followed by persistent facial diplegia.[2] Feeding difficulties lasting 15 months were present in one and MG was unrecognized at birth in one of the two mothers. The third child had no neonatal symptoms but he had a "hypotonic face with a high arched palate . . ." and later required speech therapy and pharyngoplasty for "unclear speech."[3] His mother was asymptomatic but subsequently had five pregnancies with fatal AMC. She had high levels of anti-AChR AB which selectively inhibited fetal AChR function with no effect on adult AChR.[3,4] It was thought that the mother developed anti-AChR AB in the latter part of her first pregnancy. The same hypothesis can be made for the child presented here, as an earlier in utero exposure to the AB might have led to more severe disease. The mother of our patient had no further pregnancies but would be at high risk of fetal complications.[3,5] What makes the facial and bulbar musculature susceptible to lasting injury when the motor end-plate is exposed to anti-AChR AB in utero is unknown. The time at which the antibodies first cross the placenta might play an important role. The recorded low CMAPs evoked from the facial muscles and the absence of decrement are compatible with a myogenic process.

Prominent facial and bulbar involvement is also encountered in patients with MG with anti-MuSK AB, but the disease mechanism is also unclear.[6,7]

Neonatal MG should be included in the differential diagnosis of a child with neonatal hypotonia with or without persistent congenital facial and bulbar paresis. The mother should have a thorough neurologic examination as well as an anti-AChR AB titer even if she is thought to be asymptomatic and even if several years have passed since the child's birth. An accurate diagnosis is important given the risk of AMC in further pregnancies.[2]

From the Unit of Pediatric Neurology and Rehabilitation, Department of Pediatrics (P.-Y.J., E.R.-P.), and Nerve-Muscle Unit, Neurology Service (T.K.), Lausanne University Hospital (CHUV); and Hôpital Régional (J.-P.M.), Sion, Switzerland.

Disclosure: The authors report no conflicts of interest.

Received February 5, 2007. Accepted in final form May 22, 2007.

Address correspondence and reprint requests to Dr. P.-Y. Jeannet, Pediatric Neuromuscular Center, Unit of Pediatric Neurology and Rehabilitation, Department of Pediatrics, CHUV, 1011 Lausanne, Switzerland; Pierre-yves.Jeannet@chuv.ch

ACKNOWLEDGMENT

The authors thank Dr. Bruno Eymard from the Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France, for input on this case and critical reading of the manuscript.

1. Engel AG, Hohlfeld R. Acquired autoimmune myasthenia gravis. In: Engel AG, Franzini-Armstrong C, eds. Myology 3rd ed. New York: McGraw-Hill Inc., 2004:1755–1790.
2. Eymard B, Morel E, Dulac, et al. Myasthenia and pregnancy: clinical and immunologic study of 42 cases (21 neonatal myasthenia cases). Rev Neurol 1989;145:696–701.
3. Brueton LA, Huson SM, Cox PM, et al. Asymptomatic maternal myasthenia as a cause of the Pena-Shokeir phenotype. Am J Med Genet 2000;92:1–6.
4. Vincent A, Newland C, Brueton L, et al. Arthrogryposis multiplex congenita with maternal autoantibodies specific for a fetal antigen. Lancet 1995;346:24–25.
5. Barnes PRJ, Kanabar DJ, Brueton L, et al. Recurrent congenital arthrogryposis leading to a diagnosis of myasthenia gravis in an initially asymptomatic mother. Neuromuscul Disord 1995;5:59–65.
6. Evoli A, Tonal PA, Padua L, et al. Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. Brain 2003;126:2304–2311.
7. Farrugia ME, Robson MD, Clover L, et al. MRI and clinical studies of facial and bulbar muscle involvement in MuSK antibody-associated myasthenia gravis. Brain 2006;129:1481–1492.

T. Obi, MD, PhD
K. Nishioka, MD
O.A. Ross, PhD
T. Terada, MD
K. Yamazaki, MD, PhD
A. Sugiura, MD
M. Takanashi, MD, PhD
K. Mizoguchi, MD, PhD
H. Mori, MD, PhD
Y. Mizuno, MD
N. Hattori, MD, PhD

CLINICOPATHOLOGIC STUDY OF A *SNCA* GENE DUPLICATION PATIENT WITH PARKINSON DISEASE AND DEMENTIA

There is evidence that α-synuclein gene (*SNCA*) point mutations and gene multiplications play a pivotal role in the development of Lewy bodies (LBs) and Lewy neuritic (LN) pathology. Dysregulation of the production/degradation of the α-synuclein protein, a major component of LBs and LNs, is speculated to result in its accumulation and produce the neuropathological features of *SNCA*-related neurodegeneration.[1]

The identification of *SNCA* multiplications in families with parkinsonism suggests that *SNCA* gene dosage may play a role in the onset of Parkinson disease (PD).[2] Most patients with *SNCA* triplication develop cognitive and autonomic dysfunction in early stage of the disease.[3,4] However, three families with *SNCA* duplication have been reported with symptoms more reminiscent of typical PD.[5,6] Interestingly, a recent study reported that one triplication (a Swedish-American pedigree) and one duplication pedigree have a common ancestor.[7] As a common mechanism of multiplications, the area including the *SNCA-MMRN1* locus could play a role in multiple copy numbers. Another multiplication family has been reported also to have the rearrangement change in

the same region indicating the *SNCA-MMRN1* locus may be fragile.[2,5-7]

With regard to clinical aspects, patients with *SNCA* duplication tend to have milder symptoms compared to those with triplication.[5-7] The onset of disease in *SNCA* duplication patients occurs approximately 15 years later (50 years of age) than that of *SNCA* triplication families (35 years of age). These features suggest that differences in genetic copy numbers could influence the clinical features of PD.

Recently, we identified two families of Japanese origin with *SNCA* duplications.[8] One patient from Family B (named as B-1) developed severe parkinsonism and dementia. These findings indicate that *SNCA* duplication also causes PD with dementia (PDD). Three copies of the locus *SNCA-MMRN1* were identified in the two duplication families. The length of this region was less than 400 kb and smaller than that reported for the previous, *SNCA* multiplication families. The patient (B-1) died of pneumonia in 2006. Herein we assessed the clinical aspects and autopsy findings and propose a relationship between *SNCA* duplication and dementia.

Clinical findings. The patient was a Japanese man. At age 47 years, the first symptom presented was a gradual-onset clumsiness of the hands. Neurologic examination showed mild masked face, hypophonic dysarthria, resting tremor of the left hand, reduced arms swing and slowing of alternating movements of the left hand, and moderate rigidity of the neck and left-side extremities. Administration of levodopa improved the parkinsonism, including tremor, bradykinesia, and rigidity. Some loss of memory and visual hallucinations appeared at age 60, and at age 61, the Mini-Mental State Examination score was 17/30. At 62 years of age, he developed fluctuation of consciousness and showed resting tremor of the left hand, moderate masked facial expression, severe hypophonic dysarthria and dysphagia, and symmetric rigidity of the neck and extremities. Brain MRI at that stage showed medial temporal lobe atrophy on both sides and widening of Sylvian fissures. A 99m-TcECD SPECT study showed hypoperfusion predominantly on both frontotemporal lobes and occipital lobes. The H/M ratio of MIBG myocardial scintigraphy was reduced (early: 1.20, late: 1.09). Polysomnography revealed abnormal behaviors in REM sleep. The apolipoprotein E phenotype was ε3/3. He became bedridden at 64 years of age and died of aspiration pneumonia at age 67 years. Consent was obtained from the relatives for autopsy examination.

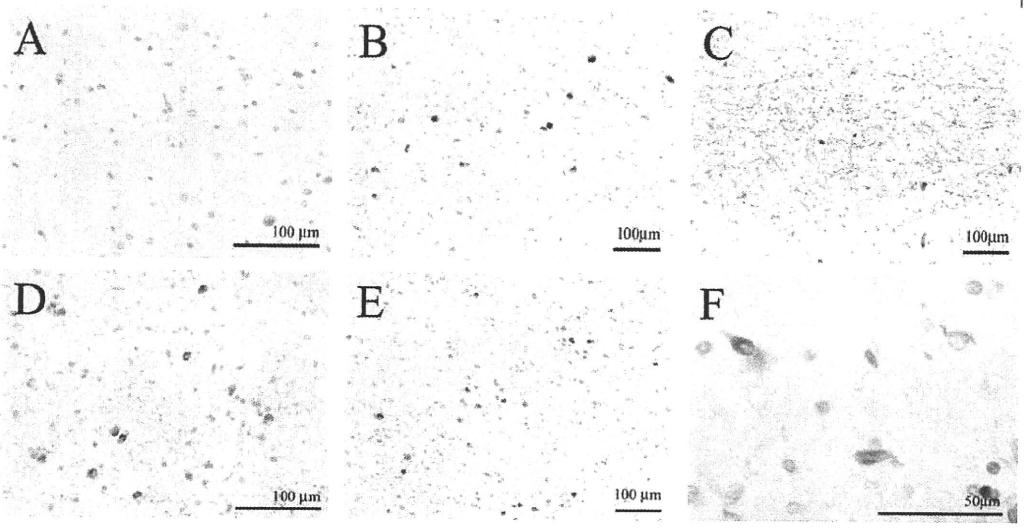
Pathologic methods. Autopsy was performed within 3 hours after death. The left side of the brain was dissected out and stored at −70 °C. The right side of the brain was fixed in neutral buffered formalin. Sections of representative areas were stained with hematoxylin-eosin (H-E), Kluever-Barrera (KB), and immunohistochemical methods. For immunohistochemical staining, we used the following primary antibodies: rabbit anti-α-synuclein polyclonal antibody (Chemicon, Temecula, CA), monoclonal antibody to α-synuclein (human, 15G7, Alexis Biochemicals, Switzerland), anti-phosphorylated α-synuclein (monoclonal, Wako , Osaka, Japan), anti-phosphorylated tau (AT8, monoclonal, Fitzgerald, Concord, MA), and anti-amyloid β 1-42 (polyclonal, IBL, Takasaki, Japan). Lewy-related pathology was assessed according to the third Consensus Guidelines for Dementia with Lewy bodies (DLB).[9]

Neuropathologic findings. The brain weight was 1350 g. Macroscopic examination showed mild frontal lobe atrophy, and severe depigmentation of the substantia nigra and locus ceruleus. Microscopically, severe loss of melanin-containing neurons and gliosis were seen in the substantia nigra pars compacta. Severe neuronal loss was also noted in the locus ceruleus, dorsal motor nucleus of the vagus nerve and the amygdala, and the nucleus basalis of Meynert (nbM). Other findings included moderate neuronal loss with gliosis in the CA2/3 in the hippocampus (figure, A), and LBs in the substantia nigra, locus ceruleus, nbM, small neurons of the oculomotor nuclei, and dorsal motor neurons of the dorsal motor nucleus of vagus nerve. Some pale bodies were present in the remaining neurons of the substantia nigra.

Immunocytochemical staining for α-synuclein showed many LBs in the cerebral cortex, and especially in the entorhinal, insular, and cingulate cortices, and in nbM, amygdala, hippocampus, and brainstem (figure, B and D). In addition, we observed an abundance of LNs in the CA2 of the hippocampus (figure, C) and many Lewy dots in the transentorhinal cortex, which were particularly evident in sections immunostained with anti-phosphorylated α-synuclein antibody (figure, E). There was a moderate deposit of LBs in the sympathetic ganglia. In contrast, only a few LBs were noted in the substantia nigra and locus ceruleus. No striking features were detected in putamen, globus pallidus, and thalamus.

Oligodendrocytes with inclusions, which were shown by α-synuclein immunohistochemistry,

Figure Hematoxylin-eosin (H-E) and anti-phosphorylated α-synuclein results



(A) Severe neuronal loss in the CA3 region of the hippocampus. H-E staining. (B) An abundance of Lewy bodies (LBs) in the CA3 region of the hippocampus. Anti-phosphorylated α-synuclein staining. (C) An abundance of Lewy neurites in the CA2 region of the hippocampus. Anti-phosphorylated α-synuclein staining. (D) Many anti-α-synuclein-positive LBs in the amygdala. Anti-phosphorylated α-synuclein staining. (E) Many LBs in transentorhinal. Anti-phosphorylated α-synuclein staining. (F) Many appearances of oligodendrocytes with inclusions in the substantia nigra. Anti-phosphorylated α-synuclein staining.

were noted in the substantia nigra and other regions including the cingulate gyrus, amygdala, nbM, transentorhinal cortex, substantia nigra, pontine base, cerebellar white matter, and spinal cord (figure, F).

The extent of Lewy-related pathology was graded as diffuse neocortical type based on the pathologic classification of DLB[9] (table). Immunohistochemical staining for tau identified few neurofibrillary tangles in the transentorhinal cortex and amygdala as well as a few diffuse-type senile plaques in the parietal cortex.

