研究成果の刊行に関する一覧表（高橋良輔）

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Short communication

Dopaminergic neuronal dysfunction associated with parkinsonism in both a Gaucher disease patient and a carrier

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Abstract

A clinical association between Gaucher disease and parkinsonism has been demonstrated. We herein report a Japanese patient with type 3 Gaucher disease who was compound heterozygous for F213I and L444P mutations in the glucocerebrosidase gene while his father was heterozygous for the L444P mutation. They both presented with parkinsonism characterized by a predominance of akinetic-rigid signs and a favorable response to anti-Parkinson therapies. We investigated the dopaminergic neuronal function using positron emission tomography (PET) with radioligands, [^{11}C] CFT and [^{11}C] raclopride. PET studies of both patients demonstrated the [^{11}C] CFT uptake to be severely decreased in the putamen and the caudate nucleus, however, the [^{11}C] raclopride uptake was normal in the basal ganglia. Although the majority of Gaucher disease patients with parkinsonism tend to be refractory to anti-Parkinson therapies. The clinical features and the findings of the PET studies suggest that patients with parkinsonism associated with the mutation in the glucocerebrosidase gene, even in heterozygosis, may be related to the presynaptic dopaminergic neuronal dysfunction reported in Parkinson's disease. A PET study to evaluate the dopaminergic neuronal function in Gaucher disease would provide both a better understanding of the effects of anti-Parkinson therapies and a help to improve our ability to make an early diagnosis of parkinsonism associated with Gaucher disease.

Keywords: Gaucher disease; Parkinsonism; Glucocerebrosidase; PET

1. Introduction

Gaucher disease is an autosomal recessive lysosomal disorder resulting from a deficiency of the lysosomal enzyme glucocerebrosidase which lead to the systemic storage of glycosphingolipids [1]. This disease is caused by mutations in the glucocerebrosidase gene located on1q21. Recent studies have revealed an association between Gaucher disease and Parkinson's disease due to a concurrence of type 1 Gaucher disease and parkinsonism and the identification of glucocerebrosidase mutations in patients with sporadic Parkinson's disease [2–7]. Initial studies of the patients affected from Gaucher disease with the parkinsonism showed that the parkinsonism was characterized by an early onset and it tended to be refractory to levodopa therapy [2,3,5], however, there is an increasing number of reports which showed parkinsonism to demonstrate the following signs of typical Parkinson disease: namely, the asymmetric onset of rigidity, resting tremor, bradykinesia, and a favorable response to Parkinson therapies [4,8]. Treatment-refractory parkinsonism suggests that mutations in the glucocerebrosidase gene may affect either postsynaptic dopaminergic neurons or both post- and presynaptic dopaminergic neurons.

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We herein investigated the dopaminergic neuronal function of the parkinsonism, responsive to levodopa therapy, in a type 3 Gaucher disease patient and his father using positron emission tomography (PET) and thus showed that our cases were involved in presynaptic dopaminergic function seen in Parkinson's disease.

2. Clinical reports

A 38-year-old Japanese man complained of difficulty in walking and a reduced speed in the normal activities of daily life. He was found to have hepatosplenomegaly at the age 6. A bone marrow analysis revealed a marked accumulation of Gaucher's cells and his glucocerebrosidase activity level was 1.8 nmol/h/mg protein (control level; 4.1–9.6 nmol/h/mg protein). He developed a generalized tonic–clonic seizure with abnormal electroencephalogram patterns at age 7 and thus was treated with anti-convulsant therapy. A neurological examination at the age 7 slowed horizontal saccadic eye movements characteristic of type 3 Gaucher disease. He was diagnosed to have type 3 Gaucher disease and thus underwent a splenectomy in early adolescence. He also suffered from spinal bone pain and abdominal pain associated with hepatomegaly at the age 28. After the initiation of enzyme replacement therapy at the age 33, the hepatomegaly and the bone pain both were improved, however, the patient gradually developed a clumsy left hand, start hesitation and freezing of gait during turning. He was unable to walk without assistance by the age 37 and thereafter presented at our hospital. His family history revealed no consanguinity and no history of Gaucher disease. A neurological examination revealed that he showed severe akinesia with a tendency to show trunk deviation to the left in the sitting position and he was unable to get up from a chair without help. He walked with a flexed posture, with small and irregular steps, while demonstrating start and turn hesitation and a reduction in his arm swing. Slurred speech, hypophonia, micrographia and generalized rigidity were also observed, as well as slowed horizontal saccadic eye movements. All other neurological examinations were unremarkable; in particular involuntary movement including tremors, and muscle strength, stretch and cutaneous plantar reflexes, co-ordination, sensory functions, or fundi and other cranial nerves were normal. A mental examination showed him to be inert. Spatial abilities were intact. His digit span was six forward, and four backward. He could repeat a seven-item name and address immediately after its oral presentation and could recall 6 of 7 items after a 5-minute delay. The Wechsler Adult Intelligence Scale (WAIS) showed verbal IQ of 60, performance IQ of 53, and full scale IQ of 52His Mini Mental State Examination (MMSE) score was 26. An electroencephalogram showed some sharp waves or spike and wave complexes over both parietal-occipital regions and abundant generalized discharges of spikes, polyspikes and slow wave complexes. Magnetic resonance imaging showed no abnormality in the brain. A slit-lamp examination and laboratory studies including thyroid function tests, serum copper and ceruloplasmin were all normal. The study of an auditory brainstem response in this patient showed no deterioration.

