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Brief Reports

A Unique Case of Cortical Myoclonus Sensitive to Visual Stimuli in the Peripersonal Space

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Video 

Abstract: **Multimodal representation of *peripersonal* or *near* space has been demonstrated in the brain of the non-human primate through invasive electrophysiological experiments. Representation of peripersonal space in the human brain has been inferred from extinction experiments and functional imaging studies. We present a unique case of lower limb myoclonus in a patient with common variable immunodeficiency which is sensitive to visual stimuli in the peripersonal space and light touch. This case provides further evidence for near space representation in the human brain. We hypothesize that somatopically organized multimodal areas exist in the human brain which code for peripersonal space.** © 2008 Movement Disorder Society

Key words: near space; peripersonal space; myoclonus; stimulus sensitive; cortical

Peripersonal space or near space is the envelope of space immediately surrounding the body.[1] Invasive electrophysiological experiments have clearly demonstrated that peripersonal space has multimodal representation in the brain of the macaque monkey.[2–5]

Until recently, the evidence for brain representation of peripersonal space in humans had been largely inferred from cross-modal extinction experiments in patients with right hemisphere lesions.[1,6,7] A recent carefully controlled functional magnetic resonance imaging (MRI) study in normal subjects provided compelling evidence for the existence of areas in the human brain which code for peripersonal space.[8] These areas overlap with those in the monkey and include frontal areas, the intraparietal sulcus, and the lateral occipital complex.[8] The anterior intraparietal sulcus was shown to have multimodal sensory properties.[8]

In the monkey model, the areas coding peripersonal space are multimodal and contain bimodal neurons which respond to light tactile stimuli and visual stimuli in the near space.[2–5] We present a unique case of cortical reflex myoclonus that provides further evidence for the representation of peripersonal space in the human brain.

CASE REPORT

This 24-year-old right handed woman has a history of common variable immunodeficiency (CVID) diagnosed in early childhood. She has a long history of opportunistic infections including bacterial meningitis at age 9, sinusitis and pneumonia. One year prior to presentation she had a splenectomy because of thrombocytopenia and leucopenia. Previously she received monthly intravenous immunoglobulin but more recently has had subcutaneous immunoglobulin three times a week.

She presented with a 1 year history of involuntary jerks of the right foot more so than the left. The jerks in the right leg could occur when her right foot approached and touched the ground when walking or when trying to place her foot on a step. She found it particularly difficult to put on her shoes as her leg would jerk when her foot approached her shoe. At the time she was constitutionally well and had no other neurological symptoms.

Salient findings on neurological examination included mild hyperreflexia in the right upper limb and slight loss of dexterity of left foot tapping. There were minimal jerks of the legs at rest. Light tactile stimulation on the right foot and lower leg often resulted in a

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myoclonic jerk of the limb. This was much less prominent on the left. A moving visual stimulus such as the examiner's hand within 30 cm of the foot or lower leg, but without necessarily touching the limb, produced a similar jerk consistently on the right and infrequently on the left (see video). A jerk could not be elicited by the mere thought of the examiner's hand near the right leg.

Routine biochemistry and screening tests for Wilson's disease were unremarkable and her full blood count revealed a borderline leukocytosis and mild thrombocytosis consistent with her history of splenectomy. Her erythrocyte sedimentation rate was 21 mm/hr and C reactive protein was 16 mg/L. MRI of the brain, including T1, T2, FLAIR, and T1 postgadolinium sequences, was normal. Electroencephalography (EEG) and EEG video telemetry were similarly normal. Cerebrospinal fluid (CSF) examination revealed 18 neutrophils, 1 lymphocyte, and 0 red cells per microliter. CSF biochemistry was normal and cultures were negative.

Further neurophysiological investigations were undertaken. Surface electromyography (EMG) over the right tibialis anterior, gastrocnemius, quadriceps, and hamstrings revealed EMG bursts of 20 to 30 ms occurring most often singularly and less often in trains of two to three lasting 20 to 50 ms, consistent with cortical myoclonus.

EMG with EEG jerk-locked back averaging was performed. Four runs of at least 150 sweeps were averaged while recording EMG from the right tibialis anterior and the EEG from C4-Fz and C3-Fz. It should be noted that myoclonus was elicited solely by presenting a moving visual stimulus near the right foot of the patient, *not touching*. A reproducible cortical waveform was demonstrated (see Fig. 1). Somatosensory evoked potentials (SEPs) from the upper limb and right lower limb were normal.

The patient was commenced on clonazepam 0.5 mg at night with significant improvement in her symptoms.

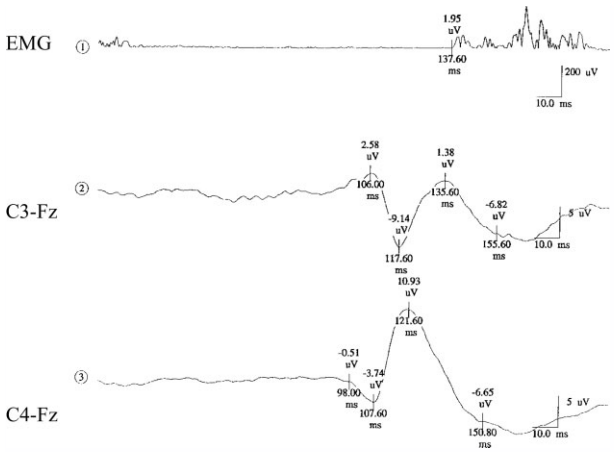

FIG. 1. The EMG jerk-locked back averaged EEG demonstrating a cortical potential. During the recording myoclonus was elicited by a nonthreatening visual stimulus in the peripersonal space of the right foot.

DISCUSSION

The multimodal representation of peripersonal space in the primate has been well documented.[2–5] The areas identified coding for peripersonal space in this primate model include the inferior portion of Area 6 (F4), the ventral intraparietal area, parietal area 7b, and the putamen.[2–5] In all of these areas bimodal neurons have been identified which respond to light touch and visual stimuli in the corresponding peripersonal space.

Experiments have been conducted previously in patients with right hemisphere lesions demonstrating that visual stimuli in the near space of the right hand or face can result in extinction of a left tactile stimulus.[1,6,7] One can speculate from these experiments that there is integration of tactile stimulation and vision in the near space; cross-modal extinction is the result of an uneven competition between the spared and affected representations of space.[1] More compelling evidence for the existence of peripersonal space representation in humans came from a recent study by Makin et al. using fMRI in healthy subjects.[8] They compared near (peripersonal space) and far visual stimuli in different situations to determine visual, proprioceptive, and tactile representations of near space. The intraparietal sulcus, frontal areas, and lateral occipital complex were shown to be responsible for the visual representation of peripersonal space.[8] The anterior intraparietal sulcus was demonstrated to have multimodal properties with representation for light touch, proprioception as well as visual stimuli in the peripersonal space.[8]

This case provides further evidence for the existence of representation of peripersonal space in the human brain and is unusual for a number of reasons. No animal or human studies previously have demonstrated representation for lower limb peripersonal space; somatotopic organization has been demonstrated in animal studies but representation for the hind limb of the monkey has not been identified.[2–5] Human studies have not demonstrated that peripersonal space is represented in both hemispheres either. The fact that both lower limbs

were affected suggests that peripersonal space representation for the lower limb may be parasagittal if a single lesion were to cause the clinical picture.

The etiology for the patient's myoclonus was unknown. Granulomatous disease similar to sarcoidosis is known to occur in CVID and central nervous system involvement may have been the cause for the presentation.[9] The CSF pleocytosis would be consistent with this and the fact that the MRI was normal does not exclude this possibility.[10] The jerk-locked back averaged EEG potential was localized centrally but it is difficult to make a clear assessment of location based on EEG alone. This form of stimulus-sensitive myoclonus has hitherto not been described; stimuli which have been commonly described to cause myoclonus include cutaneous, stretch, loud noise (startle), and visual threat,[11] but this was a case of nonthreatening visual stimuli in the near space resulting in myoclonus.

The myoclonus was clearly of cortical origin as the results of the jerk-locked back averaging demonstrate. Cortical myoclonus is thought to result from pathological hyperexcitability of sensorimotor cortical areas.[12–16] Ashby et al. provided compelling evidence by extensive invasive neurophysiological investigations in a patient with focal cortical myoclonus that the pathologic hyperexcitability was confined to an appropriate of primary motor cortex in that particular case. The patient's myoclonus resolved when the area of cortex was resected.[12] Giant SEPs have been associated with cortical myoclonus and are thought to result from the cortical hyperexcitability which occurs in this condition.[16] Early components of the SEP are not pathologically enlarged which indicates that disinhibition of thalamocortical projections is not involved, but rather pathologically enhanced cortico-cortical projections.[16] Researchers have studied somatosensory evoked fields (SEFs) with magnetoencephalography in patients with cortical myoclonus and giant SEPs.[13–15] Coregistration with MRI has been used to localize the earliest component of the SEF which is pathologically enlarged (P25m). This component has been localized to the sensory cortex in some patients and the motor cortex or even premotor areas in others.[13–15] It is likely that hyperexcitability need only occur in part of the circuit involved in the generation of myoclonus for cortical myoclonus to occur. A hyperexcitable sensory cortex may result in myoclonus through projections directly onto spinal interneurons or may drive the motor cortex through connections with the precentral gyrus. Hyperexcitability in motor areas may result in myoclonus more directly through descending corticospinal tracts.

Visual stimuli in the peripersonal space and light tactile stimulation produced myoclonus of the lower limb of equal magnitude while myoclonus largely did not occur at other times. As mentioned previously, brain representation of peripersonal space in the monkey is multimodal with areas containing bimodal neurons responding to similar stimuli. We hypothesize that hyperexcitability of analogous areas in the human was responsible for the myoclonus in this case. The clinical picture was not suggestive of sensorimotor cortex hyperexcitability as the primary problem given that myoclonus was not occurring in other situations such as at rest, on action, or provoked by other stimuli. Also, visual stimuli in the near space have not been previously reported to result in myoclonus, despite numerous reports of patients with cortical myoclonus having demonstrable sensorimotor hyperexcitability in the form of giant SEPs.

There is evidence both from human and animal experiments that the representation of peripersonal space is "hardwired," i.e. cannot be modulated through learning.[1] It was of interest that in our case that the patient imagining a visual stimulus in the near space could not provoke myoclonus, and she was not consciously able to inhibit the production of myoclonus by visual stimuli in the near space.

In conclusion, this unique case of cortical myoclonus provides further evidence for the existence of multimodal representation of peripersonal space in humans. The clinical picture and neurophysiology is suggestive that the pathology selectively affects multimodal areas coding for lower limb peripersonal space.

LEGENDS TO THE VIDEO

Segment 1. Patient attempting to put on shoes.
Segment 2. At rest.
Segment 3. Tactile stimulation with eyes closed.
Segment 4. Visual stimuli in the peripersonal space.

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Responsiveness to Levodopa in Epsilon-Sarcoglycan Deletions

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Video 

Abstract: **Myoclonus-dystonia (M-D) is characterized by early-onset myoclonus and dystonia, and is often due to mutations in the epsilon-sarcoglycan gene (SCGE) at locus 7q21. The pathogenesis of M-D is poorly understood, and in a murine knockout model, dopaminergic hyperactivity has been postulated as a mechanism. We present two unrelated individuals with M-D due to SCGE deletions who displayed a robust and sustained response to levodopa (L-dopa) treatment. In contrast to using dopamine blocking agents suggested by the hyperdopaminergic knockout model, we propose that a trial of L-dopa may be considered in patients with myoclonus-dystonia.** © 2009 Movement Disorder Society

Key words: myoclonus; dystonia; Epsilon-Sarcoglycan; Levodopa

Myoclonus-dystonia (M-D) is characterized by early-onset dystonia and myoclonus, and is often responsive to alcohol.[1,2] A large proportion of M-D is caused by mutations in the epsilon-sarcoglycan gene (SCGE).[3,4] Clonazepam,[5] sodium oxybate,[6,7] valproate,[8] levetiracetam,[9] L-5-H-tryptophan[10] and deep brain stimulation[5,11,12] may improve symptoms, but efficacy may be limited, or side effects too great.[13] Dopaminergic agents are not typically included in the

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treatment regimen, and their role is unclear. Based on a knockout murine model demonstrating striatal hyperdopaminergia, it has been suggested that dopamine blocking agents may be used for the treatment of this condition.[14] Contrary to what this model predicts, we report two children with M-D due to exonic deletions of SCGE who had significant improvement of their myoclonus with levodopa (L-dopa).

METHODS

Two patients were systematically examined and videotaped, and maximal medical treatment was undertaken. Informed consent was obtained. The SCGE gene was sequenced, and screening for deletions was performed using quantitative duplex PCR assay for all coding exons as previously described.[15]

RESULTS

Patient 1 is a 17-year-old right-handed boy, who was born at 35 weeks via uncomplicated vaginal delivery. He was noted at birth to have macrocephaly and low set ears, with low birth weight and height. He had mild developmental delay initially but caught up quickly with his psychomotor milestones and intelligence was normal. He had a single febrile seizure at 15 months. He received growth hormone for persistent low growth from age 6 to 15 years. Motoric development was normal until age 12, when trunk and leg myoclonus developed, which led to frequent falls when walking. The movements were initially noted to be purely myoclonus, and he was given the diagnosis of primary essential reticular myoclonus. Within 2 years, he also developed dystonic posturing of his trunk, arms and legs, and myoclonus involving his voice, arms and neck which was worse with action. In addition to falls, there was frequent jerking at rest, difficulty writing and pouring. His symptoms progressed over several months and then stabilized. Response to alcohol is unknown.

Evaluation included unremarkable MRIs of brain and spine, normal EEG, muscle biopsy, serum amino and urine organic acids, EMG/NCS, brainstem auditory evoked potentials, somatosensory evoked potentials and visual evoked potentials. Ophthalmologic examination was negative for Kayser-Fleischer rings. Testing for mucolipidosis I and II was reportedly negative. Commercially available genetic testing was negative for Fragile X, Lafora disease (PME1, PME2A, and PME2B), DYT1 GAG deletion and dopa-responsive dystonia (DRD) exonic mutations (GCH-1) (Athena diagnostics, Worcester, MA; Massachusetts General Hospital Neurogenetics, DNA diagnostic lab, Charlestown, MA). Chromosomal analysis (Genzyme genetics, Cambridge, MA) was also normal.

The patient's paternal grandmother has late-onset parkinsonism responsive to pramipexole. Both parents and three siblings are asymptomatic. Valproic acid markedly improved the myoclonus but induced hyperammonemia. Clonidine, zonisamide, and levetiracetam did not improve symptoms. Clonazepam 1.5 mg/day may have modestly improved his myoclonus, and tremor was slightly improved with 10 mg/day trihexyphenidyl.

At age 15 (segment 1), on 1.5 mg/day of clonazepam and 10 mg/day of trihexyphenidyl, he was noted on exam to be bright and outgoing, to have short stature and diffuse myoclonus of his neck, arms, trunk and legs at rest and with action, and which was not worse with startle or tactile stimuli. He had dystonic posturing in the right arm when writing; gait was characterized by trunk flexion, and dystonic leg posturing, with superimposed myoclonic jerks. Mental status, cranial nerves, strength, reflexes and sensory testing were normal. There was no parkinsonism or cerebellar dysfunction.

Screening for deletions of SCGE was performed as previously described,[15] and demonstrated deletions of exons 2 to 5. The deletion was also observed in the asymptomatic father, consistent with maternal imprinting of the SGCE gene.

Because of the prominent dystonia, poor growth and family history of parkinsonism, he was treated with empiric carbidopa/L-dopa in addition to trihexiphenidyl and clonazepam. At 300 mg/day of L-dopa, there was significant improvement in jerking. At 800 mg/day, his myoclonus markedly improved such that he was able to sit still and pour, could write better, and was only falling infrequently, twice per month on average versus several times per day before L-dopa therapy. Of note, as his truncal myoclonus alleviated, truncal and right leg dystonia were more noticeable (segment 2). Increase of trihexyphenidyl further improved truncal and leg dystonia. He is currently maintained on a regimen of 800 mg/day of L-dopa, 19 mg/day of trihexiphenidyl and 1.5 mg/day of clonazepam, and has not developed motor fluctuations or dyskinesias after 3 years of therapy.

