Movement Disorders
Vol. 24, No. 1, 2009, pp. 104–137

Brief Reports

Genotype–Phenotype Correlates in Taiwanese Patients with Early-Onset Recessive Parkinsonism

Ming-Jen Lee, MD, PhD,[1] Ignacio F. Mata, PhD,[2] Chin-Hsien Lin, MD,[3] Kai-Yuan Tzen, MD,[4] Sarah J. Lincoln, BSc,[2] Rebecca Bounds, BSc,[2] Paul J. Lockhart, PhD,[5] Mary M. Hulihan, MSc,[2] Matthew J. Farrer, PhD,[2] and Ruey-Meei Wu, MD, PhD[3]*

[1]*Department of Medical Genetics, National Taiwan University Hospital and College of Medicine, National Taiwan University, Taipei, Taiwan;* [2]*Laboratory of Neurogenetics, Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, Florida, USA;* [3]*Department of Neurology, National Taiwan University Hospital and College of Medicine, National Taiwan University, Taipei, Taiwan;* [4]*Department of Nuclear Medicine, National Taiwan University Hospital and College of Medicine, National Taiwan University, Taipei, Taiwan;* [5]*Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Melbourne, Victoria, Australia*

Abstract: **We screened for mutations in the *PARKIN*, *DJ-1*, and *PINK1* genes in a Taiwanese cohort (68 probands; 58 sporadic and 10 familial) with early-onset parkinsonism (EOP, onset <50 years of age). We identified 9 patients harboring mutations in *PARKIN* (three compound heterozygous and six single heterozygous carriers), 3 patients with heterozygous *PINK1* mutations (including two novel substitutions M341I and P209A), and no *DJ-1* mutations. Our frequencies of *PARKIN* (two allele mutation, 4.4%; single allele, 8.8%) and *PINK1* (single heterozygous, 4.4%) mutations in Taiwanese–Chinese are similar to those in Caucasian and other Asian EOP patients. Although the role of heterozygosity of recessive genes in EOP remains to be resolved, molecular analysis and functional imaging will play a decisive role in differential diagnosis and determined therapeutic strategy.** © 2008 Movement Disorder Society

Key words: *PARKIN*; *DJ-1*; *PINK1*; early-onset parkinsonism; Parkinson's disease; Taiwanese

Parkinson's disease (PD) is the second most common neurodegenerative disorder, clinically characterized by resting tremor, bradykinesia, cogwheel rigidity, and postural instability. Increasing evidence for the importance of molecular genetic defects associated with early-onset parkinsonism (EOP, age of onset <50 years) and causative mutations in several genes have been identified (reviewed by Farrer, 2006[1]).

Mutations in the *PARKIN* gene are the most common cause of autosomal recessive EOP. Up to 50% of autosomal recessive and 15 to 20% of sporadic early-onset patients harbor mutations in *PARKIN*.[2] Mutations in the *DJ-1* gene also cause EOP, but appear to be rare in Caucasians with a mutation frequency of only 1 to 2%.[3] Recently, mutations in PTEN-induced protein kinase 1 (*PINK1*) were identified as a cause for autosomal recessive PD[4] and also associated with sporadic EOP.[5] Herein, we assess the mutation frequency of the *PARKIN*, *DJ-1*, and *PINK1* genes and comment on the apparent effects of single mutant alleles in a series of Taiwanese patients with EOP.

PATIENTS AND METHODS

Sixty-eight patients (26 male, 42 female) of ethnic Taiwanese/Chinese descent were recruited in this study. The mean age at onset was 40.1 ± 7.0 years (range, 18–49 years) and the mean disease duration 9.5 ± 6.0 years (range, 1–26 years). All had an age of onset less than 50 years and developed the cardinal features for a clinical diagnosis of possible or probable PD.[6] A family history was regarded as positive if the affected proband had a relative with parkinsonism within three degrees of relationship. Ten probands filled the criteria for a positive family history of PD (10/68, 14.7%). The study was reviewed by the institutional ethics board committee of the National Taiwan University Hospital.

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

*Correspondence to: Dr. Ruey-Meei Wu, Department of Neurology, National Taiwan University Hospital, Taipei 100, Taiwan. E-mail: robinwu@ntu.edu.tw

Received 17 August 2007; Revised 10 February 2008; Accepted 31 March 2008

Published online 12 November 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22093

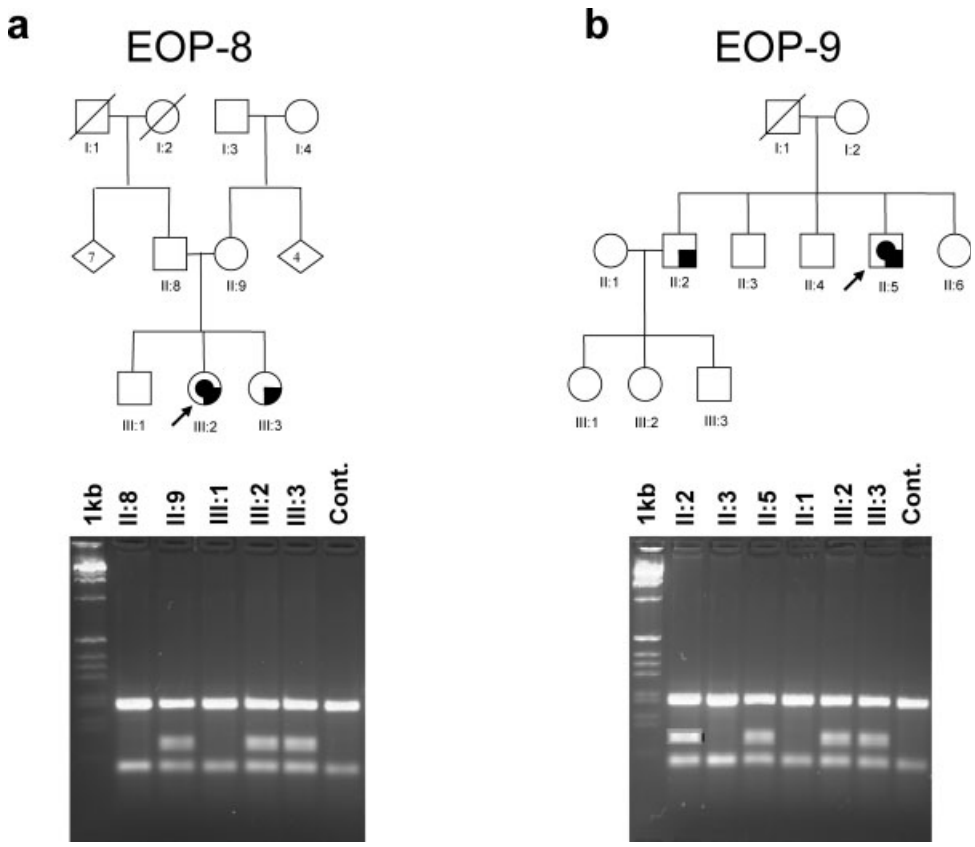

FIG. 1. Two family pedigrees of Taiwanese patients with *PARKIN* mutations, families EOP-8 (**a**) and EOP-9 (**b**). The missense mutation C441R, occurred in two distinct families (EOP-8 and EOP-9), which resulted in creation of a restriction site for *Hha I* (lower panel, a and b) (symbols: square = male; circle = female; slash = deceased; arrowhead = proband; ◔, and ◪ = Parkinsonism; ◉ = concomitant dystonia).

Molecular Analysis (See Supplementary Material)

Blood was collected and EBV-transformed cell lines were established using standard methods. Messenger RNA was reverse transcribed into cDNA and then, the *PARKIN*, *DJ-1*, and *PINK1* cDNAs were sequenced. Semiquantitative analysis was also performed to assess larger genomic deletions/duplications. For PINK1 copy number analysis, exon 1 was coamplified with an anonymous reference sequence on chromosome 18, and then the Hex-labeled products from the log-linear amplification phase were separated on an ABI 3100. Any variant identified was confirmed using a sequence-based screening method or a restriction digest assay, as shown in Figure 1. A subset of the patients screened for PARKIN or DJ-1 mutations have been reported previously.[7–9]

RESULTS

Using quantitative PCR and direct sequencing, 9 patients were found to harbor a *PARKIN* mutation (three compound heterozygous (3/68, 4.4%) and six single heterozygous (6/68, 8.8%) changes) (Table 1). Four of these patients with *PARKIN* mutations have been reported previously.[8,9] Gene dosage screening identified five exon deletions and one exon duplication in six of the index patients (Table 1). Four missense mutations of the *PARKIN* gene were found in six patients (R396G, Y267H, G284R, C441R, see Table 1), with R396G and C441R occurring twice in two apparently unrelated families. Two of these missense mutations, Y267H (EOP-3) and R396G (EOP-2/4), are novel. The substitution of the amino acids changes of the electric charge and/or the polarity of the side chain. Both the Y267H and G284R are conserved among the species. The C441R is located at the IRB domain. These findings suggest that the missense mutations might be pathologic to the PARKIN protein.

Probands in families EOP-8 and EOP-9 (Table 1) harbored the compound heterozygous mutation (del. ex4/C441R). In family EOP-8, the C441R substitution occurred in the index case (III:2, Fig. 1a), her younger

TABLE 1. *Clinical summary for patients with mutations from the Taiwanese cohort (68 probands) associated with autosomal recessive or early-onset Parkinsonism*

Patient/family	Mutations	Age at onset (yr)	Disease duration (yr)	Clinical features	Respons to L-dopa	Family history	Dyskinesia	Motor fluctuation	Other manifestations
EOP-1	PARKIN (dup. Ex6/wt)	49	2	B, R, A, PT	+	−	−	−	Anxiety
EOP-2*	PARKIN (R396G/wt)	44	7	B, R, A	+	−	−	−	
EOP-3	PARKIN (Y267H/wt)	44	3	RT, PT, AT, A, R, B	+	−			ET since age 10, anxiety
EOP-4*	PARKIN (R396G/wt)	40	12	B, RT, PI, A	+	−	−	−	Also has a PINK1silent polymorphism (Ex4/Het A804G/L268L)
EOP-5*	PARKIN (del. Ex2-3/wt)	19	21	R, PT	+	+	+	+	Suicide attempts, foot dystonia, anxiety
EOP-6*	PARKIN (del. Ex2/G284R)	32	14	B, R	+	+	−	−	
EOP-7	PARKIN (del. Ex5/wt)	18	3	B, PT, RT, R, A	No use	−	−	−	ET and foot dystonia
EOP-8	PARKIN (del. Ex4/C441R)	30	1	B, R, A	+	+	−	−	Foot dystonia, anxiety
EOP-9	PARKIN (del. Ex4/C441R)	23	26	B, AT, PT, RT, R, PI	+	+	+	+	Foot dystonia, right pallidotomy
EOP-10	PINK1 (M341I/wt)	35	14	RT, R, PI, A	+	−	+	+	Dystonia
EOP-11	PINK1 (M341I/wt)	39	11	B, RT, R, PI,A	+	−	−	−	Dystonia, died at 50 years
EOP-12	PINK1 (P209A/wt)	30	7	B, R, A	+	−	−	−	

dup., duplication; del., deletion; Ex, exon; +, positive; −, negative; A, asymmetry; AT, action tremor; B, bradykinesia; PI, postural instability; PT, postural tremor; R, rigidity; RT, resting tremor; MSA, multiple system atrophy; ET, essential tremor; *reported previously.[9]

sister (III:3) and her unaffected mother (II:9). Both of her parents are healthy at the study time. The same mutation also occurred in the affected individuals II:2 and II:5 of family EOP-9 (Fig. 1b). However, neither their deceased father (I:1, Fig. 1b) nor their elderly mother (I:2, Fig. 1b), who should harbor a single heterozygous mutation, had the cardinal features of PD. Both the 38-year-old daughter (III:2, Fig. 1b) and the 35-year-old son (III:3) of Patient II:2 had the missense mutation but without PD symptoms.

In *DJ-1* and *PINK1* cDNA analysis, there was no alternative exon splicing or deletion/duplication; however, four sequence variants of *PINK1* gene were identified. These consisted of three single heterozygous missense mutations (two with 1023G > A (M341I), and one with 625C > G (P209A)) and one synonymous change (804A > G (L268L)) (Table 1). The missense mutations M341I and P209A are novel and located in the putative kinase domain of the PINK1 protein. The M341I mutation occurred in two sporadic cases without a family history of disease. None of the aforementioned sequence variants in *PARKIN* and *PINKI* genes were identified in 300 population-matched healthy samples.

DISCUSSION

This is the first comprehensive study of mutation frequency for the three recessive PD genes, *PARKIN, PINK1*, and *DJ-1*, in a Taiwanese cohort. Mutations in the *PARKIN* gene were most common (two allele mutant, 4.4%, and one single allele, 8.8%), mutations in *PINK1* were less common (one single allele, 4.4%), and mutation in *DJ-1* was not observed.

The clinical features of the patients with *PARKIN* mutations were consistent with the previous reports except a low frequency of the occurrence in dyskinesia.[10,11] This may be due to the fact that our patients with *PARKIN* mutation were treated with very low dose of levodopa (<205 mg/days) even over a 10-year duration of disease. The *PARKIN* mutation frequencies for our cohort are in good agreement with those in previous studies of sporadic EOP in European and North American cohorts.[10,12] Reviewing of the literature, the mutation frequency of the *PARKIN* gene in ethnic Chinese is ~15%.[13–19] However, variations in mutation frequency may result from ethnic differences, study design limitations, or the methodology used.

The frequency of *PINK1* mutations in Caucasian EOP patients is 1 to 7%.[4,20–22] In 80 Asian EOP patients, Tan et al. reported that the mutation fre-

quency of *PINK1* gene is 2.5% for homozygous and 1.3% for single heterozygous mutations.[23] Fung et al. identified one single heterozygous *PINK1* mutation in 73 Taiwanese EOP patients (1.4%).[24] Recently, the mutation frequency was reported to be 5.1% in familial cases and 2% in sporadic cases in Taiwan.[25] In this study, three heterozygous *PINK1* mutations were found in 68 EOP patients, giving a mutation frequency of 4.4%. Thus, the mutation frequency for the *PINK1* gene in Asian populations is probably less than 5%, which is in line with the findings in Caucasians.[12] To date, *DJ-1* mutations, which are exceedingly rare in Caucasians, have not been identified in Asians. The original study design, which includes a comprehensive and simultaneous analysis of the three recessive genes in cDNA samples obtained from patients, allow investigating the possible occurrence of a digenic/polygenic inheritance, as suggested in the recent report of a family with single heterozygous mutations in both *PINK1* and *DJ-1* genes.[26] The negative results of the study exclude a digenic inheritance in this cohort.

The percentage of PD patients with only one detectable mutant *PARKIN* allele is high (Foroud et al., 60.2%[27]; Poorkaj et al., 70%[28]). The identification of affected heterozygous carriers in population- and family-based studies has led to the hypothesis that one mutant *PARKIN* or *PINK1* allele may contribute to risk.[29,30] The pathogenicity of single missense mutations is also supported by large family-based studies in which heterozygous carriers manifested early signs of parkinsonism, and clinically has been supported by PET analysis.[31] However, many of these studies were biased as they only assessed affected subjects and had no normal population controls. In addition, their conclusions were based on single, albeit large families in which a shared genetic or environment component may also contribute to disease (even monogenic Parkinsonism is age-associated and thus multifactorial). It is more problematic that the association observed between *PARKIN* and/or *PINK1* heterozygosity and disease has been used to imply causation.

A few single heterozygous mutations were identified in this study. Four of the 68 probands had compound heterozygous mutation in the *PARKIN* gene sufficient to cause PD, whereas five probands were carriers of a single heterozygous mutation, but their contribution to early onset disease is equivocal at best. It is noteworthy that the parents and parental siblings in these families, who may also be *PARKIN* carriers, were clinically normal. It is also dubious whether any of the novel *PINK1* mutations we reported here were sufficient in the heterozygous state to cause disease, as the parents of the probands harboring *PINK1* M341I and P209A coding substitutions were still healthy in their seventh and eighth decades (examined by local doctors). These observation suggested that the possibility of pathogenesis due to a single heterozygous mutation is rather low, a conclusion rather different from that in prior haploinsufficiency studies. Additional modifier genes may contribute to disease susceptibility, perhaps in combination with environmental toxin exposures.

Acknowledgments: We would like to thank the patients and Minnie Schreiber for technical assistance. Funding was provided by the Morris K. Udall Centre of Excellence for Parkinson's disease research (Genetic Core) at Mayo Jacksonville (P50 NS40256), the National Science Council, Taiwan (NSC93-2314-B002-241 and NSC 94-2314-B-002-036), and the National Taiwan University Hospital (NTUH.94A10). We are indebt to Dr. Owen Ross for his critical reading of the manuscript.

REFERENCES

1. Farrer MJ. Genetics of Parkinson disease: paradigm shifts and future prospects. Nat Rev Genet 2006;7:306–318.
2. Lucking CB, Durr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. French Parkinson's disease genetics study group. N Engl J Med 2000;342:1560–1567.
3. Hedrich K, Djarmati A, Schafer N, et al. DJ-1 (PARK7) mutations are less frequent than Parkin (PARK2) mutations in early-onset Parkinson disease. Neurology 2004;62:389–394.
4. Valente EM, Salvi S, Ialongo T, et al. PINK1 mutations are associated with sporadic early-onset parkinsonism. Ann Neurol 2004;56:336–341.
5. Valente EM, Abou-Sleiman PM, Caputo V, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 2004;304:1158–1160.
6. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Arch Neurol 1999;56:33–39.
7. Lockhart PJ, Bounds R, Hulihan M, Kachergus J, et al. Lack of mutations in DJ-1 in a cohort of Taiwanese ethnic Chinese with early-onset parkinsonism. Mov Disord 2004;19:1065–1069.
8. Wu RM, Shan DE, Sun CM, et al. Clinical, 18F-dopa PET, and genetic analysis of an ethnic Chinese kindred with early-onset parkinsonism and parkin gene mutations. Mov Disord 2002;17: 670–675.
9. Wu RM, Bounds R, Lincoln S, et al. Parkin mutations and early-onset Parkinsonism in a Taiwanese Cohort. Arch Neurol 2005; 62:82–87.
10. Lohmann E, Periquet M, Bonifati V, et al. How much phenotypic variation can be attributed to parkin genotype? Ann Neurol 2003; 54:176–185.
11. Mata IF, Lockhart PJ, Farrer MJ. Parkin genetics: one model for Parkinson's disease. Hum Mol Genet 2004;13:R127–R133.
12. Klein C, Djarmati A, Hedrich K, et al. PINK1, Parkin, and DJ-1 mutations in Italian patients with early-onset parkinsonism. Eur J Hum Genet 2005;13:1086–1093.
13. Bia H, Shao M, Dong X, et al. Preliminary studies on parkin gene deletion at exons 1 to 6 in Chinese patients with praecox Parkinson's disease. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2000;17:323–325.

