 1 (IGF-1) system (IIS)—encompassing insulin, IGF-1, and IGF binding proteins (IGFBPs)—performs important neuromodulatory functions in the central nervous system (CNS).[3–5] Abnormalities in the IIS signaling pathway were thought to play a part in the physiopathological processes of various neurodegenerative disorders, including Alzheimer's disease, SCAs, and Huntington disease through different mechanisms.[3,4,6–8]

Chronic treatment with IGF-1 improved neurological deficits in neurotoxic and transgenic animal models of ataxia.[9,10] In transgenic animal models of other polyQ disorders, there was also evidence of involvement of signaling components of the IIS in the modulation of mutant proteins and disease phenotype.[11,12] On clinical grounds, altered serum levels of IGF-1 and IGFBPs have been reported in patients with late onset cerebellar ataxia (LOCA).[13]

Bearing in mind the evidence pointing toward the potential alteration of the IIS in polyQ disorders, we aimed to investigate the biomarker profile of serum IIS components in SCA3, considering their relationship with clinical, molecular, and neuroimaging findings.

Patients and Methods

Design and Subjects

A case–control study was performed between May and October of 2007 with 46 molecularly confirmed SCA3 patients from the neurogenetics clinic of Hospital de Clínicas de Porto Alegre (HCPA) and 42 healthy, nonrelated individuals with similar age, gender, and environmental characteristics. Nutritional and mood evaluation were undertaken to control for confounding factors. The *ATXN3* expanded regions were analyzed as previously described.[14] Subjects diagnosed with

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other neurological, endocrine, renal, or hepatic disorders were excluded. The study was approved by the local Ethics Committee and all subjects gave their informed written consent.

Clinical Evaluation

Two clinical ataxia scales were applied: the SARA[15] and NESSCA scores.[16] Disease duration and age at onset were informed by patients and their relatives. All subjects completed the beck depression inventory.[17]

Magnetic Resonance Imaging (MRI)

MRI was done using 1.5 T system. Sagittal T1-weighted images (repetition time [TR] = 2,000 ms and echo time [TE] = 3.45 ms)—slice thickness of 1 mm, pixel size of 0.49 mm—were performed. The normalized volumes of the brainstem, mesencephalus, pontine tegmentum, basis of pons, medulla oblongata, and cerebellum of SCA3 patients were measured on fluid-attenuated inversion recovery, using semiautomated segmentation techniques and voxel count volumetry using the software ImageJ. More detailed procedures of this technique were described elsewere.[18]

Samples and Assays

Serum was obtained by blood centrifugation at 6,000g for 5 min, frozen immediately, and stored at $-70°C$ until analyses. All samples were obtained between 10 AM and 4 PM and all subjects were under fasting conditions. Serum IGF-1 (DuoSet R&D System), IGFBP-1 (Medix Biochemica, Finland), and IGFBP-3 (Mediagnost, Germany) were measured in duplicate by enzyme-linked immunosorbent assay. Serum insulin (Roche Diagnostics, Germany) was determined by electrochemiluminescence. We utilized the *HOMA Calculator v2.2.2* to determine HOMA2 parameters.

Statistical Analysis

All variables in the study showed a normal distribution on a one-sample Kolmogorov-Smirnov test, except HOMA2-%S, which was log transformed. Comparisons of IIS component levels between cases and controls were performed using a two-tailed unpaired Student's t test. The univariate general linear model was utilized to control for confounding factors that were significantly correlated with the outcomes. Gender was analyzed with a χ^2 test and all correlations with the Pearson correlation test followed by a linear regression model. Statistical significance was defined as $P < 0.05$.

Results

Demographic data of cases and controls are shown in Table 1. MRI was obtained randomly in 26 (14 females) of these SCA3 patients, whose demographic characteristics were similar to those found in the overall group of patients.

Table 1. Demographics of the enrolled individuals

	Controls	SCA3 Patients	
	Mean (SD)	Mean (SD)	P
N	42	46	NA
Age (yr)	45.5 (12)	44.5(11)	0.683
Gender (M/F)	16/26	22/24	0.357
Glucose (mg/dl)	86.7 (6.5)	85.9 (12.6)	0.785
Total cholesterol (mg/dl)	206 (35)	188 (38)	0.085
HDL (mg/dl)	55 (14)	58 (11)	0.352
LDL (mg/dl)	124 (39)	108 (28)	0.098
VLDL (mg/dl)	25 (10.9)	16.1 (8.7)	0.003**
Triglycerides (mg/dl)	125(54)	80 (43)	0.003**
Creatinine (mg/dl)	0.77 (0.15)	0.79 (0.2)	0.728
Albumin (g/dl)	4.6 (0.28)	4.7 (0.25)	0.369
Total bilirubin (mg/dl)	0.64 (0.35)	0.58 (0.2)	0.486
Prothrombin time (INR)	1.01(0.06)	1.03 (0.06)	0.39
BMI (kg/m^2)	27.1 (4.5)	24.4 (4.1)	0.01**[a]
BDI	10.5 (9)	16 (11)	0.005**
Disease duration (yr)		9.97 (6.6)	NA
Age at onset (yr)		34.5 (10)	NA
CAG(n)		73.3 (3)	NA
NESSCA		17.5 (6.3)	NA
SARA		14.4 (7.6)	NA

[a]Corrected for age.
NA, not applicable.
**$P < 0.01$.

Insulin/IGF-1 System

Differences regarding serum concentrations of IGF-1, IGFBP-1, IGFBP-3, IGF-1:IGFBP-3 molar ratio, insulin, and HOMA analysis between cases and controls are shown in Table 2.

Total IGF-1 serum levels did not differ between groups ($P = 0.550$; corrected for age) nor correlated with clinical or molecular variables. IGF-1 was inversely correlated with medulla oblongata ($R = -0.489$; $P = 0.011$) and basis of pons volumetry ($R = -0.439$; $P = 0.025$) on MRI (Supporting Information Figs. 1 and 2).

IGFBP-1 serum levels were higher in patients with SCA3 than in controls ($P = 0.001$; corrected for BMI). IGFBP-1 levels were correlated significantly with CAG expanded repeats ($R = 0.451$; $P = 0.003$; Fig. 1)—which was the only factor independently associated in the linear regression model ($R = 0.452$; $P = 0.006$). IGFBP-1 did not correlate with MRI volumetries.

IGFBP-3 levels were lower ($P = 0.001$) and the IGF-1:IGFBP-3 molar ratio (an indirect form of measuring free IGF-1) was higher ($P = 0.039$; corrected for age and gender) in SCA3 patients than in controls. Neither IGFBP-3 nor IGF-1:IGFBP-3 molar ratio independently correlated significantly with any clinical, molecular, or MRI volumetries variables.

Insulin levels were lower in cases than in controls ($P = 0.027$, after correction for body mass index [BMI]). Age at onset correlated directly with insulin levels ($R = 0.510$; $P = 0.0001$) and was the only factor independently associated in the linear regression model with insulin ($R = 0.365$; $P = 0.026$; Fig. 2). When we considered age of onset as a dependent factor, both

Table 2. Serum levels of insulin/insulin-like growth factor 1 system components in SCA3

	Controls	SCA3 Patients	
	Mean (SD)	Mean (SD)	P
IGF-1 (ηg/mL)	117.4 (36.3)	114.5 (32.2)	0.550[a]
IGFBP-1 (ηg/mL)	1.32 (0.98)	2.67 (1.8)	0.001**[b]
IGFBP-3 (μg/mL)	2.01 (0.36)	1.4 (0.8)	0.001**
IGF-1/IGFBP-3 molar ratio	0.23 (0.12)	0.36 (0.24)	0.039*[a,c]
Insulin (μIU/mL)	9.5 (6)	6.2 (3.5)	0.027*[b]
HOMA2-%B	92.9 (50.5)	83.9 (35)	0.637
Log (HOMA2-%S)	4.35 (0.63)	4.8 (0.55)	0.003[b]
HOMA2-IR index	1.3 (0.8)	0.9 (0.5)	0.022[b]

[a]Corrected for age.
[b]Corrected for body mass index.
[c]Corrected for gender.
*$P < 0.05$; **$P < 0.01$.

insulin ($R = 0.404$; $P = 0.012$) and CAG expanded repeat ($R = -0.738$; $P = 0.0001$) influenced the variable independently. Insulin levels did not correlate with MRI volumetries.

HOMA analysis was performed to examine the steady-state β-cell function (HOMA2-%B), peripheral insulin sensitivity (HOMA2-%S), and resistance (HOMA2-IR index). HOMA2-%B (β-cell function) was similar between groups ($P = 0.637$), whereas the log (HOMA2-%S) (insulin sensitivity) was higher ($P = 0.003$; corrected for BMI), and HOMA2-IR index (insulin resistance) was lower ($P = 0.022$; corrected for BMI) in patients with SCA3 than in control participants. Log (HOMA2-%S) and HOMA2-IR index correlated with age at onset ($R = -0.444$; $P = 0.003$ and $R = 0.492$; $P = 0.001$, respectively; Fig. 2), which was the only factor independently associated with these variables in the linear regression model ($R = -0.408$; $P = 0.014$ and $R = 0.378$; $P = 0.021$, respectively). When age at onset was considered as the dependent

factor, HOMA2-%S ($R = -0.408$; $P = 0.014$) or HOMA2-IR index ($R = 0.378$; $P = 0.021$) and CAG expanded repeat ($R = -0.703$; $P = 0.0001$) influenced the variable independently. None of the HOMA parameters correlated with MRI volumetries.

Discussion

The present results indicated significant changes in various constituents of the IIS in SCA3 patients, pointing toward IGFBP-1 as a disease biomarker and a possible disease modifier effect related to insulin sensitivity.

We found no differences in serum IGF-1 levels in SCA3 patients; however, we observed that IGF-1 serum levels were inversely correlated with the volume of medulla oblongata and basis pontis, two brain structures that are primarily affected in SCA3.