Molecular analysis. *SNCA* duplication in Family B was confirmed and refined with copy number variation analysis using the Affymetrix 250K SNP array (Affymetrix, Santa Clara, CA), quantitative real time PCR (ABI prism 7700 sequence detector; Applied Biosystems, Foster City, CA), and FISH analysis as reported previously.[7,8]

Discussion. Recent studies have reported the presence of abundant LBs in the cerebral cortex of PDD, indicating that these neuropathologic features are similar to those of DLB.[10,11] The pathologic features of *SNCA* triplication families (Iowa and Swedish-American pedigree) have been described previously.[3,4,12] These findings indicated that severe neuronal loss and gliosis in the hippocampus (CA2/3) is a striking feature. Severe degeneration of the substantia nigra and locus ceruleus was also common and numerous LBs appeared throughout the brain including the cerebral cortex. The pathology was described as diffuse LB disease, which corresponds to the designation of diffuse neocortical type in DLB. In our patient with *SNCA* duplication, the distribution of LBs also corresponds to diffuse neocortical type based on the pathologic classification of DLB.[9] Oligodendrocytes with inclusions were also seen in our patient, similar to those patients with *SNCA* triplication.[12,13]

These findings suggest that the pathomechanism of *SNCA-MMRN1* multiplication could have a common pathway in families, based on the

Table Pattern of Lewy-related pathology in brainstem, limbic, and neocortical regions

Brainstem regions			Basal forebrain/limbic regions				Neocortical regions		
IX-X	LC	SN	nbM	Amygdala	Transentorhinal	Cingulate	Temporal	Frontal	Parietal
3	3	2	3	3	3	3	2	2	0

IX = 9th cranial nerve nucleus; X = 10th cranial nerve nucleus; LC = locus ceruleus; SN = substantia nigra; nbM = nucleus basalis of Meynert; 1 = mild; 2 = moderate; 3 = severe; 4 = very severe.

overexpression of the α-synuclein protein in the blood and brain of patients with triplication.[3,14] This gain of function mechanism causing abundant α-synuclein expression could explain the presence of LBs in many areas of the brain. In advanced stages, patients with *SNCA* multiplication perhaps tend to display abundant LBs, in association with severe parkinsonism and dementia, with the pathologic features relating directly to the clinical symptoms.

Apart from the copy number of *SNCA*, other factors such as aging are inextricably involved in the age at onset of dementia. However, the important message is that not only *SNCA* triplication but also *SNCA* duplication could induce severe dementia. Thus, multiple copies of the *SNCA-MMRN1* region are an important cause of parkinsonism with dementia.

From the Department of Neurology (T.O., T.T., K.Y., A.S., K.M.), Shizuoka Institute of Epilepsy and Neurological Disorders; Department of Neurology (K.N., T.M., H.M., Y.M., N.H.), Juntendo University School of Medicine, Tokyo, Japan; and Department of Neuroscience (O.A.R.), Mayo Clinic College of Medicine, Jacksonville, FL.

Disclosure: The authors report no conflicts of interest.

Received December 31, 2006. Accepted in final form May 25, 2007.

Address correspondence and reprint requests to Prof. Nobutaka Hattori, Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo, Tokyo 113-8421, Japan; nhattori@med.juntendo.ac.jp

ACKNOWLEDGMENT

The authors thank Masako Itaya for preparing and staining the brain tissue samples.

1. Spillantini MG, Schmidt ML, Lee VM, et al. Alpha-synuclein in Lewy bodies. Nature 1997;388:839–840.
2. Singleton AB, Farrer M, Johnson J, et al. alpha-Synuclein locus triplication causes Parkinson's disease. Science 2003;302:841.
3. Farrer M, Kachergus J, Forno L, et al. Comparison of kindreds with parkinsonism and alpha-synuclein genomic multiplications. Ann Neurol 2004;55:174–179.
4. Muenter MD, Forno LS, Hornykiewicz O, et al. Hereditary form of parkinsonism–dementia. Ann Neurol 1998;43:768–781.
5. Ibanez P, Bonnet AM, Debarges B, et al. Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. Lancet 2004;364:1169–1171.
6. Chartier-Harlin MC, Kachergus J, Roumier C, et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 2004;364:1167–1169.
7. Fuchs J, Nilsson C, Kachergus J, et al. Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication. Neurology 2007;68:916–922.
8. Nishioka K, Hayashi S, Farrer MJ, et al. Clinical heterogeneity of alpha-synuclein gene duplication in Parkinson's disease. Ann Neurol 2006;59:298–309.
9. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005;65:1863–1872.
10. Mattila PM, Rinne JO, Helenius H, et al. Alpha-synuclein-immunoreactive cortical Lewy bodies are associated with cognitive impairment in Parkinson's disease. Acta Neuropathol (Berl) 2000;100:285–290.
11. Mori H. Pathological substrate of dementia in Parkinson's disease–its relation to DLB and DLBD. Parkinsonism Relat Disord 2005;11 Suppl 1:S41–45.
12. Gwinn-Hardy K, Mehta ND, Farrer M, et al. Distinctive neuropathology revealed by alpha-synuclein antibodies in hereditary parkinsonism and dementia linked to chromosome 4p. Acta Neuropathol (Berl) 2000;99:663–672.
13. Wakabayashi K, Yoshimoto M, Tsuji S, et al. Alpha-synuclein immunoreactivity in glial cytoplasmic inclusions in multiple system atrophy. Neurosci Lett 1998;249:180–182.
14. Miller DW, Hague SM, Clarimon J, et al. Alpha-synuclein in blood and brain from familial Parkinson disease with SNCA locus triplication. Neurology 2004;62:1835–1838.

Y.P. Ning, MD, PhD
K. Kanai, MD, PhD
H. Tomiyama, MD
Y. Li, MD
M. Funayama, PhD
H. Yoshino, BS
S. Sato, MD, PhD
M. Asahina, MD, PhD
S. Kuwabara, MD, PhD
A. Takeda, MD, PhD
T. Hattori, MD, PhD
Y. Mizuno, MD
N. Hattori, MD, PhD

PARK9-LINKED PARKINSONISM IN EASTERN ASIA: MUTATION DETECTION IN *ATP13A2* AND CLINICAL PHENOTYPE

PARK9, a form of autosomal recessive parkinsonism, or Kufor-Rakeb syndrome (KRS), is characterized by subacute or slowly progressive, juvenile-onset, levodopa-responsive parkinsonism, pyramidal signs, dementia, and supranuclear gaze palsy.[1-5] Recently, *ATP13A2* was identified as the causative gene for *PARK9* in Chilean and Jordanian families.[4] This gene contains 29 exons encoding a lysosomal type 5 P-type ATPase. Six mutations have been reported in only five probands so far.[4,5] Here, we describe a Japanese patient with KRS with a novel mutation who developed early onset parkinsonism, dementia, and other features. We also describe PET findings of *PARK9*-linked parkinsonism.

Methods. Haplotype analysis was conducted in 117 (mainly Japanese) patients with early onset (≤50, 26.8 ± 11.7 years, mean ± SD) parkinsonism. Among them, 14 patients had dementia. Patients who exhibited homozygosity on *PARK9* locus by haplotype analysis underwent direct sequencing for all 29 exons (e-Methods on the *Neurology*® Web site at www.neurology.org); the remaining patients underwent direct sequencing for exons 13, 16, and 26, in which mutations have been identified.[4] The methods of direct sequencing, sequences of the primers, and PCR conditions are available (table e-1). The study was approved by the ethics committee of Juntendo University and all subjects gave informed consent.

Results. Twenty-eight of 117 patients exhibited homozygosity on *PARK9* locus. Among them, we found a Japanese proband (Family A) with a novel homozygous F182L (c.546C>A) mutation (figure e-1A). The consanguineous parents and the other two unaffected siblings had heterozygous F182L mutation. This mutation was not detected by direct sequencing of exon 6 in 300 chromosomes of normal controls.

Haplotype analysis showed homozygosity spanning the *PARK7* and *PARK9* regions (figure e-1B) in the proband and heterozygosity in her parents and the other two unaffected siblings. No causative mutation was detected in *DJ-1* and *PINK1* in all patients.

The clinical features of the proband, a 43-year-old woman, are described in the table, the e-Case report, the video, and figure e-2. Neuroimaging showed several interesting findings: MRI showed diffuse brain and spinal cord atrophy, and ^{18}F-dopa PET study revealed reduced uptake in the striatum bilaterally (figure e-2).

Discussion. The cardinal features and diffuse brain atrophy of the proband closely resembled previously reported ones.[1-5] Therefore, it was possible that this patient was given a diagnosis of KRS clinically. Genetically, phenylalanine-182 is highly conserved throughout most species (figure e-1C). It has been reported that missense mutations in the loop between the transmembrane segment of the membrane protein (including *ATP13A2*) could affect disease phenotype significantly.[5] These findings and absence of F182L in normal controls support that the homozygous F182L mutation causes KRS.

Our findings of *ATP13A2* mutation in a Japanese family together with the reported Jordanian, Chilean, Brazilian, and Italian cases suggest that *PARK9* exists worldwide though rearrangements could not be excluded.[1-5] The role of a single heterozygous mutation remains unclear, although two symptomatic Italians and two asymptomatic Brazilian and four asymptomatic Japanese carriers have been reported.[5]

The clinical symptoms of our patient were similar to those reported previously.[1-5] However, there were also some different findings. Our patient was comparatively older at onset (22 years), without subacute onset like the Brazilian with homozygous missense mutation,[5] a slower progression rate compared with the Jordanian family (time of progression to bed-ridden state = 20 years vs 12 months),[1,3,4] and no apparent motor fluctuation. Our patient also showed inconsistent levodopa responsiveness with severe drug-induced psychosis and amyotrophy. These differences might be due to the different mutation types (such as missense/truncation mutations) or the different mutation localization.

A new interesting aspect of our report is neuroimaging in KRS. Although peripheral neuropathy was not apparent (e-Case report), our patient had generalized brain and spinal cord atrophy on MRI, which might reflect pyramidal tract degeneration and also multisystemic neurodegeneration in KRS by *ATP13A2* mutation. The pyramidal symptoms and weakness of the lower limbs, described previously in patients with KRS,[1] also could be caused by spinal cord atrophy.

PET findings of patients with levodopa-responsive autosomal recessive parkinsonism with *parkin*, *PINK1*, or *GBA* single heterozygous mutation indicate presynaptic dopaminergic dysfunction

Supplemental data at www.neurology.org

Table Clinical features of patients with Kufor-Rakeb syndrome[1-5]

Origin of family	Japanese	Chilean				Jordanian				Brazilian	Italian	Italian
Patient	A	II-8	II-9	II-10	II-11	V44	V48	V49	V53	BR-3042	VE-29	PK-69-01
Zygosity	Homo	Comp hetero				Homo				Homo	Hetero	Hetero
Mutation	F182L	1019GfsX1021/1306+5G→A				552LfsX788				G504R	T12M	G533R
Age at onset, y	22	18	17	15	12	12	15	13	12	12	30	40
Disease duration, y	21	27	26	26	26	24	19	18	11	10	5	16
Initial symptoms	G	B, M	B, R	B, M	D	B, M, R	B, R	M, R	B, R	B	N/A	N/A
Clinical signs												
Increased muscle tone	+	+	+	+	+	+	+	+	+	+	+	+
Babinski sign	+	+	−	+	+	+	+	+	+	−	−	−
Palmomental reflex	+	+	−	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Tremor	+	+	+	+	+	−	−	−	−	−	+	−
Rigidity	+	+	+	+	+	+	+	+	+	+	+	+
Bradykinesia	+	+	+	+	+	+	+	+	+	+	+	+
Slowed saccade eye movement												
Vertical	+	N/A	−	+	+	+	+	+	+	N/A	N/A	N/A
Horizontal	−	N/A	−	+	+	+	+	−	−	N/A	N/A	N/A
Supranuclear upgaze palsy	+	+	−	+	+	+	+	+	+	+	−	−
FFF mini-myoclonus	+	+	−	+	+	+	+	+	+	−	N/A	N/A
Hallucination	+	+	+	−	−	+	+	+	+	+	−	+
Dementia (MMSE)	15/30	N/A	19/30	15/30	9/28	14/30	2/30	13/30	2/30	−	−	−
Response to anti-PD drugs												
Trihexyphenidyl	N/A	+	+	+	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Levodopa	+	N/A	No tolerance	No tolerance	N/A	+	+	+	+	+	+	+

Homo = homozygous; Comp hetero = compound heterozygous; Hetero = heterozygous; B = bradykinesia; M = mental retardation; R = rigidity; D = developmental disturbance; G = gait disturbance; FFF mini-myoclonus = facial-faucial-finger mini-myoclonus; MMSE = Mini-Mental State Examination; PD = Parkinson disease; − = absent; + = present; N/A = not assessed.

in striatonigral system, in contrast to postsynaptic dysfunction in multiple system atrophy and progressive supranuclear palsy without levodopa responsiveness.[6] ^{18}F-dopa PET scan of our patient with levodopa-responsive parkinsonism with homozygous *ATP13A2* mutation also showed a presynaptic pattern often observed in idiopathic PD.

Intriguingly, the *GBA* gene encoding lysosomal enzyme was reported to be associated with synucleinopathies such as Lewy body diseases. Since the lysosomal degradation pathway can clear α-synuclein aggregates,[7] lysosomal dysfunction by *ATP13A2* or *GBA* mutation could be important in the pathogenesis of parkinsonism.

Altogether, our findings expand the phenotypic spectrum associated with *PARK9*-linked parkinsonism into multiple-system disorders. Furthermore, functional analysis of *ATP13A2* could open a new therapeutic window in widespread neurodegenerative disorders.

