SHORT REPORT

Prevalence of the *LRRK2* G2019S mutation in a UK community based idiopathic Parkinson's disease cohort

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The *LRRK2* G2019S mutation is the commonest genetic cause of Parkinson's disease (PD) identified to date, although estimates of its prevalence in idiopathic disease vary considerably. Our objectives were to determine G2019S mutation frequency in an unselected, community based cohort of idiopathic PD cases from the UK and to describe phenotypic characteristics among carriers. The mutation was present in two of 519 cases (0.4%) and none of 887 control individuals. The true prevalence of the mutation in idiopathic disease, its penetrance, and the phenotypic heterogeneity of associated cases have important implications for genetic screening in the clinical field.

Parkinson's disease (PD) is a common neurodegenerative disease, which clinically manifests as a movement disorder accompanied to a variable extent by cognitive, affective, and psychiatric symptoms. Pathologically, PD is defined by a loss of dopaminergic neurones in the nigrostriatal tract with the formation of α-synuclein positive Lewy bodies within the substantia nigra. Although in the vast majority of cases, the aetiology is likely to be multifactorial, several causative genes have been identified in rare Mendelian forms of PD. The most prevalent to date is leucine rich repeat kinase 2 (*LRRK2*)[1] or dardarin,[2] which is estimated to account for 5–7% of familial PD cases.[3 4] Of the several pathogenic mutations identified within this gene, 6055G→A is by far the commonest, accounting not only for the majority of familial *LRRK2* associated cases, but also a significant number of idiopathic cases.[5–13] The mutation is located within exon 41 and results in a substitution of glycine for serine at amino acid position 2019 (G2019S). This substitution occurs within a highly conserved ''activation segment'' of the protein and is postulated to result in a gain of function of its kinase activity, compatible with its autosomal dominant mode of transmission.[3]

The identification of pathogenic *LRRK2* mutations in significant numbers of idiopathic PD (IPD) cases is clinically highly important, prompting the question as to whether genetic testing should be made more widely available in PD. In order to address this question, it is crucial to refine our estimates of the frequency of *LRRK2* mutations in IPD, and to clarify whether certain phenotypic characteristics can be used to identify suspected carriers for screening. We therefore evaluated the frequency of the *LRRK2* G2019S substitution and its associated clinical characteristics in a large unselected cohort of patients with IPD and a control population in Cambridgeshire.

METHODS

Our study population consisted of 538 patients with IPD and 909 controls of white ancestry. Controls included 146 spouses of patients with PD and 763 parents of patients with multiple sclerosis. Cases included 160 patients involved in a population based epidemiological study of incident PD within Cambridgeshire[14] and 378 consecutive prevalent cases attending our research clinic, which recruits patients from community based sources in East Anglia including the UK Parkinson's Disease Society (UKPDS) as well as those referred by general practitioners, neurologists, and geriatricians, hence providing a relatively unbiased sample of patients. All cases were diagnosed according to UKPDS Brain Bank criteria, although family history was not used as an exclusion criterion. All patients had undergone at least one clinical assessment through their participation in our longitudinal epidemiological studies, comprising a detailed history of their disease, a full clinical examination, the Unified Parkinson's Disease Rating Scale (UPDRS), the Beck Depression Inventory, the Mini Mental State Examination (MMSE) and tests from the computerised Cambridge Neuropsychological Test Automated Battery (CANTAB), designed to discriminate between temporal and frontal cognitive deficits.[15] In the patients, the male to female ratio was 3:2, mean age of disease onset was 63 years (range 25–91), mean UPDRS score was 24, and 14% reported a family history of one or more first degree relatives with parkinsonian symptoms. Ethics approval for this study was granted by the local research ethics committee (Cambridge, UK).