IGFBP-3 levels—which binds more than 80% of peripheral IGF-1 and increases its half life[19]—were lower, while an indirect measure of free IGF-1 levels, the IGF-1:IGFBP-3 molar ratio, was higher in SCA3 patients than in controls. Higher levels of free IGF-1 may have opposite consequences: the peptide might either be early metabolized, being in insufficient levels for an adequate transport to the CNS, or might be coupled to the higher expressed IGFBP-1 increasing its transport to target brain areas, as this binding protein is known to participate in tissue allocation of circulating IGF-1.[20]

IGFBP-1 levels were higher in SCA3 patients than in controls and were independently related only to CAG expanded repeats, with more severe mutations leading to higher levels of IGFBP-1. These results point toward IGFBP-1 as a possible biomarker of the disease, therefore, the actual link between this biomarker and neuropathology should be put in perspective. Mutated ataxin-3 could interfere with IGFBP-1 synthesis or metabolism due to its ubiquitin-related properties and to its disruptive effects on gene transcription.[2] As ataxin-3 is also
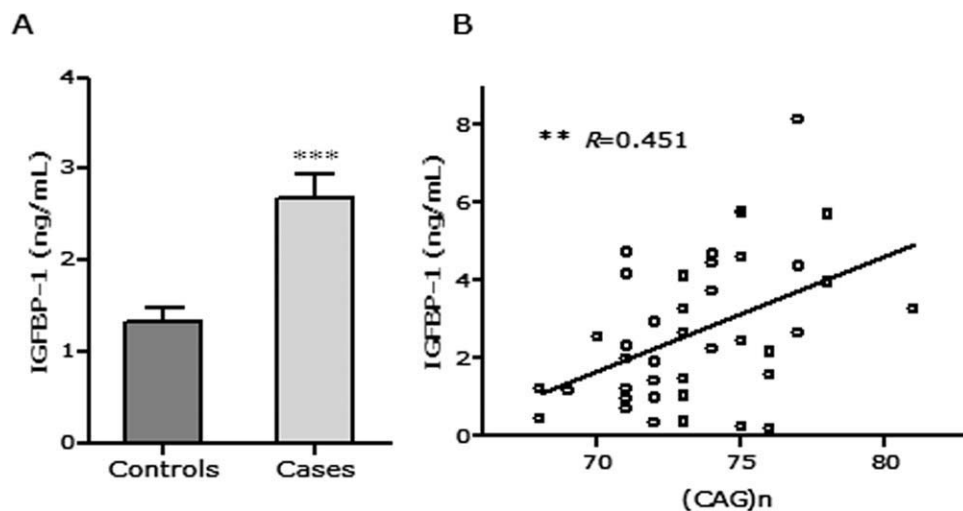

FIG. 1. IGFBP-1 as a biomarker of SCA3. **A**: Analysis of IGFBP-1 serum levels in SCA3 patients and controls corrected for BMI. **B**: CAG expanded repeat (CAG)n simple correlation with IGFBP-1 serum levels. Values are given as means and error bars represent standard error (SEM); **$P < 0.01$; ***$P < 0.001$.
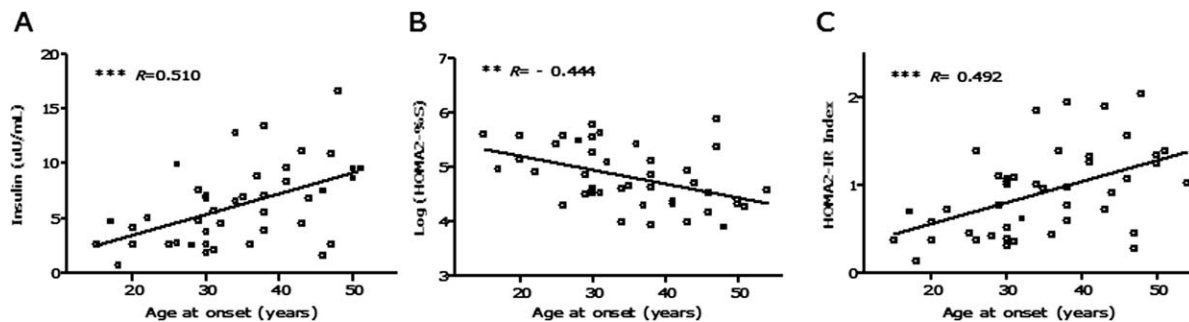
FIG. 2. Correlation between insulin homeostasis parameters with SCA3 age at onset. Age at onset simple correlation with insulin serum levels (**A**), sensitivity (**B**)—log (HOMA2-%S)—and resistance (HOMA2-IR) index (**C**).**$P < 0.01$; ***$P < 0.001$.

associated with endoplasmic reticulum (ER) stress, and ER stress is in turn related to increased IGFBP-1 production in liver,[21] thus, higher IGFBP-1 levels may constitute a marker of ER stress due the mutated ataxin-3.

We found lower serum levels of insulin in SCA-3 patients when compared with controls, even when corrected for BMI. These findings are in agreement with those previously described in patients with LOCA.[13] According to our data, insulin levels were independently and directly associated with age at onset but, interestingly, were not correlated with CAG expanded repeats. Because we found different levels of insulin, but no changes in glucose levels, we studied the peripheral insulin sensitivity. SCA3 patients had higher insulin sensitivity and lower resistance index than the control group, with a normal steady state β-cell function. This higher sensitivity to insulin was, again, inversely associated only with age at onset. According to these data, SCA3 patients seemed to show an increased peripheral sensitivity to insulin and, in consequence, a reduction in serum levels of insulin. Higher sensitivity to insulin and lower insulin levels were both related to earlier disease onset.

In polyQ disorders, the strongest determinant of age at onset is the number of CAG expanded repeats of the mutated protein.[22] As our cross-sectional study was performed years after the clinical onset of SCA3, we were not able to determine whether insulin sensitivity was indeed influencing age at onset or whether the opposite occurred.

Conclusions

As SCAs are rare and slow progressive disorders, the identification of biomarkers with clinical relevance could help to shorten trials and reduce the number of patients needed. In this study, we found a potential role of IGFBP-1 as a serum biomarker and a possible disease-modifying mechanism related to insulin sensitivity in SCA3, which deserves future investigations. ■

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Clinical Correlates of Quality of Life in Tourette Syndrome

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ABSTRACT

Tourette syndrome (TS) is a neurodevelopmental disorder involving tics, which is frequently accompanied by comorbid obsessive compulsive (OCD) or attention deficit hyperactivity disorder (ADHD). Individuals with TS often report poor quality of life (QoL) in comparison with the general population. This study investigated the clinical correlates of QoL in young people with TS using a self-report multidimensional QoL measure, and a range of clinical scales used to assess tic severity and the symptoms of anxiety, depression, OCD, ADHD and other emotional and behavioral symptoms. Symptoms of depression, OCD, and ADHD appeared to have a widespread negative impact on QoL, but poorer QoL was not associated with increased tic severity. Greater emotional and behavioral difficulties, including symptoms of OCD, were among the best predictors of poor QoL in young people with TS. © 2010 Movement Disorder Society

Key Words: Tourette syndrome; tics; quality of life; obsessive compulsive disorder; attention deficit hyperactivity disorder

Tourette syndrome (TS) involves multiple motor and one or more vocal (phonic) tics. Comorbid attention deficit hyperactivity disorder (ADHD) and obsessive–compulsive disorder (OCD) are common. Studies have shown that the TS can be associated with poor quality of life (QoL). Specifically, there is an evidence that aspects of social life and relationships may be particularly vulnerable in TS.[1,2] In this study we aimed to determine which clinical variables most influence QoL of young people with TS.

Previous studies suggest that influences on QoL in TS include tic severity,[1,3–5] anxiety, and depression.[1,4,6] Symptoms of OCD and ADHD may also be important.[3] In fact, one study reported a relationship between these symptoms and QoL but not between tic severity and QoL.[7] Knowledge of the important clinical determinants of QoL in young people with TS can encourage interventions effective in maintaining their psychological well-being.

We aimed to expand research in this area by focusing on a clinic-based population, using a measure that provided a multidimensional assessment of QoL from the individual's own perspective, The Youth Quality of Life Instrument-Research version (YQOL-R).[8,9] A self-report measure of QoL was selected, as studies in TS have shown that proxy-rated measures may not correlate with a young person's own assessment of their QoL.[5] In addition, this measure contained items to assess social aspects of QoL, which may be particularly relevant to TS.[1–3] Although a disease-specific instrument is available for adults,[10] the current study used a generic QoL measure designed for use with young people so that comparisons could be made with a healthy control group. Inclusion of a range of clinical scales to assess tic severity and symptoms of anxiety, depression, OCD, and ADHD allowed the investigation of whether particular clinical symptoms appeared to exert diverse or more specific impacts on aspects of QoL related to the self, environment, and relationships.

Patients and Methods

Participants

Fifty young people with TS according to DSM-IV-TR criteria were enrolled (44 males; mean age 13.26

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years; SD 2.32). Thirty were taking medication: 15 pimozide (6 plus fluoxetine, 1 plus clonidine, 1 plus fluoxetine and clonidine); 7 aripiprazole (1 plus fluoxetine); 6 risperidone (1 plus fluoxetine) and 2 sulpiride.

Procedure

The study received approval from a local ethics committee. Participants were consecutively recruited from patients attending the Neuropediatric Unit, University of Catania. Assessments were performed by fully qualified child and adolescent psychiatrists with extensive experience in TS, and data were collected at the same point in time. The QoL scale was completed along with six clinical measures. These were the YQOL–R,[8,9] the Yale Global Tic Severity Rating Scale (YGTSS),[11] the Multidimensional Anxiety Scale for Children (MASC),[12] the Child Depression Inventory (CDI),[13] Conner's ADHD/DSMV-IV Scale (CADS),[14] the Yale-Brown Obsessive Compulsive Scale (Y-BOCS),[15] and the Child Behavior Checklist (CBCL).[16]

Measures

YQOL-R

The YQOL–R contains "perceptual" items, which involve things that are only known by the adolescent him/herself (e.g., I feel that life is worthwhile), and "contextual" items, which are potentially verifiable (e.g., during the past month, how often did you spend time with a friend having a good time outside of school?).

The 41 "perceptual items" generate scores for four domains. Each item is rated from 0 to 10. The self domain (14 items: score range 0–140) provides a perspective on the adolescent's sense of the person that they are (e.g., "I feel good about myself"). The relationships domain (14 items: score range 0–140) assesses family and peer relationships (e.g., "I am happy with the friends I have"). The environment domain contains 10 items (score range 0–100), including "I feel my life is full of interesting things to do" and the general QoL domain (score range 0–30) contains three broader items (e.g., "I enjoy life"). The scores for these domains are combined to give a total score of 410.

The 15 "contextual items" are used as individual indicators. These allow the assessment of more objective factors that may affect perceived QoL. For all items other than the reverse scored items D4, D6, D7, D8, D9, and D10, low scores indicate a low QoL.

YGTSS

The YGTSS is a reliable clinician rated interview, whereby the clinician notes the presence of tics based on child and parent report and behavioral observation, and then rates the severity of these in terms of number, frequency, intensity, complexity, and interference.

MASC

The MASC is a validated scale, which assesses anxiety disorders in children and adolescents. It contains three subscales (physical, harm, and social), which are combined to generate a total score.

CDI

The CDI is a self-rated instrument, which allows the diagnosis of major depressive or dysthymic disorder in children and adolescents. It has been extensively validated and is designed for children and adolescents aged 7 to 17 years.

CADS

The CADS is a validated self- and proxy-rated (parent and teacher) scale used with 12 to 18 year olds. It is used to diagnose ADHD and can allow discrimination between the subtypes predominantly inattentive, hyperactive–impulsive, and combined attention-deficit/hyperactivity disorder.

Children's Y-BOCS

The Y-BOCS is a reliable instrument used to assess the severity of obsessive-compulsive symptoms in children. The clinician initially notes the presence of obsessions and compulsions based on observation and child and parent report and then rates the severity of these symptoms in terms of number, frequency, intensity, resistance, and interference.

CBCL

The CBCL is a validated parent-rated scale assessing the frequency and intensity of behavioral and emotional difficulties shown by a child over the preceding 6 months. It consists of eight syndrome scales (withdrawn, somatic complaints, anxious/depressed, social problems, thought problems, attention problems, delinquent behavior, and aggressive behavior) and two composite scales (externalizing and internalizing problems).

Data Analysis

Correlations were calculated between scores on the QoL measure and clinical scales. A stepwise regression was then conducted to identify the best predictors of QoL score.