From the Department of Neurology (Y.P.N., H.T., Y.L., S.S., N.H.) and Research Institute for Diseases of Old Ages (M.F., H.Y., Y.M., N.H.), Juntendo University School of Medicine, Tokyo; Department of Neurology (K.K., M.A., S.K., T.H.), Graduate School of Medicine, Chiba University; and Department of Neurology (A.T.), Tohoku University School of Medicine, Miyagi, Japan.

Disclosure: The authors report no conflicts of interest.

Received April 11, 2007. Accepted in final form September 7, 2007.

Address correspondence and reprint requests to Prof. Nobutaka Hattori, Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan; nhattori@med.juntendo.ac.jp

1. Najim al-Din AS, Wriekat A, Mubaidin A, et al. Pallido-pyramidal degeneration, supranuclear upgaze paresis and dementia: Kufor-Rakeb syndrome. Acta Neurol Scand 1994;89:347–352.
2. Hampshire DJ, Roberts E, Crow Y, et al. Kufor-Rakeb syndrome, pallido-pyramidal degeneration with su-

pranuclear upgaze paresis and dementia, maps to 1p36. J Med Genet 2001;38:680–682.
3. Williams DR, Hadeed A, al-Din AS, et al. Kufor Rakeb disease: autosomal recessive, levodopa-responsive parkinsonism with pyramidal degeneration, supranuclear gaze palsy, and dementia. Mov Disord 2005;20:1264–1271.
4. Ramirez A, Heimbach A, Grundemann J, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 p-type ATPase. Nat Genet 2006;38:1184–1191.
5. Di Fonzo Chien HF, Socal M, et al. ATP13A2 missense mutations in juvenile parkinsonism and young onset Parkinson disease. Neurology 2007;68:1557–1562.
6. Kohno S, Shirakawa K, Ouchi Y, et al. Dopaminergic neuronal dysfunction associated with parkinsonism in both a Gaucher disease patient and a carrier. J Neurol Sci 2007;252:181–184.
7. Meredith GE, Totterdell S, Petroske E, et al. Lysosomal malfunction accompanies alpha-synuclein aggregation in a progressive mouse model of Parkinson's disease. Brain Res 2002;956:156–165.

E. Ohta, MD, PhD
T. Nagasaka, MD, PhD
K. Shindo, MD, PhD
S. Toma, MD, PhD
K. Nagasaka, MD
K. Ohta, MD, PhD
Z. Shiozawa, MD, PhD

NEUROFERRITINOPATHY IN A JAPANESE FAMILY WITH A DUPLICATION IN THE FERRITIN LIGHT CHAIN GENE

Neuroferritinopathy is a rare autosomal dominant movement disorder with the deposition of iron and ferritin within the basal ganglia. Four different pathogenic mutations in the ferritin light polypeptide (FTL) gene have been reported.[1-4] The variety of its clinical features makes the diagnosis of neuroferritinopathy difficult. In this study we investigated a Japanese family with neuroferritinopathy to clarify the phenotypic and genetic spectrum of neuroferritinopathy.

Proband. A 42-year-old Japanese man first developed hand tremors in his middle teens. He noticed his right foot dragging at age 35, and generalized hypotonia, hyperextensibility, aphonia, micrographia, hyperreflexia, and cognitive impairment (IQ = 66) at age 42. His unsteady gait with long steps, with his arms and legs dangling, seemed to be due mainly to hypotonus. Rigidity, spasticity, dystonia, or chorea were not observed. His serum ferritin concentration was 5 μg/L (normal = 33 to 330). A brain MRI revealed bilateral symmetric cystic changes of the pallidum and the striatum. Hyperintense lesions in the T2-weighted imaging involved the thalamus, dentate nucleus, and substantia nigra.

The proband's mother had developed hand tremors at age 10. She presented with difficulty walking at age 35 and developed cognitive impairment and akinetic mutism, and died at age 64. Her CT imaging showed cystic changes of the pallidum and the striatum. None of the proband's relatives, except for his mother, had any neurologic symptoms.

Methods. After informed consent was obtained, genomic DNA was extracted from a blood sample of the proband and was amplified by PCR. The entire coding region of the FTL gene was sequenced using a BigDye Terminator Cycle Sequencing Kit according to the manufacturer's protocol. In order to confirm the mutation, the PCR-RFLP assay was developed with AciI. We have not performed genetic testing in any asymptomatic family member because informed consent was not obtained.

Results. In exon 4 of the *FTL* gene, duplication of the 469–484 sequence was found (figure, A). The mutation replaces the C-terminal 14 amino acid residues with a novel 23 amino acid sequence (figure, B). This mutation is described as c.469_484dup16nt (p.Leu162Arg*fs*X185) in standard genetic nomenclature. The mutation was not found in 20 control chromosomes and after BLASTN searching of International Nucleotide Sequence Database Collaboration (INSDC). The mutation creates the gain of an AciI restriction site, proven by PCR–restriction fragment length polymorphism analysis (figure, C).

Discussion. Neuroferritinopathy was first reported in 2001. The original mutation, an insertion of adenine in position 460–461 (460InsA), has been found mainly in cases of neuroferritinopathy of the north of England.[1] The insertion of a dinucleotide, thymine and cytosine, in position 498–499 was detected in a French family.[2] The insertion of a cytosine in position 646–647 was reported in a family of French Canadian and Dutch ancestry.[3] A missense mutation in position 474 of guanine to adenine was found in a family of Gypsy ancestry.[4] In this study, we found a novel mutation, the duplication of the 469–484 sequence of the *FTL* gene in a Japanese family. This is the first family with neuroferritinopathy of non-European origin. The deceased proband's mother was undoubtedly affected by neuroferritinopathy based on her clinical features and CT findings. All of her relatives, except for the proband, had no neurologic symptoms. Considering the high penetration of neuroferritinopathy,[5] we suspect that a new genetic mutation in the *FTL*

A reassessment of risks and benefits of dopamine agonists in Parkinson's disease

Angelo Antonini, Eduardo Tolosa, Yoshikuni Mizuno, Mitsutoshi Yamamoto, Werner H Poewe

Neurologists have several choices of drugs that have been shown to be effective for the treatment of the symptoms of Parkinson's disease. Among the first options are the dopamine agonists, which are commonly used both as an early monotherapy and as an adjunct therapy to levodopa. However, before starting any treatment, the overall benefit-to-risk ratio to individual patients must be considered. For the dopamine agonists, the available evidence on their symptomatic efficacy, effect on long-term levodopa-related motor complications, putative effect on progression of disease, and adverse event profile must be taken into account. Recently, the ocurrence of adverse events such as leg oedema, daytime somnolence, impulse control disorders, and fibrosis have increasingly been recognised. The risks of these potentially serious adverse events must therefore be taken into account and treatment decisions should be based on considerations of risks versus benefits for individual patients.

Lancet Neurol 2009; 8: 929–37
Published Online August 25, 2009
DOI:10.1016/S1474-4422(09)70225-X

Parkinson Institute, Istituti Clinici di Perfezionamento, Milan, Italy (A Antonini MD); Neurology Service, Hospital Clinic, University of Barcelona and Centro de Investigación Biomedica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Barcelona, Spain (E Tolosa MD); Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan (Y Mizuno MD); Department of Neurology, Kagawa Prefectural Central Hospital, Kagawa, Japan (M Yamamoto MD); and Department of Neurology, Medical University of Innsbruck, Austria (W H Poewe MD)

Correspondence to:
Werner H Poewe, Department of Neurology, Medical University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria
werner.poewe@i-med.ac.at

Introduction

The use of oral dopamine agonists for the treatment of Parkinson's disease dates back to the 1970s when the ergolinic drug bromocriptine was first introduced. The first generation of dopamine agonists were all ergot derivatives and their pharmacological profile differed from that of levodopa in several ways. For example, ergot derivatives had a longer half-life than levodopa and had a differential affinity primarily to D1-like and D2-like dopamine receptors. Although more than 50 years have passed since the non-ergot agonist apomorphine was first reported to exert strong antiparkinsonian effects,[1,2] most of the currently used non-ergot dopamine agonists have entered the clinic more recently and include pramipexole, ropinirole, rotigotine, and piribedil (table 1).

The symptomatic efficacy of dopamine agonists to treat Parkinson's disease is firmly established and several studies have also shown that early use of these drugs as initial monotherapy is associated with a reduced long-term incidence of motor complications (ie, motor fluctuations and dyskinesia) compared with levodopa.[3–7] Although this evidence has led to dopamine agonists being classified as first-line options for initial monotherapy in early Parkinson's disease in many national and international guidelines,[8,9] there have also been recent concerns about the safety profile of these drugs in the longer term. These concerns are related to the risk of developing impulse control disorders, peripheral oedema, daytime somnolence, and heart valve fibrosis. The recognition of the risk of cardiac fibrotic valvulopathies with pergolide and cabergoline[10] has caused regulatory authorities in many countries to restrict the use of these drugs to second-line options with specialised cardiac safety monitoring.

In this Review, we first outline the benefits of using dopamine agonists in the management of Parkinson's disease. We then discuss recent evidence on each of the potential risks, outline the consequences for the management of Parkinson's disease, and provide recommendations for clinical neurologists on how to individualise treatment decisions based on considerations of their risks versus benefits.

Benefits of dopamine agonists in the medical management of Parkinson's disease

Early monotherapy

On the basis of a consistent body of evidence from randomised controlled trials, the dopamine agonists dihydroergocryptine, pergolide, pramipexole, and ropinirole[11–18] have all been shown to be effective as monotherapy in early Parkinson's disease.[8] The evidence for efficacy was less strong for bromocriptine, cabergoline, and lisuride as there have not been randomised trials of these compounds in early Parkinson's disease.[19–22] In recent placebo-controlled trials, rotigotine[23,24] and piribedil[25] have been shown to be efficacious in early disease. Of note, cabergoline,[7] pergolide,[6] pramipexole,[4,5] and ropinirole[3] have also been tested against levodopa in large randomised trials. The results from these trials have all shown a significantly reduced risk of motor complications compared with levodopa, in particular dyskinesias, over double-blind follow-up periods of up to 5 years. However, the effect on symptoms as assessed with the unified Parkinson's disease rating scale (UPDRS) was consistently greater for levodopa (table 2).

Continuous dopaminergic stimulation and reduced risk of motor complications

The exact reason why initial monotherapy with a dopamine agonist is associated with a reduced risk for motor complications, in particular dyskinesias, compared with levodopa is not fully understood. The most popular hypothesis is that therapies with long half-lives provide more continuous stimulation of brain dopaminergic receptors and that such continuous stimulation is key to the reduced risk of motor complications with longer-acting drugs compared with short-acting drugs, such as levodopa. Short-acting drugs induce discontinuous or pulsatile stimulation, which is associated with altered gene expression and firing patterns in basal ganglia output neurons, particularly of the direct pathway, that cause development of levodopa-induced dyskinesias.[26–28] This concept is supported by data from several preclinical studies in primates treated with MPTP (1-methyl-4-

	D2/D3 receptor affinity	D1 receptor affinity	NE receptor affinity	5-HT$_{2B}$ receptor affinity	Half-life (h)
Ergot agonists					
Bromocriptine	D2	–	+	+/–	3–6
Cabergoline	D3>D2	–	+	+	65
Dihydroergocriptine	D2	+/–	+	+	12–16
Lisuride	D2	–	+	+*	2–3
Pergolide	D3>D2	+	+	+	15–20
Non-ergot agonists					
Apomorphine	D3>D2	+	–	–	0·5
Piribedil	D3>D2	–	+/–	–	20
Pramipexole	D3>D2	–	+/–	–	10
Ropinirole	D3>D2	–	–	–	6
Rotigotine	D3>D2	+	–	–	5–7†

–=no affinity. +=high affinity. +/–=moderate affinity. NE=norepinephrine. *Antagonist. †After transdermal application.

Table 1: **Pharmacological properties of the dopamine agonists**

	Number on levodopa (agonist)	Duration (years)	ΔUPDRS part III score		Dyskinesia (% of patients)		Wearing-off (% of patients)	
			Levodopa	Agonist	Levodopa	Agonist	Levodopa	Agonist
Levodopa vs ropinrole[3]	89 (179)	5	–4·8±8·3	–0·8±10·1	45	20	34	23
Levodopa vs pramipexole[4]	151 (150)	2	–7·3±8·6	–3·4±8·6	30·7	9·9	38·0	23·8
Levodopa vs pergolide[6]	146 (148)	3	–2·8±7·8	2·8±9·8	26·0	8·2	43·8	30·6*

ΔUPDRS=change in unified Parkinson's disease rating scale. *Frequency of motor complications (fluctuations plus dyskinesia).

