Frequency and Phenotypes of LRRK2 G2019S Mutation in Italian Patients with Parkinson's Disease

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Abstract: **To evaluate the frequency of the LRRK2 G2019S mutation in Italy, we tested 1,072 probands with Parkinson's disease (PD; 822 sporadic and 250 familial): 20 patients (1.9%) carried the G2019S mutation, 11 patients (1.3%) were sporadic, and 9 (4.3%) had a positive family history. Considering only probands with autosomal dominant inheritance, the G2019S frequency raises to 5.2%. All presented a typical phenotype with variable onset and shared the common ancestral haplotype. Mutation frequency raised from 1.2% in early onset PD to 4.0% in late onset PD.** © 2006 Movement Disorder Society

Key words: Parkinson's disease; LRRK2; Dardarin; mutation screening; Italy

Parkinson's disease (PD) is a frequent neurodegenerative disorder, with age-related prevalence reaching 2% in the seventh decade of life. Although most cases are sporadic, a subset of patients reports a positive family history. Several mendelian genes cause autosomal dominant or recessive forms of Parkinsonism, but the mutation rate is very low.[1] The sole exception is represented by a unique mutation in the *LRRK2* gene (6055G>A, resulting in a G2019S substitution in the MAPKKK domain of the protein), which accounts for 0.5% to 2% of PD and up to 8% of patients with definite family history.[2–10] The G2019S mutation is inherited in an autosomal dominant manner with age-related penetrance[5] and has been detected in patients from several countries of Europe, Africa, and America. Regardless of geographic origin and ethnicity, all mutation carriers share an ancestral haplotype spanning 60 to 150 kb, indicating an ancient founder effect.[5,9] The phenotype is indistinguishable from idiopathic PD but presents wide variability in age at onset, ranging from 38 to 80 years.[3,10] To evaluate the frequency and phenotypic spectrum of G2019S in Italy, the mutation was searched for in 1,072 Italian patients with early- and late-onset PD.

PATIENTS AND METHODS

The 1,072 patients were examined by neurologists from 10 movement disorder units in Northern, Central, and Southern Italy and received a diagnosis of clinically definite PD according to the UK Brain Bank Criteria. All underwent a detailed interview to disclose family history for PD or other movement disorders. Patients with evidence of secondary Parkinsonism or with atypical features such as early dementia, ophthalmoplegia, early autonomic failure, and pyramidal signs were excluded. A total of 300 unrelated Italian healthy controls were also included in the study. Written informed consent was obtained for all subjects.

Analysis of the G2019S mutation was performed using a denaturing high-performance liquid chromatography–based approach. After polymerase chain reaction amplification of exon 41 of LRRK2 gene,[4] fragments were analyzed on a WAVE DNA Fragment Analysis System (Transgenomic, Crewe, UK), using a buffer-B start concentration of 54% and an oven temperature of 59.1°C. These conditions were verified by analyzing a positive control known to carry the G2019S mutation. Samples with an abnormal elution profile were directly

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sequenced in both directions. Microsatellite markers and single nucleotide polymorphisms spanning the founder haplotype were genotyped as described by Kachergus and colleagues.[5]

RESULTS

We tested 1,072 probands (651 men and 421 women) with clinically definite PD. Mean age of onset was 50.7 ± 11.3 years (range, 16–85 years), and mean age at examination was 61.4 ± 12.0 years (range, 21–95 years). There were 564 patients who had early-onset PD (85 with onset <35 years and 479 between 36 and 50), 408 patients who had onset between 50 and 65, and the remaining 100 had onset >65 years. A total of 822 patients were sporadic, 207 had a positive family history for PD, and 43 reported a family history of tremor without a precise diagnosis. Among the 207 familial cases, 85 had autosomal dominant inheritance (a parent or offspring with PD), 52 reported a family history compatible with autosomal recessive inheritance (an affected sibling with healthy parents), whereas in 70 cases the mode of inheritance was probably autosomal dominant but could not be specified.

We identified the G2019S heterozygous mutation in 20 probands from Northern (n = 5), Central (n = 7), Southern Italy (n = 5), and Islands (Sicily and Sardinia, n = 3): 11 patients were sporadic, 9 reported at least 1 affected relative. Inheritance was autosomal dominant in 8 families, whereas 1 proband had 1 affected sibling. Only 1 affected relative (the daughter of proband #10) was available for clinical examination and genetic testing. She also carried the G2019S mutation, which was not detected in any of 300 Italian controls. No other exon 41 mutations were found in patients or controls.