14. Lu CS, Chou YH, Weng YH, Chen RS. Genetic and DAT imaging studies of familial parkinsonism in a Taiwanese cohort. J Neural Transm Suppl 2006;90:235–240.
15. Peng R, Gou Y, Yuan Q, et al. Mutation screening and association analysis of the parkin gene in Parkinson's disease patients from South-West China. Eur Neurol 2003;49:85–89.
16. Tang B, Liu S, Yan X, et al. Analysis of the parkin gene deletion mutations in Chinese patients with Parkinson's disease. Zhonghua Nei Ke Za Zhi 2001;40:799–801.
17. Xu Y, Liu Z, Wang Y, Tao E, Chen G, Chen B. A new point mutation on exon 2 of parkin gene in Parkinson's disease. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2002;19:409–411.
18. Wang T, Liang Z, Sun S, et al. Point mutation in the parkin gene on patients with Parkinson's disease. J Huazhong Univ Sci Technol Med Sci 2003;23:145–147.
19. Wang T, Liang Z, Sun S, et al. A novel point mutation in parkin gene was identified in an early-onset case of Parkinson's disease. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2003;20:111–113.
20. Healy DG, bou-Sleiman PM, Gibson JM, et al. PINK1 (PARK6) associated Parkinson disease in Ireland. Neurology 2004;63: 1486–1488.
21. Rohe CF, Montagna P, Breedveld G, et al. Homozygous PINK1 C-terminus mutation causing early-onset parkinsonism. Ann Neurol 2004;56:427–431.
22. Rogaeva E, Johnson J, Lang AE, et al. Analysis of the PINK1 gene in a large cohort of cases with Parkinson disease. Arch Neurol 2004;61:1898–1904.
23. Tan EK, Yew K, Chua E, et al. PINK1 mutations in sporadic early-onset Parkinson's disease. Mov Disord 2006;21:789–793.
24. Fung HC, Chen CM, Hardy J, et al. Analysis of the PINK1 gene in a cohort of patients with sporadic early-onset parkinsonism in Taiwan 1. Neurosci Lett 2006;394:33–36.
25. Weng YH, Chou YH, Wu WS, et al. PINK1 mutation in Taiwanese early-onset parkinsonism: clinical, genetic, and dopamine transporter studies. J Neurol 2007;254:1347–1355.
26. Tang B, Xiong H, Sun P, et al. Association of PINK1 and DJ-1 confers digenic inheritance of early-onset Parkinson's disease. Hum Mol Genet 2006;15:1816–1825.
27. Foroud T, Uniacke SK, Liu L, et al. Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease. Neurology 2003;60:796–801.
28. Poorkaj P, Nutt JG, James D, et al. parkin mutation analysis in clinic patients with early-onset Parkinson [corrected] disease. Am J Med Genet A 2004;129:44–50.
29. Lesage S, Magali P, Lohmann E, et al. Deletion of the parkin and PACRG gene promoter in early-onset parkinsonism. Hum Mutat 2007;28:27–32.
30. Clark LN, Afridi S, Karlins E, et al. Case-control study of the parkin gene in early-onset Parkinson disease. Arch Neurol 2006;63:548–452.
31. Hedrich K, Hagenah J, Djarmati A, et al. Clinical spectrum of homozygous and heterozygous PINK1 mutations in a large German family with Parkinson disease: role of a single hit? Arch Neurol 2006;63:833–838.

Prevalence of Unilateral Tremor in Autosomal Dominant Essential Tremor

Fenna Phibbs, MD,[1] John Y. Fang, MD,[1] Michael K. Cooper, MD,[1] David P. Charles, MD,[1] Thomas L. Davis, MD,[1] and Peter Hedera, MD[1,2]*

[1]Department of Neurology, Vanderbilt University, Nashville, Tennessee; [2]Center for Human Genetics Research, Vanderbilt University, Nashville, Tennessee

Abstract: **The presence of bilateral arm tremor is a key diagnostic feature of essential tremor (ET). We analyzed the presence of unilateral arm tremor in familial ET cohort of 133 autosomal dominant ET kindreds with 412 affected individuals. Inclusion criteria in patients with unilateral arm postural and/or kinetic tremor required the duration of tremor for at least 5 years, without hypokinetic-rigid syndrome, dystonic posturing, or history of sudden onset of tremor. Only subjects with at least one living first degree relative who met diagnostic criteria for definite ET were included. Eighteen subjects met the inclusion criteria and five had postural tremor only, while the majority (13/18) had a combination of postural and kinetic tremor. Our data shows that unilateral tremor associated with ET is relatively rare and can be identified in 4.4% patients in a cohort of familial ET.** © 2008 Movement Disorder Society

Key words: essential tremor; diagnosis; Parkinson disease

The presence of bilateral, predominantly symmetrical postural and/or kinetic tremor involving mostly upper extremities and the absence of other movement abnormalities, such as dystonia or parkinsonism support the diagnosis of essential tremor (ET).[1,2] ET is exclusively a clinical diagnosis and there is an ongoing debate regarding the definition of the phenotype of this most common movement disorder.[3,4] ET likely represents a heterogeneous condition, contributing to the controversy surrounding its diagnostic criteria.[5–7] The differences in the stringency of diagnostic definition are reflected in a considerable variability of detected ET prevalence.[8,9]

*Correspondence to: Dr. Peter Hedera, MD, Department of Neurology, Vanderbilt University, 465 21st Avenue South, 6140 MRB III, Nashville, TN 37232-8552, USA.
E-mail: peter.hedera@vanderbilt.edu

Received 6 November 2007; Revised 3 March 2008; Accepted 4 April 2008

Published online 30 October 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22113

Unilateral upper extremity tremor is not typical for ET and it may raise concerns for an alternative diagnosis, especially Parkinson disease or dystonic tremor.[1] Even though some diagnostic criteria classify patients with unilateral arm tremor as having possible ET, the true prevalence of this finding is unknown due to the absence of an accepted diagnostic marker for ET. Invoking Occam's razor, we hypothesized that patients with an atypical unilateral tremor but first degree relatives with a classic presentation of ET have a high likelihood of suffering the same genetic condition. We have used this approach in our cohort of ET kindreds and analyzed the frequency of unilateral arm tremor in patients, whose diagnosis of ET was supported by positive family history indicating autosomal dominant inheritance.

PATIENTS AND METHODS

We included 412 individuals with tremor from 133 kindreds who were classified as having definite or probable ET by previously published criteria.[1,2] Individuals with bilateral postural and kinetic arm tremor with or without asymmetry for more than 5 years, absence of additional neurologic abnormalities, absent history of exposure to tremorogenic drugs before the onset of symptoms, and without history and examination suggestive of psychogenic tremor or sudden onset with a stepwise deterioration were classified as definite ET. Individuals with duration of tremor less than 5 years or tremor isolated to a body part, such as voice or head tremor, or a unilateral postural or kinetic arm tremor were classified as probable ET. Furthermore, subjects with unilateral tremor were included only if they did not have signs of hypokinetic-rigid syndrome and failed dopaminergic challenge. A failed response to dopaminergic stimulation was defined as no improvement of tremor following a 2 week course of at least 600 mg per day of levodopa. Several patients were also unsuccessfully challenged with other dopaminergic agonists, but we required a negative L-dopa trial.

Patients with unilateral tremor were selected for this study if they experienced postural and/or kinetic tremor affecting only one arm that was at least of moderate severity, lasting at least 5 years, and nonresponsive to dopaminergic challenge. We excluded patients who had a resting tremor associated with their postural and/or kinetic tremor. Additional criteria for diagnosis encompassed absence of rigidity/bradykinesia, dystonic posturing, or a sensory trick or null point to stop tremor. We also excluded patients with a task-specific tremor or a history of sudden onset. Furthermore, we included only subjects in whom the diagnosis of ET was further supported by a positive family history defined as the presence of least one living first degree relative (parent, sibling, offspring) who met diagnostic criteria for definite ET based on the presence of bilateral tremor, confirmed by a member of our movement disorders group. Individuals with unilateral tremor without a known family history or with only historical evidence of additional relatives with otherwise typical ET were excluded.

Tremor was quantified using the NIH ET consortium grading and the rating scale from the Washington Heights-Inswood Genetic Study of Essential Tremor (WHIGET) (0[normal]–3 score assigned for resting and postural tremor, and 0–4 for finger to nose, pouring water, drinking water, drinking with a spoon, and drawing a spiral tasks).[10–12] Patients with slight isolated postural tremor (scores 0 and 1) were not included; however, we permitted a barely noticeable tremor in unaffected arm in patients who were classified as having unilateral tremor. Furthermore, the degree of disability was also judged by self-reporting of questions adapted from the Tremor disability questionnaire (0[normal]–100).[12]

RESULTS

Eighteen subjects from 17 families with autosomal dominant ET met the inclusion criteria; their clinical and demographic characteristics are summarized in the Table 1. The majority (16/18) had a strictly unilateral tremor, while two had a minimal, intermittent, fine postural tremor affecting the contralateral upper extremity. Both of these individuals were examined several times within the 3-year period and this fine contralateral tremor was not present on each occasion. Furthermore, the degree of observed tremor in the contralateral extremity was not severe enough to warrant the diagnosis of definite ET, defined as the occurrence of bilateral tremor. Isolated postural tremor was observed in five patients and 13 had a combination of postural and kinetic tremor. None of these patients had signs of tremor affecting other body segments, including absent head or voice tremor.

Eight individuals with unilateral tremor had affected offspring with otherwise typical bilateral arm tremor, six individuals with unilateral tremor had an affected parent with definite ET, and four had affected siblings with definite ET and additional clinical and historical evidence of AD ET. The tendency for a familial occurrence of unilateral tremor associated

TABLE 1. *Demographic and clinical characteristics of patients with unilateral tremor*

Gender	Age (yr)	Age of tremor onset (yr)	Duration of tremor (yr)	WHIGET scores		Self-reported disability
				R	L	
M	30	22	8	2	0	10
M	32	18	14	14	0[a,b]	20
M	40	22	18	15	0	28
M	44	35	9	22	0	47
M	44	30	14	12	0	29
M	44	31	13	0	14	13
M	49	40	9	0	15[b]	33
M	52	30	22	2	0	5
M	60	35	25	0	23	25
M	63	55	8	0	18	7
F	33	20	13	15	0	25
F	37	18	19	15	0	21
F	38	30	8	2	0	10
F	40	30	10	0	7	15
F	41	20	21	21	0[a]	25
F	41	32	9	0	2	0
F	48	40	8	0	2	12
F	50	39	11	0	8[b]	22

[a]Fine isolated postural tremor (WHIGET score 1) was intermittently observed in these two patients.
[b]Left-handed individual.

with autosomal dominant ET does not appear to be very strong. We have identified only one instance where a parent diagnosed as definite ET reported a previous history of a long-term of unilateral tremor and had an affected child who manifested unilateral tremor at the time of the diagnosis. Only one mid-size pedigree contained two individuals each with unilateral tremor.

DISCUSSION

Our data show that individuals with unilateral tremor can be identified in 4.4% of patients with autosomal dominant ET. However, our diagnostic approach, requiring the support of the diagnosis of ET by the presence of otherwise typical bilateral ET in first degree relatives may underestimate the actual prevalence of a long-standing unilateral tremor in ET patients. We used this additional diagnostic criterion to further strengthen the diagnosis of ET with the assumption that an overlap of two different tremor-causing movement disorders is relatively rare.

A recent study suggested that 37% of patients were misdiagnosed as ET and one of the most common factor associated with misdiagnosis was the presence of a unilateral tremor.[13] Unilateral, particularly resting, tremor may suggest the diagnosis of PD. ET is heterogeneous condition and may also coexist with PD.[14,15] Moreover, we excluded individuals who showed a combination of resting, postural and kinetic tremor, even though they failed a previous dopaminergic challenge to minimize a possibility of including patients with tremor-dominant, slowly progressive Parkinson's disease. This likely further underestimates the frequency of unilateral tremor in ET, because the presence of resting tremor has been previously reported as relatively common in patients with ET, especially in advanced stages of the disease.[16] None of 17 kindreds containing individuals with unilateral PD had any evidence for the coexistence of both disorders in these families.

Most published diagnostic criteria emphasize the presence of predominantly symmetrical tremor affecting the upper extremities, even though some degree of asymmetry in not uncommon in definite ET.[1,2,17] Analysis of the motor phenotype in a cohort of 487 patients with ET found asymmetry of tremor in 47% of patients and unilateral tremor in 10% of their patients.[18] However, this study also included patients with shorter duration of tremor, and only 27% of all patients met conservative and definite diagnostic criteria for ET. We also identified an additional 23 individuals from our cohort of familial ET who met diagnostic criteria for definite ET, but reported a previous history of unilateral arm tremor ranging from 11 to 33 years before the onset of contralateral tremor. We did not include them in our analysis because recall of tremor history is many times not reliable and we did not want to overestimate the frequency of unilateral tremor in confirmed ET.[19]

In conclusion, unilateral arm tremor is relatively rare in ET but this diagnosis should be strongly considered if other conditions, especially PD and dystonia, can be excluded on clinical grounds.

Acknowledgments: We thank the members of the families described here, whose help and participation made this work possible. This work was supported by NIH K08NS42743 to PH. PH had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. Jankovic J. Essential tremor: clinical characteristics. Neurology 2000;54(Suppl):S21–S25.
2. Deuschl G, Bain P, Brin M, an Ad Hoc Scientific Committee. Consensus statement of the movement disorder society on tremor. Mov Disord 1998;13(Suppl 3):2–23.
3. Elble RJ, Tremor Research Group. Report from a U.S. conference on essential tremor. Mov Disord 2006;21:2052–2061.
4. Chouinard S, Louis ED, Fahn S. Agreement among movement disorders specialists on the clinical diagnosis of essential tremor. Mov Disord 1997;12:973–976.
5. Jankovic J. Essential tremor: a heterogeneous disorder. Mov Disord 2002;17:638–644.
6. Elbe RJ. Essential tremor is a monosymptomatic disorder. Mov Disord 2002;17:633–637.
7. Louis ED, Ford B, Barnes LF. Clinical subtypes of essential tremor. Arch Neurol 2000;57:1194–1198.
8. Louis ED, Ottman R, Hauser WA. How common is the most common adult movement disorder? Estimates of prevalence of essential tremor throughout the world. Mov Disord 1998;13:5–10.
9. Findley LJ. Epidemiology and genetics of essential tremor. Neurology 2000;54(Suppl):S8–S13.
10. Brain PG, Findley LJ, Atchinson P. Assessing tremor severity. J Neurol Neurosurg Psychiatry 1993;56:868–873.
11. Louis ED, Barnes L, Wendt KJ, et al. A teaching videotape for the assessment of essential tremor. Mov Disord 2001;16:89–93.
12. Louis ED, Yousefzadeh E, Barnes LF, Yu Q, Pullman SL, Wendt KJ. Validation of a portable instrument for assessing tremor severity in epidemiologic field studies. Mov Disord 2000;15:95–102.
13. Jain S, Lo SE, Louis ED. Common misdiagnosis of a common neurological disorder. How are we misdiagnosing essential tremor? Arch Neurol 2006;63:1100–1104.
14. Shahed J, Jankovic J. Exploring the relationship between essential tremor and Parkinson's disease. Parkinsonism Relat Disord 2007;13:67–76.
15. Rajput AH, Rozdilsky B, Ang L, Rajput A. Significance of Parkinsonian manifestation in essential tremor. Can J Neurol Sci 1993;20:114–117.
16. Koller WC, Rubino FA. Combined resting postural tremors. Arch Neurol 1985;42:683–684.
17. Louis ED, Wendt KJ, Pullman SL, Ford B. Is essential tremor symmetric? Observational data from a community-based study of essential tremor. Arch Neurol 1998;55:1553–1559.
18. Whaley NR, Putzke JD, Baba Y, Wzsolek ZK, Uitti RJ. Essential tremor: phenotypic expression in a clinical cohort. Parkinsonism Relat Disord 2007;13:333–339.
19. Busenbark K, Barnes P, Lyons K, Ince D, Villagra F, Koller W. Accuracy of reported family histories of essential tremor. Neurology 1996;47:264–265.

Serotonin and Dopamine Transporter Genes Do Not Influence Depression in Parkinson's Disease

Nadeeka N.W. Dissanayaka, BSc (Hons),[1,2]
Peter A. Silburn, PhD, FRACP,[1,2]
John D. O'Sullivan, MD, FRACP,[1]
and George D. Mellick, PhD[2*]

[1]School of Medicine, University of Queensland, Brisbane, Australia; [2]Eskitis Institute for Cell and Molecular Therapies, School of Biomolecular and Biomedical Sciences, Griffith University, Brisbane, Queensland, Australia

Abstract: **Altered levels of the neurotransmitters dopamine and serotonin are observed in both Parkinson's disease (PD) and depression. Therefore, the neurotransmitter transporter genes, *SLC6A3* (dopamine) and *SLC6A4* (serotonin) are candidates for depression in PD. We genotyped 24 tagging SNPs together with VNTRs and the *SLC6A4* LPR polymorphism in 190 PD patients categorised according to lifetime history of depression. Log-additive, dominant and recessive statistical models were constructed. No significant genotype or haplotype associations were observed suggesting that common genetic variables around the dopamine and serotonin transporter genes do not play a significant role in the etiology of depression in PD.** © 2008 Movement Disorder Society

Key words: *SLC6A4*; *SLC6A3*; depression; Parkinson's disease; haplotypes; genetic variants

Depression is a common nonmotor manifestation of Parkinson's disease (PD).[1] As is the case for depression in the general population, the etiological factors leading to depression in PD are poorly understood, although a genetic contribution is proposed. Although there is a substantial literature around genetic association studies of depression,[2] there have been few investigations of depression in the context of PD. As neurotransmitter transporters are important regulators of neurotransmitter levels in the brain, their genetic variants are considered potential risk factors for depression and modifiers of treatment responses to pharmacological interventions that act on the transmitter pathways.

*Correspondence to: George D. Mellick, Eskitis Institute for Cell and Molecular Therapies, School of Biomolecular and Biomedical Sciences, Griffith University, Nathan, QLD4111, Australia.
E-mail: g.mellick@griffith.edu.au

Received 27 February 2008; Revised 2 April 2008; Accepted 17 April 2008

Published online 30 October 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22134

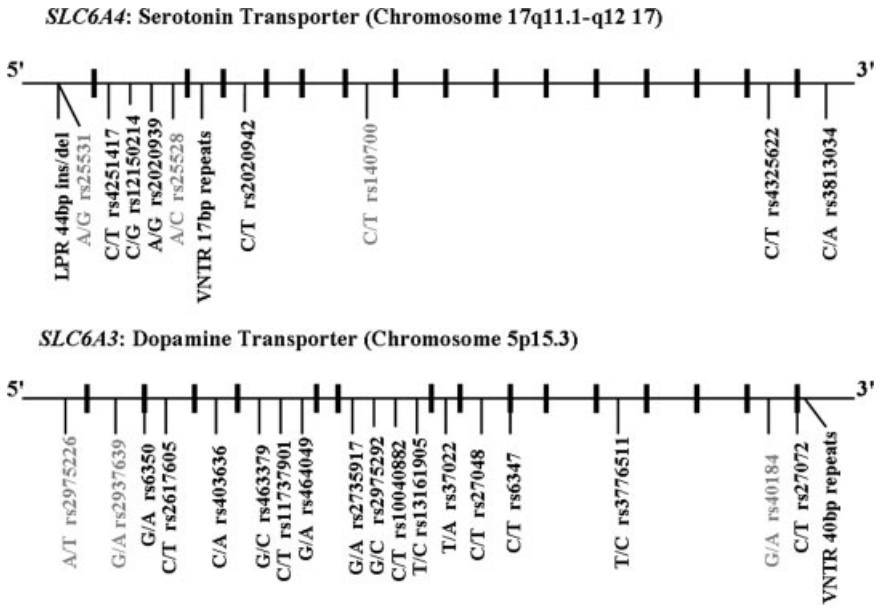

FIG. 1. Selected genetic variations of the serotonin (*SLC6A4*) and dopamine (*SLC6A3*) transporter genes. The vertical thick black lines represent the exons. The diagram is not to scale.