Table 2: **Results of the main trials of levodopa versus dopamine agonists in early Parkinson's disease**

phenyl-1,2,3,6-tetrahydropyridine), which all showed that treatment with a long-acting dopamine agonist was associated with reduced dyskinesias compared with levodopa.[29–32] Additionally, results from clinical studies with continuous subcutaneous infusion of apomorphine[33] or lisuride[34] or with continuous enteral infusions of levodopa[35,36] have also shown fewer pre-existing levodopa-induced dyskinesias. There are, however, no studies that indicate that continuous delivery of levodopa from the start of treatment could prevent the development of dyskinesias. Studies that have used either slow-release levodopa or combinations of levodopa with the catechol-O-methyltransferase inhibitor entacapone have not shown decreased incidences of motor complications over periods of up to 5 years.[37] On the contrary, results from the recent STRIDE-PD (STalevo Reduction In Dyskinesia Evaluation-Parkinson's Disease) trial, which compared initial levodopa plus carbidopa therapy with and without entacapone, have shown that early entacapone use is associated with shorter latency to the development of dyskinesias and, overall, increased dyskinesia rates.[38]

On the other hand, the 4-year and 5-year levodopa-controlled monotherapy trials with pramipexole[5] and ropinirole[3] both show that dyskinesia rates remain lower for these two drugs than for levodopa monotherapy even after most patients had received levodopa as an adjunct therapy to maintain symptomatic control. Recently, 6-year follow-up data for 222 of 301 patients originally randomised in the CALM-PD (Long Term Impact of Initiating Pramipexole Versus Levodopa in Early Parkinson's Disease) trial continued to show reduced overall dyskinesia rates for those patients initially randomised to pramipexole.[39] A similar trend was observed at a 10-year follow-up time point for the ropinirole versus levodopa cohort, but this observation was based on less than 20% of the original study cohort remaining available for assessment.[40] However, when analysing disabling dyskinesias separately, their rate was similar at 4 years in the pramipexole versus levodopa trial regardless of which drug was chosen for initial therapy.[39] Moreover, the 14-year results of the UK bromocriptine trial suggest that the initially lower incidence of motor complications in patients who started on the agonist was not maintained in the long term compared with those who started on levodopa, whereas initial levodopa therapy resulted in better symptomatic control throughout the observational period.[41] Although the conclusions to be drawn from these data are limited by the small number of patients who were evaluable after 14 years, they are consistent with the understanding that there is an eventual need for levodopa in almost all patients with Parkinson's disease. The results from these studies suggest that differences in initial risk of dyskinesia progressively diminish when patients are followed-up in the long term.[42]

Neuroprotection

Halting or slowing disease progression remains a key unmet need in the management of Parkinson's disease, and dopamine agonists have been extensively studied for their putative neuroprotective effects. Several mechanisms have been proposed and include indirect antioxidant effects via activation of presynaptic dopamine autoreceptors, leading to reduced dopamine turnover, as well as antiapoptotic effects via activation of intracellular kinase systems (see elsewhere for review[43]). On the basis of preclinical studies showing potential neuroprotective activity of dopamine agonists, several clinical trials were undertaken that used surrogate biomarkers of nigrostriatal function as primary end-points to try to avoid confounding symptomatic effects that would hinder the interpretation of their putative disease-modifying effects.

The REAL-PET study (ReQuip as Early Therapy versus L-dopa using Positron Emission Tomography) compared ropinirole with levodopa using striatal uptake of ^{18}F-fluorodopa on PET imaging as the primary endpoint.[44] For patients who had imaging in the CALM-PD trial (CALM-PD-CIT), pramipexole was compared with levodopa using β-CIT uptake on SPECT

imaging (single photon emission computed tomography)[45] as the primary outcome measure. Both studies showed that treatment with the dopamine agonist was associated with a significant decrease in the rate of decline of the imaging biomarker compared with that seen for levodopa. However, as neither study included a placebo group, they cannot conclusively indicate whether the results reflect "protection" provided by the agonist or "toxicity" caused by levodopa. The study results could also have been due to differences in the pharmacological effect of these drugs on the biomarker rather than on cell survival or function.[46] However, data from a recent study (INSPECT; Investigating Effects of Short-Term Treatment With Pramipexole or Levodopa on ^{123}I-β-CIT and SPECT Imaging in Early Parkinson's) indicated that short-term pramipexole therapy did not modify dopamine transporter binding measured with β-CIT SPECT.[47] Nevertheless, this neuroimaging biomarker might be insufficiently sensitive to detect the effect of an intervention on disease progression in the long term.[42,46,48,49]

More recently, delayed-start designs for trials assessing disease-modifying versus symptomatic effects have been used to investigate disease modification in Parkinson's disease.[50,51] In a trial with a delayed-start design, patients are randomised to receive the study drug or placebo for a fixed time interval (eg, 9 months), after which patients in the placebo group are switched to the study drug and followed for another fixed time interval. Whereas differences between the two groups at the end of the placebo-controlled phase can be explained by both symptomatic and/or disease-modifying effects, a difference that is sustained until the end of the second phase, when patients in both groups are receiving the same drug, is assumed to indicate a true disease-modifying effect. A recent trial of this design showed benefits of early versus delayed treatment with the monoamine oxidase B inhibitor rasagiline in a large cohort of patients with early Parkinson's disease;[51,52] furthermore, a trial combining a delayed-start design with dopamine transporter binding visualised on SPECT imaging as a surrogate marker for disease progression is underway to assess the disease-modifying effects of pramipexole (the PROUD study; Assessment of Potential ImPact of PRamipexole On Underlying Disease).[53]

Adjunct therapy in advanced Parkinson's disease

Several dopamine agonists extend "on" time and reduce "off" time disability when used as an adjunct therapy in patients treated with levodopa who have motor fluctuations. Such efficacy probably reflects the longer half-life of drugs such as pergolide, pramipexole, and ropinirole, for which class I evidence from randomised placebo-controlled trials in Parkinson's disease is available. The efficacy of apomorphine injections or infusions[54,55] or transdermal rotigotine[56,57] is mainly associated with their "on-demand" delivery (ie, apomorphine rescue injections) or continuous delivery (ie, rotigotine patch, apomorphine infusions). When patients with advanced Parkinson's disease who are treated with levodopa receive an agonist to reduce "off" episodes, dyskinesia can emerge or, if already present, can worsen. This risk can usually be controlled by reducing the pre-existing total daily dose of levodopa.[58,59]

Risks associated with dopamine agonist use in Parkinson's disease

The acute side-effects of dopamine agonists are similar to those observed with levodopa and include nausea, vomiting, and postural hypotension. These adverse events tend to occur with the initiation of treatment and tend to abate as tolerance to the drug develops.[60] Discontinuation rates associated with adverse events did not differ between different dopamine agonists and levodopa in randomised double-blind trials.[19,61] However, compared with levodopa, dopamine agonists are associated with a higher rate of some dopaminergic side-effects, including hallucinations, somnolence, and sudden-onset sleep, as well as impulse control disorders (figure).[62] Additionally, certain adverse reactions, including peripheral oedema and fibrotic reactions, seem to be specifically associated with the use of dopamine agonists and are not seen with other dopaminergic therapies. Peripheral oedema can be induced by all types of dopamine agonists, and possibly levodopa, whereas fibrosis seems mainly to be caused by ergot derivatives.

Peripheral oedema

Oedema is a poorly understood complication of agonist therapy in Parkinson's disease. This adverse event has probably been under-reported for a long time and can occur with both ergot and non-ergot therapy. A post-hoc analysis of the CALM-PD trial recently found the 4-year incidence of leg oedema to be 45% in patients who were initially randomised to pramipexole.[63] Although this percentage is higher than previous estimates,[3,4] the authors note that oedema seemed to emerge after at least 2 years of therapy. Thus, earlier studies, which tended to be of shorter duration, are likely to have missed this complication. Leg oedema also occurs as a complication of ergot-derived dopamine agonists,[64] or even levodopa, which suggests a class effect. This hypothesis would seem plausible as dopamine is an important regulator of the sympathetic nervous system, aldosterone secretion, and ATP-mediated sodium and potassium channels.

Daytime sleepiness and sleep attacks

Depending on the trial design and criteria used, excessive daytime sleepiness was found in 16–51% of patients with Parkinson's disease (in studies that included more than 90 individuals; table 3). Sudden-onset sleep as a side-effect

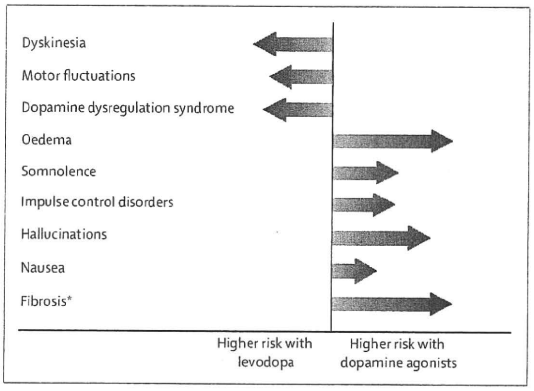


Figure: **Risk of motor complications and other adverse events with dopamine agonists versus levodopa**
The length of the arrows indicates the relative extent of risk. *Ergot agonists *vs* levodopa (see text).

of non-ergolinic dopamine agonists such as pramipexole and ropinirole was first reported by Frucht and co-workers[79] and might occur in 4–6% of patients with Parkinson's disease who have had dopaminergic therapy.[68,72] Both excessive daytime sleepiness and sudden-onset sleep can be induced by all types of currently used dopamine agonists and also, more rarely, by levodopa.[80] In a questionnaire-based survey of 2952 patients with Parkinson's disease, 22 of 769 (2·9%) patients on levodopa monotherapy had had sudden-onset sleep; occurrence was higher in patients on dopamine agonist monotherapy (7 of 131; 5·3%) and highest (137 of 1869; 7·3%) in patients on combined treatment with levodopa plus a dopamine agonist.[72]

Impulse control disorders

Dopaminergic therapy for Parkinson's disease can also be associated with behavioural abnormalities, which include dopamine dysregulation syndrome, abnormal repetitive non-goal oriented behaviours (punding), and reward or incentive-based compulsive actions. Collectively, these abnormalities are common and can have major and sometimes devastating psychosocial consequences. Whereas dopamine dysregulation syndrome and punding have been primarily associated with high-dose levodopa or continuous subcutaneous apomorphine treatment, impulse control disorders seem to be mainly associated with dopamine agonists. Impulse control disorders are characterised by a failure to resist an impulse, drive, or temptation to carry out an act that is harmful to oneself or others.[81] In Parkinson's disease, typical impulse control disorders include hypersexuality, gambling, compulsive shopping, and compulsive eating. In three systematic studies of the prevalence of impulse control disorders in Parkinson's disease,[82–84] 6–7% of patients across all three studies met criteria for one of these disorders. In the first study, 18 of 272 (6·6%) patients with Parkinson's disease met criteria for one of these disorders at some point during the course of the disease, including 11 of 272 (4·0%) patients with an active impulse control disorder.[82] Compulsive gambling and compulsive sexual behaviour were equally common. There were no differences between the different dopamine agonists in their association with impulse control disorders (p=0·21). In another study of 297 patients, the lifetime prevalence of pathological hypersexuality was 2·4% (7 patients) and compulsive shopping was 0·7% (2 patients).[83] When including data on pathological gambling,[85] the lifetime prevalence of impulse dyscontrol was 6·1% in all patients with Parkinson's disease, which increased to 13·7% in patients on dopamine agonists. A recent study of more than 3000 patients published in preliminary form has reported even higher figures: 17·1% of patients receiving dopamine agonists had at least one type of impulse control disorder and about a third of these patients had more than one of these disorders.[86] Recently, Cilia and co-workers[87] used functional imaging to show abnormal resting state dysfunction of the mesocorticolimbic network in patients with Parkinson's disease with pathological gambling, suggesting a drug-induced overstimulation of reward-related neuronal systems.

Fibrotic reactions

In contrast to all of the above-mentioned adverse events associated with dopamine agonists, the development of pleural, pericardial, peritoneal, or valvular fibrosis seems to be specifically associated with ergot compounds. Retroperitoneal and pleuropulmonary fibrosis are often irreversible after drug withdrawal and can be fatal.[88] Case reports of pleuropulmonary fibrosis with bromocriptine were published soon after its introduction,[89–95] and the same complication was subsequently reported for pergolide[96] and cabergoline, for which there have also been rare reports of constrictive pericardial fibrosis.[97] Pleuropulmonary fibrosis can develop as late as 11 years after initiation of pergolide therapy,[98] highlighting the need for maintained vigilance during chronic therapy for Parkinson's disease with ergolinic agonists.

A possible link between pergolide and valvular heart disease was first reported by Pritchett and co-workers[99] and was subsequently strongly suggested by echocardiographic studies.[100–102] Further reports also implicated cabergoline, and more recently bromocriptine, although there is no firm evidence for cardiac valvular changes induced by lisuride at present.[103] A meta-analysis[104] of heart valve abnormalities in patients with Parkinson's disease treated with dopamine agonists indicated a high rate of moderate valvular changes in patients treated with pergolide or cabergoline. This report included data from seven cross-sectional studies, including 477 patients treated with these ergolinic dopamine agonists, 127 patients with the non-ergot dopamine agonists ropinirole and pramipexole, and 364 control patients. Moderate to severe valvular changes were detected in

26% of patients treated with pergolide and cabergoline, 10% of patients treated with non-ergot agonists, and 10% of control patients. Severe valvulopathy was observed in less than 1% of patients in both cross-sectional and observational studies. Overall, the severity of ergot-induced vavulopathies tends to correlate with cumulative doses.[101,105] On the basis of such findings, the European Medicines Agency has added new warnings and contraindications to the product information of cabergoline and pergolide to reduce the risk of fibrosis.