Patient 2 is a 12 year-old right-handed girl who was adopted at birth. She is the product of a normal pregnancy and delivery and her biological parents were thought to have been heavy alcohol users. She had a normal psychomotor development, and is of normal intelligence. At the age of 7, irregular distal tremor was observed in both hands. The tremor markedly

worsened after her menarche at age 11. Over the following months, irregular "lightning-like" movements of her arms and legs, more prominent on the right side were seen, and difficulty with writing emerged. After age 12, the symptoms stabilized. Examination was notable for writer's cramp, mild head tilt and action-induced myoclonus. She had no parkinsonism or cerebellar dysfunction. Her mental status, cranial nerves, strength, reflexes, sensory exam, stance and gait were normal.

Examinations included unremarkable MRI of brain, CBC, comprehensive metabolic panel, normal thyroid function, ESR, negative ANA and anti-DNA antibodies and had undetectable Lyme titers. She had normal serum copper and ceruloplasmin levels and an ophthalmologic examination did not reveal Kayser-Fleischer rings. Genetic testing for DYT1 mutations was normal, as well as sequencing for GCH-1 (Massachusetts General Hospital Neurogenetics, DNA diagnostic lab, Charlestown, MA).

A deletion of exon 5 of the SCGE gene was demonstrated when using quantitative duplex PCR assay for all coding exons. At the age of 12, she was started on carbidopa/L-dopa. At 300 mg/day, her myoclonus markedly improved in frequency and amplitude. She was able to write for prolonged periods of time and her head tilt was less noticeable. She has been on 350 mg/day of L-dopa for the past 3 years with sustained marked improvement in her symptoms, and without side effects including motor fluctuations and dyskinesias. She, however, continues to have very fine small amplitude myoclonic jerks resembling postural tremor in both hands, and mild writer's cramp at the end of the day.

DISCUSSION

We present two patients with M-D and SCGE deletions who responded to L-dopa therapy. The mechanism of the L-dopa-responsiveness is unclear. Parkinsonian features of reduced armswing and postural instability responsive to L-dopa therapy were reported in one SCGE mutation positive individual with low CSF HVA levels, and rest tremor and reduced armswing in another without CSF testing.[16] Hjermind et al. reported good response to carbidopa/L-dopa in one of nine mutation carrying family members, but attributed this to a possible placebo effect as the minority of individuals with the same mutation sustained this benefit.[17] Epsilon-sarcoglycan is expressed in midbrain monoaminergic neurons although its precise relation to dopamine modulation is unclear.[18] Yokoi et al. found an inverse correlation of dopamine and 5-HT metabolites in the SCGE knockout mouse model of M-D,[14] theorized a hyperdopaminergic state in the syndrome, and hypothesized that L-dopa treatment may not only be ineffective, but may worsen symptoms. However, to our knowledge, L-dopa has not been demonstrated to make M-D worse,[4,19] may improve symptoms[16,17] and in our case series, it was clearly beneficial.

Although M-D as a phenotype of DRD was reported in one patient with a GCH1 mutation,[20] we do not believe the response to L-dopa suggests that our patients had DRD. There is only a single report to date, and our patients did not demonstrate the characteristic dramatic response to low doses of L-dopa. Further, screening for GCH1 mutations was negative in both children, although this mutation testing alone does not exclude DRD.

Patient 1 had an unusual presentation. Although growth deformities are seen as part of the phenotype of DRD,[21] and growth retardation, mild developmental delay and facial dysmorphism have been described in patients with large deletions of the 7q21 locus that included contiguous genes in M-D patients,[22,23] to our knowledge this is the first report of these as part of the M-D phenotype due to deletions restricted to SCGE. In addition falls are an uncommon manifestation of myoclonus in SCGE mutation carriers.[17]

In summary, with the limitations that two single cases pose, we infer that it is possible that in a subset of patients with myoclonus dystonia, there is a dopamine dysregulation component that could be at least partially alleviated with L-dopa. The L-dopa responsiveness is probably independent to the specific mutation, as both patients had different deletions, although it appears that the phenotype of patients with small mutations is similar to those with large ones.[24] We cannot explain the heterogeneity in response to L-dopa, as this varies among family members and is not genotype specific. We suggest that a trial of this well-tolerated medication may be considered in some individuals with M-D.

LEGENDS TO THE VIDEO

Segment 1. ("8/13/2004, Before L-dopa"). This segment shows the baseline neurological examination of our patient. A general view at rest shows generalized myoclonus that is also evident with arms outstretched. There is no evidence of bradykinesia when performing fast finger taps and hang grips. Action myoclonus is seen on foot taps and alternating heel and toe taps.

Writer's cramp and leg dystonia present during writing. There is frequent generalized myoclonus that triggers a fall while walking.

Segment 2. ("3/4/2005, On 800 mg/day L-dopa"). This segment shows the improvement in his symptoms after treatment with L-dopa. Generalized myoclonus is much reduced at rest, and with actions such as finger taps, hand grips and alternating heel and toe taps. Writer's cramp persisted, but gait was much improved with a decrease in the amplitude of myoclonus and avoidance of falls. Truncal dystonia was more noticeable after treatment with L-dopa.

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Genetic Association Study of the P-Type ATPase *ATP13A2* in Late-Onset Parkinson's Disease

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Abstract: **A role of *ATP13A2* in early-onset Parkinsonism (EOP) has been proposed. Conversely, the contribution of this ATPase to late-onset Parkinson's disease (PD) remains unexplored. We therefore conducted a case–control association study in this age-of-onset group with PD. The initial sample was of German origin and consisted of 220 patients with late-onset PD (mean age of onset 60.1 years) and 232 age-matched unrelated controls. Five single nucleotide polymorphisms (SNPs) covering *ATP13A2* and its common haplotypes were genotyped. The overall association results in this sample were negative. Interestingly, gender stratification gave a positive result for SNP *rs11203280* (P_{UNC} = 0.016) in men. This result could not be reproduced in a replication sample of German and Serbian origin composed of 161 patients with late-onset PD (mean age of onset 51.7 years) and 150 age- and ethnic-matched controls. In conclusion, we found no consistent evidence for an association between *ATP13A2* and late-onset PD.** © 2008 Movement Disorder Society

Key words: *ATP13A2*; Parkinson's disease; association study; Parkinsonism

Parkinson's disease (PD) and Parkinsonism are a heterogeneous group of common neurodegenerative disorders with a prevalence of ~3% in people over the age of 65 years. Both diseases show several common clinical and pathological features, such as hypokinetic-rigid syndrome, tremor at rest, and nigral degeneration.[1–3] Regarding the age of disease onset, the term early-onset Parkinsonism (EOP) describes a group of patients with onset of symptoms before the age of 40 years.[4]

Several common key factors have been involved in the molecular etiology of PD and Parkinsonism, including enhanced oxidative stress, mitochondrial dysfunction, accumulation of aberrant or misfolded proteins, and dysfunction of the ubiquitin-proteasome system (UPS) and the lysosome.[5–7] In this regard, we have identified loss-of-function mutations in *ATP13A2*, a mainly neuronal P-type ATPase gene, the gene product of which is localized in the lysosome. Initially, mutations were found in 2 families affected by a rare form of EOP with dementia called Kufor-Rakeb disease (KRD).[8] However, the clinical characterization of the KRD phenotype showed additional features beyond the spectrum of "pure" PD. Nevertheless, the presence of levodopa-responsive Parkinsonism and the increased expression of *ATP13A2* in the brain of patients with idiopathic PD raised the question whether variants in this gene could also be associated with idiopathic PD.[8–10] This issue has attracted further attention by the identification of a homozygous missense mutation and two heterozygous mutations in EOP patients.[11] However, whether *ATP13A2* also contributes to the pathogenesis of late-onset PD is currently unknown. Therefore, we investigated this question in 2 independent case–control samples of late-onset PD by a systematic single nucleotide polymorphism (SNP)-based association study. Here, we present the results of five SNPs, which capture the predicted common haplotype variations of *ATP13A2* locus.

MATERIAL AND METHODS

Patients

After obtaining informed consent, all patients underwent a standardized neurological examination by a

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movement disorder specialist. The study was approved by the local university ethics committees. The diagnosis of PD was based on the UK Brain Bank diagnostic criteria (with the exception that positive family history was not regarded an exclusion criterion).[12] Patients were collected at movement disorders centres in Bonn, Cologne, Lübeck, and Belgrade and represent consecutive series, not originating in a geographic isolate. The initial case–control sample was collected in Bonn and Cologne. Patients were included in this study only if the onset of symptoms was over the age of 40 years and they had German background (i.e., both parents were born in Germany). Controls of this sample were included if their age was over 40 years and they had a German background. A replication sample of German and Serbian origin was collected in Lübeck and Belgrade. Family history was regarded positive if Parkinsonism was reported for 1 first- or second-degree relative. PD was rated using the Unified Parkinson's Disease Rating Scale (UPDRS) III. A more detailed description of both patient and control samples including their ethnic background is found in Table 1.

SNP Selection

Haplotype-tagging (ht-)SNPs were selected from the HapMap database (http://www.hapmap.org; version July 2006) to discriminate between all predicted common haplotypes (with an estimated frequency >5%) within haplotype blocks in the Central European sample. Haplotype blocks were defined as regions in which >85% of total haplotype diversity is covered by common haplotypes, using the program hapblock.[13]

Genetic Analysis

Initial SNP-genotyping on genomic DNA was performed on a TaqMan™ platform with assays designed by Applied Biosystems. The SNP *rs3754511* was genotyped using pyrosequencing. The genotyping of the replication sample was performed by RFLP for SNPs *rs2871775* and *rs3754511*, using *Hpa*I and *Msp*I restriction enzymes, respectively. Genotyping of *rs3738815* and *rs11203280* was carried out on a LightCycler (Roche Diagnostics). All primer sequences and experimental conditions are available from the authors.

Statistics

First, quality control was applied to the dataset. Thus, individuals displaying a low call rate (one missing SNP) were removed from the analysis. SNPs passed the quality control when showing a call rate higher than 95%. The exact test described and implemented by Wigginton et al. was used to check for Hardy–Weinberg equilibrium (HWE).[14] Distribution of genotypes was consistent with HWE in both groups of cases and controls. Genotypes and allele distributions were compared between cases and controls for all SNPs using the global genotype test and Armitage trend test, genotypic test, dominant test, recessive test. To perform case–control tests on the distribution of a probabilistically inferred set, the haplotypes standard EM algorithm was used. All analyses were performed using the free open source PLINK v0.99r developed by Purcell et al.[15]

Power Calculation

A power analysis was performed with the Genetic Power calculator.[16] We estimate that, under the assumption of complete LD between the marker tested and the disease-causing variant, we had 77% power to detect a true difference in allele frequency between the 220 patients with PD and 232 controls (i.e., in the first sample) with a single-marker association analysis ($\alpha = 0.05$), further assuming a frequency of the disease-associated allele A of 0.25, a relative risk of 1.5 for genotype Aa and of 2.25 for genotype AA, and a prevalence of PD in the general population over the age of 50 years of 1.5%. Using these parameters with both samples together, the estimated power increases to 95%.

RESULTS

The initial genotyping involved seven SNPs covering the entire *ATP13A2* locus. The sample used for the first stage of our association analysis included a total of 452 unrelated individuals from Germany (Table 1). Although two SNPs failed the quality control and were omitted from the analysis, the remaining five SNPs described >90% of the most common haplotypes. Therefore, genotypic distributions between cases and control subjects were compared for these five SNPs. These comparisons were performed using different test models including a recessive model assuming homozygocity, dominant model, genotypic model, and additive model. Regardless which test was performed, the results for the first sample did not support a significant allelic or genotypic association with PD with a minimal *P*-value for *rs11203280* of $P_{UNC} = 0.134$ (Table 2). Interestingly, the same SNP showed a significant association in the men subgroup with an uncorrected *P*-value of $P_{UNC} = 0.016$. However, this result was

TABLE 1. *General characteristics of the case–control dataset*

	Patient data							Control data					
			Ethnic background		Sex				Ethnic background		Sex		
Sample origin	Inclusion criteria*	No. of patients	German	Serbian	Men	Women	Age of onset ± S.D.	Inclusion criteria	No. of controls	German	Serbian	Men	Women	Age ± S.D.
Bonn and Cologne, Germany	UBBCDC	220	–	–	153	67	60.1 ± 9 (41–85)	Population from University Hospital Bonn	232	–	–	105	127	69.6 ± 11.7 (41–99)
Luebeck, Germany	UBBCDC	161	123	38	126	35	51.7 ± 10.3 (40–80)	Healthy volunteers	150	119	31	97	53	53.9 ± 10,8 (40–90)

Age and standard deviation (S.D.) are given in years.
*Daniel and Lees.[12]
UBBCDC, UK Brain Bank Clinical Diagnostic Criteria.

not significant after permutation-based correction for the number of test performed ($P > 0.05$).

This finding prompted us to analyse a second independent sample of cases and controls; especially, as the initial sample had a reduced power to detect a true allelic difference. Thus, we genotyped the same five SNPs in a cohort containing 311 individuals of German and Serbian background (Table 1). The analysis revealed no association between this second sample and PD and no gender effect was observed in this second cohort (*rs11203280* of $P_{UNC} = 0.389$ overall and $P_{UNC} = 0.570$ in men; Table 2). As expected, the analysis of both samples combined showed no association with PD (data not shown). To avoid any bias in the analysis because of population stratification in the second sample, we merged the German individuals from both samples to determine whether these initially observed differences were specific to German patients with PD (Table 2). However, no positive association emerged from this analysis in either the complete sample and in the German men subgroup, best *P*-value at *rs11203280* of $P_{UNC} = 0.514$ and $P_{UNC} = 0.084$, respectively.

DISCUSSION

We present here the data of a systematic case–control association study in 2 independent late-onset PD samples. The overall results of our analysis did not support an association between *ATP13A2* and PD. Interestingly, gender stratification of the initial sample showed a significant association for men with PD in the single marker analysis. Unfortunately, this finding did not withstand a correction for multiple testing. Nevertheless, we decided to test the veracity of this finding by increasing the power of detection of our study. Therefore, we analyzed a second independent sample of individuals with German and Serbian background. However, the gender effect could neither be replicated in the second sample nor in both samples combined. Furthermore, no gender effect was observed in the analysis of all German individuals from both samples. To confirm our findings, we analyzed the raw data for the *ATP13A2* locus contained in two different genome wide association studies (GWA).[17,18] This analysis revealed that all SNPs from our study were contained in one of the GWA,[17] and that they showed no association between this ATPase gene and PD, either (data not shown). Interestingly, the cohort analyzed in this GWA was composed of white individuals from USA with an age of onset of PD ranging from 55 to 84 years. Thus, our

TABLE 2. *Statistical analysis of SNPs with differential allele distribution between men and women*

	All				Men				Women			
	Allele distribution (%)				Allele distribution (%)				Allele distribution (%)			
	Patients A|B	Controls A|B	*P*-value	OR (95% CI)	Patients A|B	Controls A|B	*P*-value	OR (95% CI)	Patients A|B	Controls A|B	*P*-value	OR (95% CI)
1st Sample	(n = 220)	(n = 232)			(n = 153)	(n = 105)			(n = 67)	(n = 127)		
rs3738815	18.5|81.5	20.1|79.9	0.540	0.9 (0.6–1.3)	17.0|83.0	23.6|76.4	0.065	0.7 (0.4–1.0)	22.0|78.0	17.0|83.0	0.270	1.3 (0.8–2.3)
rs1203280	30.6|69.4	35.1|64.9	0.134	0.8 (0.6–1.1)	29.8|70.2	39.5|60.5	**0.016**	0.6 (0.4–0.9)	32.3|67.7	31.5|68.5	0.871	1.0 (0.7–1.6)
2nd Sample	(n = 161)	(n = 150)			(n = 126)	(n = 97)			(n = 35)	(n = 53)		
rs3738815	20.2|79.8	19.3|80.7	0.785	1.1 (0.7–1.6)	21.8|78.2	21.1|78.9	0.860	1.0 (0.7–1.6)	14.3|85.7	16.0|84.0	0.727	0.9 (0.4–2.0)
rs1203280	33.5|66.5	30.3|69.7	0.389	1.2 (0.8–1.6)	36.1|63.9	33.5|66.5	0.570	1.1 (0.8–1.7)	24.3|75.7	24.5|75.5	0.969	1.0 (0.4–2.0)
1st + 2nd Sample (Germans only)	(n = 343)	(n = 351)			(n = 241)	(n = 171)			(n = 102)	(n = 180)		
rs3738815	19.0|81.0	20.3|79.7	0.550	0.9 (0.7–1.2)	18.9|81.1	23.8|76.2	0.084	0.7 (0.5–1.0)	19.3|80.7	16.9|83.1	0.472	1.2 (0.8–1.8)
rs1203280	31.9|68.1	33.5|66.5	0.514	0.9 (0.7–1.2)	32.9|67.1	37.7|62.3	0.140	0.8 (0.6–1.1)	29.5|70.5	29.4|70.6	0.989	1.0 (0.7–1.5)

n indicates the number of tested individuals.
P-values were obtained using the Armitage Trend Test. Results shown here are not corrected for multiple testing.
Positive uncorrected *P*-value is shown in bold.
CI, confidence interval.

data combined with the results from Fung et al. strongly argue for a lack of association between *ATP13A2* and late-onset PD, at least in European and US populations.