The serotonin transporter (5HTT) gene (*SLC6A4*), located at the chromosome 17q11.1–17q12, has been widely studied as a candidate gene for depression in the general population.[2–4] The dopamine transporter (DAT) gene (*SLC6A3*), located at chromosome 5p15.3, has also been studied as a candidate gene for PD and psychiatric disorders.[5,6] In all cases the focus of these studies has been on isolated polymorphism. Only three previously published studies have examined genetic risk factors for depression in PD; all focused on the 5HTT-LPR, were of limited sample size, and were equivocal in conclusion.[7–9] The aim of the current study was to conduct a comprehensive, haplotype tagging approach to determine whether common genetic variation around the *SLC6A4* and *SLC6A3* loci influence risk for depression in PD.

METHODS

Patients with PD, of European Caucasian ethnicity, were recruited from public and private Neurology clinics in Brisbane, Australia. Informed consent was obtained from all participants and the project protocol was approved by Human Research Ethics Committees at the participating institutions. A diagnosis of idiopathic PD according to the United Kingdom Brain Bank Criteria[10] was made by specialist Movement Disorders Neurologists. A validated depression screening method based on the Geriatric Depression Scale (GDS-15) and three screening questions was administered.[11] Patients with a diagnosis of dementia or those who were unable to complete the questionnaire were excluded.

The patients who scored an optimal discriminatory value of >6 in the GDS-15 were classified into the "currently depressed" group and the patients who had a history of having been treated for depression were classified into the "previously depressed" group. The patients with a score of <5 (GDS-15 screening cut-off), with no history of treatment for depression and no reported depressive symptoms during their lifetime were included in the "never depressed" group.[11] In this way we could focus on two extreme phenotypes of "currently depressed" and "never depressed". Of 324 PD patients originally available for analysis, 176 (54%) showed a history of depression ("previously" or "currently" depressed) and 98 (30%) were classified as "never depressed". Fifty (16%) of the participants scored within the gray area of uncertain phenotype with regard to depression and were excluded from further analysis in this study. Ninety-five "never depressed" PD patients (M = 59; F = 36; Mean age ± SD = 69.9 ± 8.0 years) were age and gender matched to 95 'depressed' PD cases. The depressed cases consisted of 36 patients who were classified as "previously depressed" and 59 who were classified as "currently depressed". The only criterion for matching was age and gender. When more than one depressed subject fitted the criteria the selection was made randomly.

TABLE 1. *The influence of common genetic variations at the serotonin (SLC6A4) and dopamine (SLC6A3) transporter genes on the risk of depression ("current" or "previous") in PD*

Gene	Variable	Minor allele frequency of controls	Model	Odds ratio (95% C.I.)	*P*-value
SLC6A4	rs4251417	13	a	0.71 (0.35–1.41)	0.33
			b	0.74 (0.36–1.51)	0.40
			c	Not determined[a]	
	rs12150214	21	a	0.65 (0.37–1.14)	0.13
			b	0.66 (0.36–1.21)	0.18
			c	3.23 (0.33–31.94)	0.32
	rs2020939	43	a	0.97 (0.64–1.48)	0.89
			b	1.20 (0.64–2.24)	0.57
			c	1.44 (0.67–3.09)	0.35
	rs2020942	38	a	1.34 (0.87–2.07)	0.18
			b	1.71 (0.92–3.15)	0.09
			c	0.91 (0.40–2.07)	0.83
	rs4325622	46	a	1.10 (0.71–1.69)	0.67
			b	1.04 (0.51–2.15)	0.91
			c	1.04 (0.51–2.15)	0.91
	rs3813034	46	a	1.05 (0.68–1.62)	0.83
			b	1.20 (0.62–2.31)	0.59
			c	1.10 (0.52–2.29)	0.81
	5HTTLPR		a	0.94 (0.61–1.44)	0.77
			b	0.92 (0.49–1.73)	0.80
			c	1.09 (0.50–2.36)	0.83
	5HTTVNTR[b]		a	1.18 (0.77–1.80)	0.44
			b	1.43 (0.77–2.64)	0.26
			c	1.02 (0.46–2.22)	0.97
SLC6A3	rs2617605	36	a	1.20 (0.79–1.82)	0.40
			b	1.20 (0.66–2.18)	0.55
			c	0.71 (0.32–1.60)	0.41
	rs403636	15	a	1.06 (0.60–1.87)	0.85
			b	1.07 (0.57–2.03)	0.83
			c	1.00 (0.13–7.36)	1.00
	rs463379	21	a	1.02 (0.62–1.67)	0.94
			b	1.18 (0.65–2.14)	0.58
			c	2.10 (0.51–8.66)	0.31
	rs11737901	35	a	0.96 (0.63–1.47)	0.85
			b	0.92 (0.51–1.64)	0.77
			c	0.98 (0.40–2.38)	0.96
	rs464049	44	a	1.03 (0.69–1.53)	0.89
			b	0.95 (0.51–1.76)	0.86
			c	0.86 (0.43–1.72)	0.66
	rs2975292	34	a	1.13 (0.73–1.73)	0.58
			b	1.50 (0.83–2.71)	0.18
			c	1.54 (0.62–3.81)	0.35
	rs10040882	24	a	1.00 (0.63–1.60)	0.99
			b	1.01 (0.56–1.81)	0.98
			c	1.01 (0.31–3.26)	0.99
	rs13161905	42	a	0.85 (0.56–1.29)	0.43
			b	0.80 (0.44–1.46)	0.47
			c	1.25 (0.56–2.76)	0.59
	rs37022	18	a	0.95 (0.55–1.65)	0.87
			b	0.99 (0.53–1.85)	0.98
			c	1.54 (0.25–9.64)	0.64
	rs27048	49	a	1.00 (0.68–1.47)	1.00
			b	0.79 (0.42–1.49)	0.46
			c	0.77 (0.40–1.50)	0.45
	rs6347	26	a	1.18 (0.74–1.88)	0.48
			b	1.42 (0.79–2.55)	0.24
			c	1.36 (0.45–4.11)	0.58
	rs3776511	20	a	0.89 (0.51–1.57)	0.70
			b	1.00 (0.53–1.90)	1.00
			c	3.78 (0.41–34.62)	0.24
	rs27072	16	a	1.43 (0.80–2.55)	0.23
			b	1.47 (0.80–2.71)	0.21
			c	0.93 (0.06–15.52)	0.96
	DATVNTR[c]		a	1.21 (0.79–1.86)	0.39
			b	1.24 (0.70–2.20)	0.47
			c	0.70 (0.27–1.83)	0.47

a, log additive model; b, dominant model; c, recessive model. All models are controlled for age and gender.

[a]Not determined due to low frequency of the homozygous alternative genotype.

[b]For the purposes of this analysis the most common 12/12 genotype was considered as the reference. Three individuals with the rare 9/12 genotype were coded with the heterozygote 10/12 genotype.

[c]For the purposes of this analysis the most common 10/10 genotype was countered as the reference. Two individuals with the 10/11 genotype was coded as heterozygous and one individual with the 9/11 and one individual with 6/9 genotype were coded as homozygous alternative allele.

Twenty-four haplotype tagging SNPs were selected from the HapMap project database (http://www.hapmap.org/); genome coverage parameters were: minor allele frequency >0.1 and r^2 value >0.9. Previously published polymorphisms were also examined (see Fig. 1). All SNPs were genotyped from genomic DNA using the Sequenom platform, at the Australian Genome Research Facility (AGRF). Other polymorphisms were genotyped using polymerase chain reaction (PCR) protocols followed by agarose gel electrophoresis (details on request). A 10% random selection of samples was genotyped in replicate for all polymorphisms for quality assurance. Polymorphisms with minor allele frequency <10%, those that failed replication or showed deviation from Hardy-Weinberg Equilibrium (HWE) were excluded from further analysis.

Linkage disequilibrium (LD) plots of the SNPs were examined using the Haploview program and correlation coefficients between variables of each gene were determined. For highly correlated SNPs (correlation coefficient >0.75), the most informative was selected and included in the ultimate haplotype analysis. Rare haplotypes (frequency <10% for *SLC6A4* and ≤5% for *SLC6A3*) were pooled. Haplotype analyses were performed using the SNPStats program (http://bioinfo.iconcologia.net/snpstats) with reference to the most frequent haplotype. Genotype associations were performed using logistic regression models adjusted for age and gender (using the SPSS 13.0 package). The log-additive, dominant, and recessive models were examined.

RESULTS

Eight of the originally selected 30 SNPs did not fulfill our study's requirements for inclusion: Two (rs6350 and rs2735917) had a minor allele frequency <10%. One (rs140700) failed genotyping quality control; and PCR products could not be produced from repeated attempts to assay the remaining five SNPs (rs40184, rs2937639, rs2975226, rs25528, and rs25531).

None of the polymorphisms showed a genotypic association with a history of depression in PD (Table 1). Similar results were observed when "currently depressed" patients were compared with the "never depressed" group. In the ultimate haplotype analysis, rs2020939 was selected to capture rs4325622 and rs3813034; rs2020942 captured the 5HTT-VNTR; and rs11737901 captured rs13161905. Four common haplotypes (frequency >10%) defined *SLC6A4* and five common (frequency >5%) haplotypes defined *SLC6A3*. There were no significant differences in haplotype frequencies between "all depressed" and "never depressed" groups; the global χ^2 probability value (*P*) for the *SLC6A4* and *SLC6A3* were 0.69 and 0.41, respectively.

DISCUSSION

This study is the first to comprehensively examine the associations between *SLC6A4* and *SLC6A3* gene polymorphisms, and depression in the context of PD. The results show that none of the examined polymorphisms contribute substantially to the risk for depression in the setting of PD. The haplotypes examined also showed no relationship to depression in PD.

This study is one of few studies to investigate potential genetic risk factors for depression in PD. Menza et al. (1999) and Mossner et al. (2001) reported that the short allele of the 5HTT-LPR is associated with depression in PD,[8,9] while Burn et al. (2006) showed no association.[7] These studies are very limited in terms of statistical power. Our current study has an increased sample size and is the first to use a comprehensive haplotype tagging strategy to study *SLC6A3* and *SLC6A4* in genetic association studies of depressive disorder, maximizing the coverage of common genetic variability at these gene loci. The use of a well validated depression recognition method is another advantage of our study. Our results support the conclusions of Burn et al. that the LPR polymorphism does not confer differential risk for depression in PD. No significant genotype or haplotype associations were revealed, supporting the conclusion that common genetic polymorphisms around these genes do not modify the risk for depression in PD.

Our study has several limitations. Firstly, we were unable to confidently assess genotypes for 6 of the 30 markers originally selected for genotyping. Regarding genotyping reliability, only one SNP failed in terms of reproducibility of genotyping scoring (rs140700) and this was excluded from analysis. The other five variables failed due to an inability to perform PCR reactions as a result of their location in difficult sequence with particularly high GC content. For two SNPs (rs2937639 and rs25531) no primers could be designed that would fulfill the Sequenom platform's requirements. For the remaining three SNPs (rs40184, 2975226, rs25528) PCR amplifications failed despite repeated attempts with redesigned primer-pairs and the use of PCR adjuvants such as 7′-deaza-dGTP and DMSO. Second, we limited our investigation to variants with a minor allele frequency of >10% and an r^2

of 0.9 for correlation to tagged variables. We can, therefore, not exclude the possibility that less common genetic variables at these loci influence depression in the setting of PD.

In conclusion, this comprehensive association study found no evidence that common genetic variables at the *SLC6A4* and *SLC6A3* loci influence the risk of depression in the setting of PD.

Acknowledgments: This study was supported financially by seed funding from Parkinson's Queensland Incorporated and a donation from Mrs Olive Miles. We thank all the patients in the study for their participation, Dr Richard Boyle for assistance in patient recruitment, Dr Greg Sutherland and Dr Gerhard Siebert for their assistance in DNA extractions and AGRF for genotyping support.

REFERENCES

1. Reijnders JS, Ehrt U, Weber WE, Aarsland D, Leentjens AF. A systematic review of prevalence studies of depression in Parkinson's disease. Mov Disord 2007;23:183–189.
2. Anguelova M, Benkelfat C, Turecki G. A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter. I. Affective disorders. Mol Psychiatry 2003;8:574–591.
3. Battersby S, Ogilvie AD, Blackwood DH, et al. Presence of multiple functional polyadenylation signals and a single nucleotide polymorphism in the 3′ untranslated region of the human serotonin transporter gene. J Neurochem 1999;72:1384–1388.
4. Wendland JR, Martin BJ, Kruse MR, Lesch KP, Murphy DL. Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. Mol Psychiatry 2006;11:224–226.
5. Ohadi M, Shirazi E, Tehranidoosti M, et al. Attention-deficit/hyperactivity disorder (ADHD) association with the DAT1 core promoter -67 T allele. Brain Res 2006;1101:1–4.
6. Vandenbergh DJ, Persico AM, Hawkins AL, et al. Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics 1992;14:1104–1106.
7. Burn DJ, Tiangyou W, Allcock LM, Davison J, Chinnery PF. Allelic variation of a functional polymorphism in the serotonin transporter gene and depression in Parkinson's disease. Parkinsonism Relat Disord 2006;12:139–141.
8. Menza MA, Palermo B, DiPaola R, Sage JI, Ricketts MH. Depression and anxiety in Parkinson's disease: possible effect of genetic variation in the serotonin transporter. J Geriatr Psychiatry Neurol 1999;12:49–52.
9. Mossner R, Henneberg A, Schmitt A, et al. Allelic variation of serotonin transporter expression is associated with depression in Parkinson's disease. Mol Psychiatry 2001;6:350–352.
10. Twelves D, Perkins KSM, Counsell C. Systematic review of incidence studies of Parkinson's disease. Mov Disord 2003;18:19–31.
11. Dissanayaka NN, Sellbach A, Matheson S, et al. Validity of hamilton depression inventory in Parkinson's disease. Mov Disord 2007;22:399–403.

Ultrasound Treatment of Cutaneous Side-Effects of Infused Apomorphine: A Randomized Controlled Pilot Study

Leon Poltawski, BSc,[1]* Hazel Edwards, MSc,[2]
Amy Todd, BSc,[1] Tim Watson, PhD,[3]
Andrew Lees, MD,[4]
Cherry Ann James, BSc[5]

[1]Division of Physiotherapy, University of Hertfordshire, UK; [2]Division of Radiography, University of Hertfordshire, UK; [3]School of Health and Emergency Professions, University of Hertfordshire, UK; [4]Reta Lila Weston Institute of Neurological Studies, University College London, London, UK; [5]Department of Neurology, University College London, London, UK

Abstract: **Apomorphine hydrochloride is a dopamine agonist used in the treatment of advanced Parkinson's disease. Its administration by subcutaneous infusions is associated with the development of nodules that may interfere with absorption of the drug. This pilot study assessed the effectiveness of ultrasound (US) in the treatment of these nodules. Twelve participants were randomly assigned to receive a course of real or sham US on an area judged unsuitable for infusion. Following treatment, no significant change was observed in measures of tissue hardness and tenderness. However, 5 of 6 participants receiving real US rated the treated area suitable for infusion compared with the 1 of 6 receiving sham US. Sonographic appearance improved in both groups, but more substantially in the real US group. Power calculations suggest a total sample size of 30 would be required to establish statistical significance. A full-scale study of the effectiveness of therapeutic US in the treatment of apomorphine nodules is warranted.** © 2008 Movement Disorder Society

Key words: Parkinson's disease; apomorphine; side effects; nodules; therapeutic ultrasound; clinical trial

Apomorphine hydrochloride has been used successfully since the 1950s in the treatment of people with later stage Parkinson's disease (PD).[1] A common side effect of delivery by infusion is the development of

*Correspondence to: Leon Poltawski, School of Health & Emergency Professions, University of Hertfordshire, Hatfield AL10 9AB, United Kingdom. E-mail: l.poltawski@herts.ac.uk

Potential conflict of interest: None reported.

Received 29 July 2008; Revised 19 August 2008; Accepted 23 August 2008

Published online 11 November 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22316

subcutaneous nodules at the site of entry.[2–4] Nodules may be tender, and profuse nodule formation is associated with extensive areas of hardened tissue, which present a physical barrier to infusion needle siting and may interfere with absorption of the drug.[5,6] Plasma apomorphine concentrations and Parkinsonian symptoms have been found to vary erratically in patients with profuse nodule formation.[7] An effective treatment for this problem is therefore desirable.

It has been suggested that therapeutic ultrasound (US) may be effective in treating apomorphine nodules,[1,8,9] but there is no published research to support its use. Reports received by our group from apomorphine users and clinicians have suggested that it can ease discomfort, soften nodules, and increase the area available for daily needle insertions. This treatment may therefore have the potential not only to reduce the side effects of apomorphine infusion but also to facilitate its absorption by promoting the resolution of nodules. The purpose of this pilot study was to investigate the effectiveness of US in the treatment of apomorphine-associated nodules and to provide data that may be used in the planning of a full-scale trial. The study design was a pilot randomized controlled clinical trial.

PATIENTS AND METHODS

Participants were recruited from a neurology clinic in University College Hospital, London. Eligible patients, those receiving apomorphine for PD and experiencing nodule formation, were invited to participate. Exclusion criteria were current treatment with, or contraindications to, therapeutic US and living too far away from the research center for domiciliary treatment to be practical. Of 27 people approached, 10 lived too far away for domiciliary treatment, 4 refused to participate, and 1 had very mild symptoms, leaving 12 people for inclusion. Written consent was obtained from all participants. The study protocol was approved by the institutional ethics committee and its scientific review panel.

Trial participants were allocated to receive either real or placebo US using computer-generated randomization. Allocation was concealed from participants and investigators involved in assessment, treatment, and data analysis. Treatment was provided with a US generator adapted to deliver real or sham (zero) US, and neither operator nor patient was aware whether real or sham US was being delivered.

The person responsible for needle siting (either participant or carer) was asked to select, by palpation, two places where they would not normally site a needle because of tissue hardening. In 11 cases, these were on the abdomen, and in one case on the thigh. On each person, one area was treated, and the other was used as a control. Each treatment consisted of a 3 MHz, 0.5 W/cm^2 continuous US beam applied for 5 min using an applicator of 4 cm^2 effective radiating area, applied with light pressure and continuous movement over a marked 16 cm^2 area, via a standard US couplant gel. These parameters were selected on the basis of published guidelines for US treatment of chronic soft tissue lesions.[10] Treatment was home-based, twice a week for 4 weeks. Participants were asked not to use any other form of nodule treatment, such as massage, on the areas selected for this study, and to avoid using them for infusions.

Several outcome measures were employed. Tissue hardness was assessed objectively using a durometer* and subjectively by asking the person responsible for setting up infusions whether they would site an infusion needle in the chosen area, using a yes/no response. Nodule tenderness was measured by pressure algometry,† and sonography‡ was used to assess the extent of tissue changes. These measures have been employed in previous studies characterizing subcutaneous soft-tissue lesions.[11–16] Preliminary investigations by our group demonstrated significant differences in hardness, tenderness, and sonographic appearance of tissue used for apomorphine infusion, when compared with normal tissue (data available from corresponding author). Sonographic changes included focal variations in echogenicity corresponding to palpable nodules, dermal thickening, and diffuse oedematous changes in the adipose tissue.[17] Pre- and post-treatment images were compared to identify the extent of change in these variables, using a scoring system in which shrinkage of nodules and normalization of dermal thickness and echogenicity were quantified. Possible scores were 0, no change; 1, partial normalization; and 2, significant or full normalization of tissue sonographic appearance.

Data were collected before each treatment, after the final treatment and at a follow-up assessment 4 weeks later. Data were also obtained from an adjacent area not used for injection, to establish normal values for each individual. Assessment was carried out by a physiotherapist and a sonographer, and treatment was provided by the physiotherapist. Statistical analysis of algometry and durometry data were carried out using SPSS 14,§ setting statistical significance at $P = 0.05$.

*Type 00 durometer (Rex Gauge Co, IL, USA).