Peripheral blood samples were collected following written informed consent, and DNA was extracted using standard phenol/chloroform methods. Individuals were screened for the G2019S mutation using a single nucleotide polymorhism based allelic discrimination *Taq*man assay by design, which uses the 5′ exonuclease activity of *Taq* polymerase to generate an allele specific fluorescent reporter signal. The signal was measured after PCR reactions on a sequence detection system (7900HT; Applied Biosystems). Carriers were then confirmed by direct sequencing. Following standard PCR reactions, amplification products were purified and then sequenced using a commercial kit (BigDye Terminator Cycle Sequencing Kit; Applied Biosystems) and electrophoresed on a DNA analyser (ABI Prism 3700; Applied Biosystems). Data were analysed using Sequencing Analysis software (version 3.7).

RESULTS

Genotyping was successful for 97.3% of our samples. Of 519 patients with IPD, two were found to be heterozygous for the G2019S mutation (0.4%). The mutation was not detected in any of 887 controls. Clinical features in the two carriers are detailed below.

Abbreviations: CANTAB, Cambridge Neuropsychological Test Automated Battery; IPD, idiopathic Parkinson's disease; MMSE, Mini Mental State Examination; PD, Parkinson's disease; UKPDS, United Kingdom Parkinson's Disease Society; UPDRS, Unified Parkinson's Disease Rating Scale

Patient 1

This woman presented at the age of 58 years with a 2 year history of tremor and sensory symptoms affecting the left hand, together with dragging of the left leg when walking. She subsequently developed increasing gait difficulty and depression. There was a dominant family history of parkinsonian symptoms, with both her mother and maternal grandmother being reported to have a shuffling gait and frequent falls in old age, although neither had a confirmed PD diagnosis. Examination of the patient 2 years after diagnosis revealed classical left sided parkinsonism with a tremor dominant motor phenotype, as determined using the method described by Jankovic *et al*.[16] Her MMSE was 30/30, but detailed neuropsychological testing revealed impaired performance on the CANTAB Tower of London task, indicating an isolated frontostriatal type deficit. Her Beck Depression score was 12, consistent with mild depression.

Patient 2

This man presented at the age of 85 years with a 1–2 year history of difficulty in walking, unsteadiness, and falls. His previous medical history included cardiac failure and depression. He was diagnosed with PD at presentation and treatment with levodopa was commenced. Three years later, he developed troublesome motor fluctuations and dyskinesias. There was no relevant family history. On examination 5 years after diagnosis, he was markedly hypomimic and hypophonic with hypersialorrhoea. He had a significant kyphoscoliosis, bilateral symmetrical bradykinesia and rigidity, and was unable to stand without assistance. Analysis of UPDRS subscores confirmed a postural instability and gait disturbance motor phenotype.[16] His Beck Depression score was 14, consistent with mild depression. Cognitive testing revealed an MMSE of 28/30 and an isolated frontostriatal-type deficit of executive function on the CANTAB tasks.

DISCUSSION

The prevalence of the G2019S mutation in our population is compared with that reported in previous screens of IPD cohorts in table 1. Combining these studies, the mutation has been found to date in 63 of 7056 cases (0.9%) and only one of 9238 screened controls. These collective data provide strong support for the hypothesis that the G2019S mutation plays a causative role in PD.

The majority of G2019S prevalence estimates in IPD are between 0.3% and 2.1%. Outlying values, such as frequencies of <0.1% in Asian cohorts[17 18] and 5.6% in a Portuguese cohort,[7] are likely to reflect differences between ethnic backgrounds. Similarly, a recent study in autosomal dominant PD has demonstrated a marked difference in G2019S mutation frequency between French and North African families (2.9% versus 41% respectively).[20] In addition, the proportion of familial cases included in idiopathic cohorts is a significant contributor to variability in frequency estimates. The exclusion of familial cases allows estimation of mutation prevalence in true sporadic disease, which is consistently <1% in all studies but one (table 1). Although this figure is lower than some authors have suggested, it seems that the G2019S mutation does have a role to play in the aetiology of sporadic PD, and indeed is unique in this respect among the genetic causes of PD identified to date.

In our study, the prevalence of the mutant allele is comparatively low, not only among sporadic cases, but also among those with a presumed dominant family history. This may be a reflection of the community based nature of our cohort, which is largely free from the selection bias that might influence those cohorts drawn from tertiary referral centres. Of course, in common with many other studies, we did not evaluate the role of *LRRK2* mutations other than G2019S, hence we may be underestimating the burden of *LRRK2* associated disease among our familial cases.