His 71-year-old father presented to our hospital in order to help his son. He also became aware of progressive difficulty of slowness during walking and developed a left clumsy hand at the age of 63. A neurological examination showed bradykinesia, symmetrical cogwheel rigidity of the upper limbs and poor backward postural reflexes. His sense of touch, vibration, position and cognitive abilities were intact. Her 65-year-old mother was asymptomatic. A neurological examination was unremarkable.

After PET studies of the proband and his farther, anti-Parkinson therapies including levodopa/carbidopa, cabergoline and selegeline HCl were initiated. The parkinsonian features in both patients showed a favorable response to the medication. The patient was able to walk without assistance and showed an improvement in both akinesia and rigidity. The Unified Parkinson disease rating scale (UPDRS) III motor score in the proband improved from 45 to 28. His father also improved from 23 to 16. During the follow-up, the proband showed a gradual appearance of a wearing-off phenomenon, motor fluctuations and levodopa-induced dyskinesia.

3. Methods and results

3.1. Molecular genetic analysis

Genomic DNA samples isolated from blood samples were subjected to restriction fragment length polymorphism (RFLP) analyses to identify any mutations in the glucocerebrosidase gene by a previous described method [9] For a molecular genetic analysis for hereditary Parkinson disease, the sequencing of the gene for α-synuclein and parkin was performed by a previously reported technique [10,11]. The genetic study demonstrated that the proband carried two known missence mutations in the glucocerebrosidase gene, L444P in exon 10 and F213I in exon 6 (Fig. 1A). The RFLP analyses of his father demonstrated a L444P mutation on the paternal allele. No mutations in the α-synuclein gene or the parkin gene were identified in the proband and his father.

3.2. PET scan

PET was performed by a high-resolution brain PET scanner (SHR12000, Hamamatsu Photonics K.K., Hamamatsu, Japan). The head of a patient was fixated using a thermoplastic face-mask enabling to fix it to the same place between separate PET measurements. First, 72 min after a bolus intravenous injection of the [^{11}C] CFT, 20-minute PET data were collected to produce a late-phase image of [^{11}C] CFT uptake [12]. Next, following three hours to allow for a decay of [^{11}C] CFT radioactivity, the same patient were scanned for 62 min after [^{11}C] raclopride injection using a

serial scans protocol [13] The final PET images were generated as semi-quantitative parametric images (a standardized uptake value image for [^{11}C] CFT, and a distribution image for [^{11}C] raclopride). Based on the regions of interest (ROIs) method, we placed the ROIs on the caudate nucleus, putamen and cerebellum on the MR images, and then transferred them onto the corresponding PET images, and finally calculated a semi-quantitative striatum/cerebellum ratio by dividing the ROI counts of either the caudate nucleus or the putamen by cerebellar counterparts. The ratio from a patient and his father was compared with the ratios from three normal control subjects and assessed statistically