This negative association between SNPs within the *ATP13A2* gene and PD is not a unique finding. Likewise, negative association was also reported for idiopathic PD and several common polymorphisms at gene loci involved in other monogenic forms of Parkinsonism,[19,20] with rare exceptions, such as common variants in α-synuclein.[21]

In conclusion, we currently cannot conclude that common variants in the *ATP13A2* locus play a major role in the molecular etiology of late-onset PD. Yet, the question whether private mutations could be responsible for the PD in this age group remains open and further studies are warranted.

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Validity of the Cornell Scale for Depression in Dementia in Parkinson's Disease with and without Cognitive Impairment

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Abstract: **Valid tools are needed to assess depression across the spectrum of cognitive impairment in Parkinson's disease (PD). The validity of the Cornell scale for depression in dementia (CSDD) was tested in a PD sample with a range of cognitive impairment. Psychiatric diagnoses were established according to DSM-IV-TR. Receiver operating characteristic curves tested the discriminant validity of the CSDD compared to the clinical diagnoses of major and minor depression. The curve for symptomatic depression had an area under the curve of 0.82. For the cut-off score ≥ 6, sensitivity was 0.83 and specificity was 0.73; for the cut-off score ≥ 8, sensitivity was 0.75 and specificity was 0.82. There was no evidence for differential measurement with respect to cognitive impairment or any other demographic or clinical variables. This study suggests that the CSDD is a valid tool for identifying depressive disorders in patients with PD across a spectrum of cognitive impairment.** © 2008 Movement Disorder Society

Key words: depression; Parkinson's disease; cognition; dementia; rating scale; Cornell scale

Depressive disturbances affect up to 50% of patients with Parkinson's disease (PD) and negatively impact quality of life.[1,2] Several studies have shown that depressive symptom rating scales are valid measures of depression in PD populations; however, depression rating scales have not been evaluated in patients with PD-dementia.[3]

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Given that dementia develops in up to 80% of patients with PD, valid tools are needed to assess depression, irrespective of the patient's cognitive status, over the course of the disease.[4,5] The Cornell Scale for Depression in Dementia (CSDD), a rater-administered instrument, has potential for this purpose; it was developed specifically for assessment of depression in dementia and has been validated in both demented and nondemented geriatric patients.[6,7] An important feature of the CSDD is that ratings are based on information obtained from clinical observation and interviews with the patient and an informant. As validation studies in PD have not been conducted, this study investigated the discriminant validity of the CSDD in a PD sample relative to a clinical assessment for depression.

SUBJECTS AND METHODS

Participants with idiopathic PD were enrolled in a longitudinal research protocol with prospective brain donation to study motor, cognitive, psychiatric, and clinical-pathological features of PD.[8] The cohort was recruited from tertiary care and community practices and included older individuals with advanced disease along with those younger and less affected who could be followed for a longer duration. Participants or their powers of attorney gave written informed consent to participate. The Johns Hopkins University Institutional Review Board approved the protocol. Only baseline data was used for this analysis. A complete CSDD and psychiatric diagnostic assessment were the only inclusion criteria for this analysis.

Psychiatric symptoms and diagnoses were assessed comprehensively by a geriatric psychiatrist and research nurse using a combination of a semi-structured clinical interview [Schedule for Clinical Interview and Diagnosis (SCID)], supplemental questions to identify disturbances not addressed by the SCID, informant interviews (family members/caregivers and clinicians), and medical records review.[9] Using this information and the mental status exam, the psychiatrist assigned final CSDD ratings and psychiatric diagnoses in consultation with the nurse. Thus, data on inter-rater reliability and length of administration for the CSDD is not available. An inclusive symptom attribution approach was used for diagnosing depression and when completing the CSDD. This approach rates all symptoms as related to depression, regardless of symptom overlap with PD or other medical conditions.[10]

Psychiatric diagnoses were established according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition Text Revision (DSM-IV-TR) criteria using all available information, but independent of CSDD ratings.[11] Diagnoses of symptomatic depression included current major depressive episode, major depressive episode in partial remission, minor depression, dysthymia, or depressive disorder not otherwise specified (NOS). Depressive disorders in full remission (asymptomatic) were classified as nondepressed. Diagnoses of clinically significant cognitive impairment, defined according to DSM-IV-TR criteria for dementia or cognitive disorder NOS, were based on the aforementioned diagnostic interview in addition to a mental status exam.

The Mini-Mental State Exam (MMSE) served as a continuous measure of global cognitive status.[12] The Unified Parkinson's Disease Rating Scale (UPDRS) motor subscale and Hoehn and Yahr (HY) stage, both rated in the "off" (end-of-dose) state, provided measures of motor deficits and disease progression, respectively.[13,14]

Analyses were conducted using STATA statistical software (Version 9.0).[15] Group differences were assessed using *t*-tests and Fischer's exact tests. Receiver operating characteristic (ROC) curves tested the discriminant validity of the CSDD, with the clinician's diagnosis providing the gold standard. The ROCCOMP command using a χ^2 statistic and logistic regressions were used to test the influence of demographic and clinical features on the area under the curve (AUC). Indices were evaluated at cut-offs defined as the maximum sum of sensitivity and specificity. Reliability was measured by Cronbach's α.

RESULTS

As shown in Table 1, 36 of the 134 participants (27%) had a symptomatic depressive disorder and 49 participants (36.6%, 95% CI: 0.28–0.45) had a cognitive impairment diagnosis. Rates of cognitive impairment were comparable between the depressed and nondepressed groups.

As expected, mean CSDD scores were higher in the depressed group, but comparable among the two depressive subgroups [mean (SD): major depression: 11.5 (6.1), minor depression: 10.3 (5.3), $P = 0.52$; major depression $>$ nondepressed: $P < 0.001$; minor depression $>$ nondepressed: $P < 0.01$] and between participants with no depression and those with a remitted depressive diagnosis [no depression: 4.7 (4.1), remitted depression: 3.9 (2.9), $P = 0.52$]. Additionally, participants with any diagnosed cognitive disturbance had higher mean CSDD scores in both depression groups (nondepressed: no impairment $<$ cognitive impairment, $P < 0.01$; depressed: no impairment $<$ cognitive impairment: $P < 0.05$).

The ROC curve to discriminate cases of symptomatic depression was characterized by an AUC of 0.82 (std. err. = 0.04, 95% CI: 0.73–0.91). As shown

TABLE 1. *Group characteristics and scale scores*

	Overall prevalence of cognitive diagnoses	
Cognitive disorder NOS	19 (14%, 95% CI: 0.09–0.21)	
Dementia	30 (22%, 95% CI: 0.16–0.30)	
	No symptomatic depressive disorder	Symptomatic depressive disorder
N	98 (73%, 95% CI: 0.65–0.80)	36 (27%, 95% CI: 0.20–0.35)
Age	67.7 (10.7, 42–90)	67.8 (9.9, 45–81)
Sex[b] (Male/Female)	70 M/28 F	17 M/19 F
Education[a] (years)	16.5 (3.4, 5–23)	15.2 (2.9, 10–24)
PD symptom duration (years)	9.4 (6.3, 1–29)	10.9 (8.1, 1–38)
Hoehn and Yahr Stage[a]	2.4 (0.7)	3.1 (1.2) (n = 34)
	I-5, I$^1/_2$-4, II-35, II$^1/_2$-29, III-19, IV-3, V-3	I-1, II-11, II$^1/_2$-5, III-5, IV-6, V-6
UPDRS-Motor ("Off")[b]	28.6 (16.1, 3–77)	38.8 (21.3, 7–78)
MMSE[a]	27.1 (3.0, 17–30) (n = 97)	25.3 (5.8, 7–30) (n = 35)
Depression diagnosis		
Major depression (n)	0	29 (22%, 95% CI: 0.15–0.30)
Minor depression (n)	0	7 (5%, 95% CI: 0.02–0.10)
Remitted depression (n)	11 (8%, 95% CI: 0.04–0.14)	0
Cognitive impairment (n)	35 (36%, 95% CI: 0.26–0.46)	14 (39%, 95% CI: 0.23–0.57)
CSDD total score[c]	4.6 (4.0, 0–20)	11.3 (5.9, 1–22)
CSDD total score, by group		
No cognitive impairment[c]	4.0 (3.3, 0–17) n = 63	9.3 (5.7, 1–22) n = 22
Any cognitive impairment[c]	5.9 (4.8, 0–20) n = 35	14.4 (4.8, 4–21) n = 14
Cognitive disorder NOS[a]	6.1 (5.3, 2–20) n = 14	12.2 (4.3, 6–18) n = 5
Dementia[c]	5.7 (4.6, 0–17) n = 21	15.7 (4.8, 4–21) n = 9
Informant type (n)		
None	19 (19%)	2 (6%)
Spouse	62 (63%)	19 (53%)
Other family member[c]	11 (11%)	14 (39%)
Paid caregiver	1 (1%)	0 (0%)
Other	5 (5%)	1 (2%)

Mean (SD, range); Two sample *t*-test: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

in Table 2, two cut-off points had near equivalent sums of sensitivity and specificity; one with higher sensitivity, the other higher specificity. Performance of the CSDD was statistically similar across cognitive impairment groups when treated as a trichotomous variable for no cognitive impairment, cognitive disorder NOS, and dementia and when treated as a dichotomous variable for no cognitive impairment versus any cognitive impairment. There were no statistically significant AUC changes after adjusting or stratifying by age, sex, education, MMSE score (treated as a continuous variable), HY stage, UPDRS motor score, or PD symptom duration. Discriminant indices were unchanged when minor depression was excluded from the analyses.

The CSDD demonstrated high internal consistency (Cronbach's $\alpha = 0.84$).

DISCUSSION

In this study, the CSDD was shown to be a valid tool for identifying clinically significant depressive disorders across the spectrum of cognitive dysfunction in

TABLE 2. *Sensitivity, specificity, positive, and negative predictive values at different scores for the Cornell Scale for Depression in Dementia*

Score	≥2	≥4	≥6*	≥8*	≥10	≥12	≥14	≥16	≥18	≥20	≥22
Sensitivity	0.97	0.89	0.83	0.75	0.56	0.44	0.39	0.31	0.17	0.06	0.03
Specificity	0.18	0.47	0.73	0.82	0.87	0.93	0.96	0.97	0.99	0.99	1.00
PPV	0.30	0.38	0.54	0.60	0.61	0.70	0.78	0.79	0.86	0.67	1.00
NPV	0.95	0.92	0.92	0.90	0.84	0.82	0.81	0.79	0.76	0.74	0.74

*Scores correspond to the maximum sun of sensitivity and specificity. This cut-off criterion is a mathematical representation of the best simultaneous combination of sensitivity and specificity that is possible given the data. It is useful in evaluating the validity of rating scales and comparing results across studies. In practice, clinicians and researchers should choose cut-off scores that reflect the purpose of depression assessment (e.g., screening program, diagnostic tool, treatment response) and the consequences of misclassification.

patients with PD. The identified cut-off scores that maximized sensitivity and specificity are similar to those reported in non-PD samples.[16,17] In addition, the reported discriminant indices are similar to other validated depressive symptom rating scales in PD, but weaker compared to validity studies of the CSDD in non-PD samples in which AUC values and sensitivities were greater than 0.90.[16–19] Finally, scale performance was not affected by cognitive impairment or any demographic or clinical variable measured in this study, a finding also observed in non-PD samples.[16] However, recruitment included patients from tertiary care practices and the high educational level of the sample are potential sources of bias.

In addition to being valid across a broad range of motor and cognitive symptom severity, the CSDD is applicable for identifying cases of major and minor depression. Yet, while CSDD scores distinguished depressed from nondepressed cases, they did not discriminate major and minor depressive disorders. We note that the diagnosis of minor depression is not validated in PD and remains a research diagnosis in DSM. However, inclusion of this subgroup in these analyses provided additional information on the ability of the CSDD to identify mild depressive disturbances. Even mild depression is clinically significant in PD as it is linked to greater physical disability and earlier initiation of symptomatic therapy for motor-related deficits.[20] However, since CSDD scores were comparable across depression subgroups, further evaluation of the ability of the CSDD to track symptom severity is needed. By contrast, the 15-item Geriatric Depression Scale discriminates major and minor depressive disorders, but its application with respect to cognitive dysfunction in PD has not been established.[18]

The lack of validated diagnostic criteria for either depressive disorders or dementia in PD limits studies in this area and the ability to compare results across studies. In keeping with the recommendations of a National Institute of Health workgroup on depression in PD, we used an inclusive approach for symptom assessment and diagnosis to enhance the sensitivity and reliability of diagnostic criteria.[10] As the original CSDD used an etiologic approach to symptom attribution, this study, in the strictest sense, reports the validity of a modified CSDD. In our study, the cognitive disorder NOS category captured cases that did not meet DSM criteria for dementia because of the absence of significant memory deficits. However, these cases would meet the criteria for dementia proposed in a recent report by the Movement Disorder Society.[21] Prominence of executive and other cognitive impairments, in comparison to memory impairment in the early to mid stages of PD dementia, complicate the direct application of DSM dementia criteria in PD.[21]

The quality of insight and awareness with respect to symptoms is often a concern when evaluating cognitively impaired patients. Interviews with informants and observation-based depressive symptom rating scales may be the most valid and reliable way to document depressive symptoms in cognitively impaired patients. However, this approach may not be satisfactory in time-limited situations. The Geriatric Depression Scale, a brief self-report instrument, is a potential alternative that has been validated in other cognitively impaired populations, but, as above, has not been studied explicitly in PD-dementia.[3,18,22] The Neuropsychiatric Inventory, a multidimensional scale also developed for use in dementia populations, requires a potentially lengthy informant interview; its validity for identifying cases of depression is not established.[3,23]

In conclusion, the CSDD holds promise as a depression screening tool for use across the spectrum of cognitive function in PD. For clinicians, the CSDD assesses relatively straightforward and observable psychopathological phenomena. An inclusive approach to symptom attribution, as used in this study, simplifies its administration and facilitates ratings by nonpsychiatric clinicians. Further study of the CSDD is needed to recommend its use as a measure of depression severity or treatment response.