The clinical features in our *LRRK2* associated patients are indistinguishable from those of typical IPD. Similar observations have been made by previous authors,[3–5 8] although it is striking that our cases are phenotypically so heterogeneous, particularly in terms of age of onset and motor phenotype. This suggests that the aetiology of *LRRK2* associated PD is not straightforward; other environmental and/or genetic factors must alter clinical expression of the disease. Such a hypothesis is in keeping with the reduced and variable penetrance observed in G2019S families.[3 5]

The question of whether genetic testing for the G2019S mutation should be offered routinely in the clinical setting remains debatable. In cases with a dominant family history, its prevalence is clearly highly significant. However, the penetrance of the condition is yet to be firmly established, which poses difficulties for genetic counselling of asymptomatic family members. The very late age of onset of one of our cases (85 years) and the recent report of a neurologically

Table 1 Studies examining the prevalence of the *LRRK2* G2019S mutation in idiopathic PD cohorts

			PD cases									Controls	
			Overall			Familial			Sporadic				
Reference	Case recruitment base	Ethnicity	n	Carr	Freq (%)	n	Carr	Freq (%)	n	Carr	Freq (%)	n	Carr
Aasly[5]	Outpatient clinic	Norwegian	435	9	2.1	65	6	9.2	370	3	0.8	519	0
Berg[6]	N/A	N/A	390	1	0.3	53	0	<1.8	337	1	0.3	1200	0
Bras[7]	Outpatient clinic	Portugese	124	7	5.6	22	2	9.1	102	5	4.9	126	0
Deng[8]	N/A	North American	326	4	1.2	150	3	2.0	176	1	0.6	130	0
Farrer[9]	Tertiary referral clinic	North American	786	4	0.5	236	4	1.7	550	0	<0.2	278	0
Gilks[10]	Tertiary referral clinic	White British	482	8	1.7	N/A	3	N/A	N/A	5	N/A	345	0
Hernandez[11]	N/A	Whiite North American	719	7	1.0	N/A	1	N/A	N/A	6	N/A	2680	0
Kay[12]	Tertiary referral clinic	North American (95% white)	1425	18	1.3	329	10	1.9	1096	8	0.7	1647	1
Zabetian[13]	Outpatient/research clinic	North American (93% European)	371	3	0.8	74	1	1.4	297	2	0.7	281	0
Tan[17]	Tertiary referral clinic	Asian†	675	0	<0.1	58	0	<1.7	617	0	<0.2	325	0
Skipper[18]	Tertiary referral clinic	Asian‡	630	0	<0.1	160*	0	<0.6	470	0	<0.2	630	0
Bialecka[19]	Outpatient clinic	Polish	174	0	<0.5	21	0	<4.7	153	0	<0.7	190	0
This study	Community/research clinic	White British	519	2	0.4	74	1	1.4	445	1	0.2	887	0

*Non-dominant family history; †88% Chinese, 7% Malay, 5% Indian; ‡84% Chinese, 8% Malay, 2% mixed Asian; Carr, carriers; Freq, frequency; N/A, not applicable.

healthy octogenarian carrying the *LRRK2* mutation[12] highlight the fact that prognosis is very uncertain for asymptomatic carriers. Among sporadic cases, G2019S is comparatively rare, and furthermore, heterogeneity of clinical presentation means that it is impossible to define a target group for screening. Undertaking widespread screening in IPD may not be cost effective. Nonetheless, on a case by case basis, screening for a single point mutation such as this is technically simple and relatively cheap, and some patients and their families may find such information helpful.

In conclusion, we found two heterozygotes for the G2019S mutation among a cohort of just over 500 community based patients with IPD, corresponding to a prevalence of 0.4%. Phenotypically, these cases resemble typical IPD but are markedly heterogeneous, including one case presenting in the ninth decade of life, and thus screening for the G2019S mutation is not straightforward.

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