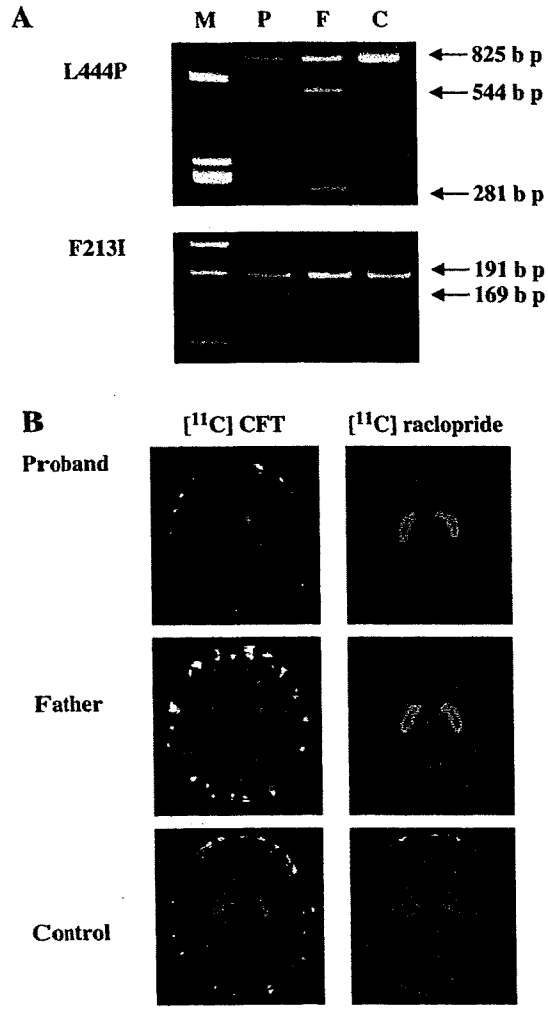

Fig. 1. A. Restriction fragment length polymorphism analyses of the L444P and the F213I mutation in the patient and his father. When the L444P mutation is present, restriction enzyme (NcII) digests an 825 bp PCR product, thus producing two fragments of 544 bp and 281 bp. When the F213I mutation is present, a restriction enzyme (AseI) digests a 191 bp PCR thereby producing two fragments of 169 bp and 22 bp M: molecular marker, P: patient, F: patient's father, C: control. B. Transaxial PET slices blended with MRI images of the proband, his father and a 38-year-old healthy man as a control.

Table 1

	Patient	His father	Normal subjects (n=3) (mean±S.D.)
[^{11}C] CFT			
Caudate nucleus/cerebellum ratio	1.82	1.56	3.83±0.07
Putamen/cerebellum ratio	1.42	1.37	3.90±0.13
[^{11}C] raclopride			
Caudate nucleus/cerebellum ratio	3.93	3.72	4.33±0.13
Putamen/cerebellum ratio	4.02	4.14	4.49±0.31

The uptake of [^{11}C] CFT in the both putamen and caudate nucleus was significantly reduced at $p<0.05$ by one sample t-test.

by one sample t-test (Table 1). The PET images of the patient and his father showed similar results. The uptake of [^{11}C] CFT in the both putamen and caudate nucleus was significantly reduced ($p<0.05$), while the [^{11}C] raclopride uptake showed a relative decrease in the same striatal regions in comparison with normal counterparts.

4. Discussion

This report documented fascinating clinical and PET findings in two patients with the same family lineage who both developed parkinsonism. The clinical features of our cases are as follows; 1) the proband with type 3 Gaucher disease and his father developed parkinsonism, 2) molecular genetic analyses in the glucocerebrosidase gene showed the proband to be compound heterozygous for L444P and F213I, while his father is heterozygous for the L444P mutation, 3) the parkinsonism showed a favorable response to anti-Parkinson therapies and 4) a dopaminergic functional neuroimaging study of both patients showed a presynaptic dopaminergic dysfunction which is normally seen in Parkinson's disease patients. Parkinsonism has been described as a rare neurological phenotype of patients with type 1 Gaucher disease [2,3] The parkinsonism in such patients is characteristically early-onset and most tend to show a poor response to levodopa therapy. The enzyme replacement therapy is not effective for the treatment of the parkinsonism in such cases. The neuropathological findings characteristic to Gaucher disease with parkinsonism showed a marked loss of dopaminergic neurons in the substantia nigra, synuclein-positive Lewy bodies and the involvement of hippocampal CA2-4 regions where glucocerebrosidase was expressed [5,14]. While the concurrence of Gaucher disease and parkinsonism could still be coincidental, the shared clinical characteristics and neuropathology of previous case reports suggest a related etiology. In our proband the diagnosis of Gaucher disease was firmly established both by a deficiency in the glucocerebrosidase activity and the gene analysis in the glucocerebrosidase gene. The first clinical manifestations including hepatosplenomegaly, slow saccadic eye movements and epilepsy preceded by osseous pain, appearing in adulthood, suggest our case to have type 3 Gaucher disease The L444P and the F213I mutations identified in our patients

are frequent in patients affected with both type 1 and 2 as well as type 3 Gaucher disease [1,9]. Although the correlation between genotypes and phenotypes in Gaucher disease is investigated, the conclusion remains elusive [1] Although many reports have demonstrated a clinical association between type 1 Gaucher disease and parkinsonism, type 3 Gaucher disease with parkinsonism is uncommon. The proband appeared to have early-onset parkinsonism which developed in his 30's as previous reports in type 1 Gaucher disease patients with parkinsonism [5]. The patient's father who carried the L444P allele developed parkinsonism in his 60's. A recent study demonstrates that parkinsonism appears to be associated with heterozygosity for a mutation in the glucocerebrosidase gene [8]. This observation indicates that the L444P mutation found in the father, even in heterozygotes, may thus be a risk factor for the development of parkinsonism. The correlation between parkinsonism as a phenotype and mutations in the glucocerebrosidase gene as a genotype has not yet been established. N370S, L444P, 84GG mutations are reported as common mutations associated with parkinsonism in Gaucher disease patients and their carriers, however, it is evident that the majority of the patients or carriers with such mutations do not always develop parkinsonism [4,5,8] An intriguing clinical feature of our patients was the fact that they had treatment-responsive parkinsonism, because initial case reports showed the parkinsonian symptoms in Gaucher disease patients to be refractory to levodopa therapy [2,3,5]. In addition, the association between a favorable response to L-Dopa and the mutation in patients with Gaucher disease has been reported in some studies. Bembi et al. reported 4 cases with a good response to L-Dopa who had either N370S, L444P or G337S mutation [4]. Goker-Alpan et al. also showed some cases with a good response to L-Dopa therapy and they had either N370S or L444P mutations, however, not all the patients with such a mutation always showed an effective response to L-Dopa therapy [8].