Results

Correlations

There were significant negative correlations between QoL total scores and scores for the MASC, CDI, CADS, Y-BOCS, CBCL, and CBCL internalizing subscale (Table1). Self-domain scores were significantly negatively related to scores on the MASC, CDI, CADS,

Table 1. Correlations between scores on clinical scales and QoL total and domain scores

Measure	Total QoL score		Self domain score		Relationships domain score		Environment domain score		General domain score	
	Sr	P	Sr	P	Sr	P	Sr	P	Sr	P
CDI	−0.511	0.000***	−0.442	0.000**	−0.557	0.000***	−0.421	0.002**	−0.413	0.003**
CADS	−0.421	0.003**	−0.374	0.009**	−0.366	0.011*	−0.392	0.006**	−0.422	0.003**
MASC-total	−0.290	0.041*	−0.342	0.015*	−0.276	0.052	−0.211	0.141	−0.256	0.072
MASC-physical	−0.143	0.323	−0.233	0.104	−0.165	0.252	−0.045	0.756	−0.136	0.347
MASC-harm	−0.246	0.085	−0.327	0.020*	−0.216	0.132	−0.168	0.244	−0.130	0.370
MASC-social	−0.245	0.087	−0.223	0.120	−0.259	0.070	−0.274	0.054	−0.302	0.033*
CBCL-total	−0.390	0.006**	−0.331	0.020*	−0.324	0.023*	−0.421	0.003**	−0.471	0.001**
CBCL-Internal	−0.348	0.014*	−0.281	0.051	−0.363	0.010*	−0.339	0.017*	−0.403	0.004**
CBCL-External	−0.275	0.055	−0.216	0.136	−0.216	0.135	−0.322	0.024*	−0.392	0.005**
Y-BOCS	−0.401	0.006**	−0.346	0.020*	−0.363	0.014*	−0.320	0.032*	−0.496	0.001*
YGTSS	−0.088	0.547	0.090	0.540	−0.187	0.197	−0.149	0.306	−0.143	0.326

*P < 0.05.
**P < 0.01.
***P < 0.001.

Sr, Spearman's rho correlation coefficient; CDI, Child Depression Inventory Score; MASC, Multi-Dimensional Anxiety Scale for Children (Total, total score; Physical, physical subscale score; Harm, harm subscale score; Social, social anxiety subscale score); CBCL, Child Behavior Checklist (Total, total score; Internal: internalizing subscale score; External, externalizing subscale score; Y-BOCS; Yale-Brown Obsessive Compulsive Scale Score; YGTSS, Yale Global Tic Severity Scale score).

Y-BOCS, CBCL, and MASC harm subscale. Environment domain scores were related to Y-BOCS and CBCL total internalizing and externalizing scores. Relationships domain scores were significantly inversely associated with scores on the CDI, CADS, Y-BOCS, CBCL, and CBCL internalizing subscale. Finally, general QoL scores were significantly related to scores on the CDI, CADS, Y-BOCS, and CBCL total and internalizing and externalizing subscales.

For contextual items, YGTSS scores were positively related to item D6 (Sr = 0.423, P = 0.002): "How often have you had serious emotional or mental health problems that you felt you needed help with?" This item was also positively related to Y-BOCS scores (Sr = 0.539, P < 0.001). Y-BOCS scores were also significantly positively related to item D1 (Sr = 0.306, P = 0.041): "How often did you have a conversation with an adult about something that is important to you?"

Multiple Stepwise Regression

Total scores for all clinical measures were entered as possible predictors into five models with total QoL scores and scores for the four QoL domains as dependent variables. This procedure was repeated using appropriate subscale scores instead of total scores for the MASC and CBCL. CBCL total scores were the best predictors for total QoL score and the environment domain, Y-BOCS scores predicted scores for the general domain, MASC harm subscale scores predicted best scores for the self domain, and CDI and Y-BOCS scores comprised the best model for the relationships domain (Table 2).

Discussion

The QoL of young people with TS appears to be significantly adversely affected by a range of clinical symptoms. Features assessed by the Y-BOCS and CBCL seemed to be most predictive of QoL score, whereas tic severity, as measured by the YGTSS, was not found to be related to QoL. Perhaps tic severity was less of a predictor of QoL in the more complex cases of TS assessed in the current sample, as other behavioral difficulties were perceived to exert a relatively greater impact on QoL. Another study reported no relationship between tic severity and QoL,[7] although this is in contrast with many other reports.[1,3–5] These discrepancies could reflect differing consideration of the role of comorbidities[4,5] or differences in the age of participants or in the measures used to assess tic severity and QoL. However, one study,[3] which reported a correlation between tic severity and QoL, used self-report measures to assess both factors, and although a self-report QoL measure was used in the current study, tic severity was assessed with a clinician rated measure. It may be that an individual's own interpretation of their tic severity could be more closely linked to their perceived QoL than clinician ratings.

Total QoL scores were lower in association with greater symptoms of anxiety and depression. Anxiety had a less widespread influence on QoL domains, but scores for the MASC harm subscale were specifically predictive of scores for the self domain. This could suggest that negative feelings about the self in TS are most closely related to perfectionism and aspects of anxious coping behavior. Depressive symptoms were linked to scores for all four QoL domains, and along with symptoms of OCD, predicted relationships domain scores. Difficulties in social interaction and relating to others

Table 2. Results of the multiple stepwise regression analysis

Dependent variable	Best predictor/s	Percentage of variance explained	Statistics
Total QoL	CBCL total ($B = -0.35$, $t = -2.56$)	11 ($R^2 = 0.13$, adj $= 0.11$)	$F(1,46) = 6.55$, $P = 0.014$
Self domain	MASC harm ($B = -0.33$, $t = -2.37$)	9 ($R^2 = 0.11$, adj $= 0.09$)	$F(1,46) = 5.63$, $P = 0.022$
Relationships domain	CDI ($B = -0.41$, $t = -3.04$)	31 ($R^2 = 0.34$, adj $= 0.31$)	$F(2,45) = 11.41$, $P < 0.001$
	Y-BOCS ($B = -0.28$, $t = -2.11$)		
Environmental domain	CBCL total ($B = -0.37$, $t = -2.70$)	12 ($R^2 = 0.14$, adj $= 0.12$)	$F(1,46) = 7.24$, $P = 0.010$
General domain	Y-BOCS ($B = -0.40$ $t = -3.00$)	15 ($R^2 = 0.16$, adj $= 0.15$)	$F(1,46) = 8.99$, $P = 0.004$

CBCL, Child Behavior Checklist; MASC, Multi-Dimensional Anxiety Scale for Children; CDI, Child Depression Inventory; Y-BOCS: Yale-Brown Obsessive Compulsive Scale.

in TS, therefore, may be linked to a combination of negative evaluation and obsessive rumination.

Symptoms of OCD were also related to scores across all QoL domains and best predicted QoL in the general domain. The positive correlation with contextual item D1 could imply that more severe symptoms of OCD may lead a young person with TS to seek social support through communication with adults. Other correlations between a contextual item and both Y-BOCS and YGTSS scores indicated that clinician ratings of symptom severity appear closely related to patients' own evaluation of their experience of TS and OCD.

The degree of emotional and behavioral problems (particularly internalizing difficulties) assessed by the CBCL was widely negatively related to QoL. This scale was predictive of environmental domain and total QoL scores. Those young people with TS whose parents rate them as exhibiting more evidence of social withdrawal, somatic complaints, and signs of anxiety and depression, are most likely to report low QoL overall. However, the range of symptoms covered by the CBCL may mean that this measure overlaps with other instruments, such as the Y-BOCS.

The limitations of this study include small sample size and the lack of a disease-specific instrument to measure QoL, which has not been developed to date. In addition, the variables included could only account for a limited amount of variance in QoL scores, the greatest being 31% for the relationships domain. Future research should seek to further explain, how complex interactions between personality, environmental, interpersonal, and cultural factors influence aspects of QoL critical to young people living with TS. ∎

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Epoetin Alfa Increases Frataxin Production in Friedreich's Ataxia Without Affecting Hematocrit

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ABSTRACT

Objective of the study was to test the efficacy, safety, and tolerability of two single doses of Epoetin alfa in patients with Friedreich's ataxia. Ten patients were treated subcutaneously with 600 IU/kg for the first dose, and 3 months later with 1200 IU/kg. Epoetin alfa had no acute effect on frataxin, whereas a delayed and sustained increase in frataxin was evident at 3 months after the first dose (+35%; $P < 0.05$), and up to 6 months after the second dose (+54%; $P < 0.001$). The treatment was well tolerated and did not affect hematocrit, cardiac function, and neurological scale. Single high dose of Epoetin alfa can produce a considerably larger and sustained effect when compared with low doses and repeated administration schemes previously adopted. In addition, no hemoglobin increase was observed, and none of our patients required phlebotomy, indicating lack of erythropoietic effect of single high dose of erythropoietin. © 2010 *Movement* Disorder Society

Key Words: Friedreich's ataxia; Epoetin alfa; erythropoietin; frataxin; iron

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Friedreich's ataxia (FA) is an autosomal recessive ataxia[1] caused by a trinucleotide GAA expansion in the first intron of the *FXN* gene.[2] The gene encodes for a 210aa mitochondrial protein called frataxin, whose mRNA and protein levels are severely reduced in FA.[3] It has been suggested that frataxin is involved in iron–sulphur cluster and heme biogenesis, iron binding/storage, and chaperone activity.[4–6]

Erythropoietin (EPO) is a glycoprotein that acts as a main regulator for erythropoiesis. Evidence suggests that both EPO and its receptor are expressed in the nervous tissue,[7,8] and neuroprotective effects have been shown in animal models of cerebral ischemic damage.[9,10]

EPO increases frataxin levels in cultured human lymphocytes from FA patients.[11] However, frataxin protein increase is not preceded by mRNA increase, suggesting that a post-transcriptional mechanism is involved.[12] Two phase II clinical trials have tested EPO in FA patients. In the first, 12 patients were treated with 5,000 IU of Epoetin beta thrice a week (TIW) for 8 weeks. This resulted in a 27% frataxin increase in peripheral blood mononuclear cells (PBMCs), reduction in oxidative stress markers, and a small clinical improvement.[13] The same group of investigators performed a second trial in which Epoetin beta was given to 8 patients at a dose of 2,000 IU TIW for 6 months. The results largely confirmed those of the first trial. However, 4 patients (50%) required repeated phlebotomy.[14]

Patients and Methods

Study Design

We designed an open-label, phase IIa clinical trial to test the efficacy of two subcutaneous single high dose of Epoetin alfa (Eprex, Janssen-Cilag, Milano, Italy). The lower dose was 600 IU/kg body weight (BW) (max 40,000 IU) and the higher dose was 1200 IU/kg (max 80,000 IU). Doses were chosen based on the previous pharmacokinetics studies[15] and on the intent to reproduce a serum EPO concentration similar to previous in vitro experiments.[11] A 3-month washout was programmed between the doses. Primary endpoint of the study was the change from baseline in frataxin levels in PBMCs at 24, 48, 96 hours, 7, 15, 30, and 60 days. Secondary endpoints were safetyand tolerability measured with clinical scale, echocardiography, and laboratory parameters. The local Ethics Committee approved the clinical trial, and it was registered at www.clinicaltrials.gov, NCT00631202.