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Zonisamide for Essential Tremor: An Evaluator-Blinded Study

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Abstract: **In this evaluator-blinded open-treatment trial, subjects with moderate/severe upper limb essential tremor were titrated to 300 mg/day zonisamide, or adjusted to a lesser dose if symptoms warranted, as monotherapy or as adjunct to stable antitremor medication, followed by a 12-week extension phase. The primary efficacy outcome variables were blinded rater videotaped/drawing tremor score changes at the Treatment and Extension visits compared to Baseline, based on Fahn-Tolosa-Marin and Postural Tremor Scales. Subjects also rated Functional Disabilities. Primary outcomes showed reduced tremor scores at the Treatment ($P < 0.00001$, n = 25) and Extension (n = 16) visits, at mean doses of 252 and 225 mg/day, respectively. Subject ratings indicated 200 mg/day was superior to 100 mg/day, whereas 300 mg/day produced no additional benefit, but instead was associated with more adverse symptoms, most commonly somnolence, poor energy, imbalance, and altered taste. Future double-blind placebo-controlled trials are warranted.** © 2008 Movement Disorder Society

Key words: essential tremor; zonisamide; clinical trial

Zonisamide is an antiepileptic medication with several mechanisms, including sodium and low-threshold calcium channel blockade, and carbonic anhydrase inhibition.[1] We noticed apparent antitremor effects in our movement disorders clinics (G.A.K., Z.V.), consistent with an early pilot trial report.[2] We therefore chose to study zonisamide for essential tremor (ET) in a pilot evaluator-blinded open-treatment clinical trial.

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TABLE 1. *Efficacy outcomes*

Scale	Baseline n = 25	Treatment n = 25	*P*	Extension n = 16	*P*
FTM A + B	34.9	26.1 (*−8.8*)	1.0×10^{-6}	24.4 (*−10.5*)	7.6×10^{-6}
Postural	25.3	19.8 (*−5.5*)	1.1×10^{-6}	17.5 (*−6.6*)	2.0×10^{-6}
Kinetic	23.2	17.3 (*−5.9*)	3.0×10^{-6}	16.0 (*−6.9*)	3.3×10^{-5}
FTM-C	16.0	11.0 (*−5.0*)	5.8×10^{-6}	10.8 (*−5.6*)	3.5×10^{-5}

Means are shown, with mean score changes from Baseline in italics. *P* values are relative to Baseline, paired Student's *t*-test.

SUBJECTS AND METHODS

Eligible subjects were at least 18 years, had definite ET,[3] with a tremor amplitude score of at least 2 in at least one hand/arm by the Fahn-Tolosa-Marin (FTM-A) scale. Subjects abstained from alcohol and caffeine on the visit day, and were taking no or stable anti-ET medications for at least 28 days before the Baseline visit, and were excluded for carbonic anhydrase inhibitor use, sulfonamide hypersensitivity, prior zonisamide use, nephrolithiasis, or hepatic disease. Other criteria were as in a previous trial.[4] The medical center's institutional review board approved the protocol, and written informed consent was obtained from all participants.

At the Screen visit (and all subsequent visits), tremor was evaluated and subjects videotaped. At the Baseline visit, 4 weeks later, 50-mg zonisamide tablets, purchased through the Veterans Affairs Pharmacy, were dispensed. Subjects were then seen at 4-week intervals for 12 weeks, while the daily dose was increased by 50 mg each 2 weeks to a target of 300 mg/day at the Treatment visit. Medication was administered BID for dosages $>$ 50 mg/day. If adverse effects occurred, the dose was reduced to a tolerated level or tapered by 50 mg each 4 days. The Treatment visit provided the outcome Efficacy measures. Subjects could then withdraw, tapering off zonisamide, or receive extended treatment for another 12 weeks if they considered zonisamide effective. Safety was assessed with laboratory evaluations, examinations, and adverse effect collection.

Tremor was assessed with FTM-A: Tremor Location/Severity,[5] rating postural and kinetic tremor amplitude in various body parts; and FTM-B: Specific Motor Tasks/Functions (writing with dominant hand, pouring water and 3 drawings with each hand). In addition, postural tremor was rated as in FTM-A but during the wing-beating position, holding a pen just above a dot, holding a cup 8.5″ above a table, holding a spoon 1″ above a table, and holding a forefinger close to a dot on a vertical surface. Subjects compared their tremor experience to Baseline in FTM-C: Functional Disabilities during everyday tasks; in a Treatment Response Scale[6] in which they rated tremor as unchanged (0) or better (+) or worse (−) to a mild (1), moderate (2), or marked (3) degree; and their Global Status Change on a visual analog scale.

Videotapes of FTM-A, postural and water-pouring tasks; and FTM-B writing/drawings from the Baseline, Treatment and Extension visits, with identifiers removed, were evaluated by two blinded movement disorder neurologists (G.K, Z.V.). If the subject did not attend the Extension visit, Visit 3 materials were substituted but not used for data analysis. Raters, who were aware they were performing zonisamide efficacy evaluations, thus received materials from three visits for every subject without knowing which visit they were rating or whether the subject attended Extension.

The primary outcomes were the blinded tremor score changes at Treatment and Extension compared to Baseline for FTM A+B; Postural Tremor score including tremor during the hands-outstretched position (from FTM-A) plus the 5 positions described above; Kinetic Tremor score including finger-to-nose (from FTM-A) and all FTM-B tasks. Subject-recorded FTM-C questionnaire changes are also reported. Comparisons employed Student's paired *t*-tests. Alpha was set at 0.05, two-sided.

Comparisons across dose (100, 200, 300 mg/day) were performed for subject-reported measures (FTM-C, Treatment Response, and Global Status Change), using parametric repeated measures analysis of variance (ANOVA). Since some subjects did not attain 300 mg/day, ANOVA-based means were computed.

RESULTS

Between November 2004 and October 2005, 25 subjects enrolled, all Caucasian, male/female: 19/6, mean (SD) tremor duration 33 (15) years, mean age 74 (9) years, prior anti-ET medications discontinued 1.3 (1.0), positive ET family history: 18/25, and positive alcohol response: 12/25. Concurrent anti-ET medications numbered 2, 1, and 0 in 2, 14, and 9 subjects, respectively.

All 25 reached the Treatment visit, mean zonisamide dose: 252 (81) mg/day; 17, 5, 1, and 2 took 300, 200, 100, and 50 mg/day, respectively. Of these, 16 entered the extension and took 225 (71) mg/day at the Extension visit.

The Spearman correlation of scores among the two blinded raters was 0.87, 0.80, and 0.91 for the FTM A+B, Postural, and Kinetic scales, respectively. Baseline Postural scores correlated with FTM A+B (0.83, $P < 0.001$). The blinded evaluators rated tremor as significantly better at the Treatment visit compared to Baseline as assessed by these scales (Table 1), with tremor rated >30% improved in 10/25, 6/25, and 11/25, respectively. Functional Disabilities (FTM-C) scores, reported by nonblinded subjects, improved comparably, with 12/25 rating >30% improved. The tremor Treatment Response was rated mildly worse by 1, unchanged by 6, and mildly, moderately, or markedly improved by 8, 7, and 3 subjects, respectively.

The 12-week extension phase was incorporated to assess whether efficacy was maintained. Nine did not enter extension due to lack of efficacy (7) or adverse effects (2). Among the remainder, comparisons between Treatment and Extension visits revealed no statistically significant change in any variable, indicating that tremor changes were stable. At the Extension visit, tremor was rated as unchanged or mildly, moderately, markedly improved by 1, 2, 9, and 4 subjects, respectively.

Subject-reported FTM-C, Treatment Response, and Global Status during dose adjustment were used to assess the dose response. Figure 1 displays ANOVA-based mean changes at 100, 200, and 300 mg/day, for all 25 subjects and for the 16 who subsequently entered the extension, presumably better responders. Pairwise comparisons for all 25 indicate that for all 3 scales 200 mg/day was superior to 100 mg/day (P = 0.0015, 0.0093, 0.0019, respectively), whereas 300 mg/day was not more effective than 200 mg/day (P = 0.83, 0.14, 0.47). Similarly, for the 16 destined to enter extension, 200 mg/day was superior to 100 mg/day (P = 0.0067, 0.0052, 0.0049), but 300 mg/day was not superior to 200 mg/day (P = 0.66, 0.27, 0.41).

At 100 mg/day, longer sleep, rhinitis, imbalance, altered taste, and poor energy was reported by 1 subject each. At 200 mg/day, 12 adverse effects were reported by 8/25 subjects including, in addition to above, imbalance (2 more), drowsiness (2), and psychomotor retardation, trouble swallowing, and diarrhea (1 each). Four subjects did not exceed 200 mg/day; of 21 who did, 15 reported 22 adverse effects, including drowsiness (6), poor energy (5), imbalance (3), altered taste (2), longer sleep, rhinitis, psychomotor retardation, diarrhea, abdominal discomfort, and nausea (1 each). In 2/25 subjects, zonisamide had to be tapered, and the dose reduced in 7/25. During extension, similar adverse effects occurred in 9/16, usually mild, but requiring dose reductions in 3 subjects.

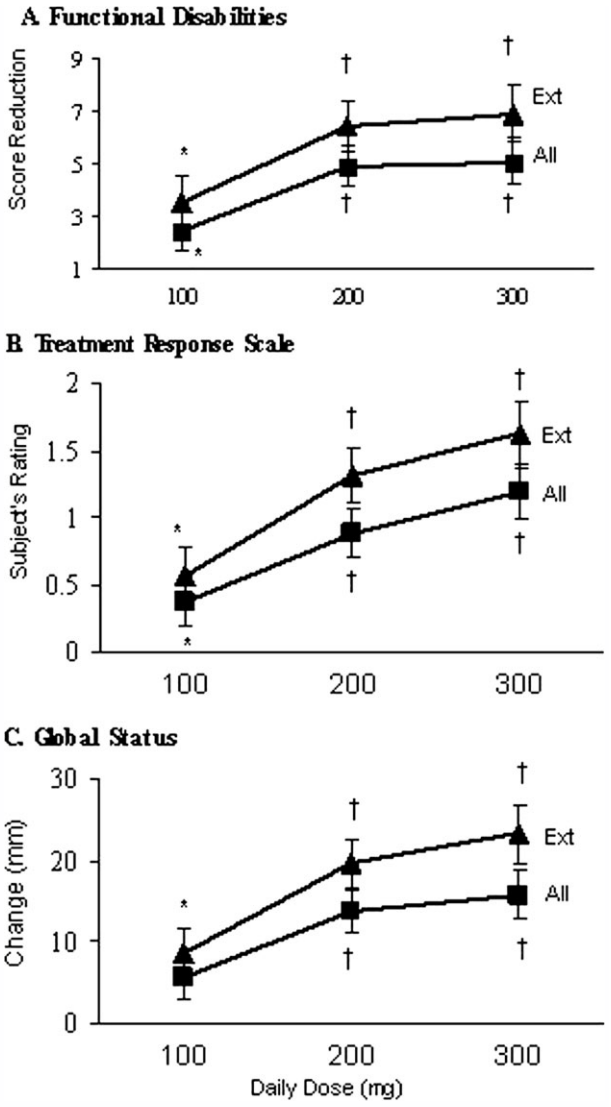

FIG. 1. Changes reported by subjects during the dose adjustment phase according to daily zonisamide dose. Means and SEMs. All, all 25 subjects; Ext, 16 subjects who subsequently entered the extension phase. *$P < 0.05$, †$P < 0.0001$.

DISCUSSION

Video and drawing ratings by two blinded evaluators in subjects taking zonisamide indicated tremor reduc-

tions in the Fahn-Tolosa-Marin Scale (FTM A+B) at the Treatment visit. In addition, tremor was rated as reduced in Postural and Kinetic subscales that we devised. Our inter-relater correlations for these scales were high, comparable to the published FTM A+B inter-rater correlation of 0.87.[7] Open-treatment Functional Disabilities (FTM-C) subject questionnaire scores also improved. The 16/25 who chose to stay on zonisamide and enter a 12-week extension phase maintained tremor reductions in the blinded ratings and subject-reported FTM-C.

Subject-reported FTM-C, Treatment Response, and Global Status indicated that 200 mg/day was superior to 100 mg/day, but 300 mg/day did not confer additional benefit. Furthermore, whereas at 200 mg/day, one-third reported adverse symptoms, usually mild, dose elevation to 250–300 mg/day induced side effects in the majority, causing half to reduce the dose acutely or later. By the Extension visit, the average zonisamide dose was 225 mg/day, compared to the original target of 300 mg/day. These observations suggest that raising the dose to 300 mg/day does not usually improve anti-ET efficacy, and is often not well tolerated.

In open-treatment observations, Morita et al.[2] reported FTM tremor score reductions in 14 subjects taking on average 136 mg/day zonisamide, and Bermejo et al.[8] judged that 9/13 patients taking on average 215 mg/day had a good response. Zesiewicz et al.[9] found in a controlled trial with a small sample size (10 per group) a reduction of postural tremor on accelerometry but no significant change in FTM scores.

In an open-treatment trial comparable to our study, Ondo titrated the dose by 50 mg/week to 200 mg/day and evaluated tremor after a 2-month plateau phase.[10] Of 22 subjects, 8 dropped out or were lost to follow-up. In the remaining 14, taking on average 176 mg/day (11 on 200 mg/day), tremor was significantly reduced, with 7/14 showing >25% tremor score reduction. Our observations, combined with Ondo's, suggest that approximately one-third (17/47) will stop zonisamide treatment early due to lack of efficacy/adverse effects, one-third will later report mild or no efficacy (13/47), and one-third will report at least moderate tremor reduction (17/47). These inferences are limited by the lack of a placebo control group, and the possibility of other time-related effects causing apparent improvements. Based upon these preliminary observations of efficacy, safety, and dose response, we suggest that zonisamide deserves further evaluation in a randomized placebo-controlled clinical trial, utilizing a slow titration of 50 mg/2 weeks and a target of 200 mg/day.

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A Novel Ferritin Light Chain Gene Mutation in a Japanese Family with Neuroferritinopathy: Description of Clinical Features and Implications for Genotype–Phenotype Correlations

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Abstract: **Neuroferritinopathy is a hereditary neurodegenerative disorder caused by mutations in the ferritin light chain gene (*FTL1*). The cardinal features are progressive movement disturbance, hypoferritinemia, and iron deposition in the brain. To date, five mutations have been described in Caucasian and Japanese families, but the genotype–phenotype correlations remain to be established. We identified a novel *FTL1* mutation (exon 4, c.641/642, 4-nucletotide duplication) in a Japanese family and compared the clinical traits with those previously reported. All mutations but one are insertions in exon 4, resulting in frameshifts. Clinical features are similar among patients with the same mutations. Middle-age onset chorea is common in patients with insertions in the 5′ portion of exon 4 including our cases, whereas patients with insertions in the 3′ portion of exon 4 develop early-onset tremor, suggesting genotype–phenotype correlations. In this family, male predominance and normal serum ferritin levels are characteristic.** © 2008 Movement Disorder Society

Key words: neuroferritinopathy; ferritin light chain; ferritin; MRI T2* sequence

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INTRODUCTION

Neuroferritinopathy (MIM 606159) is a progressive movement disorder caused by mutations in the ferritin light chain gene (*FTL1*).[1] The mutation is assumed to disturb the normal structure and functions of ferritin.[2] Serum ferritin level is usually markedly decreased. Abnormal iron and ferritin depositions are observed in the brain, particularly in the basal ganglia.[3–5] The disorder typically presents as middle-age onset chorea and is often misdiagnosed as Huntington disease.[6] To date, five different mutations have been reported in Caucasian and Japanese patients,[1,3,4,7–10] but the precise genotype–phenotype correlation has not been fully understood. Here, we report on a novel mutation identified in a Japanese family with neuroferritinopathy, describe the clinical features, and discuss the genotype–phenotype correlations.