We evaluated the dopaminergic function of our patients using neuroimaging techniques with a PET system [^{11}C] CFT, a dopamine transporter probe, allows us to study the integrity of the presynaptic dopaminergic system [12]. [^{11}C] raclopride, a low affinity dopaminergic D2 receptor ligand, has been used to study the post synaptic dopaminergic function [13]. The PET study with the combined use of [^{11}C] CFT and [^{11}C] raclopride in our patients who both carried the L444P mutation allele showed a presynaptic dopaminergic dysfunction The mutation in the glucocerebrosidase gene, even in heterozygosis, may be associated with the presynaptic dopaminergic neuronal dysfunction which shares a common pathogenesis to Parkinson's disease. It is not clear why the parkinsonism associated with mutations in the glucocerebrosidase gene shows such variation in the responsiveness to levodopa therapy. We speculate that at the onset of the parkinsonism, this mutation may be associated with a dysfunction of presynaptic dopaminergic neuron and then, during the progression of the parkinsonism, the patient may develop dysfunction of postsynaptic dopaminergic neurons resulting in the poor responsiveness to levodopa therapy. Another possibility may be that other genetic or environmental factors may interact with the glucocerebrosidase gene thus resulting in the development of variation in the responsiveness to anti-Parkinson therapy. It is important to be aware of the association between Gaucher disease and parkinsonism. We should therefore investigate parkinsonian symptoms in not only probands of Gaucher disease but also their family members. A PET study to evaluate pre- and postsynaptic dopaminergic neuronal function provides an excellent understanding of an association with Gaucher disease and Parkinson's disease.

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Screening for Lrrk2 G2019S and Clinical Comparison of Tunisian and North American Caucasian Parkinson's Disease Families

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Abstract: Mutations in the leucine-rich repeat kinase-2 gene (*LRRK2*) are responsible for some forms of familial as well as sporadic Parkinson's disease (PD). The purpose of this study was to examine the frequency of a single pathogenic mutation (6055G>A) in the kinase domain of this gene in United States and Tunisian familial PD and to compare clinical characteristics between patients with and without the mutation. Standardized case report forms were used for clinical and demographic data collection. We investigated the frequency of the most common substitution of *LRRK2* (G2019S, 6055G>A) and its impact on epidemiological and phenotypic features. The frequency of mutations in Tunisian families was 42% (38/91) and in U.S. families 2.6% (1/39), with the unique opportunity to compare homozygous (n = 23) and heterozygous (n = 109) Tunisian carriers of G2019S substitutions. Individuals with G2019S substitutions had an older age at onset but few other differences compared with families negative for the substitution. Patients with *LRRK2* mutations had typical clinical features of PD. Comparisons between individuals with heterozygous and homozygous *LRRK2* mutations suggested that gene dosage was not correlated with phenotypic differences; however, the estimated penetrance was greater in homozygotes across all age groups. © 2006 Movement Disorder Society

Key words: Parkinson disease; genetics; LRRK2; G2019S; PARK8

Six genes have been implicated in specific forms of Parkinson's disease (PD).[1] The PARK8 locus was first identified in a Japanese family with autosomal-dominant PD.[2] The leucine-rich repeat kinase 2 (*LRRK2*) gene was associated with PD and cloned in 2004.[3,4] Several pathogenic mutations have since been reported in other populations.[3,5–16] A high frequency of the *LRRK2* G2019S 6055G>A mutation has been reported in North Africans (37%) and Ashkenazi Jews (30% in familial PD and 13% in sporadic), and there is evidence for a common founder.[10,11,17–20] Clinical features are similar to classic PD. The pathological features are heterogeneous but

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most often resemble sporadic PD, with loss of neurons in the substantia nigra and the presence of Lewy bodies.[3,4,8,16,21–23]

In Tunisia due to the sociocultural conditions and especially to a high rate of consanguineous marriage, the frequency of neurodegenerative disorders including PD is higher than in other countries.[24] Unique geographic and sociocultural factors have created favorable circumstances for the study of genetic diseases in this country such as the large family sizes, low rates of migration, small size of the country, and the availability of good neurological clinical expertise.