Patients

Ten patients were enrolled in the study after giving informed consent (Table 1); 1 patient withdrew his consent 30 days after the first administration and was

Table 1. Patient characteristics

Patient	Age	Sex	Age at onset	Disease duration	GAA1	GAA2	ICARS baseline	ICARS frataxin peak*
1	19	M	8	11	709	830	56	61
2	29	F	23	6	800	1000	36	39
3	30	F	18	12	600	1267	58	56
4	25	M	14	11	958	958	51	48
5	43	M	23	20	200	421	25	25
6	22	F	11	11	489	1022	39	36
7	22	M	15	7	734	934	49	52
8	19	M	9	10	752	1041	66	–
9	31	F	15	16	355	561	68	78
10	40	F	28	12	580	780	55	45
Mean	29 ± 8.2		17.2 ± 6.4	11.8 ± 4.2	602.8 ± 232	863.7 ± 253.9	48.6 ± 13.1	48.9 ± 15.4

Mean ± SD for the 9 patients who completed the study.
*ICARS performed 6 months after the second Epoetin alfa administration (month 9 in Fig. 1).
GAA1, triplet repeat number in the minor allele; GAA2, triplet repeat number in the major allele; ICARS, International Cooperative Ataxia Rating Scale.[15]

not considered for endpoint evaluation. Inclusion criteria were clinical and molecular diagnosis of FA (18–50 years of age). Exclusion criteria were idebenone treatment, wheelchair use, renal, hepatic or hematological disease, positive thrombosis history, hypertension, acute disease, pregnancy, and breastfeeding.

Quantitative Analysis of Frataxin and EPO

PBMCs were extracted from 15 mL of ethylenediaminetetraacetic acid (EDTA)-anticoagulated whole blood using Leucosep tubes (Greiner Bio-one, Germany). PBMCs were lysed, and total protein was measured using the bicinchoninic acid (BCA) assay. About 7.125 µg of each protein extract was analyzed in duplicate with lateral-flow immunoassay[16] and calibrated using frataxin protein standard (kit and Hamamatsu ICA-1000 scanner; Mitosciences, Eugene, OR). Preliminary test showed an intra-assay and interassay coefficient of variability both <5%. Data from 31 carriers and 19 control individuals were analyzed in parallel. Serum concentrations of EPO were measured using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN).

Secondary Outcomes

Laboratory parameters were monitored at screening, baseline, and 7, 15, 30, and 60 days after each administration and comprised hematology, urine examination, coagulation, and a serum routine biochemistry with iron, ferritin and transferrin determination. Adverse events and blood pressure were monitored at each visit. Electrocardiograms were performed at screening and 30 days after each administration. The International Cooperative Ataxia Rating Scale (ICARS)[17] was measured at baseline, 7 and 30 days after each administration, and 6 months after the last dose.

Conventional M-mode, two-dimensional imaging, and quantitative regional strain and strain rate were used to evaluate cardiac morphology and function. Echocardiography was performed at baseline and 6 months after the second EPO administration.

Statistical Analysis

Statistical analysis for continuous variables was conducted by two-way ANOVA for repeated measures. P values of less than 0.05 were considered statistically significant. Posthoc analysis was performed with the Dunnett's test to compare basal levels with different time points (*Graphpad Prism 5.0c*).

Results

Frataxin

We did not observe significant variations of frataxin in the 24 hours to 60 days interval, either after the first or the second EPO administration. Peaks were observed 96 hours after the first dose (mean increase 8.9%; $P = 0.47$) and 48 hours after the second (16.3%; $P = 0.34$). Surprisingly, when analyzing frataxin levels throughout the study, we found a slow and sustained increase (Fig. 1). Basal frataxin was 12.7 ± 3 pg/µg total protein and rose to 17.1 ± 5.8 pg/µg (35% relative increase from baseline; $P < 0.05$) 3 months after the first Epoetin alfa administration. After the second injection, frataxin increased to 18.3 ± 7 pg/µg (44% relative increase compared with baseline; $P < 0.01$) 2 months later. Given this unexpected delayed effect of Epoetin alfa, we decided to extend the observation period. At 6 months from the second administration, we found frataxin to be 19.5 ± 5.4 pg/µg (54% relative increase from baseline; range 14–144; $P < 0.001$). At 12 months, frataxin values were no longer significantly higher than baseline (16.1 ± 9 pg/µg; $P > 0.05$).
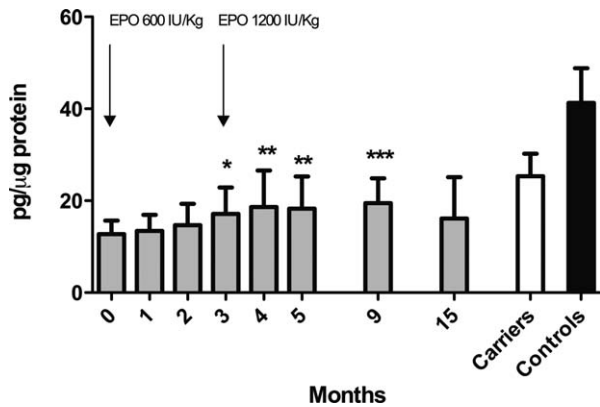
FIG. 1. Frataxin levels after two single doses of Epoetin alfa. Frataxin levels are shown as pg/μg of total protein extract from the patients who completed the study (n = 9, gray bars), carriers (white bar; n = 31; 25.3 ± 4.9 pg/μg total protein), and healthy controls (black bar; n = 19; 41.3 ± 7.5 pg/μg total protein). All values are mean ± standard deviation (statistical significance compared with baseline = *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Serum EPO

Twenty-four hours after the first administration, serum EPO increased from 11.2 ± 8.6 mIU/mL at baseline to 1153 ± 354 mIU/mL (Supporting Information Figure). EPO then decreased to basal levels 7 days after injection. The second injection produced similar results (19.7 ± 19.6 and 3343 ± 657 mIU/mL).

Iron

Total iron decreased from 75.0 ± 39.1 μg/dl at baseline to 44.4 ± 24.4 μg/dl ($P < 0.05$) at 7 days and returned to 81.1 ± 31.5 μg/dl at 30 days. The second injection produced similar results (83.6 ± 46.5, 31.0 ± 14.4, 76.8 ± 34.3 μg/dl; $P < 0.01$). Ferritin decreased from 104.9 ± 98.7 ng/mL at baseline to 18.5 ± 12.4 ng/mL ($P < 0.001$) at 7 days after the first dose and returned to 73.4 ± 79.2 ng/mL at 30 days. The higher dose caused similar results (70.3 ± 90.2, 30.5 ± 41.4, 62.1 ± 77.6 ng/mL, $P < 0.01$). Transferrin remained stable for the same time points (first dose 250.1 ± 58.68, 273.4 ± 48.5, 244.6 ± 58.8 mg/dl; second dose 285.1 ± 53.0, 273.8 ± 64.9, 288.1 ± 52.3 mg/dl, $P = $ NS). Mean transferrin saturation decreased by 44.6% ($P < 0.01$) 7 days after the first and 63.2% ($P < 0.001$) 7 days after the second dose, when compared with baseline.

Secondary Outcomes

A total of 10 grade 1 adverse events were recorded after the lower dose and two after the higher dose (myalgia, flu-like syndrome, hypotension, nausea, itching and reaction at injection site, headache, nocturnal sweating, and extremities warm feeling). Blood pressure and electrocardiograms showed no change during the study. Hematocrit, erythrocytes, hemoglobin, and all other laboratory safety parameters were not influenced by treatment. Echocardiographic measures showed no change from baseline. As a prototype of myocardial con-

tractility, the left ventricle strain was −19.9 ± 5.1% at basal and −18.5 ± 6.7% at 6 months ($P = $ NS). ICARS was unchanged during the study (Table 1; baseline 48.6 ± 13.1 vs. 48.9 ± 15.4 at frataxin peak, $P = $ NS).

Discussion

In vivo, our study did not replicate the previous in vitro findings of an acute increase in frataxin after EPO stimulation. Although the lack of statistical significance of the early increase we observed may be because of the small sample size, any clinical significance of such a small change is likely to be limited. In contrast, an unexpected delayed effect of Epoetin alfa on frataxin levels in PBMCs was observed 3 months after 600 IU/kg of Epoetin alfa (35% increase). The second injection caused an additional increase in frataxin up to 54% above baseline. A carry-over effect cannot be excluded and could be responsible of the observed effect. Interestingly, the increase was evident after serum EPO returned to basal levels, suggesting that a direct stimulation of the EPO receptor is not involved in the delayed effect. Previous clinical trials[13,14] were designed with continuous low dose administration, and the interval between EPO administration and frataxin increase could not be assessed. We demonstrate that single high dose of EPO can produce a considerably larger and sustained effect when compared with low TIW doses. In addition, no hemoglobin increase was observed, and none of our patients required phlebotomy, indicating lack of erythropoietic effect of single high dose of EPO. In contrast, our study failed to demonstrate a clinical improvement that was reported in the past trials. This could be explained by a lower sensitivity of ICARS, compared with Friedreich Ataxia Rating Scale (FARS) or the scale for the assessment and rating of ataxia (SARA) scale, or by the presence of a learning effect of repeated measuring in the previous trials.

Exact effect of EPO on frataxin is unknown. EPO administration is known to reduce circulating hepcidin,[18] and as a consequence ferroportin inhibition is released and iron stores are reduced.[19] In this study, EPO reduced transferrin saturation, indicating iron redistribution from peripheral tissues to the bone marrow. The absence of a clear hematopoietic effect remains unclear. Perhaps, very high single EPO doses may have a very strong and rapid relocating effect on iron, but repeated dosing may be necessary to obtain a hematopoietic effect. This iron relocating effect may be a clue to explain the delay in frataxin increase. The sustained effect of EPO remains obscure, and long-lasting post-transcriptional effects cannot be excluded.

In conclusion, it is possible to achieve a considerable increase in frataxin using a very simple administration scheme of Epoetin alfa with no hematological side effects. In the absence of a control group, the present data should still be regarded as preliminary until a

randomized, placebo-controlled trial, has been performed. ■

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Somatosensory Temporal Discrimination Tested in Patients Receiving Botulinum Toxin Injection for Cervical Dystonia

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ABSTRACT

We designed this study to find out more about the relationship between the sensory effects of Botulinum toxin type A (BTX) and the clinical benefits of BTX therapy in patients with cervical dystonia (CD). In 24 patients with CD, we tested sensory temporal discrimination (STD) in the affected and two unaffected body regions (neck, hand, and eye) before and 1 month after BTX injection. In 8 out of the 24 patients with CD, STDT values were tested bilaterally in the three body regions before, 1 and 2 months after BTX injection. As expected, STD testing disclosed altered STD threshold values in all three body regions tested (affected and unaffected by dystonic spasms) in patients with CD. STD threshold values remained unchanged at all time points of the follow-up in all CD patients. The lack of BTX-induced effects on STD thresholds suggests that STD recruits neural structures uninvolved in muscle spindle afferent activation. © 2010 *Movement* Disorder Society

Key Words: botulinum toxin; cervical dystonia; somatosensory temporal discrimination

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Introduction

Botulinum toxin (BTX) type A is widely used to treat neurological conditions characterized by increased muscle activity.[1–3] BTX acts peripherally by inhibiting acetylcholine release from the presynaptic neuromuscular terminals.[4,5] Animal experiments and human studies in patients with dystonia show that BTX acts also at intrafusal fibre level thereby reducing muscle spindle afferent discharges.[6,7]

Investigating possible BTX-induced effects on sensory functions, Walsh and Hutchinson[8] found that BTX therapy partly reversed the abnormal spatial discrimination in patients with dystonia. Dystonia also leads to abnormal somatosensory temporal discrimination (STD).[9–12] Whether BTX restores the altered STD threshold (STDT) in patients with dystonia is still unknown.