FAMILY

Patient 1: Proband

Patient 1 (Fig. 1A, V-6) was healthy until age 40 when he noticed clumsiness of his left arm. At the age of 42, his neurological evaluation revealed choreoathetosis and occasional dystonia in his left arm, tremor in his left leg, and dyskinesia of his tongue and left face. Motor examination found normal strength with hypotonia in the left upper and lower limbs. The findings of cognitive function tests were normal. He developed emotional lability and depression and was hospitalized in the psychiatric department of our hospital to prevent him from committing suicide.

His serum ferritin level was 46 ng/mL (normal range 40–200). T2-weighted magnetic resonance imaging (MRI) showed hyperintense areas rimmed with hypointense areas in the bilateral globus pallidus and putamen, suggesting cystic degeneration with abnormal mineral deposition (Fig. 1B). T2* imaging revealed a more widespread distribution of abnormal mineral deposition in the putamen, pallidum, internal capsule, thalamus, caudate head, red nucleus, substantia nigra, and dentate nucleus.

Haloperidol, trihexyphenidyl, tiapride, and benzodiazepine have been administered, which have not improved his movement disturbance, whereas an antidepressant has been helpful for treating depression. After he was diagnosed as having neuroferritinopathy, monthly venesections (400 mL/mo) have been con-

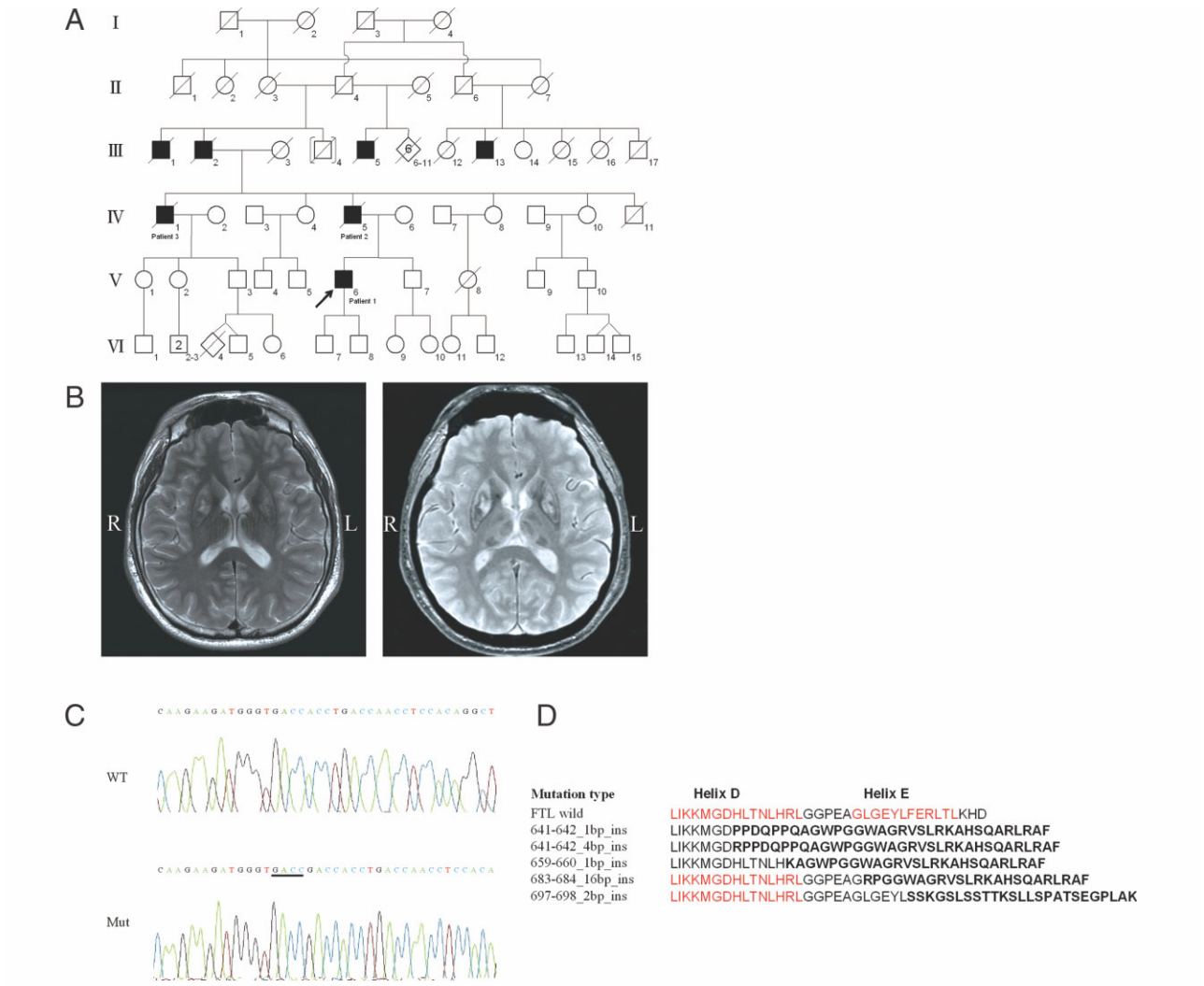

FIG. 1. (**A**) Pedigree of family. Square symbols represent males; circles, females; diamond shape, sex unknown; and filled symbols, affected individuals. The arrow indicates the proband. The symbol/indicates deceased individuals, and d-shows the age at death. (**B**) MRI study of proband (Patient 1). Conventional T2-weighted MR image (left) shows bilateral hyperintensity T2 signal areas rimmed with hypointensity T2 signal areas in the globus pallidus and putamen. The T2* sequence (right) reveals more widespread hypointensity T2 signal areas in the globus pallidus, putamen, caudate head, and thalamus in this slice. (**C**) Wild-type sequence (top) and mutant sequence (bottom) of *FTL1* showing 4-bp duplication (underlined). (**D**) Translated amino acid sequences of the C-terminal portions of wild-type and mutant *FTL1*. Amino acid sequences composing helical structures are shown in red. Substituted amino acid sequences by frameshift mutations are shown in bold.

ducted for 2 months, but to date his clinical conditions have remained unchanged.

Patient 2

Patient 2 (Fig. 1A, IV-5), the proband's father, showed rubbing movement of his left fingers at the age of 49. One year later, he noticed involuntary movements of his face, left arm, and left leg. At the age of 52, neurological examination showed dyskinesia of his face and tongue, choreoathetosis of his fingers and toes, and hypotonia in all extremities. The Wechsler Adult Intelligence Scale-Revised (WAIS-R) showed a mild cognitive impairment (IQ 78). His serum ferritin level was 84 ng/mL (normal range 40–200). T2-weighted MRI showed hyperintense areas in the bilateral globus pallidus, and in later MRI studies, the lesions extended over the putamen and globus pallidus, with mild atrophy of the caudate head.

Several drugs including haloperidol, trihexyphenidyl, tiapride, sulpiride, and benzodiazepine were administered, but none improved his condition. These involuntary movements predominantly on his left side worsened, extending to all limbs, with clear asymmetry remaining throughout the clinical course. The patient died of aspiration pneumonia at the age of 65.

Patient 3

Patient 3 (Fig. 1A, IV-1), the proband's uncle, developed involuntary movements at the age of 51. He first noticed the involuntary opening and closing movements of his right hand, and consequently the rubbing movement of his right toes and clumsiness of his tongue. One year later, involuntary movements spread to all his extremities. He also complained of forgetfulness and difficulty in speech and swallowing. He was examined at the age of 56, and various involuntary movements were observed; dyskinesia of the face and tongue, choreoathetosis of the left arm, fine postural tremor of fingers of both hands, involuntary opening and closing movements of both hands, and quick rubbing movements of the right toes. He also showed dysarthria and dysphagia. Muscle tone decreased in his upper extremities. WAIS-R revealed a mild cognitive impairment (IQ 83). His serum ferritin was not measured. His brain computed tomography (CT) showed hypodense areas in the bilateral globus pallidus and putamen. He was treated with haloperidol, tiapride, and trihexyphenidyl; however, they did not improve his symptoms. He died of asphyxiation by food at the age of 62.

Other Members

III-1 and III-2 developed involuntary movements at the age of 60. III-2 committed suicide at the age of 67. III-5 developed choreoathetosis of his right arm. III-13 showed involuntary movements of his eyelids and extremities at the age of 51, and his brain CT showed hypodense areas in the bilateral globus pallidus and putamen. The proband's aunts in their sixth decade (IV-4, IV-8, and IV-10) presently do not show any involuntary movements.

METHODS

After informed consent was obtained, genomic DNA was extracted from peripheral blood leukocytes following standard protocols. DNA was amplified and sequenced, using the primer pairs of FTL1a/F, FTL1a/R,

TABLE 1. *Genetic and clinical features of neuroferritinopathy*

Mutation						Clinical features													
Exon	Position*	Type	Effect	Ethnic background	Number of patients (Male : Female)	Age at onset (yr)	Chorea	Dystonia	Orolingual dyskinesia	Tremor	Bradykinesia	Hypotonia	Ataxia	Dysphonia	Pyramidal sign	Cognitive decline	Psychiatric problems	Reference
3	c.485	G to A	missense	Portuguese (gypsy ancestry)	1(1:0)[a]	13					+	–	+		+	+	+	7
4	c.641/642	1-bp insertion	frameshift	French Canadian and Dutch ancestry	2(1:1) proband sister	63 49	+	+	+	–	–	+	+	+	+	–	+	3
												–			+	–	+	
		4-bp insertion	frameshift	Japanese	7(7:0) Patient 1 Patient 2 Patient 3	41 49 51	+	+	+	+	–	+	–	–	+/–	–	+	This study
							+	–	+	–	–	+	–	–	+	+	–	
							+	–	+	+	–	+	–	–	–	+	–	
	c.659/660	1-bp insertion	frameshift	English and French	40(20:20)[b]	13–63	70%	82.5%	65%	5%	35%		0%	45%	0%	41.7%	12.5%	8
	c.683/684	16-bp insertion	frameshift	Japanese	2(1:1) proband mother	midteens 10	–	–		+		+		+		+		9
										+	+					+		
	c.697/698	2-bp insertion	frameshift	French	11(7:4)	20 (proband)		+	+	+	+		+		+	+	+	4

*cDNA positions are in accordance with NM-000146 in GenBank.
[a]In addition to one patient, there are two asymptomatic carriers, his mother and younger brother, in the family.
[b]One female of 41 subjects with the mutation was an asymptomatic carrier.

FTL1b/F, FTL1b/R, FTL2/F, FTL2/R, FTL3/F, FTL3/R, FTL4/F, and FTL4/R in accordance with a previous report.[1] The polymerase chain reaction products of exon 4 of *FTL1* were subcloned into a pGEM®-T Easy vector (Promega, WI) and sequenced.

RESULTS

Nucleotide sequence analysis of *FTL1* revealed a heterozygous mutation in exon 4, that is, a four-nucleotide duplication at c.641/642 (codon 148/149) (Fig. 1C). The duplication results in a frameshift of the predicted amino acid sequence with a disruption near the carboxyl terminus. The heterozygous mutation was found in all the patients examined (III-13, IV-1, IV-5, and V-6), whereas the mutation was not found in members who married into the family (III-3 and IV-6).

DISCUSSION

To date, five different *FTL1* mutations have been reported,[1,3,4,7–10] and the mutation in this family is a novel mutation. With one exception, all reported mutations, including that found in this study, are insertions in exon 4. A missense mutation in exon 3 was reported in one family.[7] All insertional mutations cause a frameshift and are predicted to produce aberrantly elongated sequences in the carboxyl terminus and to disarrange a hydrophobic residue, which is assumed to disrupt the iron storage function of ferritin.[2]

In this family, clinical features are markedly similar with little variation: middle-age onset chorea in unilateral extremities, orolingual dyskinesia, and hypotonia are the common findings. Dystonia, mild cognitive impairment, and psychiatric problems are also observed in some patients, whereas Parkinsonism and cerebellar ataxia are absent.

As summarized in Table 1, considerably broad ranges of symptoms of neuroferritinopathy have been reported. However, the cardinal features and age of onset show relatively little variation among the patients in individual families. In the families with insertions at c.641/642 in exon 4 (families of this report and French Canadian and Dutch ancestry[3]), the ages of onset are 41 to 63 and the clinical presentations are characterized by middle-age onset chorea, orolingual dyskinesia, and mild cognitive impairment, which are also the cardinal features of many patients with an adenine insertion at c.659/660 in exon 4.[1,8,10] In contrast, in the families with insertions in the 3′ portion of exon 4 (at c.683/684[9] and c.697/698[4]), early-onset tremor, gait disturbance, and cognitive decline are commonly observed. Although all the insertional mutations in exon 4 disrupt normal structure of the terminal portion of the ferritin light chain, the mutations in the 5′ portion of exon 4 (at c.641/c642 and c.659/660) result in disruption of the last portion of helix D and the whole length of helix E, but the mutations in the 3′ portion of exon 4 (at c.683/684 and c.697/698) leave helix D intact and only disrupt helix E (Fig. 1D). Intact helix D structure is assumed to facilitate incorporation of mutant ferritin light chain into ferritin, which may disturb ferritin function, leading to severe phenotype.[2] Thus, the difference in the effect of insertional mutations on the helix D might be related to the diversities in the clinical presentations. In addition to the insertional mutations, a missense mutation (A96T) has been described in a family.[7] The proband had hypoferritinemia and exhibited distinctive symptoms including ataxia, bradykinesic-rigid syndrome and episodic psychosis in adolescence, but without involuntary movements. MRI showed distinctive lesions limited to globus pallidum. Taken together, these observations provide distinct genotype–phenotype correlations in neuroferritinopathies.

Intriguingly, all the patients are male in this family. Although mutational analyses were not carried out on female members who may carry the mutant gene, the observations raise the possibility that women might be less vulnerable to abnormal iron deposition owing to menstruation. Maciel et al. also suggested this possibility, referring to an asymptomatic female carrier.[7] In addition, serum ferritin level was within the normal range in this family. Chinnery et al. reported that serum ferritin level was normal in 18% of the male patients and 77% of the premenopausal patients in their study.[8] A normal serum ferritin level suggests the formation of a relatively stable ferritin structure and presumably mildly impaired ferritin functions, but the precise mechanisms underlying the development of neuroferritinopathy await further investigation.

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Ikeda, Kanazawa, Tsuji. 3. Manuscript: A. Writing of first draft: Kubota; B. Review and Critique: Ichikawa, Momose, Goto, Igeta, Hashida, Yoshida, Ikeda, Kanazawa, Tsuji.

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Effects of Inhibitory rTMS on Bladder Function in Parkinson's Disease Patients

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Abstract: **Patients affected by Parkinson's disease (PD) may present with lower urinary tract (LUT) dysfunction characterized by involuntary detrusor overactivity. We evaluated possible impact of a 2-week course of low frequency 1 Hz repetitive transcranial magnetic stimulation (rTMS) on LUT behavior in eight advanced PD patients complaining of urinary disturbances. We tested the effects of rTMS measuring urodynamic examination and the International Prostate Symptoms Score (IPSS) questionnaire, used for evaluation of subjective LUTS. rTMS was able to improve temporarily LUT behavior in PD patients, increasing bladder capacity and the first sensation of filling phase. Moreover, a reduction of IPSS score was noticed, due to an improvement on filling phase symptoms. The beneficial effects assessed with the IPSS lasted for up to 2 weeks after the end of the stimulation. rTMS seems to be an effective, noninvasive alternative treatment for PD patients with urinary disturbances.**

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Key words: bladder functions; transcranial magnetic stimulation; Parkinson's disease; rTMS; urodynamic

It is well known that two thirds of patients affected by Parkinson's disease (PD) complain of urinary disturbances such as urinary urgency, increased urinary frequency or urinary incontinence progressively worsening with disease stage.[1] These symptoms have a certain impact on daily activities and quality of life of PD patients.[2] Urodynamic studies performed in these patients showed a pattern of involuntary detrusor over-

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TABLE 1. *Clinical characteristic of Parkinson's disease patients*

Patients	1	2	3	4	5	6	7	8
Gender	F	F	M	F	M	F	M	M
Age (yr) 70	52	61	64	59	73	75	69	
Disease duration (yr)	11	9	8	10	12	7	12	9
Hoehn and Yahr stage in on condition	2.5	3	2.5	2	2.5	2	3	2.5
L-dopa equivalents therapy (mg/day)	1100	950	800	1000	1150	750	1000	950
UPDRS section III in on condition (pre-/post-rTMS)	17/19	21/20	18/17	20/22	24/21	14/15	26/29	18/21
UPDRS section IV (Items 32–39)	1	3	1	1	2	1	4	4

activity evident during the storage phase.[3] However, the current pharmacological treatment of lower urinary tract (LUT) dysfunction in PD is largely unsatisfactory, with variable results obtained with dopaminergic antiparkinsonian treatment.[4] Therefore, alternative approaches could be potentially useful to control these symptoms. At this regard, in a recent study repetitive transcranial magnetic stimulation (rTMS) has been found to influence bladder activity in multiple sclerosis patients.[5]

Therefore, the aim of this study was to evaluate the possible impact of a 2-week course of low frequency 1 Hz rTMS, a procedure known to inhibit cortical excitability,[6] on LUT behavior in a population of advanced PD patients complaining of urinary disturbances. We speculated that long lasting inhibitory modulation of cortical circuits involved in sustained detrusorial muscle overactivity may reduce the previously described bladder dysfunction in PD and thus partially normalize urinary disturbances.