The current study originated as a linkage study of familial PD from Tunisia and United States. A whole genome scan identified a significant linkage peak on chromosome 12 in Tunisian families. Further genotyping in this region was carried out, specifically for G2019S, a substitution reported with high frequency in PD patients from North Africa. The main objectives of this study were to compare the frequency of the G2019S substitution in Tunisian and U.S. familial PD groups, and the phenotypic features of patients sharing the mutation (G2019S+) with those without the mutation (G2019S-).

PATIENTS AND METHODS

Study Populations

Tunisian PD patients were recruited from the Institut National de Neurologie, Tunis, which provides a specialized neurological service to the entire country. The U.S. patients were recruited from four sites: Emory University, Atlanta, Georgia; Struthers Parkinson's Center, Minneapolis, Minnesota; Baylor College of Medicine, Houston, Texas; and the University of Kansas Medical Center, Kansas City, Kansas. Study sites obtained local ethics committee or investigational review board approval before beginning subject recruitment. Subjects were informed of all aspects pertaining to their participation in the study and gave either written or proxy consent.

Standardized recruitment methodology was used to collect multiplex PD families. The proband was examined at the study site, and additional family members were recruited by means of the proband. Inclusion criteria were age at assessment older than 18 years, at least 1 other affected first- to third-degree blood relative consenting to participate (excluding a monozygotic twin), and diagnosis of PD according to United Kingdom PD Society Brain Bank (UKPDS) criteria.[25]

Physical examinations were performed by neurologists specialized in movement disorders at each site. Individuals were diagnosed as "affected" if they satisfied the UKPDS criteria, "unaffected" if all signs of parkinsonism were absent, and "uncertain" if only one parkinsonian sign or abnormal feature was present. Standardized case report forms were used for clinical and demographic data collection, although some Tunisian patients completed only an earlier, simplified version. Investigators from the United States (R.L.W.) and United Kingdom (D.B./J.S.) re-examined a random subsample of 34 Tunisian patients from 15 families (16% of recruited families) and their diagnoses were 100% concordant with the diagnoses made by the Tunisian neurologists.

Duration of disease was calculated by subtracting the age at onset (AAO) from the age at examination. For Tunisia, when AAO was unknown, it was estimated from patient history. The most commonly used anti-PD medications in Tunisia, that is, amantadine, trihexyphenidyl, and benserazide, did not have levodopa equivalents, so the quantitative amounts could not be reported.[26]

Genotyping

DNA was extracted by standard procedures from a peripheral venous blood sample.[27] The genome scan was carried out using 1,116 microsatellite markers spaced an average of 4 centimorgans across the genome.[28]

All individuals were genotyped by GlaxoSmithKline (GSK) for the *LRRK2* 6055G>A (G2019S) mutation and 34 additional single nucleotide polymorphisms (SNPs) in the *LRRK2* region. Twenty-five–nanogram aliquots of the DNA samples were arrayed into 96-well microtiter plates. Genotyping was performed by a modification of the single base chain extension assay.[29] After genotyping, the data were scored using a modification of Spotfire Decision Site version 7.3. Genotypes for assays passing quality control tests were exported to an analysis database.

Analysis

MERLIN[30] was used for nonparametric multi-point linkage analysis (Tunisia and United States) and FASTLINK[31] MLINK version 5.1 for parametric analysis (Tunisia). The model used for parametric analysis was dominant inheritance with disease gene frequency 0.0001 and penetrance based on AAO in years (<35 = 0.2, 35–44 = 0.35, 45–54 = 0.5, 55–64 = 0.71, and >74 = 1.0).

Statistical analyses were conducted in STATA version 8.0. The nonparametric Mann–Whitney unpaired test was used to calculate differences in continuous variables between groups, as they were not normally distributed. The Pearson's χ^2 test was used to detect differences among categorical variables. The nonparametric Wil-

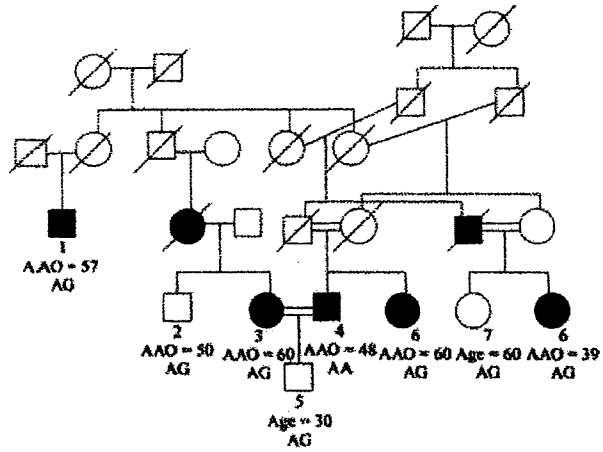

FIG. 1. An example of a Tunisian pedigree containing Parkinson's disease (PD) patients with and without G2019S mutations.

coxon sign rank test was used to test for differences within each population between score ratings by the examiner and patient on the Schwab and England Activities of Daily Living scale.

