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Role of *LRRK2* variant p.Gly2019Ser in patients with Parkinsonism

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***Background & objectives*: Parkinsonian disorder, including Parkinson's disease (PD), is an aetiologically complex neurodegenerative disorder. Mutations in leucine-rich repeat kinase 2 (*LRRK2*) gene have been implicated in an autosomal dominant form of PD with variable penetrance. The identification of a common *LRRK2* variant (p.Gly2019Ser) in dementia with Lewy bodies indicated its potential role in Parkinsonian disorder. The current study was aimed to identify the p.Gly2019Ser variant in Indian patients with Parkinsonian disorder.**

***Methods*: The patient group consisting of 412 classical PD patients, 107 PD patients with cognitive impairment, 107 patients with Parkinson plus syndrome and 200 unrelated controls were recruited from eastern part of India. The allele representing p.Gly2019Ser variant was screened by polymerase chain reaction followed by restriction fragment length polymorphism analysis.**

***Results*: The p.Gly2019Ser variant was identified in an East Indian young-onset female PD patient in a heterozygous state having several motor and autonomic problems without disturbed cognition. Her younger brother, sister and elder son harbouring the same mutation were asymptomatic carriers for the variant. However, the influence of *DNM3* on decreased disease onset in this family was not clear.**

***Interpretation & conclusions*: Identification of the p.Gly2019Ser variant in only one patient among a large number of Indian patients (n=626) with Parkinsonian disorder in our study suggests a limited role of the *LRRK2* variant towards disease pathogenesis.**

Key words Gly2019Ser mutation - *LRRK2* - Parkinson plus - Parkinson's disease - parkinsonism

Parkinsonism is an umbrella term that includes a large number of neurological disorders, of which Parkinson's disease (PD) is the most common. Other Parkinsonian disorders show some clinical features overlapping with PD and others, including bilateral and symmetric onset, lack of long-term benefit of levodopa, absence of rest tremor and early dementia. PD is the second most common aetiologically complex neurodegenerative disorder and is characterized by tremor, rigidity, bradykinesia and postural instability[1]. About 10 per cent of patients with PD are familial and result from mutations in causal genes[2]. The *LRRK2*

(leucine-rich repeat kinase 2) gene has been implicated in an autosomal dominant form and often sporadic PD. *LRRK2,* containing 51 exons, encodes a 280 kDa multifunctional protein with multiple domains, which can phosphorylate itself as well as other PD-associated proteins such as parkin and alpha-synuclein (SNCA)[1]. More than 100 nucleotide variants have been reported in the *LRRK2* gene in the Parkinson Disease Mutation Database (*http://www.molgen.vib-ua.be/PDMutDB*), among which p.Gly2019Ser (c.6055G>A) is the most common and well-characterized mutation[1]. The age of onset differs greatly between p.Gly2019Ser carriers across the world[1]. A single nucleotide polymorphism (SNP) in dynamin 3 (*DNM3)* (*i.e.,* rs2421947) has been reported as a modifier of age of onset in LRRK2 Gly2019Ser Parkinsonism[3]. LRRK2 interacts with dynamin superfamily GTPases (Dnm1, Dnm2 and Dnm3) to regulate membrane dynamics for endocytosis and mitochondrial morphology[4]. However, no significant alteration in interaction between p.Gly2019Ser and Dnm1 compared to wild-type LRRK2 has been reported[4].

The p.Gly2019Ser variant has also been identified in dementia patients with Lewy bodies (DLB) in Caucasians[5]. Experimental evidence suggests that abnormal substrate phosphorylation and cytotoxicity by p.Gly2019Ser are responsible for irreversible neuronal loss - a common pathology for Parkinsonism[6].

Genetic screening of *LRRK2* was independently performed from three geographical regions of India (north, south and east)[7-9]. The screening of common pathogenic mutations (*viz*., p.Arg1441Gly, p.Arg1441Cys, p.Arg1441His, p.Tyr1699Cys and p.Gly2019Ser) and a risk variant common among Asians (p.Gly2019Ser) was not observed in the eastern Indian cohort[7]. However, the presence of the Gly2019Ser variant in *LRRK2* has been reported in a single PD patient in a heterozygous state in only one study[9]. The present study was conducted to specifically look for this mutation which is common in other world populations, in eastern Indian patients with PD and Parkinson plus, to assess the role of *LRRK2* in patients with a wide spectrum of Parkinsonian phenotypes.