†Type II pressure algometer (Somedic, Hörby, Sweden).

‡Micromaxx 3.4.1 ultrasound unit with 13-6 MHz linear transducer (Sonosite, Hitchin, UK).

§SPSS Inc, Chicago, USA.

TABLE 1. *Characteristics of study participants*

Parameter	Real US group	Sham US group
Sample size	6	6
Sex	2 M, 4 F	2 M, 4 F
Age (yr)	65 (51–72)	64 (47–75)
Time since PD diagnosis (yr)	22 (13–32)	18 (9–25)
Hoehn & Yahr stage (when off)	4 (all)	4 (3–4)
Time on apomorphine (yr)	5 (2–10)	4 (0.25–7)
Infusion rate (mg/hr)	5.6 (5.0–7.0)	6.4 (3.5–13.8)
Infusion time (hr/day)	14 (12–15)	15 (10–24)
Infusion dose (mg/day)	77 (66–103)	73 (47–130)
Infusion site	A=5, T=1, O=1	A=6

Values are given as mean (range). One participant used several sites for infusion. A, abdomen; T, thigh; O, other sites (lower back and posterior shoulder).

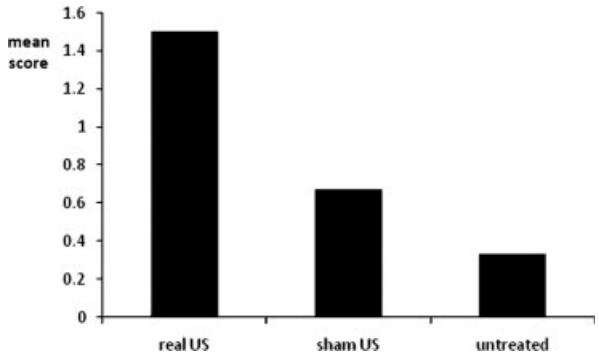

FIG. 1. Mean sonographic improvement score. 0, no improvement; 1, partial improvement; 2, substantial improvement/full normalization of sonographic appearance.

The study sample was too small for formal statistical analysis of sonographic images and the dichotomous response regarding needle siting, and so simple pre- and post-treatment comparisons were made on these variables.

RESULTS

Assessments and treatments took place during the period July to November 2007. Demographic and clinical data for the participants are provided in Table 1. All participants completed the full course of treatment, and infusion rates remained constant during the trial. Several participants inadvertently sited a needle in a marked site during the follow-up period, and so data from this period were not included in the analysis.

Paired *t*-tests demonstrated no significant differences between the real and sham US groups for durometer and algometer values at baseline. Durometry and algometry data were analyzed using a repeated measures ANOVA, which showed no differences over the course of treatment within the real and sham US groups, either for durometry ($df = 8$, $F = 0.863$, $P = 0.555$) or algometry ($df = 8$, $F = 1.578$, $P = 0.162$). Given these findings, no statistical tests for differences between these groups and between them and the control (untreated) areas were attempted on these measures.

The area's suitability for needle siting was assessed by the person responsible for siting. All selected areas were initially judged unsuitable. After treatment, 5 of 6 of the real US group and 1 of 6 of the sham US group said their treated area was now suitable for needle siting. However, 2 of 6 of each group said their control (untreated) area had also become suitable for siting.

Sonographic assessment scores indicated that normalization of tissue appearance was greatest in the tissue treated with real US (see Fig. 1). Five out of six of this group improved, with substantial improvement in four cases. Sham US and untreated tissue also showed some improvement, although it was less substantial than in the real US group.

DISCUSSION

Although average durometry values did not change significantly during the treatment period, most participants in the real US group judged that the treated tissue had softened sufficiently to be used for needle siting. Practical difficulties encountered developing a reproducible durometry technique may account for this discrepancy. Arguably, greater credence should be given to participant opinion because the primary aim of the therapy is to increase the area available for needle placement. The lack of significant differences in algometry readings may be because treatment areas were selected on the basis of their hardness rather than tenderness. Preliminary work suggested that harder areas comprise mostly older nodules, which tend to be less tender than younger ones. The small sample size may also account for the lack of statistically significant change in durometry and algometry.

Sonographic data were suggestive of a therapeutic effect. Reductions in nodule size and dermal thickness were observed, and transitions toward a normal sonographic appearance arguably indicate normalization of tissue structures. However, this interpretation may only be verified by histological correlation. Sonographic normalization did not always coincide with palpable tissue softening, suggesting that in some cases tissue structure may improve, but not sufficiently to enable injection. In some cases, there was evidence of improvement in untreated tissue. This was to be

expected given participants' comments that some nodules resolve spontaneously. Therapeutic US may accelerate this process. It may also be more effective in some presentations than others, or using different treatment parameters.

Were suitability for needle siting to be used as the primary outcome measure, published tables of sample sizes required for binary outcome measures[18] suggest that a full-scale trial would require 30 participants to provide a statistically significant result. A larger trial would enable subgroup analysis to identify prognostic factors for this form of treatment. The study sample was drawn from a single neurology clinic, but the demographic and clinical characteristics of the sample are similar to those reported in other studies.[2,3,19,20]

CONCLUSIONS

Data from this study are encouraging and suggest that a larger scale trial may be justified. Despite its subjectivity, user opinion of a treated area's suitability for injection may be the most practical and clinically meaningful outcome measure. Sonography may help identify variations in tissue response to treatment. A pragmatic trial may be the most appropriate, including other forms of treatment such as massage, and treating a larger area of affected tissue.

Acknowledgments: Financial assistance for this study was provided by the Parkinson's Disease Society and Britannia Pharmaceuticals Ltd. We thank all those who took part in this study, and to Karen Shaw. Thanks to Sonosite, UK, for their assistance in the provision of sonography equipment.

REFERENCES

1. Bowron A. Practical considerations in the use of apomorphine injectable. Neurology 2004;62(6 Suppl 4):S32–S36.
2. Deleu D, Hanssens Y, Northway MG. Subcutaneous apomorphine—an evidence-based review of its use in Parkinson's disease. Drugs Aging 2004;21:687–709.
3. Hagell P, Odin P. Apomorphine in the treatment of Parkinson's disease. J Neurosci Nurs 2001;33:21–38.
4. Pietz K, Hagell P, Odin P. Subcutaneous apomorphine in late stage Parkinson's disease: a long term follow up. J Neurol Neurosurg Psychiatry 1998;65:709–716.
5. Stocchi F, Farina C, Nordera G, Ruggieri S. Implantable venous access system for apomorphine infusion in complicated Parkinson's disease. Mov Disord 2001;14:358.
6. Nicolle E, Pollak P, Serre-Debeauvais F, et al. Pharmacokinetics of apomorphine in Parkisonian patients. Fundam Clin Pharmacol 1993;7:245–252.
7. Manson AJ, Hanagasi H, Turner K, et al. Intravenous apomorphine therapy in Parkinson's disease: clinical and pharmacokinetic observations. Brain 2001;124(Part 2):331–340.
8. Colzi A, Turner K, Lees A. Continuous subcutaneous waking day apomorphine in the long term treatment of levodopa induced interdose dyskinesias in Parkinson's disease. J Neurol Neurosurg Psychiatry 1998;64:573–576.
9. Lees A, Turner K. Apomorphine for Parkinson's disease. Pract Neurol 2002:280–286.
10. Watson T. The role of electrotherapy in contemporary physiotherapy practice. Man Ther 2000;5:132–141.
11. Fischer A. Tissue compliance meter for objective, quantitative documentation of soft tissue consistency and pathology. Arch Phys Med Rehabil 1987;68:122–125.
12. Roberts KL. Reliability and validity of an instrument to measure tissue hardness in breasts. Australian J Adv Nurs 1998;16:19–23.
13. Fischer A. Algometry in diagnosis of musculoskeletal pain and evaluation of treatment outcome: an update. J Musculoskeletal Pain 1998;6:5–32.
14. Nussbaum E, Downes L. Reliability of clinical pressure-pain algometric measurements obtained on consecutive days. Phys Ther 1998;78:160–169.
15. Beggs I. Ultrasound of soft tissue masses. Imaging 2002;14:202–208.
16. Nessi R, Betti R, Bencini PL, Crosti C, Blanc M, Uslenghi C. Ultrasonography of nodular and infiltrative lesions of the skin and subcutaneous tissues. J Clin Ultrasound 1990;18:103–109.
17. Edwards H, Poltawski L, Todd A. Sonographic characterisation of tissue changes associated with infused apomorphine hydrochloride: a case series. Ultrasound 2008;16:155–159.
18. Campbell MJ, Julious SA, Altman DG. Estimating sample sizes for binary, ordered categorical, and continuous outcomes in two group comparisons. BMJ 1995;311:1145–1148.
19. Garcia Ruiz PJ, Sesar Ignacio A, Ares Pensado B, et al. Efficacy of long-term continuous subcutaneous apomorphine infusion in advanced Parkinson's disease with motor fluctuations: a multicenter study. Mov Disord 2008;23:1130–1136.
20. Katzenschlager R, Hughes A, Evans A, et al. Continuous subcutaneous apomorphine therapy improves dyskinesias in Parkinson's disease: a prospective study using single-dose challenges. Mov Disord 2005;20:151–157.

Essential Tremor Might Be Less Frequent Than Parkinson's Disease in North Israel Arab Villages

Amir Glik, MD,[1] Magdalena Masarwa, MD,[1] Amin Abuful, MN,[2] Amar Deeb, BN,[2] Rosa Strugatsky, MD,[1] Lindsay A. Farrer, MD, PhD,[3,4,5,6] Robert P. Friedland, MD,[7] and Rivka Inzelberg, MD[1,8]*

[1]*Department of Neurology and The Sagol Neuroscience Center, Sheba Medical Center, Tel Hashomer, Israel;* [2]*Department of Neurology, Tel Aviv University, Ramat Aviv, Israel;* [3]*Department of Medicine (Genetics Program), Boston University Schools of Medicine and Public Health, Boston, Massachusetts, USA;* [4]*Department of Neurology, Boston University Schools of Medicine and Public Health, Boston, Massachusetts, USA;* [5]*Department of Genetics and Genomics, Boston University Schools of Medicine and Public Health, Boston, Massachusetts, USA;* [6]*Department of Epidemiology and Biostatistics, Boston University Schools of Medicine and Public Health, Boston, Massachusetts, USA;* [7]*Department of Neurology, Laboratory of Neurogeriatrics, Case Western Reserve University, School of Medicine, Cleveland, Ohio, USA;* [8]*Department of Neurology, Rappaport Faculty of Medicine, Technion, Haifa, Israel*

Abstract: **Essential tremor (ET) is much more prevalent than Parkinson's disease (PD) in Western countries. We estimated ET and PD prevalence in Wadi Ara Arabic villages in Northern Israel. In this door-to-door survey, all consenting residents aged ≥65 years were systematically examined by an Arabic speaking team. No prescreening questionnaires were used. A random sample of 900 subjects [437 males, mean age (SD) = 72.6 years (6.6)] of the 2,163 eligible residents were evaluated. Sixteen subjects had an action, intentional tremor. Tremor prevalence was estimated as 1.78% (95% CI 1.1–2.87). Nine of these had another likely cause of tremor. Only 7 patients were diagnosed as ET [prevalence 0.78% (95% CI 0.38–1.6)]. PD was diagnosed in 13 subjects. PD prevalence was 1.44% (95% CI 0.84–2.45). ET is unusually uncommon in this population and possibly even less frequent than PD. The PD prevalence in Wadi Ara is similar to that reported in Western countries.** © 2008 Movement Disorder Society

Key words: epidemiology; prevalence studies; tremor; Parkinson's disease; Arabic

*Correspondence to: Dr. Rivka Inzelberg, Department of Neurology, The Sagol Neuroscience Center, Sheba Medical Center, Tel Hashomer, 52621, Israel.
E-mail: inzelber@post.tau.ac.il or rivka.inzelberg@gmail.com

Potential conflict of interest: None reported.
The first two authors have equally contributed to this study.
Received 20 May 2008; Accepted 27 August 2008
Published online 29 September 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22324

Both essential tremor (ET) and Parkinson's disease (PD) are among the most frequent movement disorders.[1,2] The incidence of both diseases increases with age.[3–6] ET affects between 12 and 23% of the elderly population versus 0.70 to 1.5% affected by PD in the same age group.[7–11] Differences in population and methodology may account for variation in prevalence estimates across studies.[10–15] Door-to-door surveys are the most appropriate way for accurately assessing the prevalence of movement disorders. The detection rate of previously undiagnosed cases can be higher.[16] Our previous observations in Wadi Ara villages in Northern Israel in a small sample of the population, suggested that ET is unusually rare.[17] The paucity of this tremor raises the question whether other movement disorders are also uncommon. We thus conducted a door-to-door study of a larger population sample and estimated the prevalence of both ET and PD.

PATIENTS AND METHODS

Wadi Ara or the Ara Valley is a rural area in Northern Israel whose inhabitants are Arabic Israeli citizens. The population aged ≥65 years included 2,163 residents on prevalence day (January 01, 2003, Israel Central Statistics Bureau). This work is part of a large-scale epidemiological study carried out in Wadi Ara, where residents aged ≥65 years are systematically consecutively approached, reply to questionnaires concerning cardiovascular risk factors, activities of daily living (ADL), life style, cognitive function and undergo full neurological examination.[17,18]

We consecutively approached village houses and examined 900 residents aged ≥65 years at prevalence day. Elderly citizens in this area reside with their children. A fluently Arabic speaking team [nurse (AA) and neurologist (MM)] examined 1 or 2 subjects in each house. The team interviewed all subjects about medical, family history, and medications. The nurse conducted cognitive evaluation, the neurologist performed full neurological examination. All information was reviewed by several neurologists (MM, RS, RI) in bimonthly consensus conferences. The study was approved by the Institutional Ethical Committee, Israel Ministry of Health, Institutional Regulatory Boards of

Case, and Boston Universities. All participants signed a written consent form. Examinations were free of charge and without asking about the health insurance status of the subject.

Diagnostic Criteria for ET

ET diagnosis was based on criteria established and validated by Louis et al.[19,20] Examination included a standardized tremor examination,[19,20] neurological examination, and the motor part of the Unified Parkinson's Disease Rating Scale (UPDRS).[21] Tremor score was calculated as the sum of scores (0–3 each item) in six tests: postural tremor and five tests with each hand (pouring water, spoon drinking, drinking water, finger-to-nose, and drawing).[19]

Diagnosis of definite ET required moderate oscillatory postural tremor usually present during examination, a moderate and clearly oscillatory kinetic tremor in at least one arm during 4 of 5 actions and tremor that by history interfered with ≥1 ADL. Diagnosis of probable ET required moderate, clearly oscillatory kinetic tremor usually present during examination and during 4 of 5 actions. Possible ET required moderate clearly oscillatory kinetic tremor during action. Exclusion criteria included cerebellar signs, parkinsonism, dystonia, peripheral neuropathy, possible drug-related tremor, hyperthyroidism, chronic alcoholism, or anxiety.

Diagnostic Criteria for Parkinson's disease

We used Gelb's criteria for PD diagnosis.[22] Group A features: resting tremor, bradykinesia, rigidity, and asymmetric onset. Group B features: prominent postural instability, freezing or hallucinations ≤3 years after onset, dementia preceding motor symptoms or in the first year, supranuclear gaze palsy, severe symptomatic dysautonomia unrelated to medication, and documentation of a condition plausibly connected to the symptoms. Possible PD was diagnosed if ≥2 Group A were present; at least 1 being tremor or bradykinesia, had either no Group B features, or in cases with symptom duration ≤3 years none of the Group B features is present and with substantial sustained documented response to levodopa (L-dopa), dopamine agonist or the patient did not have an adequate trial of L-dopa or dopamine agonist. Probable PD was diagnosed when ≥3 Group A features and none of the Group B features were present (symptoms ≥3 years) and with substantial sustained documented response to L-dopa or dopamine agonist. Since definite PD requires autopsy confirmation, we had no definite PD cases.

RESULTS

Of the 951 subjects that were approached, 918 accepted to participate in the study (refusal rate 3.5%). Exclusion causes (n = 18): recent head trauma (n = 2), recent ischemic stroke (n = 3), end-stage renal failure (n = 2), metastatic carcinoma (n = 2), systemic disease (n = 7), and severe depression (n = 2). The cohort consisted of 900 subjects [437 men, mean age (SD) 72.6 years (6.6)]. The mean age (SD) of men was 72.7 years (7) and of women was 72.4 years (6.2). The target population aged ≥65 years counts 2,163 residents. The proportion of subjects aged ≥75 years was similar in the target population [n = 709 (33%)] and our cohort [n = 313, (35%); (χ^2 = 1.054, d.f. 1, $P >$ 0.1)]. The gender distribution of the target population included 52% men versus 49% men in the examined population (χ^2 = 2.9, d.f. = 1, P = 0.09).

A postural, kinetic, and clearly oscillatory tremor of moderate amplitude was observed in 16 subjects [8 men, mean age (SD) 74.4 years (6.3), range 66–85]. Tremor prevalence was 1.78% (95% CI 1.1–2.87). Of these, 9 had another possible tremor cause: aminophylline treatment (n = 8) and severe renal failure (n = 1) [5 men, mean age (SD) 74.9 years (6), range 66–83, tremor score 2–20]. One patient had head tremor (83 years, male). None reported a family history of tremor. In all, tremor severity was enough to raise the differential diagnosis of ET. We could not exclude ET with superimposed drug-induced tremor since these two types of tremor were similar. Only 7 subjects [3 men, mean age (SD) 73.9 years (7.3), range 67–85, tremor score 4–25] had a tremor compatible with the possible ET without any additional cause. If all cases with additional causes of tremor would be excluded, the prevalence of ET would be 0.78% (95% CI 0.38–1.6).

Table 1 shows the age-stratified distribution of tremor cases. Prevalence was higher (not significantly) in older age strata (χ^2 = 1.905, d.f. = 2, $P >$ 0.1 all tremor cases; χ^2 = 1.052, d.f. = 2, $P >$ 0.1 possible ET).

PD was diagnosed in 13 subjects [6 men, age (SD) 72.3 years (4.9), range 65–81 years; Hoehn and Yahr stage II (n = 3), III (n = 5), IV and V (n = 5), UPDRS scores: range 10–89]. PD prevalence was estimated as 1.44% (95% CI 0.84–2.45). In addition, one more patient was diagnosed as drug-induced parkinsonism. Two subjects were diagnosed as possible PD and 11 as probable PD. Three subjects were newly diagnosed by our team.