RESULTS

The linkage analysis included 197 affected, 326 unaffected, and 17 diagnostically uncertain individuals from 80 Tunisian families; and 83 affected, 33 unaffected, and 3 uncertain individuals from 39 U.S. families. *LRRK2* genotyping included an additional 11 Tunisian families (4 affected, 11 unaffected, 10 uncertain) with at least 2 affected, or 1 affected and 1 diagnostically uncertain individual. Uncertain subjects were most commonly diagnosed as essential tremor (ET). All Tunisian participants were of Arab–Berber ethnicity, with the exception of 1 family originated from Turkey and 2 from Southern Europe. All U.S. families were Caucasian.

The maximum logarithm of odds (LOD) scores for Tunisia on chromosome 12 were 3.64 (MERLIN) and 2.75 (MLINK), located at marker D12S1681. The 1-LOD drop region spanned chromosome 12p11.22 – q13.1 (D12S1057 to D12S368). The U.S. collection did not have a peak in the 1-LOD drop region (LOD = 0).

Of 39 U.S. families, 1 (2.6%) had two affected sisters heterozygous for the G2019S substitution. No unaffected U.S. individuals had the substitution. Of 91 Tunisian families, 38 (42%) had at least one G2019S+ affected individual, and 1 additional family had a G2019S+ uncertain individual.

An example of a G2019S+ Tunisian pedigree with is shown in Figure 1. A total of 73 affected, 1 uncertain, and 58 unaffected individuals were G2019S+. Of these, 20 affected patients from 15 families and 3 unaffected individuals from 2 families were homozygous, comprising 17% of G2019S+ patients. The mean AAO (range) in years for affected individuals was AA = 56 (30–82), AG = 60 (31–87), and GG = 48 (9–85). "G" represents the normal allele and the single nucleotide replacement, with "A" the mutation. The AAO was not significantly different between affected individuals with heterozygous and homozygous mutations ($P = 0.1$).

To further evaluate the effect of the mutation status on the penetrance of PD in the Tunisian families, we have calculated the penetrance within G2019S+ individuals and for those with homozygous and heterozygous mutations. Penetrance was calculated by dividing the number of affected subjects by the total number of G2019S+ affected and unaffected individuals within each age category (Table 1).[11] The penetrance for homozygotes was consistently higher than heterozygotes across all age groups.

Of 39 G2019S+ Tunisian families, 9 families had G2019S+ (n = 13) affected individuals also had G2019S− (n = 13) affected individuals. The mean AAO within these families was 55 years in G2019S+ and 66 years in G2019S− affecteds.

TABLE 1. *Penetrance among* LRRK2 *6055G>A (G2019S) mutation carriers, calculated by dividing the number of affected G2019S+ by the total number of G2019S+ in each age category*

	All G2019S+			Homozygotes (AA)			Heterozygotes (AG)		
Age, yr	Aff	Unaff	Penetrance	Aff	Unaff	Penetrance	Aff	Unaff	Penetrance
<35	3	4	0.43	1	0	1.00	2	4	0.33
35–44	11	9	0.55	4	1	0.80	7	8	0.47
45–54	9	11	0.45	2	1	0.67	7	10	0.41
55–64	23	10	0.70	9	0	1.00	14	10	0.58
65–74	21	17	0.55	3	1	0.75	18	16	0.53
>74	6	7	0.46	1	0	1.00	5	7	0.42

The age was defined as age at onset for affected individuals and age at examination for unaffected individuals, in years. Aff, affected with PD; Unaff, unaffected with PD; PD, Parkinson's disease.

Basic demographic characteristics are shown in Table 2, stratified by population and G2019S status. Affected individuals were, on average, older than unaffected individuals within each study population and genetic subtype. It also appears there is an excess of females represented within the G2019S+ Tunisian individuals, compared to the normally observed male preponderance in PD seen in G2019S− individuals in this population.

The clinical characteristics of Tunisian patients with and without the *LRRK2* mutations were compared (Table 3). The U.S. subjects were not stratified, because only two individuals were G2019S+. The mixed type of PD (tremor plus another symptom) was only applied in Tunisia. In the United States, 71.1% of patients were tremor-dominant, and in Tunisia, 90.4% (G2019S+) and 80.5% (G2019S−) were either mixed or tremor-dominant. In general, there were no obvious clinical differences between G2019S+ and G2019S− patients. The only significant difference was an older AAO in G2019S+ patients ($P = 0.0001$).