In addition to treatment of Parkinson's disease, dopamine agonists are also first-line therapies for restless legs syndrome. The smaller doses of cabergoline or pergolide used in patients with this syndrome, compared with dose levels used in patients with Parkinson's disease, might explain the absence of reports of cardiac valvulopathy in such patients. Neither cabergoline nor pergolide are approved for use in restless legs syndrome, and the non-ergot pramipexole and ropinirole are the only dopamine agonists currently licensed for this indication, making it unlikely that cardiac valvulopathy will become a serious drug-induced complication in restless legs syndrome.

The mechanisms underlying cardiac valvulopathy induced by cabergoline or pergolide are likely to involve agonism of these two drugs at the 5-HT_{2B} receptor subtype, which is expressed on heart valve leaflets.[106] This action might induce fibroblast mitogenesis, thereby inducing thickening, retraction, and stiffening of valves and causing incomplete leaflet coaptation and valvular regurgitation. The extent to which fibrotic valvular changes are reversible after discontinuation, and whether switching to a non-ergot agonist prevents further progression, is being studied. Preliminary evidence from a prospective 2-year follow-up study of 21 patients who had developed moderate to severe valvulopathy during ergot therapy and were switched to non-ergot agonists showed regression of valve regurgitation in 13 patients. In 46 patients in the same study who had continued therapy on cabergoline and pergolide, there was an 8% per year incidence of new cases of moderate to severe regurgitation in any heart valve (Antonini A, unpublished). Although the observation of regression of valve regurgitation in more than half of the patients who replaced ergot with non-ergot agonists is encouraging, persistence of abnormalities in a large proportion of the patients is worrisome. This persistence could be due to flow turbulence induced by the valve dysfunction, which might contribute to worsening fibrosis even when the drug causing the complications is withdrawn.

Risks versus benefits of dopamine agonists in clinical practice

As a class, dopamine agonists have been successfully used for many years as an adjunct therapy to levodopa in patients who develop motor complications, and have an important role as initial monotherapy, particularly in younger patients. Over the past decade, the driving force for the increased prominence of dopamine agonist monotherapy has been its documented benefits in reducing the risk of dyskinesias compared with levodopa.[3,5] As motor complications are often difficult to manage, contribute substantially to overall disability and cost of illness,[107] and contribute to poor quality of life in Parkinson's disease,[108] this benefit is considered by many to outweigh both the inferior symptomatic efficacy and the specific risks associated with dopamine agonist therapy compared with levodopa. However, excessive daytime sleepiness, sudden-onset sleep, and impulse control disorders can have serious consequences both for the patient and their social relations. The need for a balanced and individualised process by which to define the best initial monotherapy strategy for any given patient is also indicated by the results of the pramipexole CALM-PD extension study.[39] After 6 years, when about 90% of patients in both groups of the original cohort were on levodopa, the outcome for overall impairment in activities of daily living was similar in the initial pramipexole and levodopa groups. The same was true for mean scores on quality of life scales and also for motor function as assessed by the UPDRS. However, motor complications, including wearing off, "on-off" effects, and dyskinesias, were more common when patients were treated initially with levodopa than with pramipexole, although disabling dyskinesias were uncommon in both groups. Epworth sleepiness scale scores were higher with initial pramipexole treatment, indicating more sleepiness in the inital pramipexole group than in the initial levodopa group.

	Number of patients (controls)	EDS screening criteria or tool	EDS in patients with PD (%)	EDS in controls (%)
van Hilten et al[65]	90 (71)	Questionnaire	44·4	31·0
Tandberg et al[66]	245 (100)	Questionnaire	15·5	1·0
Ondo et al[67]	303 (··)	ESS >10	50·2	··
Hobson et al[68]	638 (··)	ESS ≥7	51·0	··
Tan et al[69]	201 (214)	ESS ≥10	19·9	9·8
Brodsky et al[70]	101 (100)	ESS ≥10	40·6	19·0
Högl et al[71]	99 (44)	ESS ≥10	33·0	11·0
Paus et al[17]	177* (··)	ESS ≥10	75·0	··
Marinus et al[73]	143 (104)	ESS >10	27·0	3·0
Kumar et al[74]	149 (115)	ESS ≥8	21·0	3·0
Monaca et al[75]	222 (··)	ESS >10	43·2	··
Ferreira et al[76]	176 (174)	ESS ≥15	33·5	16·1
Verbaan et al[77]	419 (150)	SCOPA-DS ≥5	43·0	10·0
Ghorayeb et al[78]	1625 (··)	ESS ≥10	29·0	··

Studies with more than 90 individuals were selected. ··=data not available. EDS=excessive daytime sleepiness. ESS=Epworth sleepiness score. PD=Parkinson's disease. SCOPA-DS=scales for outcomes in PD-daytime sleepiness. *2952 patients were screened by telephone interview and 177 were identified to have EDS; after assessment, there were 133 (75%) with an ESS of ≥10.

Table 3: **Frequency of EDS in individuals with Parkinson's disease and controls**

Search strategy and selection criteria

References for this Review were identified through searches of PubMed (from 1966 to June, 2009) and the Cochrane Library (1948 to June, 2009) with the search terms "apomorphine", "bromocriptine", "cabergoline", "dihydroergocryptine", "dopamine agonist", "dyskinesia", "fibrosis", "impulse control disorders", "motor complications", "(o)edema", "pergolide", "piribedil", "pramipexole", "ropinirole", "rotigotine", "sleep", "somnolence", and "cardiac valvulopathy". Only peer-reviewed papers published in English were considered. Additionally, reference lists in published reviews were checked for relevant references, regulatory publications issued by the European Committee of Proprietary Medicinal Products and the US Food and Drugs Administration were reviewed, and recent releases of information (ie, congress abstracts and press releases) from major Parkinson's disease clinical trials were considered.

As there have been no head-to-head studies of dopamine agonists, there is little information available to help choose which agonist to use, and physicians will need to take into account factors such as the patient's lifestyle. However, the ergot-derived dopamine agonists are associated with an additional risk of potentially life-threatening side-effects.[103] Because of these effects, pergolide and cabergoline should be second-line therapies. Moreover, the need for these ergot-derived dopamine agonists has largely been superseded by the newer agonists pramipexole and ropinirole, which seem to have equal efficacy and do not seem to have fibrotic side-effects.[103] As one of the main reasons for prescribing cabergoline was its long half-life and its associated benefits with compliance, recent developments in once-daily formulations for both ropinirole and pramipexole mean that this drug might no longer be required.

Conclusions

The choice of drug when initiating therapy in early Parkinson's disease depends on several factors, including level of disability, age, comorbidities, and cognitive status, as well as the different risk profiles of the available drugs. Most commonly, initial drug therapy is with dopaminergic drugs, although amantadine or anticholinergic drugs might still be considered to be appropriate in a few cases. None of the available drugs has yet been proven to delay the progression of disability unequivocally and in a clinically meaningful way, although a recent study suggested evidence for disease-modifying potential of rasagiline.[109] Therefore, selection among the available dopaminergic drug classes will largely depend on symptomatic efficacy and the degree of motor impairment. Monoamine oxidase B inhibitors have a lesser effect on the main motor features and are therefore an option for patients with mild disability, whereas dopamine agonists and levodopa are needed for patients with greater impairment or whose professional work and performance on activities of daily living will be affected. Levodopa remains the gold standard for symptomatic efficacy and should not be withheld from patients in whom sufficient symptomatic control cannot be otherwise obtained. Whether or not a dopamine agonist should be used as early monotherapy will largely depend on the perceived risk of dyskinesias—for which younger age is a major determinant. The use of ergolinic dopaminergic agonists such as pergolide or cabergoline in early Parkinson's disease monotherapy is no longer recommended because of their poor overall benefit to risk ratio when compared with initial levodopa monotherapy, based on their additional risk of potentially life-threatening pleuropulmonary or cardiac valvular fibrosis. However, dopamine agonists are also first-line drugs as an adjunct therapy in patients treated with levodopa who have motor fluctuations, and ergolinic agonists might still be needed for this indication in countries where newer drugs are not readily accessible.

A clinician's judgment on the different risk profiles of monoamine oxidase B inhibitors, dopamine agonists, and levodopa, as well as expectations about effect size, will substantially affect the final decision on which drug to use. This decision should be based on careful and judicious counselling of patients and caregivers about the benefit to risk ratios of the different drug options and should remain an individualised decision for every new patient.

Contributors

WHP and AA developed the concept of this Review, provided the first draft, contributed to multiple revisions, and were responsible for the final version of the manuscript. ET and YM reviewed the initial draft, and discussed and reviewed subsequent drafts. MY participated in the planning of the Review, and discussed, reviewed, and revised the manuscript.

Conflicts of interest

AA has received funding as an adviser and speaker in sponsored symposia from Boehringer Ingelheim, GE Pharmaceuticals, GlaxoSmithKline, Novartis, Solvay, UCB, and Valeant. ET has participated on advisory boards for UCB and Boehringer Ingelheim, and has received honoraria for lecturing in symposia from Boehringer Ingelheim, GlaxoSmithKline, Lundbeck, Teva, and UCB. WHP has received lecture fees and consulting honoraria from Boehringer Ingelheim, GlaxoSmithKline, Lundbeck, Merck Serono, Novartis, Solvay, Teva, and UCB. YM and MY have no conflicts of interest.

Acknowledgments

Editorial assistance was provided by Anita Chadha-Patel, who was supported by an unrestricted grant from the Neureca Foundation for movement disorders, Milan, Italy.

References

1 Struppler A, von Uexkull T. Studies of mechanism of action of apomorphine on Parkinson's tremor. *Z Klein Med* 1953; 152: 46–57.

2 Schwab RS, Amador LV, Lettvin JY. Apomorphine in Parkinson's disease. *Trans Am Neurol Assoc* 1951; 56: 251–53.

3 Rascol O, Brooks DJ, Korczyn AD, De Deyn PP, Clarke CE, Lang AE; for the 056 Study Group. A five-year study of the incidence of dyskinesia in patients with early Parkinson's disease who were treated with ropinirole or levodopa. *N Engl J Med* 2000; 342: 1484–91.

4 Parkinson Study Group. Pramipexole vs levodopa as initial treatment for Parkinson disease: a randomized controlled trial. *JAMA* 2000; **284**: 1931–38.

5 Holloway RG, Shoulson I, Fahn S, et al. Pramipexole vs levodopa as initial treatment for Parkinson disease: a 4-year randomized controlled trial. *Arch Neurol* 2004; **61**: 1044–53.

6 Oertel WH, Wolters E, Sampaio C, et al. Pergolide versus levodopa monotherapy in early Parkinson's disease patients: the PELMOPET study. *Mov Disord* 2005; **21**: 343–53.

7 Bracco F, Battaglia A, Chouza C, et al. The long-acting dopamine receptor agonist cabergoline in early Parkinson's disease: final results of a 5-year, double-blind, levodopa-controlled study. *CNS Drugs* 2004; **18**: 733–46.

8 Horstink M, Tolosa E, Bonuccelli U, et al. Review of the therapeutic management of Parkinson's disease. Report of a joint task force of the European Federation of Neurological Societies and the Movement Disorder Society-European Section. Part I: early (uncomplicated) Parkinson's disease. *Eur J Neurol* 2006; **13**: 1170–85.

9 Miyasaki JM, Martin W, Suchowersky O, Weiner WJ, Lang AE. Practice parameter: initiation of treatment for Parkinson's disease: an evidence-based review. Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2002; **58**: 11–17.

10 Horvath J, Fross RD, Kleiner-Fisman G, et al. Severe multivalvular heart disease: a new complication of the ergot derivative dopamine agonists. *Mov Disord* 2004; **19**: 656–62.

11 Bergamasco B, Frattola L, Muratorio A, Piccoli F, Mailland F, Parnetti L. Alpha-dihydroergocryptine in the treatment of de novo parkinsonian patients: results of a multicentre, randomized, double-blind, placebo-controlled study. *Acta Neurol Scand* 2000; **101**: 372–80.

12 Barone P, Bravi D, Bermejo-Pareja F, et al; for the Pergolide Monotherapy Study Group. Pergolide monotherapy in the treatment of early PD: a randomized, controlled study. *Neurology* 1999; **53**: 573–79.

13 Shannon KM, Bennett JP Jr, Friedman JH; for the Pramipexole Study Group. Efficacy of pramipexole, a novel dopamine agonist, as monotherapy in mild to moderate Parkinson's disease. *Neurology* 1997; **49**: 724–28.

14 Adler CH, Sethi DK, Hauser RA, et al. Ropinirole for the treatment of early Parkinson's disease. *Neurology* 1997; **49**: 393–99.

15 DA agonists—non-ergot derivatives. Ropinirole: management of Parkinson's disease. *Mov Disord* 2002; **17** (suppl 4): S98–102.

16 DA agonists—non-ergot derivatives. Pramipexole: management of Parkinson's disease. *Mov Disord* 2002; **17** (suppl 4): S93–97.

17 DA agonists—non-ergot derivatives. Piribedil: management of Parkinson's disease. *Mov Disord* 2002; **17** (suppl 4): S90–92.