We designed this study to find out more about the relationship between the sensory effects of BTX and the clinical benefits of BTX injected into the neck muscles for CD. To do so, in patients with CD, we tested STD in the affected and two unaffected body regions (neck, hand, and eye) before and about 1 month after BTX injection. In a further group of patients, we also tested STDT values bilaterally in the three body regions at baseline, 1 and 2 months after BTX. We also sought a possible correlation between changes in STDT values after BTX treatment and changes in clinical severity scores for dystonia.

Subjects and Methods

Subjects

We recruited 24 outpatients with primary CD (17 women; mean age: 59.5 ± 2.5 years, range 35–83 years, mean education level: 11.0 ± 1.2 years). Three patients were naive to BTX treatment (Table 1). In a first group of 16 patients, STDT values were tested on one side in three body regions before and 1 month after BTX injection in the neck muscles. As a control group, we recruited 16 age-matched healthy control subjects (11 women, mean age 59.06 ± 3.4 years, range 35–81; education level: 10.2 ± 1.3 years). In the second group of 8 patients, STDT values were tested bilaterally in three body regions before, 1 and 2 months after BTX. The severity of motor impairment was assessed with the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS). Clinical assessment was performed at baseline and 1 month after treatment in the group of patients tested unilaterally (n= 16); and at baseline, 1 and 2 months after BTX in the group of patients tested bilaterally (n = 8).[13] All patients were treated with BTX type A injected into the dystonic neck muscles (BOTOX, Allergan; DYSPORT, Ipsen).

Stimuli and STD Procedure

Somatosensory discrimination was tested by delivering paired stimuli starting at an interstimulus interval (ISI) of 0 msec, followed by progressively increasing ISIs (in 10 msec steps).[11] The surface skin electrode was applied to the neck, the index finger (hand), and near the orbit (eye). Subjects had to report whether they perceived a single stimulus or two temporally separated stimuli. The first of three consecutive ISIs at which participants recognized the stimuli as temporally separated was considered the STDT. Each session comprised four separate blocks. The STDT for each stimulated body site was defined as the average of four STDT values, one for each block, and was entered in the data analysis.[9,10]

Statistical Analysis

Wilcoxon's test was used to analyze changes in severity scores before and after BTX therapy in the group of 16 patients tested unilaterally. Friedman's repeated measures ANOVA was used to analyze changes in severity scores before, 1 and 2 months after BTX in the 8 patients tested bilaterally. Repeated-measures ANOVA with factor "Time"(before and after treatment) and "Body part" (neck, hand, and eye) was used to compare data obtained from patients at baseline and after BTX treatment. Between-group repeated measures ANOVA with factor "Time" (before, 1 and 2 months after BTX), factor "side" (left and right body side), and "body region" (neck, hand and eye) was used to compare STDT changes in patients naive and non-naive to BTX tested bilaterally. STDT values in patients and healthy subjects were compared in a between-group repeated measures ANOVA with factor "Body part" as the main factor. Spearman's correlation coefficient was used for assessing possible correlations between clinical features and changes in STDT values in patients. Tukey Honest Significance Difference Test was used for post hoc analysis. Values are expressed as means and standard error (SE).

Results

After patients with CD received BTX therapy, their clinical severity scores decreased significantly ($Z = 3.34$, Asymp Sig. = 0.0008). Friedman's repeated measures ANOVA for clinical severity scores in the subgroup of CD patients tested with a longer follow-up post BTX also showed a significant improvement of motor symptoms after BTX ($\chi2 = 14.25$, Asymp Sig. = 0.001).

The intensity of electrical stimuli for STDT testing was statistically unchanged after BTX ("hand" pre-BTX vs. post-BTX: 7 ± 4 vs.7 ± 3mA, $P = 0.27$; "neck" pre-BTX vs. post-BTX: 4 ± 0.9 vs. 4 ± 1mA, $P = 0.14$; "eye" pre-BTX vs. post-BTX: 3 ± 0.8 vs. 3 ± 0.7 mA; $P = 0.17$).

As expected, STDT values in the affected and two unaffected body regions tested were higher in patients than in healthy subjects. Between-group repeated measures ANOVA comparing STDT values in the group of CD patients (pre-BTX) and in the healthy subjects showed a

Table 1. Demographic and clinical features of patients with cervical dystonia studied

Nr	Age onset	Duration	Spread	BTX duration	Severity pre-BTX	Severity post-BTX
1	56	3	no	2	11	11
2	43	3	no	3	7	5
3	51	30	OMD 61 yr	11	11	6
4	53	30	no	18	11	7
5	40	32	no	12	9	7
6	60	17	OMD 60 yr	9	12	8
7	21	18	no	15	7	3
8	46	8	no	5	10	8
9	42	20	WC 43 yr	14	24	20
10	47	4	no	2	8	5
11	65	11	no	8	4	2
12	51	6	no	2	10	5
13	34	22	no	14	6	4
14	27	31	no	6	10	10
15	51	4	no	3	8	6
16	23	22	Arm 23 yr	2	10	6
17	34	1	no	0	14	6
18	47	5	no	0	40	39
19	53	4	no	2	20	11
20	41	18	no	4	36	23
21	28	30	no	7	31	15
22	35	35	no	3	31	12
23	42	22	no	4	23	10
24	48	14	no	0	28	17
mean	43.25	16.25		6.08	15.88	10.25
SE	2.33	2.28		1.09	2.14	1.65

significant factor "group" ($F(1,30) = 26.24$; $P = 0.0001$) a significant factor "Body part" ($F(1,2) = 3.76$; $P = 0.02$) but a nonsignificant interaction of factor "group" and "Body part" ($F(2,60) = 0.77$; $P = 0.46$) (Fig. 1).

BTX left the altered STDT in the three body regions tested unchanged. Repeated-measures ANOVA showed no significant effect of the factor "Time: pre-BTX vs. post-BTX" ($F(1,15) = 2.23$; $P = 0.15$), a significant effect of the factor "Body part" ($F(2,30) = 4.08$; $P = 0.02$) but no significant interaction between factors "Time" and "Body part" ($F(2,30) = 0.27$; $P = 0.76$). STDT values in the CD group were higher in the "hand" than in the "eye" and "neck" and remained unchanged after BTX (Fig. 2).

BTX left the altered STDT unchanged also in the group of patients tested bilaterally independently on whether the patients were naive or not to BTX at all time points tested after BTX. Between-group repeated-measures, ANOVA showed no significant effect of the factor "Time" ($F(2,10) = 1.44$; $P = 0.28$), no significant effect of factor "Group" ($F(1,5) = 1.41$; $P = 0.28$), no significant effect of factor "side" ($F(1,5) = 2.43$; $P = 0.17$), a significant effect of the factor "Body part" ($F(2,10) = 7.11$; $P = 0.01$), but no significant interaction between main factors ($P > 0.05$).

No correlation was found between disease duration and severity scores or between changes in severity scores and differences in the STDT values at baseline and after BTX treatment in patients with CD in the three body parts ($P > 0.05$).

Discussion

The novel finding in our study is that clinically effective BTX injected in the dystonic neck muscles in CD patients failed to restore STDT values in both affected and unaffected body regions.
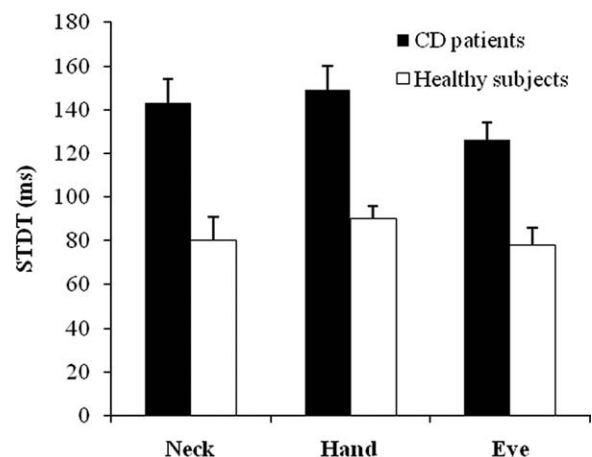

FIG. 1. Somatosensory temporal discrimination thresholds tested in three body parts (neck, hand, and eye) in patients with cervical dystonia (CD) and healthy subjects. Each column represents mean value; the bars represent standard error.
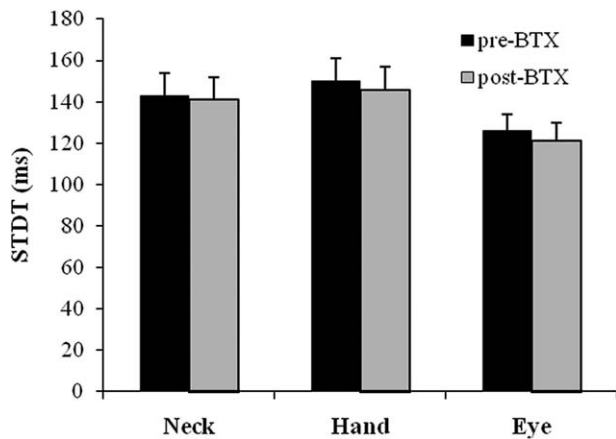
FIG. 2. Somatosensory temporal discrimination thresholds tested in three body parts (neck, hand, and eye) in patients with cervical dystonia (CD) before and 1 month after botulinum toxin (BTX) injected in the neck muscles. Each column represents mean value; the bars represent standard error.

afferent inflow. Because STDTs remained unchanged after BTX, we conjecture that STD recruits neural structures uninvolved in muscle spindle afferent activation and that cortical remodelling possibly present after BTX in CD patients does not influence STDTs. Previous studies in patients with dystonia suggested that BTX can partially restore changes in the somatotopy of the sensory-motor cortex investigated with neurophysiological techniques.[20,21] If we consider this latter hypothesis, our finding that BTX left STDT values unchanged in the three body regions tested (affected and unaffected by the dystonic motor symptoms) suggests that cortical integration of STDT processes does not depend on the size of the cortical map related to the three body regions.

Whether BTX acts on other types of sensory afferents, besides Ia fibre and gamma motor endings, remains an open question.

