Frequency of the *LRRK2* G2019S mutation in South African patients with Parkinson's disease

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Abstract

G2019S in *LRRK2* is the most common mutation associated with Parkinson's disease (PD). Highest frequencies are in North African Arabic (30-41%) and Ashkenazi Jewish (6-30%) populations, mostly due to founder effects. Here, we investigated the frequency of G2019S in 647 unrelated South African PD patients from different ancestral origins. It was found in only 1.2% (8/647) of patients. Notably, none of the 91 individuals of African ancestry had G2019S. It was present in 1.9% (3/154) and 1% (5/493) of early- and late-onset cases, respectively. The frequency of G2019S exhibits ethnic-specific differences, and warrants further study in sub-Saharan African populations.

Keywords Parkinson's disease; *LRRK2* gene; G2019S mutation; phenotype; South African patients

Introduction

A recent report on global prevalence, disability and deaths related to Parkinson's disease (PD), estimated that the number of people affected by this disorder has doubled from 1990 to 2016 [1]. This trend is likely to persist as life expectancy increases globally. PD is characterised neuropathologically by a loss of dopaminergic neurons in the substantia nigra and clinically by rigidity, bradykinesia and resting tremor. Mutations in the Leucine-Rich Repeat Kinase 2 gene (*LRRK2*) are considered to be the most common cause of sporadic and familial (autosomal dominant) forms of PD [2, 3].

Several pathogenic mutations have been identified, the most frequent being G2019S, which occurs in the kinase domain, and is postulated to cause upregulation of kinase activity [4]. In patients of European ancestry, G2019S has been shown to account for roughly 4% of familial PD and 1% of sporadic PD cases [5]. G2019S is more prevalent in late-onset cases, although it has been found in early-onset cases as well as controls [6]. Variation in G2019S frequency is influenced by ethnic and geographical origin, with the highest frequencies in North African Arabic (30-41%), and Ashkenazi Jewish (6-30%) populations [5, 7]. The frequency is relatively lower in North American (1-3%) European (1-4%) and Asian (<1%) populations [5]. The origin of G2019S is proposed to be associated with a common founder effect in North Africa, which spread globally through migration of the Ashkenazi Jewish population [8].

Studies focusing on genetic causes of PD in Africa are very limited. A recent review identified only 11 genetic studies conducted in Sub-Saharan Africa (SSA) [9]. Among the studies that screened for G2019S in SSA PD patients, the largest sample size reported (205 PD patients) refers to a South African study, in which only four patients were found to harbour the mutation [10]. In the present study, we investigated the frequency of G2019S in an extended group of South African PD probands from different ancestral origins.

Materials and methods

Study participants A total of 647 unrelated PD patients were recruited from various neurology clinics around the country but were predominantly from the Western Cape Province. Patients met the UK Parkinson's Disease Society Brain Bank Research Criteria for diagnosis of PD. Demographic characteristics are shown in Supplementary Table S1. Mixed ancestry refers to a population unique to South Africa resulting from an admixture of individuals of African, European and Asian ancestral origins [11]. G2019S was previously screened in 205 of these 647 PD patients [10]. These patients were included in the present study.

Genetic analysis PCR amplification and high-resolution melt (HRM) analysis were done as reported previously [10]. Following HRM, all samples exhibiting altered melting curves were Sanger sequenced at the Central Analytical Facilities (Stellenbosch University, South Africa). Sequences were analysed using BioEdit software, version 7.0.5 [12].

Results

The heterozygous and homozygous forms of G2019S were clearly distinguishable from the wild-type allele using HRM (Supplementary Figure S1). Our study revealed that only 1.2% (8/647) of the patients harboured G2019S. The observed frequencies per ethnic group, with 95% confidence intervals, are shown in Supplementary Table S2. Four of these individuals had been reported previously [10]. Of the eight, seven are Ashkenazi Jewish and one is of Mixed ancestry but had a German grandfather (Table 1). None of these patients had neuropathological confirmation of their diagnosis.