18 DA agonists—ergot derivatives. Pergolide: management of Parkinson's disease. *Mov Disord* 2002; **17** (suppl 4): S79–82.

19 Goetz CG, Poewe W, Rascol O, Sampaio C. Evidence-based medical review update: pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. *Mov Disord* 2005; **20**: 523–39.

20 Montastruc JL, Rascol O, Senard JM, Rascol O. A randomised controlled study comparing bromocriptine to which levodopa was later added, with levodopa alone in previously untreated patients with Parkinson's disease: a five year follow up. *J Neurol Neurosurg Psychiatry* 1994; **57**: 1034–38.

21 Rinne UK, Bracco F, Chouza C, et al; for the PKDS009 Study Group. Early treatment of Parkinson's disease with cabergoline delays the onset of motor complications. Results of a double-blind levodopa controlled trial. *Drugs* 1998; **55** (suppl 1): 23–30.

22 Rinne UK. Lisuride, a dopamine agonist in the treatment of early Parkinson's disease. *Neurology* 1989; **39**: 336–39.

23 Jankovic J, Watts RL, Martin W, Boroojerdi B. Transdermal rotigotine: double-blind, placebo-controlled trial in Parkinson disease. *Arch Neurol* 2007; **64**: 676–82.

24 Watts RL, Jankovic J, Waters C, Rajput A, Boroojerdi B, Rao J. Randomized, blind, controlled trial of transdermal rotigotine in early Parkinson disease. *Neurology* 2007; **68**: 272–76.

25 Rascol O, Dubois B, Caldas AC, Senn S, Del Signore S, Lees A. Early piribedil monotherapy of Parkinson's disease: a planned seven-month report of the REGAIN study. *Mov Disord* 2006; **21**: 2110–15.

26 Olanow CW, Obeso JA, Stocchi F. Continuous dopamine-receptor treatment of Parkinson's disease: scientific rationale and clinical implications. *Lancet Neurol* 2006; **5**: 677–87.

27 Olanow CW, Obeso JA, Stocchi F. Drug insight: continuous dopaminergic stimulation in the treatment of Parkinson's disease. *Nat Clin Pract Neurol* 2006; **2**: 382–92.

28 Cenci MA. Dopamine dysregulation of movement control in L-DOPA-induced dyskinesia. *Trends Neurosci* 2007; **30**: 236–43.

29 Bedard PJ, Di Paolo T, Falardeau P, Boucher R. Chronic treatment with L-DOPA, but not bromocriptine induces dyskinesia in MPTP-parkinsonian monkeys. Correlation with [3H]spiperone binding. *Brain Res* 1986; **379**: 294–99.

30 Maratos EC, Jackson MJ, Pearce RK, Jenner P. Antiparkinsonian activity and dyskinesia risk of ropinirole and L-DOPA combination therapy in drug naive MPTP-lesioned common marmosets (Callithrix jacchus). *Mov Disord* 2001; **16**: 631–41.

31 Smith LA, Tel BC, Jackson MJ, et al. Repeated administration of piribedil induces less dyskinesia than L-dopa in MPTP-treated common marmosets: a behavioural and biochemical investigation. *Mov Disord* 2002; **17**: 887–901.

32 Maratos EC, Jackson MJ, Pearce RK, Cannizzaro C, Jenner P. Both short- and long-acting D-1/D-2 dopamine agonists induce less dyskinesia than L-DOPA in the MPTP-lesioned common marmoset (Callithrix jacchus). *Exp Neurol* 2003; **179**: 90–102.

33 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**: 151–57.

34 Stocchi F, Ruggieri S, Vacca L, Olanow CW. Prospective randomized trial of lisuride infusion versus oral levodopa in patients with Parkinson's disease. *Brain* 2002; **125**: 2058–66.

35 Stocchi F, Vacca L, Ruggieri S, Olanow CW. Intermittent vs continuous levodopa administration in patients with advanced Parkinson disease: a clinical and pharmacokinetic study. *Arch Neurol* 2005; **62**: 905–10.

36 Antonini A, Isaias IU, Canesi M, et al. Duodenal levodopa infusion for advanced Parkinson's disease: 12-month treatment outcome. *Mov Disord* 2007; **22**: 1145–49.

37 Koller WC, Hutton JT, Tolosa E, Capilldeo R; for the Carbidopa/Levodopa Study Group. Immediate-release and controlled-release carbidopa/levodopa in PD: a 5-year randomized multicenter study. *Neurology* 1999; **53**: 1012–19.

38 PDtrials. Primary objective of STRIDE-PD study was not achieved. http://www.pdtrials.org/en/clinical_research_news/release/pr_1236180097 (accessed Aug 17, 2009).

39 Parkinson Study Group CALM Cohort Investigators. Long-term effect of initiating pramipexole vs levodopa in early Parkinson disease. *Arch Neurol* 2009; **66**: 563–70.

40 Hauser RA, Rascol O, Korczyn AD, et al. Ten-year follow-up of Parkinson's disease patients randomized to initial therapy with ropinirole or levodopa. *Mov Disord* 2007; **22**: 2409–17.

41 Katzenschlager R, Head J, Schrag A, Ben-Shlomo Y, Evans A, Lees AJ. Fourteen-year final report of the randomized PDRG-UK trial comparing three initial treatments in PD. *Neurology* 2008; **71**: 474–80.

42 European Medicines Agency. Guideline on clinical investigation of medicinal products in the treatment of Parkinson's disease (CHMP/563/95 Rev.1). July, 2008. http://www.emea.europa.eu/pdfs/human/ewp/056395en.pdf (accessed Aug 7, 2009).

43 Olanow CW. Can we achieve neuroprotection with currently available anti-parkinsonian interventions? *Neurology* 2009; **72**: S59–64.

44 Whone AL, Remy P, Davis MR, et al. The REAL-PET study: Slower progression in early Parkinson's disease treated with ropinirole compared with levodopa. *Neurology* 2002; **58** (suppl 3): A82.

45 Dopamine transporter brain imaging to assess the effects of pramipexole vs levodopa on Parkinson disease progression. *JAMA* 2002; **287**: 1653–61.

46 Ahlskog JE. Slowing Parkinson's disease progression: recent dopamine agonist trials. *Neurology* 2003; **60**: 381–89.

47 Jennings D, Tabamo R, Seibyl J, Marek K. InSPECT: investigating the effect of short-term treatment with pramipexole or levodopa on 123b-CIT and SPECT imaging. *Mov Disord* 2007; **22** (suppl 16): S143 (abstract).

48 Ravina B, Eidelberg D, Ahlskog JE, et al. The role of radiotracer imaging in Parkinson disease. *Neurology* 2005; **64**: 208–15.

49 Marek K, Jennings D, Tamagnan G, Seibyl J. Biomarkers for Parkinson's disease: tools to assess Parkinson's disease onset and progression. *Ann Neurol* 2008; **64** (suppl 2): S111–21.

50 Parkinson Study Group. A controlled, randomized, delayed-start study of rasagiline in early Parkinson disease. *Arch Neurol* 2004; **61**: 561–66.

51 Olanow CW, Hauser RA, Jankovic J, et al. A randomized, double-blind, placebo-controlled, delayed start study to assess rasagiline as a disease modifying therapy in Parkinson's disease (the ADAGIO study): rationale, design, and baseline characteristics. *Mov Disord* 2008; **23**: 2194–201.

52 Olanow CW, Rascol O. Early rasagiline treatment slows UPDRS decline in the ADAGIO delayed start study. *Ann Neurol* 2008; **64** (suppl 12): S68 (abstract).

53 Schapira A, Hsu HH, Scrine MF, Gordon KL, Marek K. PROUD: The impact of early vs delayed treatment with pramipexole on new onset Parkinson's disease. *Mov Disord* 2008; **23** (suppl 1): S194 (abstract).

54 Poewe W, Kleedorfer B, Wagner M, Bosch S, Schelosky L. Continuous subcutaneous apomorphine infusions for fluctuating Parkinson's disease. Long-term follow-up in 18 patients. *Adv Neurol* 1993; **60**: 656–59.

55 Dewey RB Jr, Hutton JT, LeWitt PA, Factor SA. A randomized, double-blind, placebo-controlled trial of subcutaneously injected apomorphine for parkinsonian off-state events. *Arch Neurol* 2001; **58**: 1385–92.

56 LeWitt PA, Lyons KE, Pahwa R. Advanced Parkinson disease treated with rotigotine transdermal system: PREFER study. *Neurology* 2007; **68**: 1262–67.

57 Poewe WH, Rascol O, Quinn N, et al. Efficacy of pramipexole and transdermal rotigotine in advanced Parkinson's disease: a double-blind, double-dummy, randomised controlled trial. *Lancet Neurol* 2007; **6**: 513–20.

58 Facca A, Sanchez-Ramos J. High-dose pergolide monotherapy in the treatment of severe levodopa-induced dyskinesias. *Mov Disord* 1996; **11**: 327–29.

59 Cristina S, Zangaglia R, Mancini F, Martignoni E, Nappi G, Pacchetti C. High-dose ropinirole in advanced Parkinson's disease with severe dyskinesias. *Clin Neuropharmacol* 2003; **26**: 146–50.

60 Yamamoto M, Schapira AH. Dopamine agonists in Parkinson's disease. *Expert Rev Neurother* 2008; **8**: 671–77.

61 Rascol O, Goetz C, Koller W, Poewe W, Sampaio C. Treatment interventions for Parkinson's disease: an evidence based assessment. *Lancet* 2002; **359**: 1589–98.

62 Stowe RL, Ives NJ, Clarke C, et al. Dopamine agonist therapy in early Parkinson's disease. *Cochrane Database Syst Rev* 2008; **2**: CD006564.

63 Biglan KM, Holloway RG Jr, McDermott MP, Richard IH. Risk factors for somnolence, edema, and hallucinations in early Parkinson disease. *Neurology* 2007; **69**: 187–95.

64 Blackard WG. Edema--an infrequently recognized complication of bromocriptine and other ergot dopaminergic drugs. *Am J Med* 1993; **94**: 445.

65 van Hilten JJ, Weggeman M, van der Velde EA, Kerkhof GA, van Dijk JG, Roos RA. Sleep, excessive daytime sleepiness and fatigue in Parkinson's disease. *J Neural Transm Park Dis Dement Sect* 1993; **5**: 235–44.

66 Tandberg E, Larsen JP, Karlsen K. Excessive daytime sleepiness and sleep benefit in Parkinson's disease: a community-based study. *Mov Disord* 1999; **14**: 922–27.

67 Ondo WG, Dat Vuong K, Khan H, Atassi F, Kwak C, Jankovic J. Daytime sleepiness and other sleep disorders in Parkinson's disease. *Neurology* 2001; **57**: 1392–96.

68 Hobson DE, Lang AE, Martin WR, Razmy A, Rivest J, Fleming J. Excessive daytime sleepiness and sudden-onset sleep in Parkinson disease: a survey by the Canadian Movement Disorders Group. *JAMA* 2002; **287**: 455–63.

69 Tan EK, Lum SY, Fook-Chong SM, et al. Evaluation of somnolence in Parkinson's disease: comparison with age- and sex-matched controls. *Neurology* 2002; **58**: 465–68.

70 Brodsky MA, Godbold J, Roth T, Olanow CW. Sleepiness in Parkinson's disease: a controlled study. *Mov Disord* 2003; **18**: 668–72.

71 Högl B, Seppi K, Brandauer E, et al. Increased daytime sleepiness in Parkinson's disease: a questionnaire survey. *Mov Disord* 2003; **18**: 319–23.

72 Paus S, Brecht HM, Koster J, Seeger G, Klockgether T, Wullner U. Sleep attacks, daytime sleepiness, and dopamine agonists in Parkinson's disease. *Mov Disord* 2003; **18**: 659–67.

73 Marinus J, Visser M, van Hilten JJ, Lammers GJ, Stiggelbout AM. Assessment of sleep and sleepiness in Parkinson disease. *Sleep* 2003; **26**: 1049–54.

74 Kumar S, Bhatia M, Behari M. Excessive daytime sleepiness in Parkinson's disease as assessed by Epworth Sleepiness Scale (ESS). *Sleep Med* 2003; **4**: 339–42.

75 Monaca C, Duhamel A, Jacquesson JM, et al. Vigilance troubles in Parkinson's disease: a subjective and objective polysomnographic study. *Sleep Med* 2006; **7**: 448–53.

76 Ferreira JJ, Desboeuf K, Galitzky M, et al. Sleep disruption, daytime somnolence and 'sleep attacks' in Parkinson's disease: a clinical survey in PD patients and age-matched healthy volunteers. *Eur J Neurol* 2006; **13**: 209–14.

77 Verbaan D, Marinus J, Visser M, van Rooden SM, Stiggelbout AM, van Hilten JJ. Patient-reported autonomic symptoms in Parkinson disease. *Neurology* 2007; **69**: 333–41.

78 Ghorayeb I, Loundou A, Auquier P, Dauvilliers Y, Bioulac B, Tison F. A nationwide survey of excessive daytime sleepiness in Parkinson's disease in France. *Mov Disord* 2007; **22**: 1567–72.

79 Frucht S, Rogers JD, Greene PE, Gordon MF, Fahn S. Falling asleep at the wheel: motor vehicle mishaps in persons taking pramipexole and ropinirole. *Neurology* 1999; **52**: 1908–10.