Also Table 1 shows the age-stratified distribution of PD cases and it is observed that the prevalence was

TABLE 1. *Stratification by age and gender; subjects with tremor and PD*

Age group (yr)	Subjects (n)	Tremor (n)	Tremor [*P** (95% CI)]	PD (n)	PD [*P** (95% CI)]
		Possible ET (n)	Possible ET [*P**(95% CI)]		
65–69	349	4	1.14 (0.45–2.91)	4	1.14 (0.45–2.91)
		2	0.57 (0.16–2.06)		
Males	172 (49%)	3	1.74 (0.59–5)	1	0.58 (0.1–3.22)
		1	0.58 (0.1–3.22)		
Females	177 (51%)	1	0.56 (0.1–3.12)	3	1.69 (0.58–4.86)
		1	0.56 (0.1–3.12)		
70–79	415	8	1.9 (0.98–3.76)	8	1.93 (0.98–3.76)
		3	0.72 (0.24–2.1)		
Males	197 (47.4%)	2	1 (0.28–3.63)	5	2.54 (1.09–5.81)
		1	0.50 (0.09–2.82)		
Females	218 (52.5%)	6	2.75 (1.27–5.87)	3	1.38 (0.47–3.97)
		2	0.92 (0.25–3.29)		
≥80	136	4	2.94 (1.15–7.32)	1	0.74 (0.13–4.06)
		2	1.47 (0.4–5.2)		
Males	68 (50%)	3	4.41 (1.51–12.18)	0	0 (0–0.54)
		1	1.47 (0.26–7.87)		
Females	68 (50%)	1	1.47 (0.26–7.87)	1	1.47 (0.26–7.87)
		1	1.47 (0.26–7.87)		
Total	900	16	1.78 (1.1–2.87)	13	1.44 (0.84–2.45)
		7	0.78 (0.38–1.6)		
Males	437 (49%)	8	1.83 (0.93–3.57)	6	1.37 (0.63–2.96)
		3	0.69 (0.24–2.0)		
Females	463 (51%)	8	1.73 (0.88–3.37)	7	1.51 (0.73–3.08)
		4	0.86 (0.33–2.19)		

Prevalence of essential tremor (ET) and Parkinson's disease (PD) stratified by age and gender. Numbers concerning ET are depicted in two rows in each cell. The upper row indicates all subjects with a postural and action tremor and the lower row indicates possible ET diagnosed by stricter criteria.

*P = Prevalence/100.

higher at the age group of 70 to 79 years; only 2 subjects were older than 80 years.

DISCUSSION

We found that ET prevalence is similar to that of PD in Wadi Ara villages. This is an unusual observation since ET is the most common adult movement disorder[19] with a crude prevalence varying between 1.3%[23] and 12.5%[10] above the age of 70 years in some populations. Other studies found a prevalence of 3.5%,[24] 3.9% (≥65 years),[25] 4.8% (≥65 years),[26] and 7%.[15] If all our tremor cases are considered as possible ET, the prevalence would be 1.78% compared with PD prevalence of 1.44%. When all the cases with an additional possible cause of tremor are excluded, ET prevalence is low (0.78%), which is about half of PD prevalence. Whereas the herein estimated prevalence of PD is similar to that observed in Western population,[1,19] but that of ET is considerably lower.[27] The rarity of ET in this door-to-door survey is compatible with our previous in-patient hospital record survey and field observations in a smaller population sample.[17,28] The aim of this study was to verify whether other movement disorders are rare in this population. Our study showed that PD is not less common than in Western populations and that ET is exceptionally uncommon.

Differences of ET prevalence in published studies may stem from methodological variations.[6,19,29] Case finding strategy is an important methodological element.[19,30] Most published studies have been carried out in two phases[12,31,32] using an initial screening step. The strength of our study is in the fact that all individuals were examined with no prior selective steps such as questionnaires, thus providing a more accurate estimate of prevalence. The rate of refusal was low, consecutive houses were approached, thus minimizing selection bias. We relied on findings at neurological examination and not patient complaints in order to prevent reporting bias. We did not find any tremor patient with a positive family history, a finding that could partly contribute to the low prevalence of ET.

In this study two (15%) of the PD cases were newly diagnosed by our team. Previous epidemiological studies have shown a considerable number of de novo cases detected,[33,34] a finding that highlights the impor-

tance of door-to-door screening in PD prevalence surveys. Studies based on known PD subjects tend to underestimate the real frequency of PD.[35]

Our ET prevalence estimate is very low. The observation that the PD prevalence in Wadi Ara is comparable with that in other populations and the fact that we examined every subject in consecutive houses decrease the likelihood that the low ET prevalence is an artifact of selection or reporting bias, health insurance status, or variable access to medical care. Factors that might "protect" the Wadi Ara inhabitants from ET remain to be elucidated.

Acknowledgments: This article is supported in part by the NIH (RO1 AG017173, R01 AG09029, RO1 HG/AG02213) the Joseph and Florence Mandel Research Fund, the Nickman Family, GOJO Corp., the Fullerton Foundation, and the Institute for the Study of Aging.

REFERENCES

1. Shahed J, Jankovic J. Exploring the relationship between essential tremor and Parkinson's disease. Parkinsonism Relat Disord 2007;13:67–76.
2. Jankovic J. Essential tremor: clinical characteristics. Neurology 2000;54:S21–S25.
3. Moghal S, Rajput AH, D'Arcy C, Rajput R. Prevalence of movement disorders in elderly community residents. Neuroepidemiology 1994;13:175–178.
4. Louis ED, Marder K, Cote L, et al. Prevalence of a history of shaking in persons 65 years of age and older: diagnostic and functional correlates. Mov Disord 1996;11:63–69.
5. Findley LJ. Epidemiology and genetics of essential tremor. Neurology 2000;54:S8–S13.
6. de Rijk MC, Tzourio C, Breteler MM, et al. Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European community concerted action on the epidemiology of Parkinson's disease. J Neurol Neurosurg Psychiatry 1997;62:10–15.
7. Trenkwalder C, Schwarz J, Gebhard J, et al. Starnberg trial on epidemiology of Parkinsonism and hypertension in the elderly. Prevalence of Parkinson's disease and related disorders assessed by a door-to-door survey of inhabitants older than 65 years. Arch Neurol 1995;52:1017–1022.
8. de Pedro-Cuesta J. Parkinson's disease occurrence in Europe. Acta Neurol Scand 1991;84:357–365.
9. Morgante L, Rocca WA, Di Rosa AE, et al. Prevalence of Parkinson's disease and other types of parkinsonism: a door-to-door survey in three Sicilian municipalities. The Sicilian Neuro-Epidemiologic Study (SNES) Group. Neurology 1992;42:1901–1907.
10. Rautakorpi I, Takala J, Marttila RJ, Sievers K, Rinne UK. Essential tremor in a Finnish population. Acta Neurol Scand 1982;66:58–67.
11. Elble RJ. Tremor in ostensibly normal elderly people. Mov Disord 1998;13:457–464.
12. Benito-Leon J, Bermejo-Pareja F, Rodriguez J, Molina JA, Gabriel R, Morales JM. Prevalence of PD and other types of parkinsonism in three elderly populations of central Spain. Mov Disord 2003;18:267–274.
13. Rajput AH, Offord KP, Beard CM, Kurland LT. Essential tremor in Rochester, Minnesota: a 45-year study. J Neurol Neurosurg Psychiatry 1984;47:466–470.
14. Brin MF, Koller W. Epidemiology and genetics of essential tremor. Mov Disord 1998;13(Suppl 3):55–63.
15. Dogu O, Sevim S, Camdeviren H, et al. Prevalence of essential tremor: door-to-door neurologic exams in Mersin Province, Turkey. Neurology 2003;61:1804–1806.
16. Claveria LE, Duarte J, Sevillano MD, et al. Prevalence of Parkinson's disease in Cantalejo, Spain: a door-to-door survey. Mov Disord 2002;17:242–249.
17. Inzelberg R, Mazarib A, Masarwa M, Abuful A, Strugatsky R, Friedland RF. Essential tremor prevalence is low in Arabic villages in Israel: door-to-door neurological examinations. J Neurol 2006;253:1557–1560.
18. Inzelberg R, Schechtman E, Abuful A, et al. Education effects on cognitive function in a healthy aged Arab population. Int Psychogeriatr 2007;19:593–603.
19. Louis ED, Ottman R, Hauser WA. How common is the most common adult movement disorder? estimates of the prevalence of essential tremor throughout the world. Mov Disord 1998;13:5–10.
20. Louis ED, Ford B, Frucht S, Barnes LF, X-Tang M, Ottman R. Risk of tremor and impairment from tremor in relatives of patients with essential tremor: a community-based family study. Ann Neurol 2001;49:761–769.
21. Fahn SE, RL. Unified Parkinson's disease rating scale. In: Fahn SM, Calne CD, Goldstein DM, editors. Recent development in Parkinson's disease, 1st ed. New York: Macmillan; 1987. p 153–164.
22. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Arch Neurol 1999;56:33–39.
23. Haerer AF, Anderson DW, Schoenberg BS. Prevalence of essential tremor. Results from the Copiah County study. Arch Neurol 1982;39:750–751.
24. Salemi G, Savettieri G, Rocca WA, et al. Prevalence of essential tremor: a door-to-door survey in Terrasini, Sicily Sicilian Neuro-Epidemiologic Study Group. Neurology 1994;44:61–64.
25. Louis ED, Marder K, Cote L, et al. Differences in the prevalence of essential tremor among elderly African Americans, whites, and Hispanics in northern Manhattan, NY. Arch Neurol 1995;52:1201–1205.
26. Benito-Leon J, Bermejo-Pareja F, Morales JM, Vega S, Molina JA. Prevalence of essential tremor in three elderly populations of central Spain. Mov Disord 2003;18:389–394.
27. Koller W, Busenbark K. Essential tremor. In: Watts R, Koller W, editors. Movement disorders: neurologic principles and practice, 1st ed. New York: McGraw Hill; 1997. p 365–385.
28. Nisipeanu P, Inzelberg R, Strugatsky R, Carasso R. Essential tremor in Jewish and Arabic population in Israel. Mov Disord 2000;15(Suppl 3):100, Abstract.
29. Louis ED, Ford B, Lee H, Andrews H, Cameron G. Diagnostic criteria for essential tremor: a population perspective. Arch Neurol 1998;55:823–828.
30. Meneghini F, Rocca WA, Anderson DW, et al. Validating screening instruments for neuroepidemiologic surveys: experience in Sicily. Sicilian Neuro-Epidemiologic Study (SNES) Group. J Clin Epidemiol 1992;45:319–331.
31. Louis ED, Ford B, Lee H, Andrews H. Does a screening questionnaire for essential tremor agree with the physician's examination? Neurology 1998;50:1351–1357.
32. Benito-Leon J, Bermejo-Pareja F, Louis ED. Incidence of essential tremor in three elderly populations of central Spain. Neurology 2005;64:1721–1725.
33. Kis B, Schrag A, Ben-Shlomo Y, et al. Novel three- stage ascertainment method: prevalence of PD and parkinsonism in South Tyrol, Italy. Neurology 2002;58:1820–1825.
34. de Rijk MC, Breteler MM, Graveland GA, et al. Prevalence of Parkinson's disease in the elderly: the Rotterdam Study. Neurology 1995;45:2143–2146.
35. Schoenberg BS, Anderson DW, Haerer AF. Prevalence of Parkinson's disease in the biracial population of Copiah County, Mississippi. Neurology 1985;35:841–845.

Increased Oxidative Stress in Patients with Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex in the Kii Peninsula, Japan

Satoru Morimoto,[1] Shigeki Kuzuhara, MD, PhD, [1,2] and Yasumasa Kokubo, MD, PhD[1]*

[1]*Department of Neurology, Mie University School of Medicine, Tsu, Japan;* [2]*Department of Neurology, National Center Hospital of Neurology and Psychiatry, Tokyo, Japan*

Abstract: **Amyotrophic lateral sclerosis and Parkinsonism-dementia complex of the Kii peninsula (Kii ALS/PDC) is an endemic and a tauopathy, which shows clinical symptoms of amyotrophy, parkinsonism, and dementia. The objective of this study was to report the role of oxidative stress on Kii ALS/PDC using biochemical analysis. Urinary 8-hydroxydeoxyguanosine (8-OHdG)/creatinine ratio was analyzed in 11 patients with Kii ALS/PDC and 8 normal controls. The mean level of urinary 8-OHdG/creatinine ratio of the patients with Kii ALS/PDC was significantly higher than that of control subjects. Oxidative stress may be implicated in pathogenesis of Kii ALS/PDC.** © 2008 Movement Disorder Society

Key words: oxidative stress; ALS; Parkinsonism-dementia complex; Kii; Guam

Amyotrophic lateral sclerosis (ALS)/Parkinsonism-dementia complex (PDC) is an endemic that exists in Guam island[1] and the Kii peninsula of Japan.[2] ALS/PDC shows clinical symptoms of parkinsonism, amyotrophy, and dementia. Neuropathologically, marked loss of nerve cells associated with abundant neurofibrillary tangles (NFTs), most predominantly in the brainstem and temporal lobe and concomitant ALS pathology involving the upper and lower motor neurons are characteristic. A family history of ALS/PDC in Kii (Kii ALS/PDC) is recorded in more than 70%.[3] Although several hypotheses regarding etiology of ALS/PDC have been proposed,[4,5] the primary cause of ALS/PDC is still unknown. Familial nature and endemic feature of ALS/PDC suggest that the reciprocal action between genetic factor and environmental factor is more likely in its pathogenesis.

Recently, Sato et al.[6] reported that urinary level of 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative damage to DNA, increased remarkably in patients with Parkinson disease, but not with multiple system atrophy. Although it is unknown whether oxidative stress participates with the pathogenesis of ALS/PDC, ALS/PDC and PD share a lot of clinical and neuropathological similarities, i.e., parkinsonism and neuronal loss of substantia nigra. On the basis of these background, we examined whether urinary 8-OHdG increase in the patients with Kii ALS/PDC.

SUBJECTS AND METHODS

We examined 11 patients with Kii ALS/PDC (mean age 72.7 ± 1.98 years, ±SEM, range 65–84) and 8 normal controls (age 74.8 ± 1.13 years, range 69–78). (Table 1) The mean disease duration of Kii ALS/PDC patients was 6.8 ± 1.0 years. Severity of patients with PDC was classified into five stages based on the Modified Hoehn–Yahr (H-Y) classification. We evaluated an ALS patient (Case 5 in Table 1) as stage V because she was bed-ridden with severe ALS symptoms. Mini-Mental State Examination (MMSE) was used to evaluate cognitive function. Smokers and obese subjects were excluded from this study because these factors may influence urinary level of 8-OHdG. This study protocol was approved by the Human Ethics Review Committee of Mie University School of Medicine and informed consent was obtained from the subjects.

Urinary 8-OHdG concentrations were measured with an enzyme-linked immunosorbent assay (ELISA) kit using a monoclonal antibody specific for 8-OHdG (New 8-OHdG Check, Japan Institute for the Control of Aging, Shizuoka, Japan). Urine samples were obtained from each individual between 10 AM and 15 PM and immediately centrifuged at 1,000*g* for 15 minutes and stored at −30°C. Patients and controls avoided physical activity in the last 24 hours before urine sampling. Primitive urine was used for measurement of 8-OHdG as it is. ELISA was carried out in plural times and in a blinded fashion, and the average value was used for statistical analysis. The sensitivity of ELISA ranged from 0.5 to 200 (ng/dL). We also measured urinary creatinine (mg/dL) to adjust for muscle mass. For statistical analysis, the Student's *t*-test was used for comparing patients group and control

*Correspondence to: Dr. Yasumasa Kokubo, Department of Neurology, Mie University School of Medicine, 2-174, Edobashi, Tsu, Mie-ken 514-8507, Japan. E-mail: kokubo-y@clin.medic.mie-u.ac.jp

Potential conflict of interest: None reported.

Received 18 April 2008; Revised 30 August 2008; Accepted 24 September 2008

Published online 29 October 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22362

TABLE 1. *Clinical characteristics of patients with Kii ALS/PDC*

	Clinical Dx	Age	Sex	Duration of the illness (yr)	Severity scale	MMSE	Urinary 8-OHdG/ creatinine ratio ± SD
Control 1	Normal	75	F	–	–	–	21.99 ± 13.94
Control 2	Normal	69	F	–	–	–	16.94 ± 9.53
Control 3	MCI	78	F	–	–	–	18.70 ± 9.66
Control 4	Normal	78	F	–	–	–	12.52 ± 6.17
Control 5	Normal	76	M	–	–	–	17.65 ± 7.16
Control 6	Normal	73	F	–	–	–	21.04 ± 4.97
Control 7	Normal	77	M	–	–	–	12.49 ± 0.11
Control 8	Normal	72	M				10.26
Case 1	PDC	68	F	7	Stage III	10/30	39.06 ± 19.26
Case 2	PDC	66	F	5	Stage IV	29/30	29.49 ± 16.14
Case 3	PDC	68	M	8	Stage IV	18/30	33.30 ± 16.74
Case 4	PDC	71	M	3	Stage V	13/30	34.19 ± 18.24
Case 5	ALS	80	F	3	Stage V	30/30	38.79 ± 7.67
Case 6	PDC	74	M	8	Stage V	0/30	12.51 ± 0.80
Case 7	PDC	75	F	3	Stage III	15/30	28.61 ± 8.92
Case 8	PDC	81	F	7	Stage II	26/30	17.72 ± 6.79
Case 9	PDC	84	F	6	Stage I	23/30	19.76 ± 3.38
Case 10	PDC	68	M	12	Stage IV	0/30	25.04 ± 5.13
Case 11	PDC	65	M	13	Stage IV	0/30	12.25 ± 1.34

MCI, mild cognitive impairment.

group. All data were expressed as mean ± SEM. A P value less than 0.05 denoted a significant difference.

RESULTS

Urinary 8-OHdG/creatinine ratio ranged from10.26 to 22.00 in normal controls and 12.25 to 39.06 in Kii ALS/PDC (Table 1). The mean urinary 8-OHdG/creatinine ratio of patients with Kii ALS/PDC was 26.43, which is higher than that of age-matched control subjects ($P < 0.05$, $P = 0.0153$). (Fig. 1A) The urinary 8-OHdG/creatinine ratio of Kii ALS/PDC patients for each Severity Scale was as follows: stage I: 19.8 ± 10.8 (value 19.76), stage II: 17.7 ± 10.8 (value 17.72), stage III: 33.8 ± 37.6 (range 28.61–39.06), stage IV: 25.0 ± 5.4 (range 12.25–33.30), and stage V: 28.50 ± 6.2 (range 12.51–38.19) (Fig. 1B). Although the urinary 8-OHdG/creatinine ratio increased along the progression of the disease, there was no significant difference between stages I, II and stages III, IV, and V ($P > 0.05$). The urinary 8-OHdG/creatinine mean ratio did not correlated with age ($P = 0.71$) and sex ($P = 0.38$) in patients with Kii ALS/PDC. There is no relationship between score of MMSE and urinary 8-OHdG/creatinine ratio ($P > 0.05$) (Fig. 2).

COMMENT

8-OHdG is produced by reaction between reactive oxygen species (ROS) and guanine residues in DNA. Urinary 8-OHdG is considered a well established biomarker of oxidative stress in DNA because the excised 8-OHdG, that is not compensated by antioxidative agents in the plasma, is excreted in urine.[7] Oxidative damage to DNA is thought to be involved in various neurodegenerative diseases such as Alzheimer disease,[8] Parkinson disease,[9] ALS,[10] and others. Sato et al.[6] reported that urinary 8-OHdG excretion correlates with the normal ageing process and H-Y staging of the patients with PD. And the mean urinary 8-OHdG/creatinine ratio is significantly higher in PD than in age-matched patients with multiple system atrophy and age-matched normal controls. They postulated that urinary 8-OHdG in PD reflects increased systemic levels of oxidative DNA damage that is caused by mitochondrial dysfunction in skeletal muscles.

This study revealed a significant correlation between urinary 8-OHdG/creatinine ratio and having Kii ALS/PDC. Although statistical significance was not apparent, urinary 8-OHdG excretion of Kii ALS/PDC increased between stages I, II and stages III, IV, and V. It also revealed that urinary 8-OHdG excretion of Kii ALS/PDC is not related with cognitive function. These results suggest that oxidative stress is implicated in ALS/PDC, and oxidative stress may affect development of the disease mainly through moderate and severe disease process.

A role of oxidative stress on pathogenesis of ALS/PDC is unknown. The result that score of MMSE did not correlate with urinary 8-OHdG/creatinine ratio may indicate that source of urinary 8-OHdG excretion does not limit to brain. Mitochondria are the most important intracellular source of ROS. In particular, systemic mitochondrial dysfunction is considered in PD, Huntington's disease, diabetes mellitus, and others. The potential mechanism for the increased 8-OHdG in Kii ALS/PDC may be systemic mitochondrial failure including brain, platelets, and skeletal muscles as suggested in PD. Systemic mitochondrial failure should be investigated in ALS/PDC.