PATIENTS AND METHODS

Eight fluctuating patients affected by idiopathic PD (see Table 1) complaining of stable although not severe urinary symptoms, such as urgency and increased day time/night time frequency defined according to the International Continence Society (ICS) standardization,[7] were enrolled for this study. Diagnosis of idiopathic PD was made according with Brain Bank Criteria.[8] The study was approved by our local ethics committee (Ref GGP05071) and all participants provided an informed consent. Exclusion criteria were: use of any drug acting on the central nervous system and/or on the LUT, except for antiparkinsonian drugs; history of urologic disorders. None of the patient was treated by anticholinergic drugs. All the subjects were evaluated with a first urodynamic session in the morning after assuming usual antiparkinsonian treatment. Then, a low frequency (1 Hz) inhibitory rTMS was applied for 2 weeks over the motor cortex maintaining stable drugs regimen.

A MagStim Rapid magnetic stimulator (Magstim Company, UK), connected with a 70-mm figure of-eight coil was used. To stimulate the pelvic floor hot spot, which is thought to be close to the leg motor hot spot[5] the coil was applied 1 cm ahead of Cz with the handle pointing backward. rTMS trains of 900 stimuli at 1 Hz and at 65% of maximal stimulator output (MSO) once a day for five consecutive days over two consecutive weeks. This intensity was well below resting motor threshold for leg muscles in all subjects and therefore did not induced any muscle twitch or discomfort.

Urodynamic evaluation, performed by two urologists, that were blind regarding the TMS protocol, was constituted by a medium filling (50 mL/min) water cystometry, followed by pressure/flow study with surface striated pelvic floor electromyography (EMG). The procedure was conducted according to the ICS recommendations. A 6-Fr transurethral double lumen catheter was used. The following urodynamic parameters were evaluated: (i) *volume* variables: first sensation of bladder filling (FSBF), neurogenic detrusor overactive contractions threshold (NDOC-t), bladder capacity (BC), postvoid residual urine, all expressed in milliliters; (ii) *pressure* variables: neurogenic detrusor overactive contractions amplitude (NDOC-a) and detrusor pressure at maximum flow (Pdet@Qmax) expressed in cm H_2O. Moreover, (iii) *maximum flow* (Qmax; mL/sec) was evaluated. Urodynamic evaluation was executed twice; before and 1 week after the end of the rTMS treatment, at the same time of the morning (9 AM).

The International Prostate Symptoms Score (IPSS) questionnaire, widely used for quantitative evaluation of subjective LUTS, was administered in coincidence with the first urodynamic evaluation, immediately after TMS chronic treatment and 2 and 4 weeks later (see Table 2). The IPSS is a seven questions questionnaire assessing LUTS symptoms of the filling (urgency, nicturia, increased daytime frequency) and of the voiding phase (incomplete empting feeling, low or intermittent flow, straining to void). It has been shown to be useful

TABLE 2. *Urodynamic measures and clinical scores before and after rTMS*

Urodynamic variables	Baseline condition	Post 1 Hz rTMS	Two weeks post 1 Hz rTMS	Four weeks post 1 Hz rTMS
Volumes	Median ± SD	Median ± SD		
First sensation (mL)	115 ± 36	185 ± 38.4*		
Bladder capacity (mL)	215 ± 40	280 ± 48.4*		
DNOC threshold (mL)	40 ± 26	25 ± 40		
Residual urine (mL)	5 ± 5	10 ± 17.4		
Pressures				
DNOC amplitude (cm H_2O)	17 ± 13.4	27 ± 11.4		
Pdet@Qmax (cm H_2O)	24.1 ± 7.7	29.0 ± 6.2		
Flow				
Q max (mL/s)	13.6 ± 3.3	12.6 ± 1.6		
Questionnaires	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
IPSS (total)	12.5 ± 1.5	8.7 ± 1.2*	8.5 ± 1.6*	11.3 ± 2.1
IPSS (filling phase symptoms)	10.3 ± 3.2	6.5 ± 2.4*	7.4 ± 2.6*	10.8 ± 4.1
IPSS (QoL)	3.8 ± 1.1	2.4 ± 1.5*	2.2 ± 1.8*	3.3 ± 1.4

*p values < 0.05.

in detecting the voiding dysfunction in patients with PD.[4]

Separate nonparametric Wilcoxon tests (using SPSS software) were applied on scores for the IPSS, volume pressure measurements. For all statistical analyses, a P value of <0.05 was considered to be significant.

RESULTS

Results are reported in Table 2. LUT symptoms were mild to moderate in all patients, according to the IPSS questionnaire score (mean ± SD = 12.5 ± 3.4). In fact, all patients had an IPSS score <19, i.e., the cut-off value above which the symptoms are considered severe. Wilcoxon tests revealed that in comparison with baseline IPSS scores were reduced immediately after rTMS ($Z = -2, 11$; $P < 0.05$) and 2 weeks after rTMS ($Z = -1, 99$; $P < 0.05$). In particular, filling (irritative) symptoms were significantly decreased immediately after rTMS ($Z = -2, 09$; $P < 0.05$) and 2 weeks after rTMS ($Z = -2, 01$; $P < 0.05$), whereas obstructive (voiding) symptoms were unchanged. Moreover, there was a significant change of quality of life (reported through the specific IPSS additional question) as assessed immediately after rTMS ($Z = -1, 89$; $P < 0.05$), and 2 weeks later ($Z = -1, 78$; $P < 0.05$), in comparison with baseline.

Urodynamic evaluations revealed that rTMS significantly ameliorated bladder volume measurements as revealed by Wilcoxon tests. Following rTMS there was an increased first sensation of bladder filling (FSBF) ($Z = -1, 84$; $P < 0.05$) and bladder capacity (BC) ($Z = -2, 07$; $P < 0.05$). Pressure measurements did not vary [detrusor pressure at maximum flow (Pdet@Qmax)]. Finally, no significant changes were observed on UPDRS (section III) score when comparing base line vs. after rTMS.

DISCUSSION

This study represents the first evidence that low frequency rTMS is able to improve LUT behavior in PD patients, increasing bladder capacity and the first sensation of filling phase.

A previous study in multiple sclerosis patients with detrusorial hypocontractility, showed that high frequency rTMS, a procedure known to increase cortical excitability, was able to improve the voiding phase, without causing effects on the filling phase.[5] It was proposed that enhancing corticospinal tract excitability, with excitatory rTMS, preferentially facilitated detrusor contraction. On the other hand, in PD LUT are mainly due to an opposite pattern of involuntary detrusor overactivity evident during the storage phase. Therefore, it is likely that in this study inhibitory rTMS may have induced an opposite modulation of the descending corticospinal tract output targeting the detrusorial muscle, resulting in a reduced bladder overactivity. Further studies directly measuring pelvic floor/detrusor muscle EMG activity before and after rTMS could be useful to better define these mechanisms.

In alternative, it is possible that current rTMS results were mediated by remote effects on subcortical regions such as the Pontin Micturition Center, site of descending excitatory projections to parasimpatic sacral centres, and/or as the periaqueductal grey, where the afferent proprioceptive projections of the bladder terminate.[9]

Additionally, a reduction of IPSS symptom score was noticed, due to an improvement on filling phase symptoms (frequency, urgency, nicturia). The beneficial effects assessed with the IPSS lasted for up to 2 weeks after the end of the stimulation period. At this regards, it has been recently demonstrated that in PD the sensation of bladder filling is processed within an insular-cingulo-frontal network.[10,11] Herzog et al.[10,11] found that subthalamic deep brain stiumulation (STN-DBS) profoundly modulates sensory processing of urinary bladder information within the primary sensory pathway of visceral afferents. Modulation of neural activity in the thalamus and the insular cortex was only present when STN-DBS was turned on. In contrast, in the STN-DBS OFF condition, they did not find a relevant modulation of these two areas by the urinary bladder state. Therefore, it is possible that rTMS may have also interfered with the efficacy of this well-established network through indirect remote effects on the thalamic nuclei or on the insular cingular cortex.

It is worthy to note that the score of IPSS final question on quality of life was reduced significantly as well, thus demonstrating that this treatment could produce a transient significant clinical impact on PD patients with LUT. Moreover, in contrast with previous investigations,[12] in this study we did not find any improvement in the UPDRS section III scores. In our opinion this could be due by the fact that we did not stimulate the hand area of the primary motor cortex, but the pelvic floor hot spot.

In conclusion inhibitory rTMS seems to be an effective, noninvasive alternative treatment for fluctuating PD patients with urinary disturbances. Moreover, this approach could be useful to treat other neurological conditions with urinary urgency.

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Author Roles: L.B., P.S., and G.K. wrote the manuscript. E.F.A., F.S., and F.P. performed the urodynamic evaluations. E.L.G. and S.T. performed the rTMS sessions. C.I. participated in the study design. Statistical analysis was conducted by L.B. and G.K.

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Haplotype Analysis of the *PARK 11* Gene, *GIGYF2*, in Sporadic Parkinson's Disease

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Abstract: **Familial Parkinsonism (*PARK*) genes are strong candidates for conferring susceptibility to common forms of PD. However, most studies to date have provided little evidence that their common variants substantially influence disease risk. Recently, mutations were described in the gene, *GIGYF2* (*TNRC15*), located at the *PARK*11 locus (2q37.1). Here, we use a haplotype tagging approach to examine common variation in the *GIGYF2* gene and PD risk. PD cases (n = 568) and age and gender-matched control subjects (n = 568) were recruited from three specialist movement disorder clinics in Brisbane (Australia) and the Australian electoral roll. Twelve tagging SNPs were assessed in all subjects and haplotype and genotype associations were explored. Overall our findings suggest that common genetic variants of *GIGYF2* do not significantly affect sporadic PD risk in Australian Caucasians.** © 2008 Movement Disorder Society

Key words: Parkinson's disease; PD-related genes; association

INTRODUCTION

Linkage studies in Parkinson's disease (PD) families have identified seven causative genes and an additional six familial Parkinsonism (*PARK*) loci. Mutations in *SNCA* (*PARK* 1 and 4), *UCHL1* (*PARK* 5), and *LRRK2* (*PARK* 8) appear to be inherited in an autosomal dominant pattern, whereas mutations in *PRKN* (*PARK* 2), *PINK1* (*PARK 6*), and *DJ1* (*PARK* 7) appeared to follow a recessive inheritance pattern. Mutations in other genes such as *OMI* (*HTRA2*, *PARK 13*), *NR4A2* (*Nurr1*), *ATP13A2*, and *GBA* also segregate with familial Parkinsonism.

In comparison, the etiology of common or sporadic forms of PD is unknown, but thought to result from complex interactions involving genetic and environmental risk factors on a background of ageing.[1–5]

Given that genetic perturbations in these genes can reproduce the PD phenotype, common genetic variations around the *PARK* genes must be viewed as strong candidates to confer risk for sporadic forms of PD. We recently tested this hypothesis by using a haplotype tagging strategy to examine all known *PARK* genes in a typical Australian case–control group.[6] Modest associations were observed for common variants around *SNCA*, *UCHL1*, *MAPT*, and *LRRK2*, whereas no associations were seen for *PRKN*, *PINK1*, *GBA*, *ATP13A2*, *HTRA2*, *NR4A2*, and *DJ1*.

Recently, heterozygous mutations in the Grb10-interacting GYF protein 2 gene (*GIGYF2*), located in the *PARK* 11 locus, were described and appeared to be inherited in an autosomal dominant pattern with incomplete penetrance.[7] Here, we extend our previous work by using a haplotype tagging approach to test whether common variants in the *GIGYF2* gene are associated with sporadic forms of PD.

SUBJECTS AND METHODS

Study Participants

The study was approved by human research ethic committees at the participating institutions. Subjects with PD (n = 568) were recruited from one private and two public movement disorders clinics in Brisbane, Australia, from 2002 to 2008. Standard criteria for the diagnosis of probable PD were used.[8] Details on exclusion criteria and recruitment of controls (n = 568) have been reported previously.[6] Cases with any atypical features or signs of an alternative akinetic-rigid syndrome were not included. Although extensive mutation screening of all genes known to result in monogenic forms of PD was not performed, all subjects were screened for the common *LRRK2* G2019S mutation and individuals positive for this mutation were excluded. Only Caucasian subjects were included in the study and each PD case was matched for age and gender to a control subject.

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TABLE 1. *Clinical, Demographic, and Exposure Information*

Factor	Cases	Controls	Comment
Individuals	568	568	
Mean age ± S.D.	72.1 ± 10.5	72.0 ± 10.5	ns[a]
Mean age at onset ± S.D.	58.6 ± 11.2		
Male/female	240/328	240/328	ns[a]
Family history of PD[d]	135/376 (26%)	99/436 (19%)	OR[b] = 1.58 (1.18–2.12) $P = 0.002$
Cigarette smoking[e]	7.6 ± 17.5	12.5 ± 23.0	F = 14.6; $P = 0.0001$[c]
Pesticide exposure[f]	80/430 (16%)	47/483 (9%)	OR[b] = 1.58 (1.18–2.12) $P = 0.002$

[a]Not significantly different.
[b]Odds Ratio (95% CI).
*ANOVA.
[d]1st or 2nd degree relative.
[e]Mean pack years (one cigarette per day for a period of a year).
[f]Positive > 26 days life exposure.

Genotyping and Data Analysis

Genetic variables were selected to "tag" the vast majority of common genetic variation in the coding region of *GIGYF2* (major allele frequencies <90%, $r^2 > 0.90$). "Haplotype-tagging" SNPs were derived from the HapMap project CEU population (Data Rel 23a/phase II Mar08, on NCBI B36 assembly (http://www.hapmap.org/)[9] using the Haploview software package running "Tagger."[10] Design and implementation of genotyping assays were carried out by the Australian Genome Research Facility in Brisbane, using the Sequenom Platform and included 10% duplicate samples. All statistical analyses were carried out in PLINK version 1.03 (http://pngu.mgh.harvard.edu/~purcell/plink/). The odds ratios (OR) for genotype associations (additive model) were calculated using logistic regression with adjustment for age, gender, cigarette smoking, and pesticide exposure. Our sample size was chosen to detect (with > 80% power) 1.5-fold or greater differences in allele frequency for all tagging variables (uncorrected $P < 0.05$).

RESULTS

Demographic and environmental exposure information are summarized in Table 1. PD patients were more likely to have a family history of PD and regular exposure to pesticides, but less exposure to cigarette smoking.