Material & Methods

A total of 626 Indian patients were recruited for this study. The patients were examined at Bangur Institute of Neurosciences (BIN), Kolkata (36 Parkinson plus patients; 412 classical PD patients; 107 PD patients with cognitive impairment); Burdwan Medical College and Hospital, Burdwan (15 Parkinson plus patients); National Neurosciences Centre Calcutta, Kolkata (38 Parkinson plus patients); Alzheimer's Related Disorder Society of India, Kolkata Chapter, Kolkata (18 Parkinson plus patients). In addition, 200 unrelated controls (mean age, 48.3±8.2 yr) with no personal or family history of Parkinsonism or any other neurological symptoms were selected from the eastern part of India for the present study. These healthy controls were unrelated family members and spouses of the patients. Their enrollment was done on the basis of disclosure about negative personal/familial history of PD along with clinical examination by a neurologist.

The study patients comprised 412 classical PD patients, 107 PD patients with cognitive impairment, 107 Parkinson plus patients from eastern India. Patients with PD having at least two of the cardinal features (tremor, rigidity, bradykinesia and postural instability) were selected. The Parkinson plus patients included DLB, progressive supranuclear palsy and multiple system atrophy. The demographic details of the patients are described in Table I.

Table I. Demographic details of the study participants (n=626) from eastern India

Category (patient studied)	Male:female	Familial:sporadic	Early onset:late onset	Age on onset (AO) (yr) mean±SD
Classical PD (412)	282:130	127:285	151:261	44.33±13.62
PD with CI (107)	75:32	22:85	20:87	54.43±14.84
Parkinson plus				
DLB (40)	34:6	7:33	3:37	60.23±10.3
PSP (55)	36:19	2:53	0:55	60.17±6.62
MSA (12)	6:6	0:12	5:7	49.08±9.41

PD with CI, Parkinson's disease with cognitive impairment and dementia; DLB, dementia with Lewy body; PSP, progressive supranuclear palsy; MSA, multiple system atrophy; early onset, AO ≤45 yr; late onset, AO >45 yr

The study protocol was approved by the Institutional Ethics Committees of the respective institutes and written informed consent was obtained from all participants.

Collection of blood samples and genomic DNA preparation: Peripheral blood samples (10 ml) were collected in ethylenediaminetetraacetic acid (EDTA) from patients and controls. Genomic DNA was prepared from fresh whole blood by a conventional salting-out method using sodium perchlorate followed by isopropanol precipitation[10]. Genomic DNA was dissolved in Tris-EDTA (TE) buffer (10 mM Tris-HCl, *p*H 8.0 and 0.1 mM EDTA).

Multiplex ligation-dependent probe amplification (MLPA) analysis: MLPA was done on 250 patients with PD with early age of onset and/or a positive family history using the *SALSA MLPA* P051 Parkinson mix 1 probemix (MRC-Holland, the Netherlands), which assays for *LRRK2* variant c.6055 G>A (p.Gly2019Ser) along with gene dosage mutations in *PRKN*, *PINK1, DJ-1* and *SNCA*.

PCR and restriction fragment length polymorphism (RFLP) analysis: The targeted region harbouring the nucleotide change c.6055 G>A (p.Gly2019Ser) in exon 41 of the *LRRK2* was amplified using a mismatched forward primer to create a *Pst*I restriction enzyme site: 5′-CATTGCAAAGATTGCTGACTGC-3′, the reverse primer: 5′-GAGGTCAGTGGTTATCCATCCT-3′[7]. The amplicon (134 bp) was digested with *Pst*I (New England Biolabs, USA) using the conditions specified by the manufacturer and electrophoresed on a seven per cent polyacrylamide gel. The wild-type amplicon (134 bp) remained undigested, whereas the mutant allele containing a *Pst*I site generated two DNA fragments (111 and 23 bp).

The SNP rs2421947 of *DNM3* was genotyped exclusively for a patient harbouring p.G2019S and for her family members by the PCR-RFLP method described above[7]. The primer set 5′-CATGTTCCCCTCTACCTGGA-3′ (forward) and 5′-TAAAGTCCTTGGCGTTTTGC-3′ (reverse) (Sigma-Aldrich, USA) was used for PCR, and the *Alw*NI (New England Biolabs, USA) restriction enzyme was used for RFLP. After digestion, the C allele produced two fragments (209 and 33 bp), whereas the G allele remained undigested (242 bp). RFLP products were separated by electrophoresis on polyacrylamide gel (7%).

DNA sequencing: Because mutation of the *PRKN* gene is the most common cause of PD, all exons of the *PRKN* gene were analyzed for the individual harbouring an *LRRK2* mutation by Sanger sequencing[11].