We first excluded the possibility that BTX injected into dystonic muscles failed to alter STDT values owing to changes in attention levels.[11] We also exclude the possibility that BTX left STDT values unchanged because we injected BTX at excessively low doses because after BTX therapy patients' clinical scores for motor deficits significantly improved. By testing patients naive to BTX therapy, taking however into account the small sample size, we believe unlikely that the lack of changes in STDT values is due to previous BTX injection-related denervation processes. By testing CD patients with a 2 months follow-up, we also exclude the possibility that STDT changes could have a different time course compared to motor symptoms relief after BTX injection. The absence of relationship between the altered STD values and the clinical benefits of BTX injected into the neck muscles provided useful information on sensory dysfunction in CD. STD is a complex physiological process that, in addition to peripheral sensory pathways, involves several cortical and subcortical circuits. Cortical and subcortical structures involved in STD include parietal cortex, prefrontal areas, subthalamus, putamen, and possibly the cerebellum.[14,15]

Unlike Walsh and Hutchinson[8] who reported a partial and transient BTX-induced change in spatial discrimination, in our patients, we failed to show BTX-induced changes in STDTs. By acting at the gamma motor endings, BTX can reduce muscle spindle afferent discharges and thereby spindle inflow to the central nervous system.[7,16] BTX could, therefore, modulate functional activity in the sensory-motor cortical areas.[17–21] This explanation agrees with Walsh and Hutchinson[8] who attributed BTX-induced changes in spatial discrimination to cortical remodeling of the upper-limb sensory areas after a change in intrafusal

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Levodopa is Not a Useful Treatment for Lesch-Nyhan Disease

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ABSTRACT

Lesch-Nyhan disease (LND) is characterized by dystonia, cognitive abnormalities, and self-injurious behavior. No effective therapies are available. LND is associated with a presynaptic dopaminergic deficit, but the reported effects of dopamine replacement therapy are conflicting. The current prospective open-label study assesses the effects of levodopa on both neurological and behavioral features of LND. All 6 study participants discontinued levodopa early, due to lack of effect and sometimes worsening of motor function. The results provide important clues for pathophysiological mechanisms and suggestions for future treatment options. ©2011 Movement Disorder Society

Key Words: Lesch-Nyhan disease; treatment; levodopa; dystonia; dyskinesias; self-injurious behavior

Introduction

Lesch-Nyhan disease (LND) is caused by a mutation in the gene encoding the purine salvage enzyme hypoxanthine-guanine phosphoribosyl transferase (HPRT).[1,2] Near-complete HPRT deficiency causes hyperuricemia and a characteristic neurobehavioral phenotype. Patients exhibit a movement disorder dominated by dystonia, chorea, and hypotonia[3]; cognitive dysfunction characterized by attentional and executive deficits; and behavioral abnormalities including self-injurious behavior.[4,5] Effective therapies for the neurobehavioral features of LND are currently lacking.[2]

Available evidence indicates that LND is associated with a presynaptic dopaminergic deficit. Postmortem human brain tissue shows a 60 to 90% decrease in basal ganglia dopamine levels, whereas other neurotransmitter systems are preserved.[6,7] In vivo PET imaging studies demonstrate a low dopamine transporter (DAT) density[8] and a decreased dopamine uptake in presynaptic terminals.[9] The relation between HPRT deficiency and dopamine dysfunction is further supported by a 50% decrease in basal ganglia dopamine content in HPRT-deficient knockout mice[10–12] and in HPRT-deficient dopaminergic cell lines.[13] Despite the loss of dopamine-related markers, there is no loss of nigral dopamine neurons in autopsy studies.[7]

These findings suggest that treatment with the dopamine precursor levodopa (L-dopa) may be helpful, but the few reported effects so far are conflicting. Motor function improved in 3 patients,[14–16] worsened in 4,[17,18] and remained unchanged in another 2.[14,19] Similar contradictory effects have been reported for the effects of L-dopa on self-injury, including improvement,[17] deterioration,[18] or no change.[17] The interpretation of these reports is hampered by lack of detailed information about treatment duration and clinical assessment. Subtle changes in specific domains of motor, cognitive, or behavioral function may have been missed. To examine more carefully the effects of L-dopa on both neurological and behavioral

Table 1. Demographics and clinical characteristics of enrolled subjects

Case	Age (yr)	Mutation	HPRT activity	Medication	Motor disorder		Self-injurous behavior
					Clinial signs	BFM	
1	27	428–432del TGCAG, insAGCAAA	<1%	Allopurinol, chlorothiazide	Dystonia, chorea, ballism, hypotonia, ophistotonus, hyperreflexia	97.5	Biting fingers, lips
2	12	IVS7+5G>A	<1%	Allopurinol, diazepam, omeprazole, fluticasone, mometason, cetirizine, levalbuterol	Dystonia, rigidity, spasticity, hyperreflexia	69.5	Biting various body parts; head banging
3	10	IVS7+5G>A	<1%	Allopurinol, diazepam,	Dystonia, hypotonia	33.5	Biting fingers, arms
4	3	508C>T	<1%	Allopurinol, gabapentin, benzodiazepine, ranitidine	Dystonia, hypotonia, ophistotonus	57.5	Biting fingers; head banging; hitting himself
5	3	10delC	N/A	Allopurinol, gabapentin, lorazepam	Dystonia, hypotonia	61.0	Biting buccal mucosa; hitting

Abbreviations used: HPRT, hypoxanthine-guanine phosphoribosyl transferase; BFM, Burke-Fahn-Marsden dystonia rating scale; N/A, not available.

features of LND, we conducted a prospective, open label, dose-escalation study.

Patients and Methods

Patients

We intended to include 10 LND patients, ranging from 2 to 40 years of age. Patients had been diagnosed with LND based on DNA mutation analysis or residual HPRT enzyme activity, and all demonstrated a clinical picture typical of LND (Table 1). Exclusion criteria included a known intolerance to L-dopa/carbidopa. Written consent was obtained from patients or their parents before inclusion. The study was approved by the local ethical committee.

Intervention

L-Dopa/carbidopa (4:1) was initiated at a low dose, typically starting at 50/12.5 mg once per day, and titrated to a maximum of 20 mg/kg L-dopa (divided 3 times per day) over 2 to 6 weeks. The study had a prospective open-label design.

Outcome Measures

The primary outcome measure was dystonia severity, as assessed in an unblinded fashion, using the Burke-Fahn-Marsden dystonia rating scale.[20] Clinical assessments were scheduled at baseline, before starting medication, and at least 1 month after reaching the target or maximally tolerated dose. Secondary outcome variables included the severity of self-injurious behaviors, determined via a weekly telephone questionnaire developed for this purpose (Supporting Information Table 1).

Results

None of the subjects completed the planned titration phase, and enrollment was terminated before the target number of 10 participants was reached. The average duration of therapy was ~2 weeks (range 1 day to 4 weeks). The average daily dose of L-dopa/carbidopa reached was 200/50 mg (range 50/12.5 to 600/150 mg) per day.

The reasons for early discontinuation are shown in Table 2. Four participants showed increased motor dysfunction, in the form of rigidity and tongue stiffness, limb flailing, and wild, agitated movements. One participant discontinued the medication after his brother developed side effects, even though he had not. There were no significant effects of L-dopa on self-injury in any participant.

Discussion

Available biochemical, functional imaging, and experimental data indicate that LND is associated with an early, relatively selective presynaptic dopaminergic deficit, and that the subsequent basal ganglia dysfunction is an important contributor to the motor, cognitive, and behavioral abnormalities.[21] The current prospective open-label study investigated the effect of L-dopa therapy on motor and behavioral abnormalities in LND. L-Dopa treatment did not prove to be beneficial in either domain and was associated with a worsening of motor function in several patients, resulting in an early termination of the study. This early L-dopa-induced exacerbation of the movement disorder provides some clues to LND pathogenesis.

As opposed to adults, where it usually results in parkinsonism, decreased dopamine in children usually causes dystonia, for which L-dopa may be beneficial. Examples include inherited deficiency of tyrosine

Table 2. Effect of L-dopa on motor function and self-injury

Case	Treatment duration	Maximum dose L-dopa/carbidopa (mg daily)	Effect on motor disorder	Effect on self-injury
1	4 weeks	600/150	Stiff arms, pronounced and less controllable movements	No obvious change
2	1 day	50/12.5	No apparent change[a]	No obvious change
3	1 day	50/12.5	Hyperactive	No obvious change
4	2 weeks	150/37.5	Twisted and stiff tongue, facial twitching, limb flailing	No obvious change
5	4 weeks	150/37.5	Stiff and agitated movements	Better mood, accompanied by less need for restraints

[a]The mother of subject #2 discontinued L-dopa because of side effects experienced by his brother, subject #3.

hydroxylase (TH), or GTP cyclohydrolase (GTP-CH).[22] Experimental studies support the concept that the age at which striatal dopamine depletion occurs has a profound influence on both motor outcome and response to L-dopa. For example, in adult rats, destruction of nigrostriatal dopamine neurons results in a motor syndrome resembling parkinsonism, but the same lesion in neonatal rats causes hyperactivity and aggressiveness.[23] And, unlike the amelioration of motor impairments observed in adult-lesioned rats, treatment of neonatally lesioned animals with L-dopa exacerbates their hyperactivity.[24] It has been proposed that differences in adaptive neuroplasticity—referring to receptor function, postreceptor signaling pathways, electrophysiological function, and synaptic changes—may account for these phenotypic differences caused by dopaminergic lesions at young compared to adult age.

Analogous adaptive processes have been suggested for the etiology of L-dopa-induced dyskinesias in advanced Parkinson's disease (PD).[25] It is tempting to speculate that similar neuroplastic changes may be responsible for the dystonia and the L-dopa-induced exacerbation in LND. Indeed, descriptions of the motor complications, by patients and their caretakers, closely resemble L-dopa-induced dyskinesias.[26] Similarly, impulse control disorders in PD, which have been regarded as the behavioral counterpart of L-dopa-induced dyskinesias,[25] might share intrinsic properties with the impulsive behaviors in LND, and it has been hypothesized that also the pathogenesis of self-injurious behavior in LND is similar to drug-induced dyskinesias.[27] The absence of major effects of L-dopa on self-injurious behavior in the current study might be explained by insufficient treatment duration, or different pathological mechanisms (and sensitivity) causing motor and behavioral symptoms in LND.

It has been hypothesized that the improvement reported in a single LND patient treated with L-dopa aged from 10 months is attributable to the (at least partial) prevention of the compensatory neuroplastic changes by restoring L-dopa levels at an early stage in neurodevelopment.[15] Unfortunately, this observation was not substantiated by objective measures. Such a preventive effect of very early L-dopa treatment cannot be ruled out but could not be confirmed in our 2 youngest subjects of 3 years of age. Another LND patient, treated after the study ended, started at 12 months of age and showed no obvious improvement of the motor disorder after a year. It should be noted that starting treatment before age 2 is not practical in LND patients, as diagnoses typically often are delayed for years.[2]

The observation that some patients with TH deficiency are acutely sensitive to L-dopa-induced hyperkinesias,[28] prompted us to start with very low doses. It could, however, be argued that the dyskinesias observed reflected an extreme sensitivity that could have been avoided by using even lower doses or slower titrations—similar to some experiences with dopa-responsive dystonia. Such an extreme sensitivity seems unlikely, for several reasons. First, again after the formal study ended, we treated an adult with dystonia due to partial HPRT deficiency with escalating doses of L-dopa for 6 months. He never benefited, but developed worsening of his motor disorder after 4 to 5 months, ultimately leading to discontinuation of the drug. Additionally, 4 classic LND patients included in the current study were given trials of dopamine agonists, starting with 0.5 mg ropinirole or 0.125 mg pramipexole daily and titrating upward. None experienced dyskinesias, but none benefited either and all discontinued. Finally, mechanistically, TH and GTP-CH deficiency are associated with a selective loss of catecholamines whereas LND causes loss of TH, aromatic L-amino acid decarboxylase, vesicular monoamine transporter, and DAT.[2] Therefore, the mechanism in LND is clearly more complex than DOPA-responsive dystonia, and responses to medications can, therefore, be expected to be different.