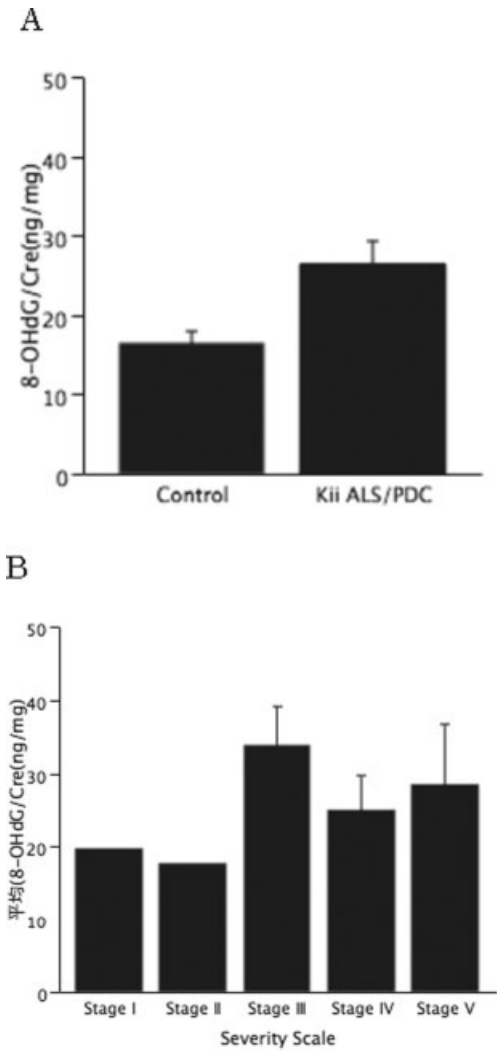

FIG. 1. (**A**) The mean urinary 8-OHdG/creatinine ratio in Kii ALS/PDC and in age-matched controls. (**B**) The mean urinary 8-OHdG/creatinine ratio in each severity stage of Kii ALS/PDC. Data are mean ± SEM. $P < 0.05$.

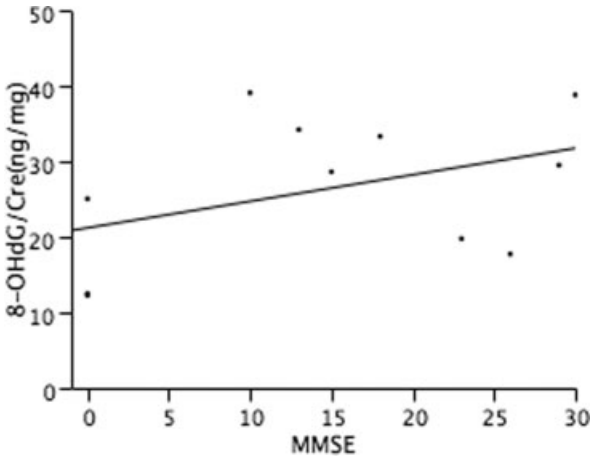

FIG. 2. The relation between urinary 8-OHdG/creatinine ratio and MMSE in Kii ALS/PDC ($P > 0.05$).

CONCLUSIONS

This study suggests that oxidative stress is implicated in ALS/PDC. 8-OHdG levels in CSF, plasma, or brain tissue should be investigated in the patients with ALS/PDC to reveal a role of oxidative stress in its pathogenesis.

Acknowledgments: This study was partly supported by a Grant-in-Aid of the Mie Medical Research Foundation; by a Grant-in-Aid of the Research Committee of CNS Degenerative Diseases, the Ministry of Health, Labor and Welfare, Japan; and by a Grant-in-Aid for the Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. We thank Dr. Akihiro Shindo for collaboration to collect urine samples of control subjects, and we also thank Professor Nobutaka Hattori, Juntendo University, Tokyo, for valuable advice for this study. All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. Hirano A, Malamud N, Elizan TS, et al. Amyotrophic lateral sclerosis and Parkinsonism-dementia complex on Guam. Further pathologic studies. Arch Neurol 1966;15:35–51.
2. Shiraki H, Yase Y. Amyotrophic lateral sclerosis in Japan. In: Vinken PJ, Bruyn GW, Klawans HL, editors. Handbook of clinical neurology, Vol. 22. Amsterdam: North Holland Publishing Company; 1975. p 353–419.
3. Kuzuhara S, Kokubo Y. Atypical Parkinsonism of Japan: amyotrophic lateral sclerosis-Parkinsonism-dementia complex of the Kii peninsula of Japan (Muro disease): An update. Mov Disord 2005;20(Suppl 12):S108–S113.
4. Cox PA, Banack SA, Murch SJ. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Cha-

morro people of Guam. Proc Natl Acad Sci USA 2003;100: 13380–13383.
5. Hermosura MC, Nayakanti H, Dorovkov MV, et al. A TRPM7 variant shows altered sensitivity to magnesium that may contribute to the pathogenesis of two Guamanian neurodegenerative disorders. Proc Natl Acad Sci USA 2005;102:11510–11515.
6. Sato S, Mizuno Y, Hattori N, et al. Urinary 8-hydroxydeoxyguanosine levels as a biomarker for progression of Parkinson disease. Neurology 2005;64:1081–1083.
7. Wu LL, Chiou CC, Chang PY, et al. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta 2004:339: 1–9.
8. Gabbita SP, Lovell MA, Markesbery WR. Increased nuclear DNA oxidation in the brain in Alzheimer's disease. J Neurochem 1998;71:2034–2040.
9. Alam ZI, Jenner A, Daniel SE, et al. Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguaninelevels in substantia nigra. J Neurochem 1997;69: 1196–1203.
10. Ihara Y, Nobukuni K, Takata H, et al. Oxidative stress and metal content in blood and cerebrospinal fluid of amyotrophic lateral sclerosis patients with and without a Cu, Zn-superoxide dismutase mutation. Neurol Res 2005;27:105–108.

Treatment of the Symptoms of Huntington's Disease: Preliminary Results Comparing Aripiprazole and Tetrabenazine

Livia Brusa, MD,[1] Antonio Orlacchio, MD, PhD,[2,3]* Vincenzo Moschella, MD,[3] Cesare Iani, MD,[1] Giorgio Bernardi, MD,[2,3,4] and Nicola Biagio Mercuri, MD[3,4]

[1]UOC Neurologia, Ospedale Sant'Eugenio, Rome, Italy; [2]Laboratorio di Neurogenetica, CERC-IRCCS Santa Lucia, Rome, Italy; [3]Dipartimento di Neuroscienze, Università di Roma "Tor Vergata", Rome, Italy; [4]Laboratorio di Neurologia Sperimentale, CERC-IRCCS Santa Lucia, Rome, Italy

Abstract: **Aripiprazole (AP), a dopamine (DA) D_2 receptor partial agonist, has recently been used to reduce schizophrenic symptoms, while tetrabenazine (TBZ), a DA depletor, has been used to treat hyperkinesias in Huntington's disease (HD). The aim of this study is to define the role of AP on chorea, motor performance, and functional disability, and to compare the effects of AP vs. TBZ in a small study of six patients with HD. Both AP and TBZ increased the Unified Huntington's Disease Rating Scale (UHDRS) chorea score in a similar way. However, AP caused less sedation and sleepiness than TBZ and was better tolerated by the patients on the trial. Moreover, AP showed a slight but not significant improvement of depression in the patients as compared to TBZ. A larger group of patients and a longer period of observation are an important prerequisite for further evaluations of AP's therapeutic use.**

Key words: Huntington's disease; aripiprazole; tetrabenazine; neuroleptics

Huntington's disease (HD) is a rare inherited neurological disorder caused by an unstable trinucleotide repeat expansion in the IT15 gene located on the short arm of chromosome 4p16.3.[1] Its main symptoms are uncontrollable abnormal movements, also known as

Dr. Brusa and Dr. Orlacchio contributed equally to this work.

*Correspondence to: Dr. Antonio Orlacchio, Laboratorio di Neurogenetica, Centro Europeo di Ricerca sul Cervello (CERC), Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Santa Lucia, 64 Via del Fosso di Fiorano, Rome 00143, Italy.
E-mail: a.orlacchio@hsantalucia.it

Potential conflict of interest: None reported.
Received 20 April 2008; Revised 6 October 2008; Accepted 9 October 2008
Published online 17 November 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22376

TABLE 1. *Modifications of the different items in HD patients caused by AP and TBZ*

	Off condition (mean ± SD)	On AP (mean ± SD)	On TBZ (mean ± SD)
UHDRS—Chorea item	10 ± 1.58	4.8 ± 1.30*	4.6 ± 1.67*
UHDRS—Parkinsonism item	24 ± 6.04	21 ± 5.83	21 ± 6.28
MMSE	20.4 ± 1.67	20.2 ± 1.78	20 ± 1.58
ESS	8.8 ± 0.83	9 ± 1	11.2 ± 1.30*,[a]
HDS	13.4 ± 3.43	11 ± 1.2	15.2* ± 4.43[a]

Main factor treatment: $P < 0.01$; Post hoc: $*P < 0.01$ vs. off treatment condition and [a]vs. AP.

HD, Huntington's disease; AP, aripiprazole; TBZ, tetrabenazine; UDHRS, Unified Huntington's Disease Rating Scale; MMSE, Mini-Mental State Examination; EES, Epworth Sleepiness scale; HDS, Hamilton Depression scale.

chorea. HD motor symptoms are treated with typical neuroleptics—mainly dopamine (DA) D2 receptor antagonists—or, more recently, with "atypical" neuroleptics. However, these drugs cause different adverse effects such as loss of voluntary movements, sedation, depression, akathisia, or tardive-dyskinesia (TD).[2] Tetrabenazine (TBZ), a monoamine-depleting agent, has a positive effect on choreic movements, but it also induces sleepiness, parkinsonism, depression, and akathisia.[3] The adverse effects caused by these agents are generally ascribed to the blocking of the DA receptors. Drug-induced adverse effects are particularly disturbing in HD patients who already experienced depression, apathy, and cognitive impairment as the disease progresses. Unlike the typical D2 antagonists, aripiprazole (AP) reduces the activity of DA D2 receptors providing a sufficient stimulation of the dopaminergic system. However, this agent has an antipsychotic effect due to the partial activation of D2 receptors, avoiding excessive receptor stimulation.[4] This mechanism of action reduces the occurrence of neuroleptic-induced TD and hyperkinetic movements during the treatment of schizophrenia and schizoaffective disorders.[5]

On the basis of these peculiar properties, this study aimed to investigate AP's potential role as a therapeutic agent in HD patients. It was found that in comparing AP and TBZ antichoreic effects, AP proved to be particularly effective in all six HD patients.

METHODS

Patients

Subjects for the trials were recruited at the *UOC Neurologia, Ospedale Sant'Eugenio,* in Rome, Italy, and written informed consent was obtained from all six HD patients. The DNA analysis for CAG expansion in the IT15 region of chromosome 4p confirmed the diagnosis in all individuals. The patients clinical characteristics are shown in Table 1 (mean age, 56.3 ± 12.4; age range, 37–76 years). Severe chorea provided adequate ground for pharmacological interventions; patients were classified at Stage 2 to 4 of the Shoulson and Fahn scale.[6] This study excluded patients with dementia, psychosis, and unsatisfactory clinical response to neuroleptics (olanzapine, clozapine, risperidone) because of disturbing extrapyramidal side effects.

Study Design

The aim of this study was to assess the clinical efficacy of AP in a group of six HD patients by comparing its effects with those of TBZ. After a three-week wash-out period from neuroleptics, patients served as their own controls in a crossover study in which the administration of two TBZ daily doses (mean final daily dose 95.83 ± 33.2) or two AP daily doses (mean final daily dose 10.76 ± 4.91) was randomly assigned to the patients for a period of three months. The dosage was titrated to a stable dosage on the basis of individual needs and complaints. All patients were examined by a neurologist blinded to the treatment assignment. Total maximal chorea and parkinsonism were rated by means of the UDHRS-specific subscores, which were administered at the end of the wash-out period and after three months of each drug treatment. Evaluations were performed in the morning, when the drug was found to be more effective. Moreover, the Clinical Global Impression scale (CGI) was obtained by interviewing caregivers and patients; depression was assessed by the Hamilton Depression scale (HDS); sleepiness was assessed using the Epworth Sleepiness scale (ESS); and the Mini-Mental State Examination (MMSE) was used to assess cognitive functions.

Statistical Analysis

The effects of TBZ, AP, and the condition without treatment on the UHDRS were assessed using a Friedman ANOVA for repeated measures, with particular

regard being made to the symptoms of chorea. Differences were considered significant at $P < 0.05$. A post hoc analysis was made using the Wilcoxon test, when required. MMSE, ESS, and HDS results were analyzed by Friedman ANOVA.

RESULTS

The maximum chorea score improved when comparing the period during which the trial drugs were administered with that of the period when no drugs were administered. No significant differences were found by comparing TBZ and AP extrapyramidal side effects (see Table 1). AP treatment indeed resulted in a reduction of 5.2 units ($P > 0$) in chorea severity and TBZ treatment caused a reduction of 5.4 units ($P > 0$). As to the ESS, patients reported an increase in sleepiness when on TBZ in comparison to the wash out and AP. Moreover, a trend toward significance was found in the assessment of depression with the HD scale when patients were on TBZ, as compared to AP. No significant changes were observed in cognitive functions when comparing the treatment conditions.

DISCUSSION

Results show that both AP and TBZ have positive effects on chorea, motor performance, and functional disability in HD patients. Interestingly, HD patients treated with AP reported less feeling of sedation and sleepiness during the wash-out period and the trials of TBZ. As established by our data, sleepiness is the most common adverse side effect of TBZ in HD patients.[3] Although a three-week period might be too short to entirely wash-out the effects of the drugs, we chose this time span so as not prolong any discomfort that the patients may endure as well as not to allow the motor symptoms to worsen.

AP is an atypical antipsychotic with an apparently unique mechanism of action on the dopaminergic system, having a partial agonist effect on D2 and 5-hydroxytryptamine-1A (5HT1A) receptors and an antagonistic effect on 5HT2A receptors.[4] Therefore, the improvement caused by AP could be ascribed to the partial stimulation of the dopaminergic system and to its effect on serotonin receptors.[7] In particular, the differential influence on the dopaminergic system that depends on the level of receptor occupancy might also explain the positive trend of this drug in the treatment of a psychiatric symptom such as depression.[8,9] AP agonistic and antagonistic effects on 5-HT receptors subtypes might affect the acetylcholine release in the ventral tegmental area and the cortex and, therefore, improve mood.[10] Moreover, in order to highlight the beneficial effects of AP compared to TBZ, it is important to note that HD long-term treatment with TBZ might cause severe depression with suicidal ideation.[11] In agreement with the present results, preliminary data showed that, besides classical neuroleptics, other partial agonists of DA D2 receptors could be useful in the treatment of chorea associated with HD.[12] Additionally, in line with the use of partial D2 agonists in hyperkinetic movements, TD patients proved to be better controlled with AP treatment.[13] Moreover, hyperkinetic movements and severe coprolalia typical of Gilles de la Tourette syndrome were also reduced by AP.[14,15]

In conclusion, although preliminary data suggest a potential beneficial effect of AP in HD patients, a full appreciation of AP therapeutic use would require a larger group of trial patients and a longer period of observation.

Contributor Roles: Research project: (1) Conception (LB, AO, NBM), (2) Organization (LB, AO, CI, GB, NBM), (3) Execution (LB, AO, VM, NBM); Statistical Analysis: (1). Design (LB, AO), (2) Execution (LB, AO, VM), (3) Review and Critique (LB, AO, CI, GB, NBM); Manuscript: (1) Writing of the first draft (LB, AO, NBM), (2) Review and Critique (LB, AO, VM, CI, GB, NBM); Administrative, technical, or material support: VM; Literature Search: LB; Funds Collection: AO, GB, NBM.

Acknowledgments: This study was supported by the Italian Ministry of Health (grants no. EBRI1.O, PS05.11, and PS05.21 to A.O.; grants no. REG.13M and RF06.70M to N.B.M.) and the Comitato Telethon Fondazione Onlus, the Amministrazione Autonoma dei Monopoli di Stato (AAMS), and the Italian city of Gubbio (grant no. GGP06209 to A.O.). We thank Michela Renna (MA) for her language advice and Antonella Borreca (BSc Hons) for her assistance.

REFERENCES

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease. Cell 1993;72:971–983.
2. Van Vugt JP, Siesling S, Vergeer M, van der Velde EA, Roos RA. Clozapine versus placebo in Huntington's disease: a double blind randomised comparative study. J Neurol Neurosurg Psychiatry 1997;63:35–39.
3. Kenney C, Hunter C, Jankovic J. Long-term tolerability of tetrabenazine in the treatment of hyperkinetic movement disorders. Mov Disord 2007;22:193–197.
4. Shapiro DA, Renock S, Arrington E, et al. Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. Neuropsychopharmacology 2003;28:1400–1411.
5. Kane JM, Carson WH, Saha AR, et al. Efficacy and safety of aripiprazole and haloperidol versus placebo in patients with schizophrenia and schizoaffective disorder. J Clin Psychiatry 2002; 63:763–771.

6. Shoulson I, Fahn S. Huntington disease: clinical care and evaluation. Neurology 1979;29:1–3.
7. Canas F. Management of agitation in the acute psychotic patient-efficacy without excessive sedation. Eur Neuropsychopharmacol 2007;17:S108–S114.
8. Hirose T, Kikuchi T. Aripiprazole, a novel antipsychotic agent: dopamine D_2 receptor partial agonist. J Med Invest 2005;52:284–290.
9. Gershon AA, Vishne T, Grunhaus L. Dopamine D2-like receptors and the antidepressant response. Biol Psychiatry 2007;61:145–153.
10. Singh AS. Does aripiprazole have a role in treating cognitive impairment in Parkinson's disease? J Neuropsychiatry Clin Neurosci 2007;19:205–206.
11. Huntington Study Group. Tetrabenazine as antichorea therapy in Huntington disease: a randomized controlled trial. Neurology 2006;66:366–372.
12. Tedroff J, Ekesbo A, Sonesson C, Waters N, Carlsson A. Long-lasting improvement following (-)-OSU6162 in a patient with Huntington's disease. Neurology 1999;53:1605–1606.
13. Duggal HS. Aripiprazole-induced improvement in tardive dyskinesia. Can J Psychiatry 2003;48:771–772.
14. Davies L, Stern JS, Agrawal N, Robertson MM. A case series of patients with Tourette's syndrome in the United Kingdom treated with aripiprazole. Hum Psychopharmacol 2006;21:447–453.
15. Ben Djebara M, Worbe Y, Schüpbach M, Hartmann A. Aripiprazole: a treatment for severe coprolalia in "refractory" Gilles de la Tourette syndrome. Mov Disord 2008;23:438–440.