Results & Discussion

The p.Gly2019Ser variant of LRRK2 was identified in only one young-onset female PD patient in a heterozygous state by MLPA analysis from eastern part of India. No additional variants were observed in other causal genes analyzed in this patient. Screening of other patients and 200 controls for the Gly2019Ser variant by RFLP did not reveal any additional mutant alleles. As shown in Figure A and B, genetic analysis by RFLP and Sanger sequencing of family members of the proband revealed the presence of the same variant in her asymptomatic brother (29 yr), younger sister (25 yr) and elder son (18 yr) but not in her mother (60 yr), elder sister (41 yr) and two paternal aunts (60 and 55 yr). This suggested that the variant allele might have been inherited from her paternal side. However, DNA from her asymptomatic father (I-1), who died at the age of 45 yr, was not available. Because there was no report on the clinical examination of the proband's father available in his lifetime, he might have had subclinical phenotypes not noticeable to a naïve person. Genotyping for the variant was not done in her two younger sons (14 and 7 yr), Figure D.

It has been reported that disease onset is accelerated by about 12.5 yr for p.Gly2019Ser carriers harbouring the GG genotype at rs2421947 of *DNM3*[3]. However, genotyping of adult family members including non-carriers, mutant individuals in the pedigree (except the brother of the patient) was found to be heterozygous for rs2421947 of *DNM3* (Figure C). Therefore, using data from a single family, it is difficult to comment on the role of *DNM3* as a genetic modifier of p.Gly2019Ser-mediated PD pathogenesis in Indians. However, our study was consistent with a study on a large number of PD patients demonstrating that disease onset was about five years earlier in women with *LRRK2* mutations[12]. Therefore, it is possible that the brother of the index case in our study may develop the disease in the future.

Our previous study on 308 PD patients and another study on 150 PD patients from eastern part of India did not identify the Gly2019Ser variant[7,13]. However, this mutation was reported previously in another female patient from north India[9]. Therefore, taking into consideration various Indian studies,