Based on the assumption that the neurobehavioral features in LND share pathophysiological mechanisms with dyskinesias in PD, treatments that have proven

beneficial for dyskinesias could be considered for LND in the near future. Examples include the glutamate antagonist amantadine,[26] or deep brain surgery (DBS). In fact, 4 LND patients who underwent DBS have been reported in the literature to date,[27,29,30] and some showed improvements in motor dysfunction and behavior. However, long-term effects are unclear, and more studies are needed before DBS can be considered effective and safe in LND.

In summary, the current study demonstrates that L-dopa is not useful for the treatment of LND and might worsen the movement disorder, but it also provides important clues for pathophysiological mechanisms and hints to future treatment options. ■

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Long-Term Follow-up of Botulinum Toxin Therapy for Focal Hand Dystonia: Outcome at 10 Years or More

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ABSTRACT

Background: Previous studies have explored the efficacy and safety of botulinum neurotoxin (BoNT) treatment for Focal hand dystonia (FHD), but none have followed a large number of patients for 10 years or more.

Methods: Retrospective study, with benefit and weakness assessed on a 0 to 4 subjective scale. Demographic, clinical and treatment characteristics were analyzed using *t* tests and Pearson correlations.

Results: Twenty FHD patients had 10 years or longer treatment. Interinjection intervals were variable. Musicians were more likely to wait longer between injections and had less complex dystonia. There was a trend for larger benefit in women and with shorter intervals. The dose increased over time. Dystonia characteristics did not predict response or side-effects, but benefit magnitude predicted longer compliance. No serious side-effects or antibody-mediated resistance occurred.

Conclusion: This is the longest reported period of BoNT treatment in the largest FHD cohort. BoNT therapy for FHD remains safe and effective after more than a decade of treatment. © 2011 *Movement* Disorder Society

Key Words: botulinum; dystonia; focal hand dystonia; safety; efficacy

Introduction

Focal hand dystonia (FHD) is a task specific focal dystonia.[1] Botulinum neurotoxin (BoNT) injection is an effective treatment,[2,3] reducing pathologic neuromuscular junction hyperactivity.[4] We previously reported the safety and effectiveness of BoNT injections for FHD in patients receiving injections for up to 6 years.[5] We continue to follow a large cohort, with 20 patients now treated for 10 years or longer.

Subjects and Methods

Patients

Patients were selected from the NIH BoNT clinic database. Diagnosis was established by initial evaluation and confirmed by ongoing observation.

BoNT Injections

Subjects returned for repeat treatment when they felt that reinjection was necessary, no more frequently than every 3 months. The initial dose and targets were based on clinical judgment,[6] with the starting dose chosen at the lower end of the range and subsequent adjustments. Injections were performed under EMG guidance, as previously described,[7] rarely supplemented by ultrasound. OnabotulinumtoxinA (Botox®, Allergan) at a concentration of 50 to 100 U/mL was used for each injection, except one single injection of Rimabotulinumtoxin B (Myobloc®, Solstice Neurosciences) in one patient.

Patient Evaluations

Muscle strength was assessed using the MRC scale. The toxin distribution and dose were adjusted based on report of weakness and benefit from previous injections. Benefit was assessed on a subjective scale from 0 to 4, based on percent restoration of normal function: 0 = none, 1 = minimal (1–25% restoration of function), 2 = mild (26–50%), 3 = moderate (51–75%), 4 = excellent (76–100%). The patients self-assessed weakness following the previous injection using a similar scale, as 0 (none), 1 (<25% reduction in normal strength), 2 (26–50%), 3 (51–75%), or 4 (76–100%). The rating procedures and treatment guidelines were consistent throughout the study, and all the information was charted in a Microsoft Access database.

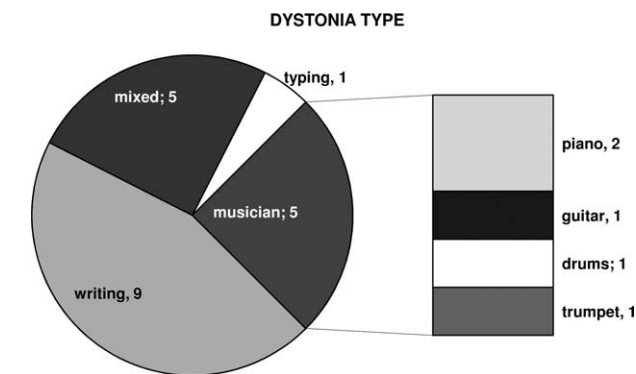
DYSTONIA TYPE

FIG. 1. Distribution of patients by FHD type.

Data Analysis

Student's *t* test and Pearson correlations were used, with $P < 0.05$ as significance threshold. All averages are presented ± standard deviation.

Results

Out of 440 patients in our database, 214 patients with FHD have been treated at least once; 20 patients continued treatment 10 years or more. Five patients had professional musician's dystonia and 15 were employed in clerical positions. Dystonia types are shown in Figure 1. Demographic and treatment characteristics are presented in Table 1.

The musicians were more likely to wait longer between injections (19.9 ± 12.4 months for musicians vs. 7.7 ± 2.3 for nonmusicians, $P < 0.002$). There was a trend for shorter interinjection intervals to be associated with higher benefit (Pearson = -0.44, $0.05 < P < 0.1$).

Most patients (11 of 20) experienced mild average benefit (grade 2). Weakness was similarly mild with 9 of 20 reporting an average grade 2 weakness, with no correlation between benefit and weakness. There was a trend towards larger benefit in women ($55.9\% \pm 15.2\%$ in women vs. $37.4\% \pm 19.5\%$ in men, $P = 0.057$).

The patients received a higher mean dose at the end of the follow-up period compared to the initial treatment (49.9 vs. 24.9 units respectively, $P < 0.00005$). Since the first dose is typically purposefully low, we repeated the analysis excluding the first injection, with similar results (49.9 vs. 31.0 units, $P < 0.002$). The benefit was higher with the last injection compared to the initial (47.3% vs. 26%, $P = 0.039$). No significant correlation was found between dose and benefit at each visit, or between dose and weakness.

To evaluate possible outcome predictors, we performed subanalyses looking at the number of muscle groups injected, divided into: supinator or pronator, hand intrinsics, forearm flexors, forearm extensors, and proximal muscles. Eighteen of twenty patients had involvement of the forearm flexors, 16 of the extensors, 9 of the intrinsic hand muscles, 6 of the pronators or supinators, and 4 of proximal arm or shoulder muscles. Most patients had involvement of more than one muscle group, average 1.7 ± 0.8 in musicians versus 3.1 ± 0.8 in nonmusicians, $P = 0.003$. This number did not correlate with either benefit or weakness.

No patients developed immunity over the duration of follow-up. All patients tolerated the discomfort of multiple injections well; none discontinued treatment due to discomfort. There were no serious adverse effects.

Eleven of the 20 patients are still receiving injections in our clinic. Two patients discontinued treatment due to insufficient response after 5 and 26 visits, respectively. Two moved out of the area and five were lost to follow-up.

We compared this group with the patients who had less than 10 years of treatment. Among the latter, a higher proportion were professional musicians (58% vs. 25%). The patients who discontinued therapy after less than 10 years had significantly lower benefit with the last injection (32% vs. 47.2%, $P < 0.005$), and the most common reason for discontinuation was insufficient benefit (62.5%).

Discussion

This is the largest FHD cohort with the longest follow-up period reported to date. Few prior reports focused on FHD; most included only a few patients in larger dystonia populations and none followed subjects for as long as 10 years.[8–11] We previously published the 6-year outcome in our cohort[5] and Marion et al. reported 9 patients followed for 5 years or more.[12] This study extends observation to a larger cohort and longer follow-up.

Our patients were demographically typical of the FHD population, with writer's cramp the most common type. There was large variability in the frequency of treatments, likely reflecting the fact that while FHD makes particular activities difficult or impossible, it is not otherwise disabling or painful. Patients therefore often tolerate the symptoms and arrange their injections based on anticipated activities. Professional musicians often timed treatments to obtain peak effect around scheduled performances. Since BoNT effects lasted on average 3 months, the long interval between injections is not related to an extended duration of action.

There was a trend for higher benefit in patients returning for treatments at shorter intervals. It is

Table 1. Patient demographics and treatment characteristics

Variable	Value
Total number of patients	20
Gender, n (%), male/female	15 (75)/5 (25)
Age at first injection (yrs, avg. ± STD)	46.6 ± 9.45
Age at dystonia onset (yrs, avg. ± STD)	37.1 ± 9.8
Duration of follow-up (yrs, avg. ± STD)	13.6 ± 2.5
Number of visits (avg. ± STD)	19.7 ± 9.9
Average dose (BoNT A units ± STD)	46.4 ± 24.6
Interinjection interval (avg. ± STD)	11.3 ± 8.8

possible that earlier reinjection enhances residual benefit from prior treatments. As noted in earlier studies,[5,13] we found no correlation between dose and benefit. Accurate selection, localization and dose adjustments are likely more important outcome determinants.[14,15]

Our cohort required a gradual increase in dose over time. This is only partly explained by the choice of a low initial dose, since the gradual increase continued over the later years of treatment. As benefit also increased, it is possible that tolerance led to less weakness with a given dose, allowing higher doses and improved benefit. This dynamic has been seen in some previous studies,[8] but not in others.[5,16]

There is a large range of response to BoNT injections. We were unable to identify factors that predict an individual's response, other than a strong tendency for women to respond better, possibly explained by a smaller muscle mass allowing the toxin to diffuse more readily to the motor endplate in women. Previous studies proposed an inverse relation between dystonia complexity and benefit, with subjects requiring injection of more muscles benefiting less.[7,17] We did not confirm this, finding no such correlation.

The professional musicians in our cohort required injection of fewer muscles, possibly reflecting the exquisite task specificity of musician's dystonia. The need to maintain finely skilled motor control and to minimize weakness is crucial for musicians,[18–20] and the fewer muscles injected might also reflect the need to minimize weakness. We also note a smaller proportion of musicians among the patients continuing treatment more than 10 years compared to the rest of our cohort, which may be indicative of a higher threshold for satisfactory benefit.

None of the patients followed for more than 10 years developed immunity despite exposure to the first Botox (Allergan) batch, which was associated with antibodies developing in 10% of cervical dystonia treatments. The newer formulation is less immunogenic,[21] and immunoresistance tends to develop in the first 4 years of treatment.[22] We show that the risk of developing immunoresistance after more than one decade of FHD treatment is low.

Among the patients who stopped BoNT therapy while under our care, the most common reason was insufficient benefit. The average benefit at the last visit before stopping was significantly lower than the average benefit in patients continuing therapy for more than 10 years, suggesting that magnitude of benefit is an important factor determining continuation of therapy.