Twelve tagging single nucleotide polymorphisms (tSNPs) covering common genetic variation across the coding region of the *GIGYF2* gene were selected from HapMap data (Fig. 1). There were no differences in haplotype frequencies between the cases and controls (global P-value = 0.13). Similarly, there were no significant individual genotypic associations. One tSNP, rs7602140 situated in the final intron of *GIGYF2*, showed a reasonable trend toward over-representation among the cases (OR = 1.24, 95% CI = 0.99–1.56, $P = 0.06$) (Table 2). For this SNP, we also genotyped an additional 432 cases (362 males, 70 females, aged 72.5 ± 10.9 years, age-at-onset 60.3 ± 11.6 years, and other demographic variables paralleling those in Table 1) and 58 unmatched controls (1 male and 57 females, aged 62.9 ± 9.3 years) recruited using an identical strategy to that outlined in the methods. This moved the putative association further toward the null (OR = 1.16, 95% CI = 0.95–1.42, $P = 0.15$).

DISCUSSION

GIGYF2 is a trinucleotide repeat-containing protein, 160 kb in size with 31 exons (27 coding) and is located at 2q37.1. The canonical transcript encodes for a 1,299 amino acid long protein that is thought to act with Grb10, a growth factor receptor-binding protein, to regulate signaling from tyrosine kinase receptors, including the insulin-like growth factor 1 (IGF1) and insulin receptors.[11] The potential neuroprotective effects of IGF1 on dopaminergic neurons, either alone[12,13] or in combination with oestrogen[14] suggested to Giovannone et al. that *GIGYF2*, situated at the *PARK* 11 locus, might be a reasonable candidate gene for further analysis. Mutations were subsequently found in 12 Italian and French familial PD patients previously linked to that region.[7]

Our findings suggest, within the power limitations of our sample size, that *GIGYF2* tSNPs are not associated with sporadic PD risk. To the best of our knowledge, this is the first study targeting *GIGYF2* as a candidate gene for PD, although tagging SNPs have been examined as part of two genome-wide association studies (GWAs) in Caucasian populations.[15,16] Fung et al. examined 25 SNPs in and around *GIGYF2* of which five were also examined in our study (rs1515961,

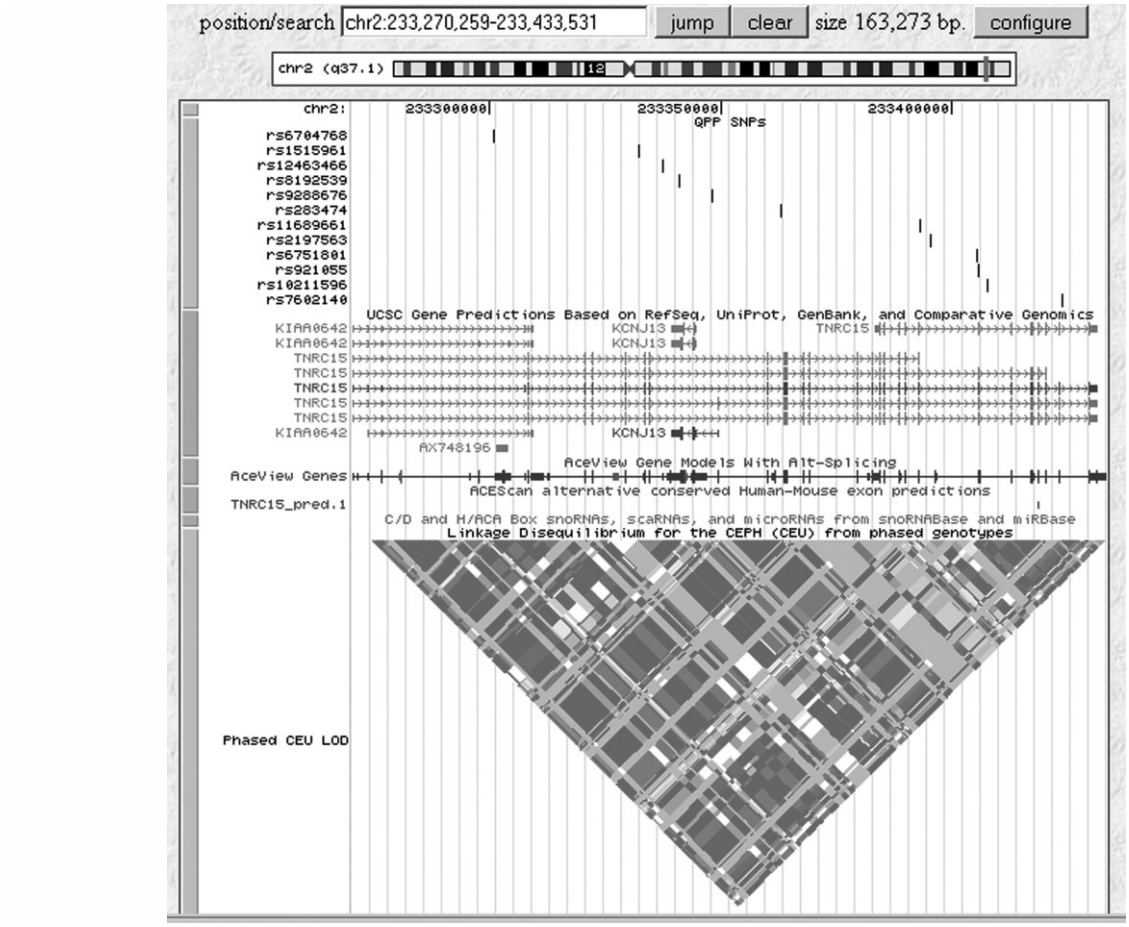

FIG. 1. Genomic features of the *GIGYF2* gene. *GIGYF2* (*TNRC15*) genomic features are illustrated using the UCSC browser (http://genome.ucsc.edu/cgi-bin/hgGateway). Gene features from top to bottom are as follows: the tSNPs assayed here; the 13 UCSC gene variants; the AceView (NCBI) alternative splicing model and the Linkage Disequilibrium Map (r^2) from the HapMap CEU (Caucasian) population.

TABLE 2. *Genotypic Associations for GIGYF2 tSNPs*

SNP	MAF cases	MAF controls	OR (95% CI)	*P*-value*
rs6704768	0.4194	0.442	0.93 (0.78–1.12)	0.44
rs1515961	0.448	0.4139	1.17 (0.98–1.39)	0.08
rs12463466	0.1128	0.1222	0.85 (0.64–1.13)	0.27
rs8192539	0.1871	0.1895	0.99 (0.79–1.24)	0.92
rs9288676	0.2098	0.2142	0.94 (0.76–1.16)	0.56
rs283474	0.3591	0.3361	1.10 (0.91–1.31)	0.32
rs11689661	0.153	0.1595	0.93 (0.73–1.18)	0.53
rs2197563	0.4286	0.4009	1.11 (0.93–1.33)	0.24
rs6751801	0.2773	0.2775	0.99 (0.81–1.20)	0.89
rs921055	0.3273	0.3668	0.86 (0.71–1.04)	0.12
rs10211596	0.4938	0.4647	1.13 (0.95–1.35)	0.16
rs7602140	0.1891	0.1561	1.24 (0.99–1.56)	0.06

*Adjusted for age, gender, cigarette smoking, and pesticide exposure.

MAF, minimum allele frequency; OR, Odds ratio, which here represents the increased risk due to possession of each copy of the alternative allele as calculated using a log-additive model.

rs8192539, rs283474, rs921055, and rs10211596). There were no associations seen for any of these SNPs. Maraganore et al. examined 12 alternative tagging SNPs to our study, whereas one, rs6738386, was in common with the Fung et al. study; again, there were no associations observed.

Consistent with the literature and our previous analyses of independent Australian case–control samples, the PD cases examined in this study were more likely than controls to report a family history of PD,[6,17] increased levels of pesticide exposure and lower frequency of cigarette smoking.[6,18–20]

The lack of association between *GIGYF2* common variants and sporadic PD is consistent with our previous findings where the majority of *PARK* genes were either not associated with PD or had very modest effects.[6]

Given the phenotypic similarities between the monogenic and common forms of PD, it is worth contemplating why we have not seen *PARK* gene effects of greater magnitude in association studies. First, if common PD pathogenesis is due to complex gene–gene and gene-environmental interactions, then individual genetic effects may be modest, variable, and highly dependent on population and environmental status. Second, sporadic forms of PD could quite easily be explained by hitherto unknown rare genetic variants. Third, a "real" effect for a common genetic variant could be diluted by cases that are highly weighted toward the phenotype by as yet unidentified causative mutation. Last, the genetic component of PD may be over estimated with the family history evidence reflecting a common environment in early life rather than shared genetics.[21]

The challenge remains for researchers to design experiments that effectively test these different hypotheses to better focus future research efforts.

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Traditional Chinese Medicine on Four Patients with Huntington's Disease

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Video 

Abstract: **Four Huntington's disease (HD) patients were treated with traditional Chinese medicines Yi-Gan San (YGS) and Chaihu-Jia-Longgu-Muli Tan (CLMT) in a cross-over manner. Two patients took YGS for 8 weeks first, and after 4 weeks of washing out, they took CLMT for 8 weeks. Two other patients took these medicines in reverse order. All patients showed a decrease in the Unified Huntington's Disease Rating Scale—motor assessment (from 106.3 ± 4.7 to 89.6 ± 5.8 as mean ± SD, P = 0.0004) by YGS treatment with no extrapiramidal symptoms or changes of cognition or ADL. Our study suggests a possibility of a new treatment for involuntary movements.** © 2009 Movement Disorder Society

Key words: movement disorder; Huntington's disease; chorea; traditional Chinese medicine; Yi-Gan San (yokukansan)

Huntington's disease (HD) is a neurodegenerative disease caused by an expanded (CAG)n repeat on the huntingtin gene,[1] which gives rise to progressive motor, cognitive, and behavioral symptoms. The chorea in HD is usually treated with antidopaminergic neuroleptics or dopamine depleting drugs with affective and cognitive side effects as well as a risk of parkinsonism.[2] We reported that the traditional Chinese medicine Yi-Gan San (YGS, Pulvis depressionis efficientiae) improved behavioral and psychological symptoms in dementia without extrapyramidal symptoms or cognitive deterioration.[3] Moreover, YGS and a YGS-similar traditional Chinese medicine Chaihu-Jia-Longgu-Muli Tan (CLMT, Formula bulpeuri cum astrea et fossileosse) were reported to suppress the increase in glutamate concentration in the hipocampal extracellular fluid during stimulation with high K^+.[4,5] Hence, we conducted a pilot study on YGS and CLMT for chorea in HD patients.

PATIENTS AND METHODS

With written, informed consent from the patients or their families, four female HD patients, A, B, C, and D were assigned to the study. Their ages were 48, 51, 52, and 68 years, and the durations of illness were 26, 11, 22, and 18 years, respectively. All patients had a family history of HD and were genetically tested showing a CAG expansion in the huntingtin gene. All the patients experienced severe involuntary movements in the form of chorea, though they took more than three kinds of neuroleptics including haloperidol. They were treated by YGS and CLMT in a cross-over manner. Namely, patients A and C took YGS for 8 weeks first (the first medication period), and after 4 weeks of washing out, they took CLMT for 8 weeks (the second medication period). Patients B and D took these medicines in reverse order. The use of medications for coexisting medical conditions was not discontinued at screening, but a change in regimen or a prescription of new drugs known to affect involuntary movements (i.e., neuroleptics) were not permitted during the study. Two blinded and fully trained neurologists evaluated their motor function using the Unified Huntington's Disease Rating Scale—motor assessment (UHDRS-m)[6] independent of each other, before and after noon, at baseline, the end of the first medication period, after the wash-out, and at the end of the second medication period. The mean value of the two observers was used for analysis. The Mini Mental State Examination (MMSE), the Barthel Index (BI), digital videodisc (DVD) recording, and laboratory tests including blood count and routine chemistry were evaluated in each period. YGS[3] and CLMT[5] contain seven and eight herbs registered in the Pharmacopoeia of Japan for each, and the medical grade of extracted granules was provided as traditional medicines "TJ-54 yokukansan (Japanese name for YGS)" and "TJ-12 saikokaryukotsuboreito (Japanese name for CLMT)" by Tsumura Co., (Tokyo, Japan). The study protocol was approved by the Insti-

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

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TABLE 1. *Change of UHDRS-m scores of each patient*

Patient	Medication in the 1st period	1st period baseline	1st period endpoint	Medication in the 2nd period	2nd period baseline	2nd period endpoint
A	YGS	112.5	86.5	CLMT	108.5	101
C	YGS	102	95	CLMT	104.5	99.5
B	CLMT	100.5	105	YGS	103.5	83
D	CLMT	108.5	98.5	YGS	107	94

There was a 4 wks washout interval between the end of the 1st medication period and the baseline of the 2nd medication period.

tutional Review Board of National Yonezawa Hospital and registered with the UMIN clinical trial registry (UMIN000000909, http://www.umin.ac.jp/ctr/index.htm).

RESULTS

UHDRS-m decreased from 106.3 ± 4.7 to 89.6 ± 5.8 (mean ± SD) in the YGS treatment period. The data for each patient was as follows: from 112.5 to 86.5 in Patient A (shown on the DVD), from 103.5 to 83 in B, from 102 to 95 in C, and from 107 to 94 in D (Table 1 and Fig. 1). UHDRS-m changed from 105.5 ± 3.8 to 101 ± 2.9 in CLMT treatment. The *P* value in the residual maximum likelihood method was 0.002, for the whole treatment 0.0004 for YGS treatment and 0.8431 for CLMT treatment (JMP 6.0.3 for Mac, SAS). All patients showed a decrease of UHDRS-m by YGS treatment, whereas three of four of the patients showed a decrease by CLMT. The MMSE and the BI scores remained at 0 and 5, respectively, in all patients. No significant change was shown in blood examinations.

DISCUSSION

YGS decreased the UHDRS-m score in all patients. The improvement of chorea was obvious in all patients as shown on the DVD movie. The change of UHDRS-m score was about 20 points as a mean. All cases in our study had a long duration of illness, so the ADL level was very low, nearly at the bed-ridden level (BI = 5). The changes of UHDRS-m were mostly observed in chorea. HD patients show variability in their symptoms during the day, thus UHDRS-m was independently evaluated by two neurologists before and after noon. Moreover, physicians, nurses, and family members frequently examined the patient state day by day, and the change was obvious to everyone in all cases. YGS has been used as a traditional medicine for more than 500 years. No serious side effect of YGS is known except for hypopotassemia due to *Glycyrrhizae radix* that occurs in about 1% of cases. Extrapyramidal symptoms were not observed both in the present and former reports. The mechanisms of YGS on HD remain unknown. Recent studies suggest that a mutant huntingtin gene causes a decrease of glutamate uptake in astrocytes, which contributes to neuronal excitotoxicity in HD.[7] YGS was reported to improve glutamate uptake of astrocytes in thiamine deficient rats.[8] Shimada Y et al. reported that an aqueous extract of the hooks and stems of *Uncaria sinensis* (Oliv.) Havil., Uncariae Uncus Cum Ramulusis in YGS protected against glutamate-induced neuronal death in cultured cerebellar granule cells.[9] They supposed that oxyindole alkaloids, such as isorhynchophylline, isocorynoxeine, and rhynchophylline, and indole alkaloids, such as hirsuteine and hirsutine, were the active components of the Uncariae.[10] In traditional Chinese medicine, both YGS and CLMT we tried in this study have been used for patients with "Gan" dysfunction.[11] Gan is one of the functional units in traditional Chinese medicine, including functions of diencephalon, basal ganglia, visual cortex, and the limbic system. The results of this and former studies of YGS may support these traditional theories, but further studies and discussions are needed to clarify the relationship.