Cardiac and Noncardiac Fibrotic Reactions Caused by Ergot-and Nonergot-Derived Dopamine Agonists

Frank Andersohn, MD[1]*
and Edeltraut Garbe, MD, PhD[1,2]

[1]Bremen Institute for Prevention Research and Social Medicine, University of Bremen, Germany; [2]Institute of Clinical Pharmacology and Toxicology, Charité Universitaetsmedizin Berlin, Germany

Abstract: **There is growing evidence that the ergot-derived dopamine agonists cabergoline and pergolide can cause fibrotic cardiac valvulopathy. Data on other fibrotic reactions and nonergot-derived dopamine agonists are sparse. Aim of this study was to investigate whether there are signals that dopamine agonists are related to cardiac and other fibrotic reactions. We identified all reports of fibrotic reactions at the heart, lung, and retroperitoneal space associated with dopamine agonists within the US Adverse Event Reporting System database. Disproportionality analyses were used to calculate adjusted reporting odds ratios (RORs). For ergot-derived dopamine agonists (bromocriptine, cabergoline, pergolide), the RORs of all reactions under study were increased, whereas no such increases were observed for nonergot-derived drugs (apomorphine, pramipexole, ropinirole, rotigotine). Fibrotic reactions due to ergot-derived dopamine agonists may not be limited to heart valves. For nonergot-derived dopamine agonists, no drug safety signals were evident.** © 2008 Movement Disorder Society

Key words: dopamine agonists; adverse effects; fibrosis; adverse drug reaction reporting systems

Case reports,[1,2] observational,[3] and clinical[4] studies suggest that the ergot-derived dopamine agonists pergolide and cabergoline can cause fibrotic reactions at the heart valves. Case reports of similar endocardial, pericardial, pleuropulmonary, and retroperitoneal reactions have been published for the ergot-derived dopa-

*Correspondence to: Dr. Frank Andersohn, Bremen Institute for Prevention Research and Social Medicine, Linzer Str. 10, D-28359 Bremen, Germany. E-mail: andersohn@bips.uni-bremen.de

Potential conflict of interest: Frank Andersohn reports no financial support or conflicts of interest. Edeltraut Garbe reports receiving consulting fees and an unrestricted grant from Bayer Schering Healthcare AG.

Received 1 August 2008; Revised 11 October 2008; Accepted 16 October 2008

Published online 17 November 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22385

mine agonists pergolide,[5] cabergoline,[6] bromocriptine,[7] and dihydroergocriptine.[8]

It has been discussed whether nonergot-derived dopamine agonists may also cause fibrotic reactions.[9] For valvular heart disease, results from different studies suggest no increased risk with the nonergot dopamine agonists ropinirole and pramipexole.[3,10] In 2003, Müller et al. however reported seven cases of pleuropulmonary changes associated with ropinirole that were submitted to the WHO Uppsala Drug Monitoring Database.[11] Single cases of adverse drug reactions (ADRs) reported within spontaneous reporting systems may, however, have occurred coincidentally during drug use and merely represent the background incidence of such events. To address this issue, disproportionality analyses have been suggested as a useful tool to analyze databases of spontaneous ADR reports.[12] No such analyses have been published for dopamine agonists and fibrotic reactions. We used the freely available US Adverse Event Reporting System (AERS) database[13] to identify reports of cardiac, pleuropulmonary, and retroperitoneal fibrotic reactions associated with ergot and nonergot-derived dopamine agonists. By calculating reporting odds ratios (ROR), an established measure of disproportionality,[14] we analysed whether safety signals were present for ergot and nonergot dopamine agonists for the respective reactions.

METHODS

Data Source and Selection of Reports

Primary data source were initial reports of ADRs submitted to the US Food and Drug Administration (FDA)[13] between January 1, 2004 and September 30, 2007. We identified all reports of fibrotic reactions where a dopamine agonist was reported as the primary or secondary suspected causal drug. Fibrotic reactions included endocardial fibrosis, heart valve regurgitation, pericardial fibrosis, pericarditis, pleural fibrosis, pleuritis, pulmonary changes including pulmonary fibrosis, interstitial lung disease, and alveolitis, or retroperitoneal fibrosis. We screened the selected reports for duplicates to exclude them from further analysis.

Statistical Analysis

Dopamine agonists were classified into ergot-derived (bromocriptine, cabergoline, pergolide) and nonergot-derived (apomorphine; ropinirole; rotigotine; pramipexole) drugs. We also classified dopamine agonists according to the last daily dose of the dopamine agonist if this information was available. We used the median daily dose of all reports as the cut-off value to define a low-medium ($\leq$median) and a high ($>$median) dose group. Cut-off values were 72 mg for apomorphine, bromocriptine 7.5 mg, cabergoline 2 mg, pergolide 1.5 mg, ropinirole 4 mg, rotigotine 4 mg, and pramipexole 2.1 mg. For each patient, we classified the outcome of the reaction in a hierarchical order (patient died; life threatening; disability; hospitalization required; other; unknown).

Patients with at least one fibrotic reaction under study were included as cases and all other patients as noncases. We used SAS PROC LOGISTIC (SAS 8.02 Institute, Cary, NC) to calculate reporting odds ratios (RORs) of fibrotic reactions for dopamine agonists as compared with other drugs. The ROR is a measure of disproportionality used for the purpose of detecting signals in spontaneous ADR reporting databases.[14] Its calculation in a spontaneous adverse event database is identical to the calculation of an odds ratio in a case-control study. It behaves similar as the odds ratio, that is, the higher the ROR, the greater the strength of the signal. We conducted multivariate analyses adjusting for sex, year of initial reporting, and age. In an additional analysis, we characterized patients according to the daily dose of the dopamine agonist. $P < 0.05$, two tailed, was considered significant, and 95% confidence intervals (CIs) were calculated for all RORs. Only significantly elevated RORs which were based $\geq$3 ADR reports were considered as safety signals.

RESULTS

We identified 816,567 reports of ADRs, reporting on a total of 780,665 patients. In 9,576 (1.2%) patients, at least one of the fibrotic reactions under study was reported. Of those, a dopamine agonist was listed in 316 cases as the primary or secondary suspected cause. After exclusion of 18 (5.7%) duplicates, 298 patients with fibrotic reactions associated with dopamine agonists were included in the analysis (Table 1). N = 268 (89.9%) cases were reported in relation to ergot-derived dopamine agonists; N = 24 (8.1%) in relation to nonergot-derived dopamine agonists; and N = 6 (2.0%) were reported for $>$1 suspected dopamine agonist. The largest number of fibrotic reactions was noted for valvular regurgitation, followed by pleural fibrosis/pleuritis and pulmonary changes. Reported case fatality was below 10% for all reactions except for pulmonary changes which were fatal or life threatening in 14 out of 47 (29.8%) cases.

While for ergot-derived dopamine agonists as a group, the RORs of all reactions under study were markedly elevated, this was not the case for nonergot-derived dopamine agonists (Table 2). For individual

TABLE 1. *Patients with fibrotic and inflammatory reactions with dopamine agonists as primary or secondary suspected drugs (N = 298)*

	Valvular regurgitation	Endocardial fibrosis	Pericardial fibrosis/pericarditis	Pleural fibrosis/pleuritis	Pulmonary changes	Retroperitoneal fibrosis
Number of patients*	170	4	27	81	47	16
Sex						
Female	84 (49.4%)	0	17 (63.0%)	25 (30.9%)	10 (21.3%)	8 (50.0%)
Male	57 (33.5%)	4 (100%)	10 (37.0%)	52 (64.2%)	30 (63.8%)	8 (50.0%)
Unknown	29 (17.1%)	0	0	4 (4.9%)	7 (14.9%)	0
Mean age in years (±StdDev)	65.0 ± 9.3	72.0 ± 4.1	65.6 ± 16.3	67.0 ± 12.4	70.0 ± 10.9	66.8 ± 8.1
Severity**						
Patient died	5 (2.9%)	0	1 (3.7%)	4 (4.9%)	7 (14.9%)	0
Life-threatening	15 (8.8%)	0	4 (14.8%)	9 (11.1%)	7 (14.9%)	3 (18.8%)
Disability	9 (5.3%)	0	0	7 (8.6%)	3 (6.4%)	1 (6.3%)
Hospitalisation required	51 (30.0%)	1 (25.0%)	18 (66.7%)	38 (46.9%)	12 (25.5%)	7 (43.8%)
Other	87 (51.2%)	3 (75.0%)	4 (14.8%)	22 (27.2%)	18 (38.3%)	5 (31.3%)
Unknown	3 (1.8%)	0	0	1 (1.2%)	0	0
Suspected dopamine agonist						
Ergot derived	159	4	25	72	37	16
Bromocriptine	3	–	2	5	5	–
Cabergoline	91	–	14	45	21	2
Pergolide	65	4	9	22	11	14
Non-ergot derived	6	–	2	9	9	–
Apomorphine	–	–	–	–	–	–
Pramipexole	2	–	1	4	3	–
Ropinirole	4	–	1	4	6	–
Rotigotine	–	–	–	1	–	–
>1 dopamine agonist suspected	5	–	–	–	1	–

*Sum over all fibrotic reactions exceeds the total number of patients ($N = 298$), as the same patient may occur in >1 reaction category.
**Categorized in a hirarchical order.

nonergot-derived dopamine agonists, there was also no increased ROR, whereas increased RORs were observed for the ergot-derived drugs bromocriptine, cabergoline, and pergolide (Table 2).

A total of 1,441 out of 3,834 (37.6%) cases of ADRs associated with dopamine agonists could be classified according to the last daily dose. For ergot-derived dopamine agonists, the RORs of all reactions except pulmonary changes were higher if used in high daily doses when compared with low daily doses. This was most evident for valvular regurgitation (low dose ROR = 79.1 (95% CI 52.7–114.5); high dose ROR = 288.1 (95% CI 210.5–390.6)) and for pleural fibrosis/pleuritis (low dose ROR = 12.3 (95% CI 7.1–19.7); high dose ROR = 38.1 (95% CI 25.2–55.8)). For nonergot-derived drugs, no influence of the dose was evident, but this analysis was limited by the low number of patients.

DISCUSSION

We identified a substantial number of fibrotic reactions associated with dopamine agonists in the US AERS database. The disproportionality analyses revealed increased RORs for ergot-derived dopamine agonists as a group, whereas no such signals were present for nonergot-derived dopamine agonists. We observed higher RORs for all reactions except pulmonary changes if ergot-derived dopamine agonists were used in high daily doses.

The RORs of valvular regurgitation were increased for pergolide and cabergoline. This ADR has been confirmed in a number of clinical and observational studies.[4] Our analyses also revealed increased RORs of nonvalvular fibrotic reactions with bromocriptine, cabergoline, and pergolide. For pleuropulmonary changes (i.e. pleural fibrosis/pleuritis and other pulmonary changes), signals were present for all ergot-derived dopamine agonists. Case reports and case series of bromocriptine induced pleuropulmonary changes have already been published in the 1980s,[15] followed by similar reports for cabergoline[16] and pergolide.[17] Rinne reported in 1981 that 7 out of 123 (5.7%) patients participating in an open uncontrolled study on long-term therapy with high daily doses of bromocriptine (20 to 90 mg) developed pleuropulmonary changes.[15] Our disproportionality analysis of the US AERS database provides additional evidence for a relationship and indicates that these reactions may have serious consequences for a substantial proportion of affected patients. From the 47 patients with pulmonary changes, about 30% had a fatal or life-threatening course.

We also identified 24 cases of fibrotic reactions associated with nonergot dopamine agonists. In con-

TABLE 2. *Adjusted* reporting odds ratios (RORs) with 95% CI for fibrotic and inflammatory reactions reported as related to dopamine agonists*

	Valvular regurgitation	Endocardial fibrosis	Pericardial fibrosis/pericarditis	Pleural fibrosis/pleuritis	Other pulmonary changes	Retroperitoneal fibrosis
Ergot-derived dopamine agonists	136.6 (113.4–163.6)	126.4 (37.1–327.1)	13.9 (9.1–20.3)	17.2 (13.4–21.8)	8.5 (5.9–11.6)	223.1 (123.4–380.7)
Bromocriptine	13.4 (3.3–35.3)	–	5.7** (0.9–17.8)	6.5 (2.3–14.2)	7.7 (2.7–17.1)	–
Cabergoline	125.4 (98.8–157.6)	–	12.1 (6.8–19.9)	17.2 (12.5–23.1)	7.8 (4.9–11.8)	45.3** (7.4–145.6)
Pergolide	322.3 (234.3–439.5)	711.6 (198.2–≥1,000)	30.8 (14.5–57.3)	28.2 (17.4–43.6)	10.7 (5.4–19.2)	978.9 (496.9–≥1,000)
Nonergot-derived dopamine agonists	1.8 (0.7–3.6)	–	0.4** (0.1–1.1)	0.7 (0.3–1.3)	0.7 (0.4–1.3)	–
Apomorphine	–	–	–	–	–	–
Pramipexole	2.1** (0.4–6.6)	–	0.7** (0.0–2.9)	1.1 (0.4–2.7)	0.8 (0.2–2.0)	–
Ropinirole	2.0 (0.6–4.6)	–	0.3** (0.0–1.3)	0.5 (0.2–1.3)	0.8 (0.3–1.7)	–
Rotigotine	–	–	–	3.6 (0.2–16.8)**	–	–

*Adjusted for age, gender, and year of reporting.
**ROR is based on <3 reports.

trast to ergot dopamine agonists, these cases gave no rise to increased RORs. Agonism at the 5-hydroxytryptamine-2B (5-HT_{2B}) receptor has been suggested as the probable mechanism for fibrotic changes at the heart valves.[18] Nonergot dopamine agonists have low affinity to the 5-HT_{2B} receptors in concordance with results from clinical studies that they do not increase the risk of cardiac valvulopathy. It is, however, unknown whether the same mechanism also applies for noncardiac fibrotic changes. Our observation that nonergot dopamine agonists do not increase RORs for noncardiac fibrotic reactions is therefore of great interest and suggests that safe use of these drugs may not be compromised by noncardiac fibrotic reactions.

Disproportionality analyses are a valuable method to evaluate the impact of single case reports within a pharmacovigilance database. It should be noted, however, that these methods cannot replace observational studies but they can only serve as an indicator for the need of such studies in terms of signal generation. Some further limitations of disproportionality analyses should be considered. For pergolide, there was an FDA alert with respect to cardiac valvulopathy in February 2003, which might have increased reporting of pergolide induced valvulopathy. It has been noted that duplication of records within the FDA AERS database might lead to false positive drug safety signals.[19] We screened all cases of fibrotic reactions associated with dopamine agonists for duplicates and excluded them in the disproportionality analyses. As we excluded only duplicate cases of fibrotic reactions due to dopamine agonists and not those due to other drugs, our analysis rather underestimates than overestimates the true RORs. The inclusion of the duplicate records did not lead to substantial differences in the RORs.

In conclusion, our analysis suggests an increased risk of nonvalvular fibrotic reactions for bromocriptine, cabergoline, and pergolide, but not for apomorphine, pramipexole, ropinirole, or rotigotine. As data on the incidence of fibrotic reactions in patients treated with the different dopamine agonists are only available for valvulopathy, observational studies addressing other fibrotic reactions are similarly needed.

Author Roles: Frank Andersohn: Conception, Organization, Execution of the Research Project. Design and Execution of Statistical Analysis. Writing of the first manuscript draft. Edeltraut Garbe: Conception and Organization of the Research Project. Design, Review and Critique of Statistical Analysis. Review and Critique of Manuscript draft.

REFERENCES

1. Pinero A, Marcos-Alberca P, Fortes J. Cabergoline-related severe restrictive mitral regurgitation. N Engl J Med 2005;353:1976–1977.
2. Van CG, Flamez A, Cosyns B, Goldstein J, Perdaens C, Schoors D. Heart valvular disease in patients with Parkinson's disease treated with high-dose pergolide. Neurology 2003;61:859–861.
3. Schade R, Andersohn F, Suissa S, Haverkamp W, Garbe E. Dopamine agonists and the risk of cardiac-valve regurgitation. N Engl J Med 2007;356:29–38.
4. Antonini A, Poewe W. Fibrotic heart-valve reactions to dopamine-agonist treatment in Parkinson's disease. Lancet Neurol 2007;6:826–829.
5. Agarwal P, Fahn S, Frucht SJ. Diagnosis and management of pergolide-induced fibrosis. Mov Disord 2004;19:699–704.
6. Guptha SH, Promnitz AD. Pleural effusion and thickening due to cabergoline use in a patient with Parkinson's disease. Eur J Intern Med 2005;16:129–131.
7. Pfitzenmeyer P, Foucher P, Dennewald G, et al. Pleuropulmonary changes induced by ergoline drugs. Eur Respir J 1996;9:1013–1019.
8. Oechsner M, Groenke L, Mueller D. Pleural fibrosis associated with dihydroergocryptine treatment. Acta Neurol Scand 2000; 101:283–285.
9. Chaudhuri KR, Dhawan V, Basu S, Jackson G, Odin P. Valvular heart disease and fibrotic reactions may be related to ergot dopamine agonists, but non-ergot agonists may also not be spared. Mov Disord 2004;19:1522–1523.
10. Junghanns S, Fuhrmann JT, Simonis G, et al. Valvular heart disease in Parkinson's disease patients treated with dopamine agonists: a reader-blinded monocenter echocardiography study. Mov Disord 2007;22:234–238.
11. Muller T, Fritze J. Fibrosis associated with dopamine agonist therapy in Parkinson's disease. Clin Neuropharmacol 2003;26: 109–111.
12. Almenoff JS, Pattishall EN, Gibbs TG, DuMouchel W, Evans SJ, Yuen N. Novel statistical tools for monitoring the safety of marketed drugs. Clin Pharmacol Ther 2007;82:157–166.
13. US Food and Drug Administration. Adverse Event Reporting System (AERS). Available at: http://www.fda.gov/cder/aers/default.htm. Accessed on July 31, 2008.
14. Rothman KJ, Lanes S, Sacks ST. The reporting odds ratio and its advantages over the proportional reporting ratio. Pharmacoepidemiol Drug Saf 2004;13:519–523.
15. Rinne UK. Pleuropulmonary changes during long-term bromocriptine treatment for Parkinson's disease. Lancet 1981;1:44–45.
16. Bhatt MH, Keenan SP, Fleetham JA, Calne DB. Pleuropulmonary disease associated with dopamine agonist therapy. Ann Neurol 1991;30:613–616.
17. Bleumink GS, van dM-E, Strijbos JH, Sanwikarja S, van Puijenbroek EP, Stricker BH. Pergolide-induced pleuropulmonary fibrosis. Clin Neuropharmacol 2002;25:290–293.
18. Roth BL. Drugs and valvular heart disease. N Engl J Med 2007; 356:6–9.
19. Hauben M, Reich L, DeMicco J, Kim K. 'Extreme duplication' in the US FDA Adverse Events Reporting System database. Drug Saf 2007;30:551–554.

Sonographic Substantia Nigra Hypoechogenicity in Polyneuropathy and Restless Legs Syndrome

Jana Godau, MD*, Almut Manz, Anne-Kathrin Wevers, Alexandra Gaenslen, MD, and Daniela Berg, MD

Department of Neurodegeneration, Hertie Institute for Clinical Brain Research, University of Tuebingen, Tuebingen, Germany

Abstract: **Substantia nigra (SN) hypoechogenicity assessed by transcranial B-mode sonography (TCS) is typical for idiopathic restless legs syndrome (RLS). Here, we investigated whether SN hypoechogenicity may differentiate between polyneuropathy (PNP) patients with and without RLS. Seventy-five patients with PNP, 65 healthy controls, and 75 patients with idiopathic RLS were investigated. A total of 41.2% patients with PNP additionally suffered from RLS. A total of 44.1% patients with PNP, 10.2% of healthy controls, and 91.2% of patients with idiopathic RLS exhibited SN hypoechogenicity. SN echogenicity did not differ significantly between PNP patients with and without RLS. Thus, TCS seems not suitable for the diagnosis of RLS in patients with PNP.** © 2008 Movement Disorder Society

Key words: ultrasound; restless legs syndrome; neuropathy

Restless legs syndrome (RLS) is, with an age-dependent prevalence of about 10%, one of the most common neurological disorders.[1,2] Often, differentiation of RLS and neuropathic discomfort is quite difficult, because on the one hand, symptoms may look very similar, and on the other hand, both syndromes may often occur combined. Polyneuropathy (PNP) has been described as a major cause of symptomatic RLS, which can be found in up to 40% of patients with primary PNP.[3–5] Cross-sectional studies demonstrated that RLS is very often overlooked in patients with PNP, which might be due to the similarities of the clinical presentation of RLS and neuropathic pain.[4] To date RLS is a purely clinical diagnosis. Polysomnography

The first two authors contributed equally to this work.