Clinical features of the 21 positive patients are shown in Table 1. Mean age at onset was 54.4 ± 12.0 years (range, 35–78 years), and mean disease duration was 9.0 ± 5.0 years. Most patients had a typical parkinsonian phenotype, with asymmetric onset, good response to therapy and absence of atypical features. Of interest, mild psychiatric disturbances unrelated to antiparkinsonian therapy (mostly anxiety and depression) were reported by approximately half of the patients, a rather higher frequency than in idiopathic PD. Cognitive impairment was evident only in the 2 patients with the oldest age at onset and was associated with relevant cortical atrophy.

The overall mutation frequency of G2019S in this Italian series is 1.9%. Among the probands with positive family history for PD, this value raises to 4.3% (9 of 207) and up to 5.2% (8 of 155), including only probands with autosomal dominant inheritance. Mutation frequency among sporadic cases is 1.3% (11 of 822).

Grouping patients on the basis of the age at onset, the mutation frequency increases from 1.2% in early onset PD (<50 years) to 4.0% in late onset PD (>65 years), with intermediate values of 2.2% in the group with age of onset between 51 and 65 years. The proportion of familial cases within each onset group is similar, ranging between 20 and 30% (Fig. 1). Although phase cannot be established in our probands, all share genotypes with the positive control known to bear the common ancestral haplotype.

DISCUSSION

The LRRK2 G2019S mutation represents the most common genetic cause of PD in different populations. Here, we screened G2019S in over 1,000 Italian probands, the largest population of index cases so far tested for this frequent mutation. We identified 20 probands carrying the G2019S mutation, with an overall frequency of 1.9%, raising to 5.2% in the group with autosomal dominant inheritance. These figures are broadly in line with several other studies[2–10] and probably represent an accurate estimate of the G2019S prevalence in Italy, as (1) the unique inclusion criterion was the diagnosis of PD, regardless of age at onset or family history; (2) the patients' group is large and encompasses a broad range of ages at onset; (3) patients originate from all Italian regions including the two major islands. However, it must be noted that PD cases with late onset (7th–8th decades) are underrepresented in our cohort, likely due to an ascertainment bias. In fact, in our experience, elderly patients are often followed up by general practitioners or referred to geriatric or neurological clinics rather than specialized movement disorder units and are generally less willing to participate to genetic research projects.

The 21 Italian mutation carriers present a typical phenotype with good response to levodopa and absence of atypical features. Mild psychiatric disturbances were found in half of the cases, whereas cognitive impairment and dementia only appeared in two octogenarian patients showing signs of cortical atrophy.[6]

Among the G2019S carriers, the age at onset was extremely variable, ranging from 35 to 78 years. Overall, mutation frequency raised significantly with increasing age at onset. As the proportion of familial cases is fairly homogeneous within age groups, this finding is likely due to the age-related penetrance of LRRK2 disease, which has been shown to increase from 17% at age 50 up to 85% at age 70 years.[5]

We conclude that the G2019S mutation is a frequent cause of PD in Italy. The phenotype indistinguishable

TABLE 1. *Clinical features of the 20 patients heterozygous for the G2019S mutation*