In a comparison of parkinsonian characteristics from Table 3 between G2019S homozygotes and heterozygotes, the only significant difference was a worse Epworth Sleepiness Scale score for homozygotes ($P = 0.005$). The mean scores were 2.3 (SD = 2.1) for homozygotes (n = 20) and 1.0 (SD = 1.4) for heterozygotes (n = 53).

Symptoms were divided into motor (dystonia, motor fluctuations, postural tremor, and rest tremor) and nonmotor (autonomic dysfunction, cognitive impairment, constipation, erectile dysfunction, hallucinations, orthostatic hypotension, thermoregulatory dysfunction, and urinary dysfunction), and compared between the United States and Tunisia, as well as G2019S substitution status (data not shown). Responses were incomplete; therefore, the reported percentages were calculated from the total possible responses in each stratum. All responding patients in the United States and Tunisia reported the presence of rest tremor, but the highest proportion of postural tremor (45%) was reported by G2019S− Tunisian patients. Nonmotor symptoms were more common in the United States than in Tunisia. Autonomic dysfunction was the most commonly reported symptom in the United States (70%) compared to 16% of Tunisian patients. Over 40% of U.S. patients reported urinary dysfunction and constipation, compared to less than 10% of Tunisian patients. None of the Tunisian patients reported cognitive impairment compared to 8% of U.S. patients, and hallucinations were rare in both populations. There were more missing responses and little variation between the G2019S subgroups within Tunisian patients.

TABLE 2. *Demographic characteristics of affected (with Parkinson's disease) and unaffected individuals with and without* LRRK2 *6055G>A (G2019S) mutation*

	United States		Tunisia						
	Affected	Affected	Unaffected	Affected	Affected	Affected	Unaffected	Unaffected	Unaffected
G2019S mutation	Het+	—	—	Homo+	Het+	—	Homo+	Het+	—
Characteristics									
N	2	81	33	20	52	128	3	55	275[a]
Families	1	38	13	15	36	66	2	27	84
Mean age at exam ±SD, yr		68.7 ± 12.0	57.7 ± 18.2	63.7 ± 12.8	68.4 ± 12.1	60.0 ± 15.8	52.8 ± 15.3	58.0 ± 15.7	53.4 ± 17.3
Median age (range), yr	73 (70–76)	69.9 (36–90)	62 (23–95)	67.4(42–87)	71.2 (38–91)	64.1 (27–87)	45.5 (43–70)	60 (23–91)	52.4 (21–90)
Sex									
Male, n (%)	0 (0)	43 (53.1)	14 (42.4)	9 (45.0)	26 (49.1)	71 (55.5)	1 (33.3)	30 (54.5)	107 (38.4)
Female, n (%)	2 (100)	38 (46.9)	19(57.6)	11 (55.0)	27 (50.9)	57 (44.5)	2 (66.7)	25 (45.5)	172 (61.7)
Ethnicity, n families	Caucasian: H=1	Caucasian: AJ=3; FN=2; FC=4, H=3; M=2	Caucasian: AJ=1; FN=1; H=3; M=1	AB=14; SE=1	AB=35; SE=1	AB=64; SE=1; TY=1	AB=2	AB= 26; SE=1	AB=81; SE=2; TY=1

Affected and unaffected status defined at the time of examination. Ethnicity is self-reported for parents or grandparents. (In the United States, most people identified themselves as Caucasian with no other specifications.)

[a]Four individuals had a missing age value.

Homo, homozygous; Het, heterozygous G2019S mutation: AB, Arab-Berber; AJ, Ashkenazi Jewish; FN, Finnish; FC, French-Canadian; H, Hispanic from North, South, or Central America; M, native or original peoples; SE, Southern Europe; TY, Turkey.

TABLE 3. *Clinical characteristics of U.S. and Tunisian Parkinson's disease patients with and without LRRK2 6055GA (G2019S) mutations*