*Correspondence to: Dr. Jana Godau, Hertie Institute for Clinical Brain Research, Hoppe-Seyler-Str. 3, 72076 Tuebingen, Germany. E-mail: jana.godau@medizin.uni-tuebingen.de

Potential conflict of interest: Nothing to report.

Received 11 October 2008; Accepted 21 October 2008

Published online 17 November 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mds.22391

TABLE 1. *Clinical data*

				Subgroups	
	Healthy controls	PNP	Idiopathic RLS	PNP+RLS	PNP−RLS
Clinical data					
N	65	68	68	28	40
Age (yr)	61 ± 9	62 ± 10	62 ± 10	58 ± 12	65 ± 9
Gender (m:f)	38:30	36:29	38:30	13:16	25:14
ASO PNP (yr)	−/−	55 ± 13	−/−	52 ± 15	58 ± 12
Duration PNP (mo)	−/−	83 ± 105	−/−	79 ± 104	86 ± 108
Serum ferritin (μg/dL)	−/−	18.7 ± 7.8	17.5 ± 8.9	20.2 ± 9.9	16.5 ± 7.8
Restless legs syndrome					
RLS (%)	−/−	41.1%	100%	100%	−/−
Idiopathic RLS (%)		10.3%	100%	24.1%	−/−
Symptomatic RLS (%)		30.8%	0%	75.9%	−/−
RLS-DI	−14.3 ± 1.6	−0.37 ± 9.3	15.3 ± 2.6	9.0 ± 3.5	−7.4 ± 5.2
ASO RLS (yr)	−/−	51 ± 17	44 ± 20	51 ± 17	−/−
Duration RLS (mo)	−/−	49 ± 111	209 ±194	49 ± 111	−/−
L-Dopa response (%)	−/−	−/−	88.3%	29.4%	−/−
RLS family history (%)	0%	5.9%	57.4%	14.2%	0%
Symptom severity scores					
IRLS	−/−	11.7 ± 14.2	24.9 ± 7.1	24.7 ± 10.2	2 ± 7.2
BDI	3.6 ± 3.5	8.8 ± 6.3	10.0 ± 7.0	10.5 ± 5.9	7.5 ± 6.4
ESS	3.5 ± 2.3	8.5 ± 5.1	8.8 ± 4.3	9.8 ± 5.0	7.5 ± 5.0
Transcranial B-mode sonography					
Sum area of SN echogenicity (cm^2)	0.31 ± 0.08	0.25 ± 0.07	0.18 ± 0.04	0.24 ± 0.08	0.25 ± 0.06
SN hypoechogenicity (%)	10.8%	44.1%	91.2%	60.8%	32.5%

PNP, polyneuropathy; RLS, Restless Legs Syndrome; PNP+RLS, patients with PNP and RLS; PNP−RLS, patients with PNP without RLS; ASO, Age at symptom onset; RLS-DI, RLS diagnostic index; IRLS, International RLS Study Group Rating Scale; BDI, Beck's Depression inventory; ESS, Epworth Sleepiness Scale; SN, substantia nigra.

(PSG) as the current "apparative gold standard" is often not conclusive, because it does not provide RLS-specific markers and PSG data on patients with PNP and symptomatic RLS is limited.[6]

Transcranial B-mode sonography (TCS) has been established as a valuable and reliable method for the diagnosis and differential diagnosis of Parkinson's disease (PD),[7] for review see Berg.[8] The characteristic finding in PD is hyperechogenicity of the substantia nigra (SN), which occurs in more than 90% of the patients. Recently, decreased SN echogenicity has been found to constitute a characteristic feature of patients with RLS.[9] When compared with healthy controls, SN hypoechogenicity discriminated RLS with high sensitivity, specificity, high positive predictive value, and high diagnostic accuracy.[10,11] These findings seem rather promising for the clinical application; however, up to now, there is no data on the potential of TCS in the differential diagnosis of RLS.

In this study, we set out to assess whether TCS assessment of SN echogenicity allows to diagnose RLS in patients with PNP.

METHODS

We investigated 75 patients diagnosed with PNP (for details see results and Table 1) presenting at our outpatients clinic, 67 healthy controls without any sign of neuropathy or RLS, and 73 patients with idiopathic RLS but without any sign of neuropathy. Both control groups were matched to the PNP group for age and sex. All subjects gave written informed consent to participate in this study, which was approved by the local ethical board.

Clinical Examination

All subjects underwent a thorough clinical and neurological examination. PNP diagnosis was defined by the typical clinical presentation and electrophysiological findings. RLS diagnosis was based on the presence of essential and additional diagnostic criteria provided by the International RLS Study Group,[12,13] diagnosis was established using the RLS diagnostic index (RLS-DI).[14] Scores > 3 points were defined as RLS in this study.

TCS

TCS was performed by an independent and experienced examiner who was blinded to the diagnoses and clinical findings. For the examination, a high-end ultrasound machine (Siemens Sonoline Elegra, Siemens, Erlangen, Germany) equipped with a 2.5 MHz transducer was used. The examination was performed

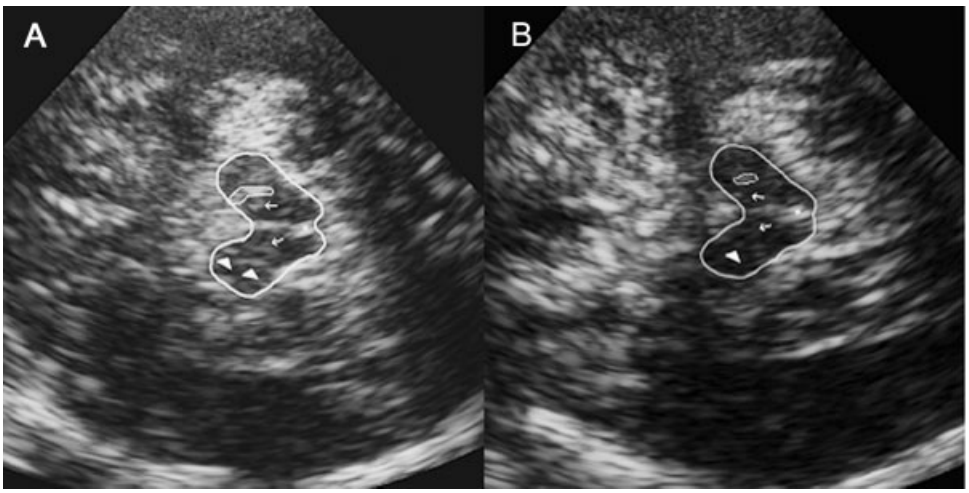

FIG. 1. Substantia nigra ultrasound images. **A**: Normal echogenicity of the substantia nigra (SN). **B**: Hypoechogenicity of the SN. The area of SN echogenicity is smaller than in A. The mesencephalic brainstem is encircled with a full line. For planimetric measurement, the echogenic signal at the anatomical site of the SN is manually encircled (dotted line). Small arrows mark the red nucleus. The aqueduct is marked with a star. The SN contraletral to application of the probe is marked with large arrows.

according to a standardized protocol as described previously.[11,15] The mesencephalic brainstem was visualized through the temporal acoustic bone window separately from both sides. Within the hypoechogenic brainstem, the echogenic area corresponding to the anatomical site of the SN was visualized and measured planimetrically (Fig. 1). The sum area of both sides was taken for further analysis.

Statistics

For determination of a cutoff value for SN hypoechogenicity, receiver operating characteristics (ROC) analysis was performed including all healthy controls and all idiopathic RLS patients (area under the curve: 0.96, 95% CI: 0.94–0.99). The resulting cutoff-value for SN hypoechogenicity as sum area of both sides was 0.23 cm^2 (showing a sensitivity of 91.2% and a specificity of 89.2% for the differentiation of both groups).

Subgroup analysis was performed for patients with PNP and RLS (PNP+RLS) vs. patients with PNP without RLS (PNP−RLS). Additionally, SN echogenicity was correlated with clinical features, including PNP subtypes, disease duration, age, age at symptom onset, serum ferritin levels, and response to dopaminergic treatment.

RESULTS

We examined 215 subjects. The three groups did not differ regarding age (Kruskal-Wallis test, $P = 0.91$) and sex ($P = 0.97$). A total of 6.5% of all subjects had no sufficient temporal acoustic bone window for clear visualization of the SN from both sides, and they were excluded from further analysis. Therefore, results of 68 patients with PNP, 65 healthy controls, and 68 patients with idiopathic RLS are reported.

Clinical Data

An overview on the clinical data of all groups is given in Table 1. A total of 41.2% of the patients with PNP were diagnosed with RLS: 2.9% with certain, 17.6% with probable, 20.6% with possible RLS; in 58.8% RLS was excluded: 4.4% had unlikely RLS, and 54.4% had no RLS according to RLS-DI. Clinical data on the subgroups of PNP patients with RLS (PNP+RLS) and without RLS (PNP–RLS) are given in Table 1.

TCS

Group Analysis

The mean sum area of SN echogenicity in patients with PNP (0.25 ± 0.07 cm^2) was significantly larger than in patients with idiopathic RLS (0.18 ± 0.04 cm^2), but significantly smaller than in healthy controls (0.31 ± 0.08 cm^2, both $P < 0.001$, Fig. 2A). A total of 44.1% of the PNP patients showed SN hypoechogenicity compared with 10.8% of the healthy control group and 91.2% of the idiopathic RLS group.

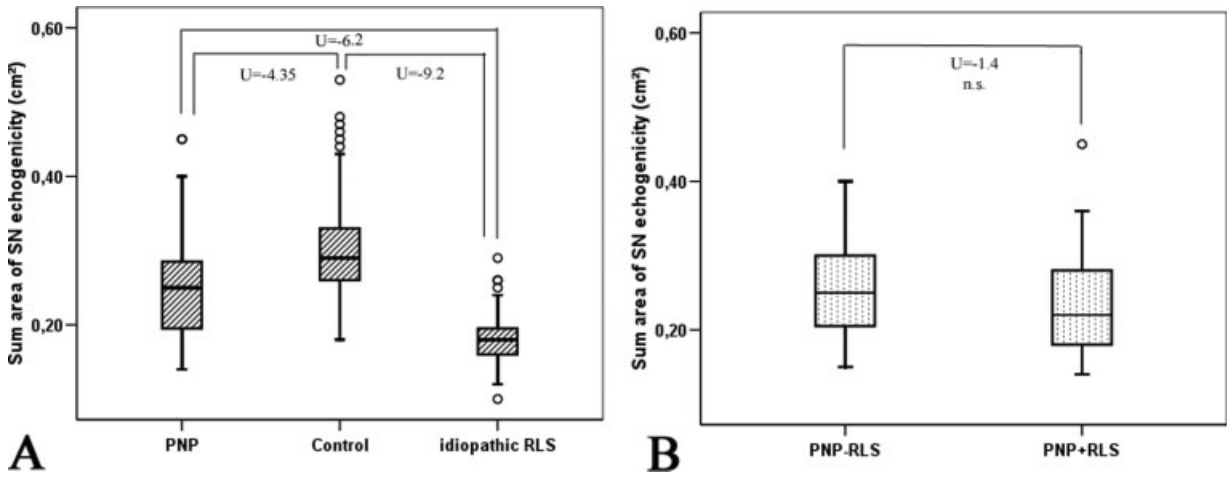

FIG. 2. Substantia nigra echogenicity. **A**: Group analysis. Differences between each pair of groups are significant at $P < 0.001$. **B**: Subgroup analysis for PNP patients with RLS (PNP+RLS) and without RLS (PNP-RLS). Differences are not significant (n.s.).

Subgroup Analysis

PNP+RLS and PNP–RLS patients did not differ significantly regarding SN area of echogenicity (0.24 ± 0.08 cm^2 vs. 0.25 ± 0.06 cm^2, $P = 0.16$, Fig. 2B) and presence of SN hypoechogenicity (60.8 vs. 32.5%, $P = 0.04$, not significant after Bonferoni correction). There were also no differences between PNP+RLS patients, who were diagnosed with either certain, probable or possible RLS (Kruskal-Wallis test, $P = 0.38$). However, RLS occurred more often when SN hypoechogenicity was present (odds ratio: OR = 4.2). Sensitivity of SN hypoechogenicity for RLS in PNP was 60.8%, specificity was 67.5%, positive predictive value was 56.7%, negative predictive value was 71.1%, and classification accuracy was 64.7% in this cohort.

Correlations

In the PNP group, SN echogenicity showed a low, but significant correlation with both the age at PNP onset ($r = 0.32$, $P = 0.01$) and the age at RLS onset ($r = 0.31$, $P = 0.01$). In the PNP-RLS group, SN echogenicity showed a moderately high correlation with age ($r = 0.55$, $P < 0.001$). In the PNP+RLS group, SN echogenicity showed a moderate association with response to treatment with levodopa ($r = 0.45$, $P = 0.004$).

SN echogenicity did not correlate with clinical severity of symptoms as measured by IRLS ($P = 0.65$), BDI ($P = 0.51$) and ESS ($P = 0.44$). There was also no association with serum ferritin levels ($P = 0.72$). We found no association neither with certain subtypes of PNP nor with certain PNP causes.

DISCUSSION

This study aimed to assess whether TCS allows to diagnose RLS in patients with PNP. A total of 41.2% of the patients with PNP were clinically diagnosed with RLS.

SN echogenicity as assessed by TCS differentiated patients with idiopathic RLS and healthy controls with high sensitivity (91.2%), specificity (89.2%), and diagnostic accuracy (AUC = 0.96). This result is consistent with results of previous studies, and therefore confirms the reliability of TCS also in the evaluation of small SN areas of echogenicity. The SN sum area of echogenicity (0.23 cm^2) for best differentiation of patients with idiopathic RLS and healthy controls was taken as the cutoff-value for SN hypoechogenicity in this study.

It was hypothesized that patients with PNP and RLS exhibit SN hypoechogenicity similar to patients with idiopathic RLS, whereas patients with PNP without RLS do not. This hypothesis could not be substantiated with our data, because there was no significant difference between PNP+RLS and PNP−RLS patients regarding SN echogenicity. However, we still found an evidence for an association of SN hypoechogenicity and RLS in patients with PNP: odds ratios showed an increased occurrence for RLS when SN hypoechogenicity was present (OR = 4.2), and SN hypoechogenicity was associated with response to dopaminergic treatment in patients with PNP. These findings underline the pathophysiological association of SN hypoechogenicity and RLS.[9–11] However, TCS seems not suitable to accurately differentiate patients with PNP with those from without RLS.

Interestingly, SN hypoechogenicity was a frequent finding in patients with PNP (44.1%), independent from the diagnosis of RLS. One problem may have been the diagnostic certainty for RLS in patients with PNP. However, the RLS-DI represents the best available clinical diagnostic standard, comprising in addition to the essential diagnostic criteria also additional diagnostic criteria. Moreover, the pathophysiological origin and clinical correlations of SN hypoechogenicity are still unclear. It has been shown in rodent and human postmortem studies that SN echogenicity is related to tissue iron content, suggesting that SN hypoechogenicity may reflect decreased tissue iron load[16–19] what is supported by the finding of reduced SN iron levels in idiopathic RLS. Surprisingly, also PNP patients without RLS showed SN hypoechogenicity more often than healthy controls. Further studies are needed to understand the pathophysiological origin of SN hypoechogenicity as well as its association with PNP. In summary, TCS seems not suitable for diagnosis of patients with RLS and PNP.

Author Roles: JG and DB designed the study, JG wrote up the manuscript. AM and AW were responsible for recruitment and clinical examination. JG and AG performed the ultrasound examinations. AW prepared the patient database, AM and JG performed the statistical analysis. All authors were involved in the correction of the manuscript. DB supervised the study and finalized the manuscript.

REFERENCES

1. Berger K, von Eckardstein A, Trenkwalder C, Rothdach A, Junker R, Weiland SK. Iron metabolism and the risk of restless legs syndrome in an elderly general population—the MEMO-Study. J Neurol 2002;249:1195–1199.
2. Hening W, Walters AS, Allen RP, Montplaisir J, Myers A, Ferini-Strambi L. Impact, diagnosis and treatment of restless legs syndrome (RLS) in a primary care population: the REST (RLS epidemiology, symptoms, and treatment) primary care study. Sleep Med 2004;5:237–246.
3. O'Keeffe ST. Secondary causes of restless legs syndrome in older people. Age Ageing 2005;34:349–352.
4. Nineb A, Rosso C, Dumurgier J, Nordine T, Lefaucheur JP, Creange A. Restless legs syndrome is frequently overlooked in patients being evaluated for polyneuropathies. Eur J Neurol 2007;14:788–792.
5. Gemignani F, Brindani F, Negrotti A, Vitetta F, Alfieri S, Marbini A. Restless legs syndrome and polyneuropathy. Mov Disord 2006;21:1254–1257.
6. Hornyak M, Trenkwalder C. Restless legs syndrome and periodic limb movement disorder in the elderly. J Psychosom Res 2004;56:543–548.
7. Gaenslen A, Unmuth B, Godau J, et al. The specificity and sensitivity of transcranial ultrasound in the differential diagnosis of Parkinson's disease: a prospective blinded study. Lancet Neurol 2008;7:417–424.
8. Berg D. Transcranial sonography in the early and differential diagnosis of Parkinson's disease. J Neural Transm Suppl 2006;70:249–254.
9. Schmidauer C, Sojer M, Seppi K, et al. Transcranial ultrasound shows nigral hypoechogenicity in restless legs syndrome. Ann Neurol 2005;58:630–634.
10. Godau J, Schweitzer KJ, Liepelt I, Gerloff C, Berg D. Substantia nigra hypoechogenicity: definition and findings in restless legs syndrome. Mov Disord 2007;22:187–192.
11. Godau J, Wevers AK, Gaenslen A, et al. Sonographic abnormalities of brainstem structures in restless legs syndrome. Sleep Med 2008;9:782–789.
12. Walters AS. Toward a better definition of the restless legs syndrome. The International Restless Legs Syndrome Study Group. Mov Disord 1995;10:634–642.
13. Allen RP, Picchietti D, Hening WA, Trenkwalder C, Walters AS, Montplaisi J. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep Med 2003;4:101–119.
14. Hogl B, Gschliesser V. RLS assessment and sleep questionnaires in practice—lessons learned from Parkinson's disease. Sleep Med 2007;8 (Suppl 2):S7–S12.
15. Berg D, Becker G. Perspectives of B-mode transcranial ultrasound. Neuroimage 2002;15:463–473.
16. Zecca L, Berg D, Arzberger T, et al. In vivo detection of iron and neuromelanin by transcranial sonography: a new approach for early detection of substantia nigra damage. Mov Disord 2005;20:1278–1285.
17. Berg D, Roggendorf W, Schroder U, et al. Echogenicity of the substantia nigra: association with increased iron content and marker for susceptibility to nigrostriatal injury. Arch Neurol 2002;59:999–1005.
18. Berg D, Grote C, Rausch WD, et al. Iron accumulation in the substantia nigra in rats visualized by ultrasound. Ultrasound Med Biol 1999;25:901–904.
19. Connor JR, Boyer PJ, Menzies SL, et al. Neuropathological examination suggests impaired brain iron acquisition in restless legs syndrome. Neurology 2003;61:304–309.