It is important to analyze the long-term outcome data for FHD separate from other dystonias, since the BoNT response rates differ. FHD has a lower overall response rate, with about 50% of patients receiving at least mild benefit compared to 80% for cervical dystonia and over 90% for blepharospasm.[23,24]

This study is limited in that it is retrospective and uncontrolled, which limits the strength of any conclusion. In addition, our primary outcome assessments are self-reported scales of benefit and weakness. All FHD research shares this limitation, as there are no widely accepted rating scales applicable to all FHD types.

Patients continued therapy for over 10 years in spite of only mild benefit, suggesting that even partial improvement may be worthwhile. BoNT injections maintained efficacy for over a decade, with good tolerability and no new side effects emergent with long-term treatment. ●

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Mitochondrial Mimicry of Multiple System Atrophy of the Cerebellar Subtype

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online video

ABSTRACT

Background: We describe a patient with clinical and radiological findings suggestive of multiple system atrophy of the cerebellar subtype (MSA-C). **Methods/Results:** Sequencing of the polymerase-γ 1 (*POLG1*) gene revealed the patient had compound heterozygous mutations of the *POLG1* gene. Muscle biopsy revealed the presence of multiple mitochondrial DNA deletions and depletion, confirming the pathogenic nature of the *POLG1* mutations. **Discussion:** This case expands the spectrum of phenotypes associated with *POLG1* mutations to include multiple system atrophy and prompts further consideration regarding whether routine screening for *POLG1* mutations is indicated in this patient population. © 2011 Movement Disorder Society

Key Words: mitochondrial disease; multiple system atrophy; polymerase gamma gene; parkinsonism; ataxia

Mitochondrial disorders can result from either primary defects in the mitochondrial DNA (mtDNA) or defects in nuclear encoded proteins that affect mtDNA structure or function. The maintenance of mtDNA replication is critically dependent upon mtDNA polymerase-γ,[1] encoded by the nuclear genes *POLG1* and *POLG2*. Mutations in *POLG1* have been described in patients with diverse clinical presentations that include parkinsonism and cerebellar ataxia.[2]

Here, for the first time, we describe a patient who presented with clinical and radiological findings suggestive of multiple system atrophy (MSA) of the cerebellar subtype (MSA-C), but was shown to have mutations of *POLG1*. This case highlights the importance of considering primary mitochondrial disorders in the differential diagnosis of parkinsonian syndromes.[3,4]

Case Report

Written informed consent was obtained from the patient to publish both video and brain imaging results for this case report. This 58-year-old woman had a progressive cerebellar syndrome. Her symptoms had started 9 years prior, with imbalance when getting out of a canoe or when walking up and down stairs. She also noted poor handwriting and mild incoordination of the hands. Her speech had become slurred. Her symptoms worsened toward the end of the day or when she was fatigued. In addition, the symptoms partially improved after excluding dietary gluten and she had lost 18 kg over the previous year. She had mild urinary incontinence when coughing. She has type II diabetes mellitus, treated with Pioglitazone. There is no history of epilepsy, cognitive problems, visual problems, stroke-like episodes, hearing problems, or menstrual disturbances. Her family history revealed that she had a sister who died at 2 years of age. This child, who was blind, was never able to roll, sit, or walk independently, and she also had intractable seizures. No diagnosis was ever established. The proband's brother has sensorineural hearing loss, glaucoma, and adult-onset diabetes mellitus requiring treatment with insulin.

On initial examination, 4 years after the onset of her symptoms, she had slight slowing of vertical saccades but a full range of eye movements and normal fundi. She had dysarthria, mild limb dysmetria that was worse on the left, mild slowing of foot taps bilaterally, and a mildly impaired tandem gait; tone and reflexes were normal with flexor plantar responses (see Supporting Information video). Investigations for coeliac

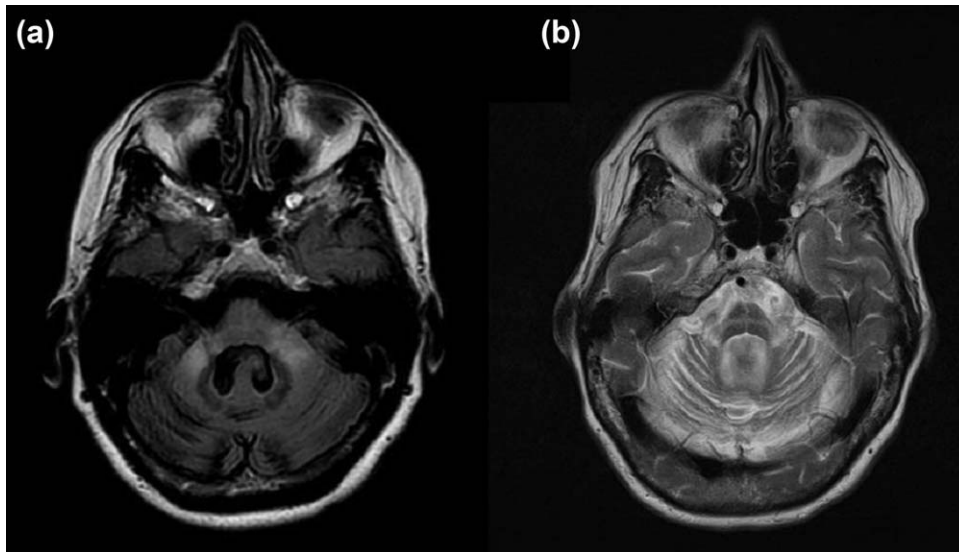--

FIG. 1. **a**: MRI FLAIR brain scan showing an enlarged fourth ventricle because of pontine and cerebellar atrophy, in association with hyperintensities in the middle cerebellar peduncles bilaterally. **b**: MRI T2-weighted brain scan demonstrating the "hot-cross bun" sign, classically associated with MSA.

disease, including a small bowel biopsy, were negative. Sensory testing was normal and nerve conduction studies were normal. In view of the significant weight loss, investigations for a paraneoplastic process were performed and the anti-Purkinje cell antibody was negative. Computed tomography of thorax and mammogram were normal. Her Vitamin B12 and E levels were normal. Metabolic studies, including plasma amino acids, urine organic acids, carnitine profile, lactate, ammonia, and leukocyte hexosaminidase A activity, were all normal. Spinocerebellar ataxia (SCA) types 1, 2, 3, 6, and 7 testing were negative. She was found to have an intermediate-range expansion of the CTG repeat of the SCA type 8 (*SCA-8*) gene, with allele sizes of ~75 and 26 CTG repeats, which were not felt to be clinically significant. Her magnetic resonance imaging (MRI) brain scan showed pontine and cerebellar atrophy with some T2 hyperintensities in the middle cerebellar peduncles (Fig.1).

Her symptoms continued to progress and 7 years after symptom onset her dysarthria and cerebellar ataxia had significantly worsened. Despite using a walker, she fell and sustained a right hip fracture. She developed postural dizziness owing to orthostatic hypotension. Moreover, her urinary urgency worsened and she developed nocturia. She had drooling of saliva and intermittent dysphagia for liquids. Examination revealed a supine blood pressure of 110/60 and 90/40 mm Hg after standing for 3 minutes, with no corresponding change in pulse rate. She had polyminimyoclonus of her outstretched hands, a positive glabellar tap and brisk deep tendon reflexes with flexor plantar responses. She was unable to walk unaided and required a wheelchair. On recent examination (9 years after onset), she had jerky saccades, marked dysarthria,

limb dysmetria, rigidity in the legs worse than in the arms, brisk reflexes, and mild bilateral bradykinesia in association with dystonic posturing of the left hand (see Supporting Information video).

Prompted by reviewing the family history, in particular the sister's clinical history that was suggestive of Alpers' syndrome, the patient was evaluated for mutations of the mitochondrial polymerase-γ 1 (*POLG1*) gene using automated DNA sequencing, and was found to carry the mutations c.2554C>T (p.R852C) and c.32G>A (p.G11D). There was no associated derangement of her liver function tests. Long-range polymerase chain reaction (PCR) analysis of a biopsy of the right vastus lateralis muscle revealed evidence of multiple mtDNA deletions, and real-time PCR analysis of the ND1/beta-globulin DNA ratio showed mtDNA depletion, confirming the pathological significance of the *POLG1* mutations. As both the proband's parents are deceased, parental genetic studies are not possible.

Discussion

We describe a patient with adult-onset, progressive cerebellar ataxia, autonomic dysfunction, mild bradykinesia, and dystonia. The ataxia initially appeared to respond to gluten exclusion from her diet, although the absence of antigliadin antibodies meant that her ataxia was unlikely to be because of gluten sensitivity.[5] She had an expanded CTG repeat of the SCA-8 gene, although this is of doubtful clinical significance, as her largest allele falls within a range that is thought not to be clinically significant.[6] The constellation of symptoms and signs, in conjunction with the MRI brain scan, are most suggestive of MSA-C.[7] However, the significant family history, presence of *POLG1*

mutations, and deletion/depletion of mtDNA in muscle suggest a mitochondrial disorder as the predominant cause of her symptoms.

To date, an association between an MSA-C phenotype and *POLG1* mutations has not been described. *POLG1* mutations have been described previously in association with infantile fatal encephalopathy and hepatopathy (Alpers' syndrome),[8,9] sensory ataxic neuropathy with ophthalmoparesis,[10,11] severe axonal neuropathy,[12] as well as adult-onset ataxia without progressive external ophthalmoparesis (PEO) or muscle involvement.[13] Ataxia has been reported secondary to either dorsal column sensory loss[13] or cerebellar dysfunction and has been associated with cerebellar atrophy on MRI.[14] *POLG1* mutations have been reported in families with PEO, ataxia, and levodopa (L-dopa)-responsive parkinsonism; pathology revealed dopaminergic cell loss in the substantia nigra but not in Lewy bodies.[15–17] Our patient has never had a trial of L-dopa. There is no link between common *POLG1* mutations and idiopathic Parkinson's disease.[18]

Both the p.R582C and the p.G11D sequence variants found in our proband have been previously reported in association with known *POLG1*-associated disease phenotypes, including Alpers' syndrome, ataxia, and neuropathy.[19,20] To our knowledge, this is the first reported case of mutations in *POLG1* causing a MSA phenotype.

Our case also illustrates the extreme clinical variability that can present within different members of the same family. The proband was a university-educated professional with no clinical symptoms until late adulthood, whereas her sister presented in the infantile period with symptoms compatible with a diagnosis of Alpers' syndrome. Although confirmatory genetic studies are not possible for the deceased sister, other cases of similar intrafamilial clinical variability have been reported.[21] We expand the spectrum of phenotypes associated with *POLG1* mutations to include MSA. Clearly blood DNA screening for *POLG1* mutations is indicated in patients with a family history suggestive of a mitochondrial disorder. Whether such screening in other patients with an MSA-C phenotype can be recommended remains to be determined.

Legends to the Video

Mitochondrial mimicry of MSA-C. The first part, recorded 4 years after onset of the disease, illustrates mild dysarthria and very mild gait ataxia. In the second part, 9 years after onset, the patient has lost weight and her speech is more dysarthric. She has limb ataxia, mild bradykinesia, and finger dystonia. She is unable to stand or walk without assistance and has a broad-based stance. ∎

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