Characteristics	United States	Tunisia	Tunisia	
	All patients	G2019S positive	G2019S negative	*P* value
N	83	73 (42[a])	128 (65[a])	—
Mean age at onset ± SD, yr	59 ± 12.6	58.9 ± 13.0	48.6 ± 18.3	0.0001
Mean duration PD ± SD, yr	9.4 ± 6.5 (0.1–30.2)	8.4 ± 6.1 (1–28)	11.3 ± 10.0 (0.2–39.8)	0.3
Type of PD, n (%)				
Akinetic-rigid	24 (28.9)	7 (9.6)	25 (19.5)	0.2
Mixed	—	59 (80.8)	89 (69.5)	
Tremor-dominant	59 (71.1)	7 (9.6)	14 (11.0)	
Hoehn & Yahr[a], Mean ± SD	2.5 ± 0.9	1.8 ± 1.0	1.8 ± 1.0	0.8
Schwab and England[a]				
Examiner-rated score, mean ± SD	79.2 ± 19.4	66.0 ± 15.3	68.2 ± 16.7	0.5
Patient-rated score, mean ± SD	82.2 ± 16.2	64.8 ± 17.0	66.6 ± 17.6	0.7
Patient vs. examiner, *P* value	0.0003	0.17	0.08	
Epworth score, mean ± SD	9.9 ± 5.1	1.4 ± 1.7	2.0 ± 2.4	0.1
Duration treatment[b], months				
Mean ± SD	67.3 ± 58.5	80.6 ± 57.6	97.3 ± 91.3	0.9
Median (range)	48 (0.03–216)	60 (1–252)	60 (1–480)	
UPDRS III[a]				
Mean ± SD	36.9 ± 16.9	48.5 ± 22.7	43.8 ± 23.3	0.3

The *P* value is to test if there are differences between G2019S-positive and -negative patients within Tunisian patients.

[a]Only some participants completed the more detailed Tunisian case-report form, N = 42 G2019-positive and N = 65 G2019S-negative.

[b]Treatment with levodopa or dopamine agonists.

PD, Parkinson's disease.

DISCUSSION

This report is one of the largest studies of patients carrying *LRRK2* G2019S substitutions, with the highest number of homozygous individuals identified to date. Comparing the clinical characteristics of these groups to G2019S− patients contributes to the understanding of the *LRRK2* mutation in PD. The G2019S substitution was more common in the Tunisian families (42%) than in the U.S. families (2.6%), in agreement with previously reported frequencies.[10,32,33]

The large number of homozygous G2019S substitutions in Tunisia is likely due to the high rate of consanguinity. Of 91 families, 67 (74%) had consanguinity and 41 (45%) of these had a consanguineous marriage between parents or grandparents of the affected individuals. A total of 18 (47%) of 38 families with heterozygous mutations and 8 (53%) of 15 with homozygous mutations had consanguineous marriages between parents or grandparents of affected individuals. No significant phenotypic differences were found between homozygotes and heterozygotes, with the exception of a worse score on the Epworth Sleepiness Scale for homozygotes. However, the G2019S− patients had the worst score, so the interpretation of this result is unclear. The AAO was not significantly different between homozygous and heterozygous individuals, although both were older at onset than G2019S− patients. This finding could be due to early-onset PD caused by other genes such as *Parkin*, although further mutation screening has not been carried out at this time.

The large Tunisian sample enabled the estimation of age-dependent penetrance of G2019S substitutions in familial PD. The estimates were from G2019S carriers and were limited to the pedigrees under study. Familial penetrance figures are likely overestimates of the population prevalence due to ascertainment bias. The penetrances found using an age-dependent dominant model were within the range of previously reported familial values.[10,11] However, when the penetrance was calculated stratifying by homozygous and heterozygous mutations, the penetrance was consistently higher in homozygotes in each age group. This finding could indicate a gene dosage effect, although AAO was not significantly different between the two groups. However, the number of homozygotes was small and the potential biases of estimating penetrance from families must be considered. Nearly half (58 [44%] of 132) of G2019S mutation carriers were asymptomatic at the time of examination. On average these individuals were younger than the affected carriers, but some individuals were older than the oldest AAO in G2019S+ patients.

LRRK2 is large and, therefore, was not fully sequenced. The 35 SNPs used, including G2019S, provided good coverage of the gene region. It remains

possible that there are other mutations in the *LRRK2* gene within the Tunisian families, although a second linkage analysis stratified by presence of G2019S substitution showed that G2019S accounted for nearly all of the chromosome 12 linkage peak. The Tunisian study had significant LOD scores greater than 3 on four chromosomes (including chromosome 12), and LOD scores greater than 2 on four additional chromosomes. The U.S. study had MERLIN LOD scores near 2 on chromosomes 1, 11, and 20.

Nine G2019S+ Tunisian families had affected individuals without G2019S substitutions. This finding may be due to another PD gene or sporadic PD. Such findings draw attention to the difficulty of genetic counseling in inbred families where coexistence of two different genetic disorders sharing similar phenotypes may be present.[34]

Of 27 individuals with uncertain PD diagnoses, including 21 ET patients, only 1 with ET was found to have a heterozygous mutation. A previous study concluded that ET was a phenotypic expression of the *LRRK2* mutation in 1 patient,[35] but our larger sample did not support this finding.

In conclusion, there were no significant differences in PD characteristics between patients with homozygous and heterozygous Lrrk2 G2019S substitutions, suggesting no gene dosage effect. However, the penetrance in homozygotes appears to be greater than in heterozygotes across age groups. There were minimal phenotypic variations between PD patients with and without G2019S substitutions.

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