Only one individual was homozygous (A/A) for the mutation (Table 1). All G2019S-carriers who reported a positive family history appear to have autosomal dominant PD (Table 1; Supplementary Figure S2 A, B, E, F and H). The mean age at onset (AAO) for the carriers was 56.6 years (SD 10.9; range 42 to 70 years). These individuals accounted for 1.9%% (3/154) of the early-onset (EOPD) cases and 1% (5/493) of the late-onset (LOPD) cases.

The clinical characteristics of the G2019S-carriers are heterogeneous (Table 2). Impaired olfaction, constipation, orthostatic hypotension, urinary dysfunction and REM sleep behaviour disorder (RBD) were observed. Interestingly, one patient presented with, and continues to have, severe lower limb dystonia and another patient presented with painful lower limb dystonia that was responsive to levodopa (Table 2). The patient with the homozygous mutation (patient ID no. 84.25) did not have a more severe phenotype or a younger AAO in comparison to the other seven patients.

Discussion

In our study, the frequency of G2019S was relatively low (1.2%) among the 647 South African study participants. Therefore, founder effects for G2019S do not appear to be applicable in this group of patients. Seven of our patients are Ashkenazi Jewish, and it should be noted that there was large-scale Jewish immigration (mainly from Lithuania) to South Africa between 1880 and 1914, and again, prior to World War 2, due to South Africa's immigration policies at the time [13, 14].

G2019S was detected in five individuals with autosomal dominant PD, and three individuals who appear to have sporadic PD (Supplementary Figure S2). The higher proportion of carriers observed with a positive family history is similar to previous reports [5, 15].

According to a recent meta-analysis on the clinical features of LRRK-related PD, heterogeneous clinical characteristics are reported to be common in these patients [16]. Although some of the clinical features observed in our patients show some similarity to previous findings, larger studies are required to confirm these observations. Of note, two of our patients presented with lower limb dystonia. G2019S-carriers have been reported to have a higher prevalence of lower limb onset of disease and manifest with the postural instability and gait difficulty PD phenotype [17, 18], however the authors are not aware of this group of patients having a higher rate of lower limb dystonia, and this warrants further investigation.

Table 1 Demographics of South African Parkinson's disease patients harbouring the *LRRK2* G2019S mutation

From: Frequency of the *LRRK2* G2019S mutation in South African patients with Parkinson's disease

Family number	Patient ID number	Zygosity	Sex	Ancestry	AAO (years)	Family history and mode of inheritance		Previously reported
ZA40	60.47	Heterozygous	Female	Mixed ancestry	47	+	AD	Yes; Bardien et al. [10]
ZA85	68.06	Heterozygous	Female	Ashkenazi Jewish	42	+	AD	Yes; Bardien et al. [10]
ZA133	81.64	Heterozygous	Female	Ashkenazi Jewish	70	–	Unknown	Yes; Bardien et al. [10]
ZA154	82.47	Heterozygous	Male	Ashkenazi Jewish	63	–	Unknown	Yes; Bardien et al. [10]
ZA206	84.25	Homozygous	Female	Ashkenazi Jewish	58	+	AD	No
ZA456	11.640	Heterozygous	Female	Ashkenazi Jewish	70	+	AD	No
ZA523	12.482	Heterozygous	Male	Ashkenazi Jewish	48	–	Unknown	No
ZA594	13.002	Heterozygous	Female	Ashkenazi Jewish	55	+	AD	No

Heterozygous, G/A; *Homozygous*, A/A; *AAO*, age at onset of PD; +, positive; −, negative; *AD*, autosomal dominant

Table 2 Clinical features of Parkinson's disease patients harbouring the *LRRK2* G2019S mutation

From: Frequency of the *LRRK2* G2019S mutation in South African patients with Parkinson's disease