80 Seppi K, Hogl B, Diem A, Peralta C, Wenning GK, Poewe W. Levodopa-induced sleepiness in the Parkinson variant of multiple system atrophy. *Mov Disord* 2006; **21**: 1281–83.

81 Voon V, Fox SH. Medication-related impulse control and repetitive behaviors in Parkinson disease. *Arch Neurol* 2007; **64**: 1089–96.

82 Weintraub D, Siderowf AD, Potenza MN, et al. Association of dopamine agonist use with impulse control disorders in Parkinson disease. *Arch Neurol* 2006; **63**: 969–73.

83 Voon V, Hassan K, Zurowski M, et al. Prevalence of repetitive and reward-seeking behaviors in Parkinson disease. *Neurology* 2006; **67**: 1254–57.

84 Isaias IU, Siri C, Cilia R, De Gaspari D, Pezzoli G, Antonini A. The relationship between impulsivity and impulse control disorders in Parkinson's disease. *Mov Disord* 2008; **23**: 411–15.

85 Voon V, Hassan K, Zurowski M, et al. Prospective prevalence of pathologic gambling and medication association in Parkinson disease. *Neurology* 2006; **66**: 1750–52.

86 Weintraub D. Dopamine and impulse control disorders in Parkinson's disease. *Ann Neurol* 2008; **64** (suppl 2): S93–100.

87 Cilia R, Siri C, Marotta G, et al. Functional abnormalities underlying pathological gambling in Parkinson disease. *Arch Neurol* 2008; **65**: 1604–11.

88 Shaunak S, Wilkins A, Pilling JB, Dick DJ. Pericardial, retroperitoneal, and pleural fibrosis induced by pergolide. *J Neurol Neurosurg Psychiatry* 1999; **66**: 79–81.

89 Bowler JV, Ormerod IE, Legg NJ. Retroperitoneal fibrosis and bromocriptine. *Lancet* 1986; **2**: 466.

90 Demonet JF, Rostin M, Dueymes JM, Ioualalen A, Montastruc JL, Rascol A. Retroperitoneal fibrosis and treatment of Parkinson's disease with high doses of bromocriptine. *Clin Neuropharmacol* 1986; **9**: 200–01.

91 Wiggins J, Skinner C. Bromocriptine induced pleuropulmonary fibrosis. *Thorax* 1986; **41**: 328–30.

92 Ward CD, Thompson J, Humby MD. Pleuropulmonary and retroperitoneal fibrosis associated with bromocriptine treatment. *J Neurol Neurosurg Psychiatry* 1987; **50**: 1706–07.

93 Kains JP, Hardy JC, Chevalier C, Collier A. Retroperitoneal fibrosis in two patients with Parkinson's disease treated with bromocriptine. *Acta Clin Belg* 1990; **45**: 306–10.

94 Todman DH, Oliver WA, Edwards RL. Pleuropulmonary fibrosis due to bromocriptine treatment for Parkinson's disease. *Clin Exp Neurol* 1990; **27**: 79–82.

95 Hely MA, Morris JG, Lawrence S, Jeremy R, Genge S. Retroperitoneal fibrosis, skin and pleuropulmonary changes associated with bromocriptine therapy. *Aust N Z J Med* 1991; **21**: 82–84.

96 Agarwal P, Fahn S, Frucht SJ. Diagnosis and management of pergolide-induced fibrosis. *Mov Disord* 2004; **19:** 699–704.

97 Townsend M, MacIver DH. Constrictive pericarditis and pleuropulmonary fibrosis secondary to cabergoline treatment for Parkinson's disease. *Heart* 2004; **90:** e47.

98 Tintner R, Manian P, Gauthier P, Jankovic J. Pleuropulmonary fibrosis after long-term treatment with the dopamine agonist pergolide for Parkinson Disease. *Arch Neurol* 2005; **62:** 1290–95.

99 Pritchett AM, Morrison JF, Edwards WD, Schaff HV, Connolly HM, Espinosa RE. Valvular heart disease in patients taking pergolide. *Mayo Clin Proc* 2002; **77:** 1280–86.

100 Van Camp G, 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:** 859–61.

101 Van Camp G, Flamez A, Cosyns B, et al. Treatment of Parkinson's disease with pergolide and relation to restrictive valvular heart disease. *Lancet* 2004; **363:** 1179–83.

102 Bleumink GS, van der Molen-Eijgenraam M, Strijbos JH, Sanwikarja S, van Puijenbroek EP, Stricker BH. Pergolide-induced pleuropulmonary fibrosis. *Clin Neuropharmacol* 2002; **25:** 290–93.

103 Antonini A, Poewe W. Fibrotic heart-valve reactions to dopamine-agonist treatment in Parkinson's disease. *Lancet Neurol* 2007; **6:** 826–29.

104 Simonis G, Fuhrmann JT, Strasser RH. Meta-analysis of heart valve abnormalities in Parkinson's disease patients treated with dopamine agonists. *Mov Disord* 2007; **22:** 1936–42.

105 Zanettini R, Antonini A, Gatto G, Gentile R, Tesei S, Pezzoli G. Valvular heart disease and the use of dopamine agonists for Parkinson's disease. *N Engl J Med* 2007; **356:** 39–46.

106 Roth BL. Drugs and valvular heart disease. *N Engl J Med* 2007; **356:** 6–9.

107 Spottke AE, Reuter M, Machat O, et al. Cost of illness and its predictors for Parkinson's disease in Germany. *Pharmacoeconomics* 2005; **23:** 817–36.

108 Chapuis S, Ouchchane L, Metz O, Gerbaud L, Durif F. Impact of the motor complications of Parkinson's disease on the quality of life. *Mov Disord* 2005; **20:** 224–30.

109 University of South Florida. Patients starting Parkinson's drug rasagaline earlier do better. http://hscweb3.hsc.usf.edu/health/now/?p=3564 (accessed Aug 18, 2009).

Fibroblast growth factor 20 gene and Parkinson's disease in the Japanese population

Wataru Satake[a,b], Ikuko Mizuta[a,c], Satoko Suzuki[a,c], Yuko Nakabayashi[a,c], Chiyomi Ito[a,c] Masahiko Watanabe[d], Atsushi Takeda[e], Kazuko Hasegawa[f], Saburo Sakoda[b] Mitsutoshi Yamamoto[g], Nobutaka Hattori[h], Miho Murata[i] and Tatsushi Toda[a,c]

[a]Division of Clinical Genetics, Department of Medical Genetics, Osaka University Graduate School of Medicine, Yamadaoka, Suita, Osaka, [b]Department of Neurology, Osaka University Graduate School of Medicine, Suita, [c]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Kawaguchi, [d]Department of Neurology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, [e]Division of Neurology, Department of Neuroscience, Tohoku University Graduate School of Medicine, Sendai, [f]Department of Neurology, National Hospital Organization, Sagamihara National Hospital, Sagamihara, [g]Department of Neurology, Kagawa Prefectural Central Hospital, Takamatsu, [h]Department of Neurology, Juntendo University School of Medicine, Tokyo and [i]Department of Neurology, Musashi Hospital, National Center of Neurology and Psychiatry, Kodaira, Japan

Correspondence to Division of Clinical Genetics, Department of Medical Genetics, Osaka University Graduate School of Medicine, 2-2-B9 Yamadaoka, Suita, Osaka 565 0871, Japan
Tel: +81 6 6879 3381; fax: +81 6 6879 3389; e-mail: toda@clgene.med.osaka-u.ac.jp

Received 3 January 2007; accepted 20 February 2007

A genetic association between the fibroblast growth factor 20 (*FGF20*) gene and Parkinson's disease has been found by the pedigree disequilibrium test. This association, however, was not replicated by a case–control association study. In order to clarify the association between the *FGF20* gene and Parkinson's disease, we attempted to replicate this association by a case–control association study using a large number of Japanese samples (1388 patients and 1891 controls). rs1721100 exhibited a significant difference in allele C versus G (P=0.0089), and in genotype CC+CG versus GG (P=0.0053). Haplotype association analysis showed that haplotype 2 was the protective haplotype for Parkinson's disease (permutation-P=0.0075). These results suggest that the *FGF20* gene is a susceptibility gene for Parkinson's disease in the Japanese population. *NeuroReport* 18:937–940 © 2007 Lippincott Williams & Wilkins.

Keywords: association, case–control study, fibroblast growth factor 20 (*FGF20*), Parkinson's disease, single nucleotide polymorphism, susceptibility gene

Introduction

Parkinson's disease (OMIM #168600) is one of the most common neurodegenerative diseases, characterized by resting tremor, cogwheel rigidity, bradykinesia, and impaired postural reflexes. These clinical features result primarily from the loss of dopaminergic neurons in the substantia nigra. Various medical treatments improve Parkinson's disease symptoms, but do little to deter disease progression [1]. In Mendelian-inherited Parkinson's disease, eight causal genes have been identified (*SNCA, parkin, UCHL1, PINK1, DJ1, LRRK2/dardarin, ATP13A2,* and *NR4A2/Nurr1*). Sporadic Parkinson's disease is a complex disorder, with multiple genetic and environmental factors influencing disease risk [2]. Identifying genetic risk factors for Parkinson's disease will be helpful in elucidating the pathogenesis of Parkinson's disease.

Genome-wide, non-parametric linkage analyses of Parkinson's disease families have revealed significant linkage in multiple chromosomal regions [3–6]. One of these prominent regions of linkage was found on chromosome 8p (LOD score 2.2 at D8S520) [6]. Subsequently, van der Walt *et al.* chose to examine the *FGF20* gene in their investigation of biological candidate genes for Parkinson's disease susceptibility in this region. The *FGF20* gene is approximately 9.3 kb (*http://genome.ucsc.edu/*), and is located approximately 6.2 Mb from a peak marker D8S520 on chromosome 8p22–p21.3 [7]. FGF20 is a neurotrophic factor that exerts strong neurotrophic properties within brain tissue, and regulates central nervous development and function [8]. FGF20 is preferentially expressed in the substantia nigra [9], and it has been reported to be involved in dopaminergic neurons survival [10]. In order to assess the genetic association of the *FGF20* gene with Parkinson's disease, they genotyped five single nucleotide polymorphisms (SNPs) [ss20399076 (rs12718379), rs1989756, rs1989754, rs1721100, and ss20399075 (rs12720208)] lying within the *FGF20* gene in 644 families from the United States, performed the pedigree disequilibrium test (PDT), the genotype PDT, the multilocus-genotype PDT, and the family-based association test, and discovered a highly significant association of Parkinson's disease with one intronic SNP, rs1989754 (P=0.0006), and two SNPs, rs1721100 (P=0.02) and rs12720208 (P=0.0008), located in the 3′ regulatory region. Furthermore, they detected a haplotype that is positively associated with risk of Parkinson's disease (P=0.0003), whereas a second haplotype was

found to be negatively associated with risk of Parkinson's disease (P=0.0009). Consequently, they concluded that the *FGF20* gene was a susceptibility gene for Parkinson's disease [11].

Subsequently, Clarimon *et al.* sought to replicate the association of the *FGF20* gene with Parkinson's disease by performing a case–control association study with four SNPs [rs1989756, rs1989754, rs1721100, and ss20399075 (rs12720208)] using Finnish and Greek samples. They found a difference in allele frequency in only rs1989754, but the difference was not significant after the Bonferroni correction. They also found no significant difference in the distribution of haplotypes between patients and controls. They hence failed to replicate the association of the *FGF20* gene with Parkinson's disease [12]. Thus, it is still controversial as to whether the *FGF20* gene is a susceptibility gene for Parkinson's disease or not. We here conducted a case–control association study using a large number of Japanese samples in order to evaluate the association of the *FGF20* gene with risk of Parkinson's disease.

Materials and methods

We recruited 1388 unrelated Parkinson's disease patients (age, 65.7±9.8; male/female ratio, 0.84) and 1891 unrelated controls (age, 48.5±17.6; male/female ratio, 1.08). The diagnosis of Parkinson's disease was based on the presence of two or more of the cardinal features of Parkinson's disease (tremor, rigidity, bradykinesia, and postural instability), according to the criteria for Parkinson's disease [13]. Patients were evaluated by certified neurologists specializing in Parkinson's disease. The average age of onset was 57.7±11.1 years. All patients and controls were of Japanese ancestry. Informed consent was obtained from each individual, and approval for the study was obtained from the University Ethical Committees. Genomic DNA was extracted from venous blood using standard procedures.

The TaqMan SNP Genotyping Assay (Applied Biosystems, Foster, California, USA) was employed for five SNPs (rs12718379, rs1989756, rs1989754, rs1721100, and rs12720208). SNP information was obtained from the dbSNP database (*http://www.ncbi.nlm.nih.gov/SNP/*) and the International HapMap Project database (*http://hapmap.org*) [14].

All statistical analyses were performed by using the software SNPAlyze (Dynacom, Japan). Genotype deviation from Hardy–Weinberg equilibrium was assessed by the χ^2 test. The statistical significance of a case–control association was evaluated by the χ^2 test, and odds ratio and its 95% confidence intervals (CIs) were calculated by the Bootstrap method. Haplotype frequencies were estimated using an expectation-maximization algorithm [15]. We evaluated pair-wise linkage disequilibrium (LD) among SNPs by D' value, and r^2 as standards for LD. Case–control haplotype analyses were carried out by calculating the permutation P-value on the basis of 10 000 replications [16].