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Sex	M	M	M	F	M	F	F	F	F	F	F	F	M	M	F	F	M	M	M	F	F
Area of origin	c	n	n	i	s	s	i	c	s	n	i	i	c	n	c	n	c	c	c	s	s
Age at onset (yr)	35	39	40	42	43	44	46	47	52	54	55	57	58	58	59	61	63	70	71	71	78
Disease duration (yr)	5	5	1	10	3	13	8	13	8	6	20	8	11	20	6	12	8	5	10	13	3
Asymmetry at onset	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Symptom of onset	b	t	r	b	b	b	t	t	b	t	r	r	r	t	b	t	b	t	r	t	t
Dystonia at onset	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sleep benefit	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Present phenotype																					
Resting tremor	—	+	+	—	—	—	+	+	+	+	—	+	—	+	+	+	—	+	+	+	+
Bradykinesia	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+
Rigidity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	+
Gait impairment	+	—	—	+	—	+	—	—	+	—	+	+	—	+	+	—	+	—	—	—	+
Orthostatic hypot.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Urinary urge	—	—	—	—	—	+	—	—	—	—	+	+	—	—	—	—	—	—	—	—	—
Incontinence	—	—	—	—	—	+	—	—	—	—	+	+	—	—	—	—	—	—	—	—	+
Hyperreflexia	+	+	—	—	—	—	+	—	+	—	—	—	+	—	—	—	—	—	—	+	—
Motor fluctuations	—	—	—	+	—	+	—	—	—	—	+	+	—	+	+	+	+	—	—	—	—
L—dopa induced dysk.	n.a.	+	n.a.	+	n.a.	+	n.a.	—	+	—	+	+	—	+	—	+	—	—	—	—	+
Psychiatric symptoms	—	a	a, pa	d	—	a, d	—	d	a	d	—	—	—	—	—	d	a, d	d	—	a, d	—
Cognitive impairment	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
LEDD	175	150	150	300	300	1244	500	500	640	450	1250	1100	754	1400	200	500	1300	375	275	318	500
UPDRSIII on	14	39	13	19	7	37	9	36	20	9	47	22	21	n.a.	19	30	23	25	10	14	n.a.
UPDRSIII off	22	n.a.	17	26	n.a.	42	n.a.	n.a.	44	n.a.	n.a.	n.a.	n.a.	26	29	n.a.	38	n.a.	n.a.	n.a.	38
H&Y score	1	2.5	1.5	3	1.5	2,5	2	2	2.5	1	4	3	2	2.5	1.5	2.5	2	2	1	2.5	2.5
Other features	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	c.atr	c.atr
Family history	—	—	—	—	ad	—	ad	—	—	ad	ad	ad	—	—	ad	—	ad	—	ad	sib	ad

Patient 7 is the daughter of Patient 11.

a, anxiety; ad, autosomal dominant; b, bradykinesia; c, central Italy; c.atr, cortical atrophy; d, depression; dysk., dyskinesias; hypot., hypotension; i, islands (Sicily and Sardinia); LEDD, levodopa equivalent daily dose; n, northern Italy; n.a., not available; pa, panic attacks; r, rigidity; s, southern Italy; sib, 1 sibling affected; t, tremor.

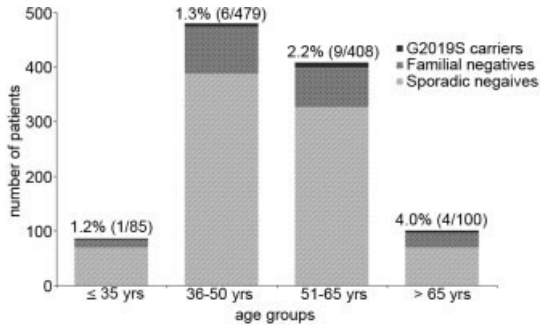

FIG. 1. Age at onset distribution of positive and negative patients. Numbers above the columns indicate the frequency of G2019S mutation within each age group (number of positive cases/total number of cases). This value increases progressively with increasing age of onset. For graphical purposes, the 43 patients with a family history of tremor have been considered as sporadic cases.

from idiopathic PD, the presence of the mutation among sporadic cases, the feasibility and cost-effectiveness of genetic testing, and the important implications in terms of counseling for carrier relatives strongly encourage the inclusion of this test among diagnostic investigations for PD.

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Cardiac ^{123}I-MIBG Scintigraphy in Patients With Essential Tremor

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Abstract: **In some cases, it is difficult to differentiate essential tremor (ET) from Parkinson's disease (PD), especially in the early stages of the disease. We investigated cardiac sympathetic dysfunction using ^{123}I-metaiodobenzylguanidine (MIBG) myocardial scintigraphy in 22 patients with ET, in comparison with early PD and tremor-dominant PD (TDPD). The mean ratio of ^{123}I-MIBG uptake in the region of interest in the heart to that in the mediastinum (H/M ratio) was significantly greater in patients with ET (1.99 ± 0.21) than in those with either TDPD (1.28 ± 0.11) or early PD (1.28 ± 0.17; each $P < 0.001$). The H/M ratio in all patients with ET was greater than two standard deviations above the range of the ratio in the patients with early PD or TDPD.** © 2006 Movement Disorder Society

Key words: cardiac MIBG; essential tremor; Parkinson's disease

Essential tremor (ET), the most common movement disorder, is characterized by a 4 to 12 Hz kinetic and postural tremor affecting the hand, head, or other parts of the body.[1] By definition, patients with ET should not have other clinical signs of parkinsonism.[2] However, patients with ET may exhibit akinesia or bradykinesia, as evidenced by increased reaction times and slow move-

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