Family number	Patient ID number	Motor symptoms						Non-motor symptoms					Surgery
		Tremor	Rigidity	Bradykinesia	Micrographia	Speech changes	Lower limb dystonia	Impaired olfaction	Constipation	Orthostatic hypotension	Urinary dysfunction	RBD	
ZA40	60.47	+	−	−	+	−	−	−	+	+	+	−	
ZA85	68.06	+	+	+	+	−	+	−	+	−	−	−	Pallidotomy
ZA133	81.64	No data available						No data available					
ZA154	82.47	+	−	−	+	+	−	−	−	+	+	+	
ZA206	84.25	+	+	+	+	−	−	−	+	−	−	+	
ZA456	11.640	+	+	+	−	−	−	+	−	−	+	−	
ZA523	12.482	+	+	+	+	+	−	+	−	−	−	−	Deep brain stimulation
ZA594	13.002	−	+	+	+	−	+	−	+	+	−	−	

RBD, REM sleep behaviour disorder; +, yes; −, no

Interestingly, to date G2019S has not been identified in a single individual of African ancestry (Supplementary Table S3). Our study represents the largest collection of SSA patients screened to date for the G2019S mutation. Sample sizes of the other studies with participants of African ancestry are relatively small, ranging from 16 [19] to 126 PD patients [20].

Our study had several limitations which included the fact that we predominantly recruited from the Western Province in South Africa which is known to not reflect the demographics of the rest of the country. For example, in this province, the Black African population comprise 35.7% whereas they are the vast majority (80.7%) for the country as a whole [21, 22]. This ascertainment bias as well as obstacles regarding recruitment of individuals from certain groups resulted in the sample size being small for patients of African (n = 91) and Asian Indian (n = 6) ancestry. The limited numbers of neurologists [9], the perceived low prevalence of PD and the belief that PD symptoms are part of normal ageing [23] possibly all contribute to the under- and mis-diagnosis of patients [9, 23].

In conclusion, our findings, along with those reported in other SSA populations, suggest that the frequency of G2019S appears to be low in SSA but more studies are needed. Further research, involving larger sample sizes, as well as screening of the entire *LRRK2* gene is required, as it is plausible that novel pathogenic mutations may be identified. As new drug targets and therapies for *LRRK2*-positive patients are becoming available [24, 25], it is important to identify a cohort of individuals who would benefit from participating in these clinical trials.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The Health Research Ethics Committee at Stellenbosch University (Protocol 2002/C059) approved this study. All procedures performed on human participants were in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki declaration and its later amendments.

References

1. Dorsey ER, Elbaz A, Nichols E, et al (2018) Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 4422:1–15. https://doi.org/10.1016/S1474-4422(18)30295-3

2. Correia Guedes L, Ferreira JJ, Rosa MM, et al (2010) Worldwide frequency of G2019S LRRK2 mutation in Parkinson's disease: a systematic review. Parkinsonism Relat Disord 16:237–42. https://doi.org/10.1016/j.parkreldis.2009.11.004

3. Lill CM (2016) Genetics of Parkinson's disease. Mol Cell Probes 30:386–396. https://doi.org/10.1016/J.MCP.2016.11.001

4. West AB, Moore DJ, Choi C, et al (2007) Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. Hum Mol Genet 16:223–232. https://doi.org/10.1093/hmg/ddl471

5. Healy DG, Falchi M, O'Sullivan SS, et al (2008) Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol 7:583–590. https://doi.org/10.1016/S1474-4422(08)70117-0

6. Lesage S, Dürr A, Tazir M, et al (2006) LRRK2 G2019S as a Cause of Parkinson's Disease in North African Arabs. N Engl J Med 354:422–423. https://doi.org/10.1056/NEJMc055540

7. Thaler A, Ash E, Gan-Or Z, et al (2009) The LRRK2 G2019S mutation as the cause of Parkinson's disease in Ashkenazi Jews. J Neural Transm 116:1473–1482. https://doi.org/10.1007/s00702-009-0303-0

8. Lesage S, Patin E, Condroyer C, et al (2010) Parkinson's disease-related LRRK2 G2019S mutation results from independent mutational events in humans. Hum Mol Genet 19:1998–2004. https://doi.org/10.1093/hmg/ddq081