Results

Two SNPs (rs1989756 and rs12720208) of the five SNPs, examined by van der Walt *et al.*, showed a monomorphism in 95 individuals drawn from the Japanese population, and therefore these two SNPs were excluded from further analysis. Three SNPs (rs1989754, rs1721100, and

Table 1 Summary of the association of three SNPs between Parkinson's disease patients and controls

SNP ID	Alleles		Genotype								Allele 1 versus Allele 2		Genotype II versus 12 + 22		Genotype II + 12 versus 22	
			Patient				Control									
	1–2	Strand[a]	1/1	1/2	2/2	Total	Frequency of allele 1	1/1	1/2	2/2	Total	Frequency of allele 1	Odds ratio (95% CI[b])	*P*-value	Odds ratio (95% CI[b])	*P*-value	Odds ratio (95% CI[b])	*P*-value
rs12718379	A-G	Reverse	249	641	481	1371	0.42	375	902	597	1874	0.44	1.11 (1.01–1.23)	0.041	1.13 (0.95–1.34)	0.19	1.16 (1.00–1.34)	0.054
rs1989754	G-C	Forward	261	628	477	1366	0.42	381	895	586	1862	0.44	1.10 (1.00–1.22)	0.055	1.09 (0.92–1.28)	0.34	1.17 (1.01–1.35)	0.040
rs1721100	C-G	Forward	270	639	458	1367	0.43	407	925	542	1874	0.46	1.14 (1.03–1.26)	0.0089	1.13 (0.94–1.36)	0.17	1.24 (1.06–1.43)	0.0053

[a]Relative to the transcriptional direction.
[b]CI, confidence intervals; SNP, single nucleotide polymorphism.

rs12720208) are included in HapMap. rs12720208 also shows no polymorphism in JPT (Japanese in Tokyo) HapMap, consistent with the genotyping results of our samples. In the *FGF20* gene region, 171 SNPs were observed in dbSNP. According to JPT HapMap, the Tagger method showed that two SNPs (rs1989754 and rs1721100) can represent the remainder of the HapMap SNPs of the *FGF20* gene region, as tag SNPs with a criteria of $r^2>0.8$ and a minor allele frequency >0.1, although the number of tag SNPs differed between JPT and CEU [CEPH (Utah residents with ancestry from northern and western Europe)] [17]. Thus, we considered that a case–control association study using three SNPs [two tag SNPs (rs1989754 and rs1721100) plus rs12718379] was appropriate for assessing the association of the *FGF20* gene with Parkinson's disease.

Table 1 shows the results of the SNP genotyping in the Parkinson's disease patients and controls. The association of rs1721100 was significant in allele 1 versus allele 2 [frequency of allele 1; 43% in patients and 46% in controls, P=0.0089, odds ratio 1.14 (95% CI, 1.03–1.26)] and in genotype 11+12 versus 22 [P=0.0053, odds ratio 1.24 (95% CI, 1.06–1.43)]. The association with rs1721100 was significant even after the Bonferroni correction (tests for three SNPs). As for rs12718379, a decrease in frequency of allele 1 was found in patients compared with controls [frequency of allele 1; 42% in patients and 44% in controls, P=0.041, odds ratio 1.11 (95% CI, 1.01–1.23)]. As for rs1989754, there was a difference in frequency of genotype 11+12 versus 22 between patients and controls [P=0.040, odds ratio 1.17 (95% CI, 1.01–1.35)]. Neither rs12718379 nor rs1989754, however, showed a significant association with Parkinson's disease after the Bonferroni correction. The genotype frequencies of all three SNPs were not significantly different from the values expected from the Hardy–Weinberg equilibrium.

We calculated the LD among the three SNPs in patients and controls. D' values (absolute value) and r^2 for pair-wise LD of controls are shown in Table 2. A high LD was detected between each pair of SNPs, and the same trend was observed in patients and in the JPT samples of the HapMap database (data not shown). These findings suggested that the three SNPs were in single LD, and we therefore performed haplotype association analysis. Haplotype frequencies of the three SNPs were estimated in patients and controls (Table 3). Two common haplotypes (haplotypes 1 and 2) covered >90% of the population haplotypes in both patients and controls. The frequency of haplotype 2 (A-G-C) was significantly less in patients than controls (38% in patients and 41% in controls, permutation-P=0.0075). This indicates that haplotype 2 is a protective haplotype for Parkinson's disease in the Japanese population. Taken together, our genetic analyses support the *FGF20* gene being a susceptibility gene for Parkinson's disease in the Japanese population.

Table 2 Linkage disequilibrium between SNPs in the *FGF20* gene

SNP ID	rs12718379	rs1989754	rs1721100
rs12718379	—		
rs1989754	0.94 (0.98)	—	
rs1721100	0.68 (0.86)	0.72 (0.88)	—

r^2 (D') values of controls are shown for each pair of single nucleotide polymorphisms (SNPs).

Discussion

Our results are consistent with the report by van der Walt *et al.* [11], which showed an association of the *FGF20* gene with risk of Parkinson's disease. The significance of the *FGF20* gene for Parkinson's disease susceptibility in our study, however, was not so strong as that shown by van der Walt *et al.* This discrepancy may result from: (i) the ethnic differences between the Japanese samples and samples from the United States; the association in the Japanese population might be smaller than in the United States, or (ii) the difference in epidemiological approaches; we performed a case–control association study by the χ^2 test in unrelated samples, while they analysed family-based samples by the PDT. rs12720208, the strongly associated SNP in the report by van der Walt *et al.*, was excluded from our study because we were not able to find polymorphism of this SNP in the Japanese samples. It is interesting that rs1721100, the most strongly associated SNP in our study, and rs12720208, however, are both located in the 3′ UTR region of the *FGF20* gene. LD indices between rs12720208 and rs1721100 showed that these two SNPs are in a single LD block (D'=1) and that the correlation was not strong (r^2=0.28) (on the basis of CEU HapMap).

On the other hand, the case–control association study by Clarimon *et al.* [12] failed to replicate the association of the *FGF20* gene with risk of Parkinson's disease, although the rs1989754 G allele frequency was higher in patients than controls in the Finnish samples (52% in patients and 42% in controls, P=0.03 before Bonferroni correction). However, as their sample size was not large enough, their study does not disprove the association of the *FGF20* gene with Parkinson's disease convincingly if the influence for Parkinson's disease in the Greek and Finnish population is to the same extent as in our Japanese sample. The sample size of their study was considerably smaller than ours (Finnish series, 144 patients and 135 controls; Greek series, 151 patients and 186 controls in their study, compared with 1388 patients and 1891 controls in our study). As mentioned in their report, their experiment had 80% power to detect

Table 3 Haplotype association analysis using three SNPs in the *FGF20* gene

	Base at SNP			Haplotype frequency		
Haplotype ID	rs12718379	rs1989754	rs1721100	Patient	Control	*P*-value
Haplotype 1	G	C	G	0.53	0.50	0.054
Haplotype 2	A	G	C	0.38	0.41	0.0075
Haplotype 3	G	C	C	0.045	0.047	0.75
Haplotype 4	A	G	G	0.035	0.028	0.11

risks from 1.7 to 3.6 in the Finnish samples and from 1.6 to 2.1 in the Greek samples, whereas the odds ratio of the *FGF20* gene in our data was 1.14. The possibility of type 2 errors in their study could not be excluded as an explanation for this negative finding. Another explanation for lack of replication could be genetic heterogeneity; there might not be an association between the *FGF20* gene and Parkinson's disease in the Greek and Finnish populations, whereas there might be in the Japanese and the United States-based population.

In this study, the three SNPs (rs12718379, rs1989754, and rs1721100) showed a difference between patients and controls to some degree. After Bonferroni correction, however, a significant association was detected only in rs1721100. The correlations between rs1721100 and the other two SNPs were not strong (r^2=0.68 with rs12718379 and 0.72 with rs1989754), which might explain the different extents of significance among the three SNPs.

The *EFHA2* gene is located 25 kb upstream of the 5′ UTR, and the *MSR1* gene is located 800 kb downstream of the *FGF20* gene. The entire region of the *FGF20* gene is within a single LD block of 20.8 kb (on the basis of JPT HapMap). Moreover, no other known genes reside within this LD block. Therefore, we concluded that our positive finding results from the association between the *FGF20* gene and Parkinson's disease.

Conclusion

We performed a case–control association study using a large number of samples (1388 Parkinson's disease patients and 1891 controls) in the Japanese population, and found a significant association of Parkinson's disease with rs1721100 and haplotype 2 (A-G-C) in the *FGF20* gene. Our results, together with those of van der Walt *et al.*, demonstrate an association of the *FGF20* gene with Parkinson's disease in two different ethnic groups. This evidence suggests the involvement of the *FGF20* gene in the pathogenesis of Parkinson's disease.

Acknowledgements

The authors are grateful to the Parkinson's disease patients who participated in this study. The authors thank Drs Akira Oka, Hidetoshi Inoko, and Katsushi Tokunaga for control samples. They also thank Dr Helena A. Popiel for editing the manuscript. This work was supported by the 21st century COE program and Research Grants (17019044 and 17590874), both from the Ministry of Education, Culture, Sports, Science and Technology of Japan; by the Grant for Research on Measures for Intractable Diseases (H17-Q-15-1) from the Ministry of Health, Labor and Welfare of Japan; and by a grant from Core Research for Evolutional Science and Technology (CREST) of the Japan Science and Technology Agency (JST).

References

1. Rascol O, Payoux P, Ory F, Ferreira JJ, Brefel-Courbon C, Montastruc JL. Limitations of current Parkinson's disease therapy. *Ann Neurol* 2003; **53**:S3–S12.
2. Warner TT, Schapira AH. Genetic and environmental factors in the cause of Parkinson's disease. *Ann Neurol* 2003; **53**:S16–S23.
3. DeStefano AL, Golbe LI, Mark MH, Lazzarini AM, Maher NE, Saint-Hilaire M, *et al.* Genome-wide scan for Parkinson's disease: the GenePD study. *Neurology* 2001; **57**:1124–1126.
4. Hicks AA, Petursson H, Jonsson T, Stefansson H, Johannsdottir HS, Sainz J, *et al.* A susceptibility gene for late-onset idiopathic Parkinson's disease. *Ann Neurol* 2002; **52**:549–555.
5. Pankratz N, Nichols WC, Uniacke SK, Halter C, Rudolph A, Shults C, *et al.* Genome screen to identify susceptibility genes for Parkinson disease in a sample without parkin mutations. *Am J Hum Genet* 2002; **71**:124–135.
6. Scott WK, Nance MA, Watts RL, Hubble JP, Koller WC, Lyons K, *et al.* Complete genomic screen in Parkinson disease: evidence for multiple genes. *JAMA* 2001; **286**:2239–2244.
7. Kirikoshi H, Sagara N, Saitoh T, Tanaka K, Sekihara H, Shiokawa K, *et al.* Molecular cloning and characterization of human FGF-20 on chromosome 8 p21.3-p22. *Biochem Biophys Res Commun* 2000; **274**:337–343.
8. Jeffers M, Shimkets R, Prayaga S, Boldog F, Yang M, Burgess C, *et al.* Identification of a novel human fibroblast growth factor and characterization of its role in oncogenesis. *Cancer Res* 2001; **61**:3131–3138.
9. Ohmachi S, Watanabe Y, Mikami T, Kusu N, Ibi T, Akaike A, *et al.* FGF-20, a novel neurotrophic factor, preferentially expressed in the substantia nigra pars compacta of rat brain. *Biochem Biophys Res Commun* 2000; **277**:355–360.
10. Ohmachi S, Mikami T, Konishi M, Miyake A, Itoh N. Preferential neurotrophic activity of fibroblast growth factor-20 for dopaminergic neurons through fibroblast growth factor receptor-1c. *J Neurosci Res* 2003; **72**:436–443.
11. Van der Walt JM, Noureddine MA, Kittappa R, Hauser MA, Scott WK, McKay R, *et al.* Fibroblast growth factor 20 polymorphisms and haplotypes strongly influence risk of Parkinson disease. *Am J Hum Genet* 2004; **74**:1121–1127.
12. Clarimon J, Xiromerisiou G, Eerola J, Gourbali V, Hellstrom O, Dardiotis E, *et al.* Lack of evidence for a genetic association between FGF20 and Parkinson's disease in Finnish and Greek patients. *BMC Neurol* 2005; **5**:11.
13. Bower JH, Maraganore DM, McDonnell SK, Rocca WA. Incidence and distribution of parkinsonism in Olmsted County, Minnesota, 1976–1990. *Neurology* 1999; **52**:1214–1220.
14. The International HapMap Consortium. The International HapMap Project. *Nature* 2003; **426**:789–796.
15. Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evol* 1995; **12**: 921–927.
16. Fallin D, Cohen A, Essioux L, Chumakov I, Blumenfeld M, Cohen D, *et al.* Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. *Genome Res* 2001; **11**:143–151.
17. De-Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nat Genet* 2005; **37**:1217–1223.