There were only four cases in this study. Under the current Japanese Pharmaceutical Affairs Law, a placebo is not available for a preliminary small study. The present study may encourage a more expanded

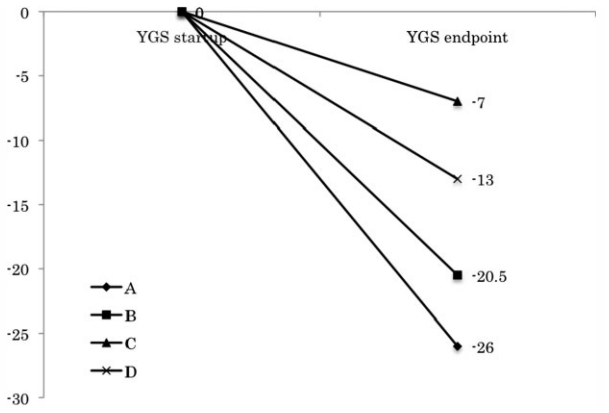

FIG. 1. The figure shows the change of UHDRS-m score in each patient between before and after the YGS treatment. UHDRS-m significantly decreased from 106.3 ± 4.7 to 89.6 ± 5.8 (mean ± SD) in the YGS treatment period.

placebo controlled study. We are now designing an expanded cross-over trial with 20 or 30 patients to investigate the effect of YGS alone on the chorea of HD. The population of HD in Japan is very limited. We hope that researchers in countries with a large HD population will take notice of our report and support our trial in the future.

LEGEND TO THE VIDEO

UHDRS-m decreased from 112.5 to 86.5 in the patient A by YGS treatment. The DVD shows the involuntary movement of the patient before and after the 8-week YGS treatment.

Author Roles: Koh Iwasaki had full access to all of the data in the study and takes responsibility for the data and the accuracy of the data analysis. K. Iwasaki, T. Satoh, T. Takahashi, H. Tago and T. Muneshige contributed to the study supervision, patient recruitment, reviewing of the data, and writing of the report. T. Satoh and T. Takahashi were clinical investigators. T. Seki, N. Yaegashi and H. Arai contributed to study supervision.

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FGF20 and Parkinson's Disease: No Evidence of Association or Pathogenicity via α-Synuclein Expression

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Abstract: **Genetic variation in *fibroblast growth factor 20* (*FGF20*) has been associated with risk of Parkinson's disease (PD). Functional evidence suggested the T allele of one SNP, rs12720208 C/T, altered PD risk by increasing FGF20 and α-synuclein protein levels. Herein we report our association study of *FGF20* and PD risk in four patient-control series (total: 1,262 patients and 1,881 controls), and measurements of FGF20 and α-synuclein protein levels in brain samples (nine patients). We found no evidence of association between *FGF20* variability and**

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

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PD risk, and no relationship between the rs12720208 genotype, FGF20 and α-synuclein protein levels. © 2009 Movement Disorder Society

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Elucidating the genetic factors involved in complex disorders such as Parkinson's disease (PD) is crucial as we move into the realm of individualized medicine. Recently, genetic association between variants in *fibroblast growth factor 20* (*FGF20* [MIM*605558]) and PD has been reported,[1–4] however further results have been conflicting.[5–7] In one of the studies, which showed a positive association between *FGF20* and PD in a series of 1,089 patients and 1,165 controls from 729 families, functional evidence was presented in support of the association findings.[4] Namely, the T allele of one single-nucleotide polymorphism (SNP) (rs12720208 C/T) associated with increased risk of PD was shown to disrupt a micro-RNA (miRNA-433) binding site. Results from in vitro (renilla luciferase assay in fibroblasts) experiments showed the rs12720208 T allele reduced binding of miRNA-433 and increased *FGF20* expression. In addition, FGF20 was reported to increase α-synuclein protein levels in dopaminergic cells. In vivo studies using brain tissue from three patients with PD suggested the T allele of rs12720208 is associated with increased protein levels of FGF20 and α-synuclein. The study proposed that the T allele of rs12720208 confers significant PD risk by promoting *FGF20* gene expression, increased FGF20 protein levels, and concomitantly α-synuclein levels.

TABLE 1. *Demographic characteristics of the four patient-control series and the combined series*

	PD patients	Controls
Irish series (174 patients, 174 controls)		
Age (yr)	61 ± 12 (33–90)	61 ± 12 (33–90)
Gender (Male)	68 (39%)	68 (39%)
Age of PD onset (yr)	49 ± 11 (18–77)	
Positive family history (%)	16	
U.S. series (420 patients, 420 controls)		
Age (yr)	72 ± 11 (29–91)	71 ± 11 (32–92)
Gender (Male)	226 (54%)	226 (54%)
Age of PD onset (yr)	62 ± 12 (16–85)	
Positive family history (%)	37	
Norwegian series (515 patients, 1138 controls)		
Age (yr)	72 ± 11 (30–99)	73 ± 11 (43–106)
Gender (Male)	310 (60%)	538 (47%)
Age of PD onset (yr)	59 ± 11 (25–88)	
Positive family history (%)	23	
North-American brain series (153 patients, 149 controls)		
Age (yr)	78 ± 7 (60–93)	75 ± 16 (27–102)
Gender (Male)	102 (67%)	77 (52%)
Age of PD onset (yr)	63 ± 10 (40–86)	
Positive family history (%)	NA	
Combined series (1262 patients, 1881 controls)		
Age (yr)	71 ± 12 (29–99)	71 ± 12 (27–106)
Gender (Male)	706 (56%)	909 (48%)
Age of PD onset (yr)	59 ± 12 (16–88)	
Positive family history (%)	NA	

The sample mean ± SD (minimum, maximum) is given for age and age of PD onset.
NA, not available.

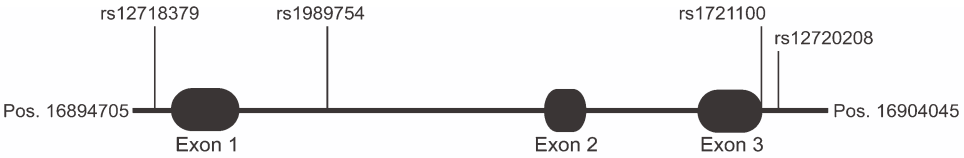

FIG. 1. Gene structure of *FGF20* on chromosome 8p22 showing the four SNPs analyzed in this study; one in the 5′UTR (rs12718379); one in the first intron (rs1989754); and two in the 3′UTR regulatory region (rs1721100, rs12720208).

Herein, we describe our association study of *FGF20* variants in four series of unrelated patients with PD and controls (total: 1,262 patients, 1,881 controls), and our assessment of *FGF20* rs12720208 genotype and human brain levels of both FGF20 and α-synuclein proteins.

PATIENTS AND METHODS

A U.S. (n = 840) and an Irish (n = 348) patient-control series, matched for age and gender, an unmatched Norwegian (n = 1,653) patient-control series, and an unmatched North-American pathological brain (n = 302) patient-control series were examined for *FGF20* association with PD (Table 1). PD diagnosis was established according to published criteria with each living patient examined by a movement disorders neurologist and the postmortem cases by experienced neuropathologists.[8] Controls were free of neurological disease or a family history of parkinsonism. The ethical committees of each institution approved the study and each living subject signed an informed consent. Brains were collected under IRB approved protocols.

DNA was extracted from blood and brain tissue using standard protocols. We genotyped four SNPs across *FGF20*, including the three SNPs associated with PD in the initial report by van der Walt et al.[2] (rs12720208, rs1721100 which was independently confirmed,[5] and rs1989754), and one SNP which yielded borderline results in the only positive replication study (rs12718379) (Fig. 1).[5] Genotyping was performed using MALDI-TOF on a Sequenom platform (>95% genotype calls). SNP genotypes were in Hardy-Weinberg equilibrium for each control population as determined using chi-square goodness of fit tests (all $P > 0.05$).[9] Linkage disequilibrium between SNPs was measured in controls by pair-wise r^2 values (Supp. Info. Table 1).[10] For the matched U.S. and Irish series, association between PD and each marker was measured by odds ratios (OR's) with 95% confidence intervals (CI's) obtained from single variable conditional logistic regression models. For the unmatched North-American pathological brain series, Norwegian patient-control series, and combined series, association between PD and each marker was measured by OR's with 95% CI's obtained from logistic regression models adjusted for age, sex, and series (combined series only). Haplotype analysis was performed using S-Plus score tests for association,[11] adjusted for age, sex, and series (combined series only); *P*-values were obtained from the asymptotic distribution of the score statistic (haplotypes <1% were not considered). Statistical sig-

TABLE 2. *Single SNP associations with PD—additive model*

	Irish series (174 patients, 174 controls)		U.S. series (420 patients, 420 controls)		Norwegian series (515 patients, 1,138 controls		North-American brain series (153 patients, 149 controls)		Combined series (1,262 patients, 1,881 controls)	
	Estimated OR (95% CI)	*P* value	Estimated OR (95% CI)	*P* value	Estimated OR (95% CI)	*P* value	Estimated OR (95% CI)	*P* value	Estimated OR (95% CI)	*P* value
rs12718379 (C)	1.35 (0.98, 1.87)	0.067	0.92 (0.76, 1.12)	0.41	0.95 (0.82, 1.11)	0.53	0.92 (0.76, 1.11)	0.38	0.97 (0.87, 1.08)	0.58
rs1989754 (G)	0.72 (0.53, 0.99)	0.043	1.01 (0.84, 1.23)	0.88	1.10 (0.94, 1.28)	0.23	1.03 (0.84, 1.25)	0.80	1.03 (0.92, 1.14)	0.62
rs1721100 (G)	1.16 (0.81, 1.64)	0.42	0.97 (0.79, 1.19)	0.75	0.89 (0.75, 1.05)	0.16	0.96 (0.78, 1.18)	0.70	0.94 (0.84, 1.06)	0.32
rs12720208 (T)	1.08 (0.62, 1.89)	0.78	1.15 (0.83, 1.58)	0.41	0.88 (0.67, 1.16)	0.37	1.13 (0.82, 1.56)	0.46	1.03 (0.86, 1.24)	0.76

Estimated odds ratios correspond to an increase of one minor allele. The choice of an additive model is based on the hypothesis and evidence from Wang et al.[4] suggesting an additive effect of the rs12720208 T allele. *P* values ≤ 0.0125 are considered statistically significant after a Bonferroni adjustment for multiple testing.

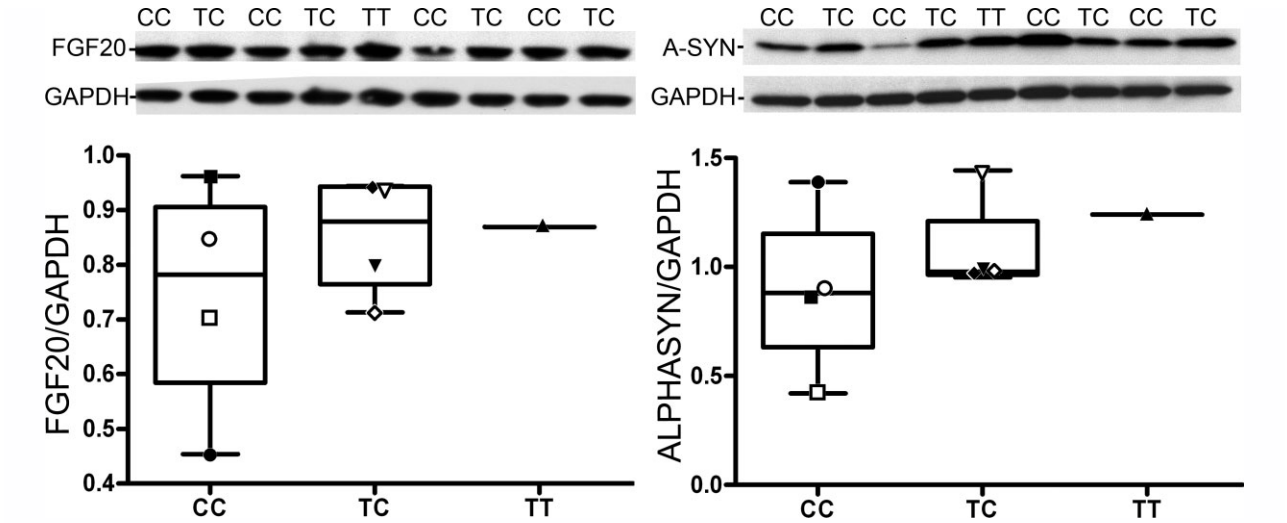

FIG. 2. Top, expression of FGF20 (*left*) and α-synuclein (*right*) in nine patients with PD. The rs12720208 genotype (CC, TC, and TT) is indicated. Bottom, relative expression levels of FGF20 and α-synuclein (normalized against GAPDH; imaging software, ImageJ/NIH) with median values, 25th–75th-tiles (boxes) and confidence intervals. Each symbol (squares, circles, and triangles) indicates normalized FGF20 (*left*) and α-synuclein (*right*) levels for one patient. There is no association between the rs12720208 genotype (CC, TC, or TT) and levels of FGF20 and α-synuclein. Comparison in individual patients shows no relationship between FGF20 and α-synuclein levels (for example, the patient with a filled square has relatively high FGF20 levels but average α-synuclein levels, the reverse being true for the patient with a filled circle).

nificance was set at the 5% level and multiple testing was adjusted for using the Bonferroni method for each family of statistical tests.

Cerebellar brain tissue from nine patients with PD was selected based on rs12720208 genotype; one homozygote TT; four heterozygotes TC; and four homozygotes CC. Total tissue lysates were prepared using RIPA extraction buffer. Protein levels were measured by Western blot using rat monoclonal anti-FGF20 antibody (R&D Systems, Minneapolis, MN) and mouse monoclonal anti-α-synuclein antibody (Invitrogen, Carlsbad, CA), and normalized to GAPDH controls.

RESULTS

Examination of the individual *FGF20* SNPs including rs12720208 (Table 2) and subsequent haplotype analysis of all four SNPs simultaneously ($P > 0.25$ in each series) revealed no significant association with PD in four separate series. Allele and genotype frequencies are given in Supporting Information Table 2. Minor allele frequencies were consistent across the four series; therefore, they were suitable for the combined analysis, which showed no association between *FGF20* variability and PD. Examination of FGF20 protein levels in nine brain samples showed no association with the rs12720208 genotype (Fig. 2). In addition, α-synuclein protein levels were not associated with FGF20 protein levels (Fig. 2).

DISCUSSION

Wang et al.[4] propose a pathomechanism for *FGF20* genetic association with PD via the over-expression of FGF20 and α-synuclein. They postulate a 3′UTR SNP (rs12720208) affects miRNA binding and results in the differential allele-specific expression of *FGF20*, thereby altering FGF20 protein levels and consequently α-synuclein. This group first proposed *FGF20* as a candidate PD gene as it was located under a linkage peak they identified in a study of small singleton and multiplex U.S. families.[1–4] Subsequent studies have shown inconsistent results, positive only in one Japanese population,[5] and negative in three Caucasian populations.[6,7] Likewise, this study did not find any association between *FGF20* genetic variability and risk of PD. In contrast to the original *FGF20* study, which used a pedigree-based analysis of familial and sporadic patients and controls,[2,4] replication studies, including ours, used an unrelated patient-control design; however, recent evidence suggests the genetic risks in sporadic PD may overlap with familial forms of the disease.[12,13] Although the pedigree-based statistical

method avoids bias from population stratification,[2,4,14] cryptic relatedness may be negligible in outbred populations.[15] Furthermore, in such populations, the classical patient-control association method may require similar or even smaller sample sizes than the pedigree-based test to assess genetic risk factors[16]; therefore, differences in statistical approach are unlikely to account for our discrepant results.

Although we can not rule out an effect of SNP rs12720208 on miRNA binding, our functional study neither showed any association between the rs12720208 genotype and levels of FGF20 or α-synuclein nor a relationship between the levels of FGF20 and α-synuclein. The considerable random variability between our nine postmortem cases in both FGF20 and α-synuclein protein expression may help explain the differences observed in brain samples from only one patient of each rs12720208 genotype (n = 3 in the original study).[4]

Our study does not support a significant role of *FGF20* variability in PD risk or a pathomechanism involving codependent FGF20 and α-synuclein protein levels. This study highlights the need for caution in interpreting association studies, even with functional data, before genetic findings have been adequately replicated.

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