9. Williams U, Bandmann O, Walker R (2018) Parkinson's Disease in Sub-Saharan Africa: A Review of Epidemiology, Genetics and Access to Care. J Mov Disord 11:53–64. https://doi.org/10.14802/jmd.17028

10. Bardien S, Marsberg A, Keyser R, et al (2010) LRRK2 G2019S mutation: Frequency and haplotype data in South African Parkinson's disease patients. J Neural Transm 117:847–853. https://doi.org/10.1007/s00702-010-0423-6

11. Patterson N, Petersen DC, van der Ross RE, et al (2010) Genetic structure of a unique admixed population: implications for medical research. Hum Mol Genet 19:411–419. https://doi.org/10.1093/hmg/ddp505

12. Hall T (1999) BioEdit : A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95-98. http://brownlab.mbio.ncsu.edu/JWB/papers/1999Hall1.pdf. Accessed 19 August 2019

13. Newman A, Evans NJ, Smith JG IS (2006) Jewish migration to South Africa : The records of the Poor Jews' Temporary Shelter. Isaac and Jessie Kaplan Centre for Jewish Studies and Research, University of Cape Town, Cape Town

14. Evans RJ (2006) The Third Reich in power, 1933-1939. Penguin Press, New York

15. Billingsley KJ, Bandres-Ciga S, Saez-Atienzar S, Singleton AB (2018) Genetic risk factors in Parkinson's disease. Cell Tissue Res 373:9–20. https://doi.org/10.1007/s00441-018-2817-y

16. Shu L, Zhang Y, Pan H, et al (2018) Clinical heterogeneity among lrrk2variants in Parkinson's disease: A Meta-analysis. Front. Aging Neurosci. 10:283

17. Alcalay RN, Mirelman A, Saunders-Pullman R, et al (2013) Parkinson disease phenotype in Ashkenazi jews with and without *LRRK2* G2019S mutations. Mov Disord 28:1966–1971. https://doi.org/10.1002/mds.25647

18. Mirelman A, Heman T, Yasinovsky K, et al (2013) Fall risk and gait in Parkinson's disease: The role of the LRRK2 G2019S mutation. Mov Disord 28:1683–1690. https://doi.org/10.1002/mds.25587

19. Mahne AC, Carr JA, Bardien S, Schutte CM (2016) Clinical findings and genetic screening for copy number variation mutations in a cohort of South African patients with Parkinson's disease. S Afr Med J 106:623. https://doi.org/10.7196/SAMJ.2016.v106i6.10340

20. Okubadejo NU, Rizig M, Ojo OO, et al (2018) Leucine Rich Repeat Kinase 2 (LRRK2) Gly2019Ser Mutation Is Absent In A Second Cohort Of Nigerian Africans With Parkinson Disease. PLoS One 363945. https://doi.org/10.1101/363945

21. Statistics South Africa (2011) Statistical release (Revised) - Census (2011). 78. https://doi.org/P0301.4. Accessed 19 August 2019

22. Statistics South Africa (2016) Community Survey 2016 Statistical Release. Stats SA Stat South Africa Community Survey 2016 Results. https://doi.org/10.1017/CBO9781107415324.004. Accessed 19 August 2019

23. Sauerbier A, Aris A, Lim EW, et al (2018) Impact of ethnicity on the natural history of Parkinson disease. Med J Aust 208:410–414. https://doi.org/10.5694/mja17.01074

24. Atashrazm F, Dzamko N (2016) LRRK2 inhibitors and their potential in the treatment of Parkinson's disease: current perspectives. Clin Pharmacol Adv Appl Volume 8:177–189. https://doi.org/10.2147/CPAA.S102191

25. Christensen KV, Smith GP, Williamson DS (2017) Development of LRRK2 Inhibitors for the Treatment of Parkinson's Disease. Prog Med Chem 56:37–80. https://doi.org/10.1016/BS.PMCH.2016.11.002