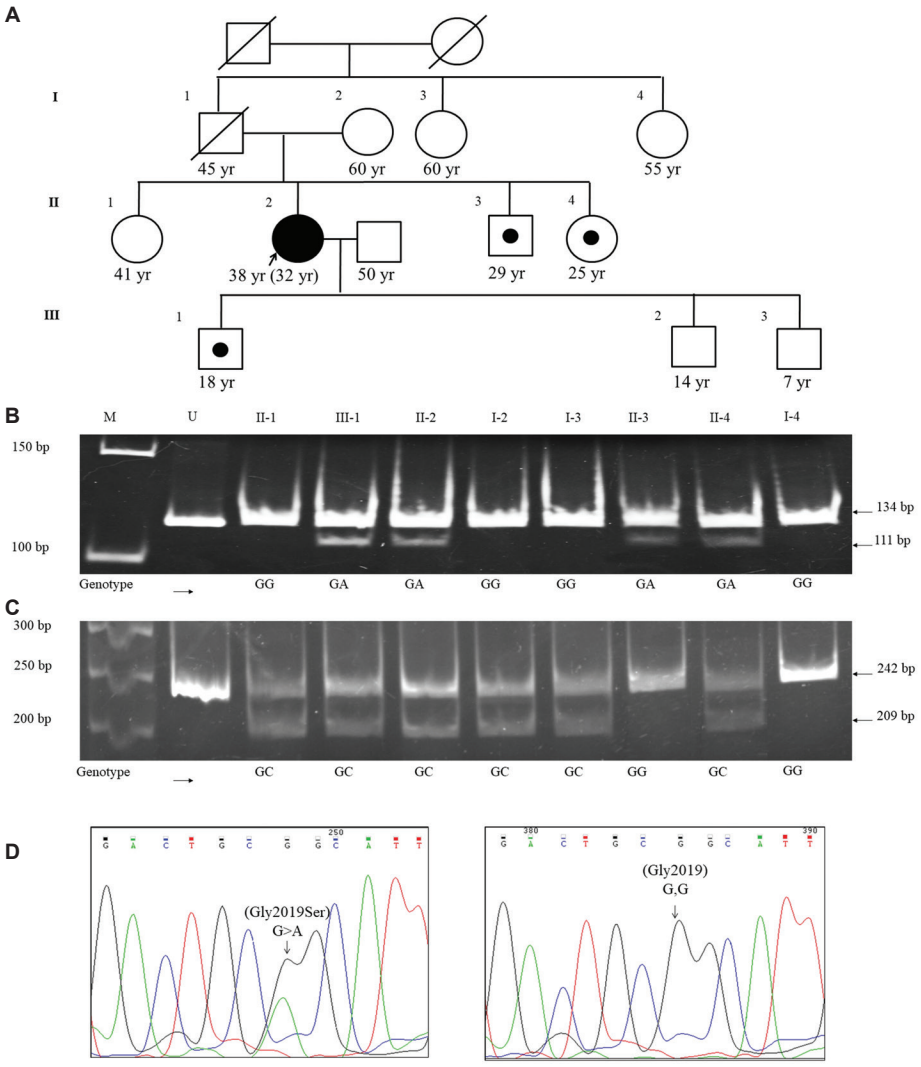

Figure. Screening the p.Gly2019Ser variant (c.6055G>A) in the leucine-rich repeat kinase 2 (*LRRK2*) gene and rs2421947 (G>C) of *DNM3* in a family affected with Parkinson's disease. (**A**) The upper panel represents a three-generation pedigree showing age and sex of each individual. The filled symbols indicate symptomatic *LRRK2* mutants; the arrow indicates the proband and a black circle within a square indicates an asymptomatic male harbouring the mutant allele. Both the current age and age at onset (in parenthesis) are given for the proband. (**B**) Segregation pattern of the *LRRK2* variant allele in family members, as represented by *Pst*I digested polymerase chain reaction products, separated by polyacrylamide gel electrophoresis. Genotypes of the index case and family members are shown. (**C**) Genotyping details of rs2421947 of *DNM3* in family members as represented by *Alw*NI digested polymerase chain reaction (PCR) products, separated as above. Lane U, undigested PCR product; lane M, 50 bp DNA ladder molecular weight marker. The sizes of the digested DNA fragments and their molecular weights are shown on the right and left side of the gels, respectively. (**D**) Chromatograms of the DNA sequence from the patient (II-2) showing the heterozygous condition of c.6055G>A of *LRRK2* and the homozygous 'wild' genotype of the mother (I-2).

p.Gly2019Ser-mediated pathogenesis may be considered a rare event (2/1996) in Indian patients with PD (Table II). The reported frequency of alleles for p.Gly2019Ser varies widely across the world[15]. Our genetic data corroborated with other Asian studies (<1%)[16].

The only female patient, in the present study harbouring the p.Gly2019Ser mutation in a heterozygous state, developed tremulousness in her left limbs (both upper and lower), slowness of activity and dragging of her left lower limb while walking at the age of 32 years. Within the next year, she also developed tremor in her right limbs, both during rest and in action. Three years later, when she visited BIN, Kolkata, for the follow up, she had whole-body rigidity, postural instability, gait disturbances and complete dependence on family members. On examination, she was found to have other clinical features including stooped

Table II. Parkinson's disease-associated pathogenic variants in *LRRK2* among Indians

Mutations screened	Demographic distribution	Patient studied	Gly2019Ser mutation/ frequency of mutation (%)	Reference
Gly2019Ser, Arg1441Cys, Arg1441Gly, Arg1441His, Ile2012Thr, Ile2020Thr	North and South	800	1 (0.125)	9
Gly2019Ser	South	140	0	8
Gly2019Ser	South	186	0	14
Arg1441Gly, Arg1441Cys, Arg1441His, Gly2019Ser, Tyr1699Cys, Ile2020Thr and Ile2012Thr	East	150	0	13
Arg1441Cys, Arg1441Gly, Arg1441His, Tyr1699Cys, Gly2019Ser and Gly2385Arg	East	308	0	7
Gly2019Ser	East	412	1 (0.243)	Present study
Total		1996	2 (0.1002)	

posture, abnormal positioning of neck (laterocolis and retrocolis), loss of facial expression, hypophonia with slurring of speech, gait disturbances, early-morning dystonia of her left foot, difficulty in turning in bed, as well as diurnal variation, insomnia and knee pain. However, she did not experience any subjective or objective memory impairment. Taking together all the clinical features, her total Unified Parkinson's Disease Rating Scale (UPDRS)[17] score was 108 in off phase [Mentation, Behaviour and Mood subscore: 2; Motor subscore: 72 and Activities of Daily Living (ADL) subscore: 34].

The patient did not take any medications since disease onset due to economic constraints. For only the last six months, she has started levodopa-carbidopa therapy with good response, as revealed by her improved UPDRS score (57 in on phase) (Mentation, Behaviour and Mood subscore: 2; Motor subscore: 34 and ADL subscore: 21).

Both features such as tremor and postural instability have been reported to be quite common disease phenotypes in carriers of the Gly2019Ser variant[1,18]. However, lower tremor scores have been reported using the UPDRS in p.Gly2019Ser carriers compared to PD patient non-carriers of this variant[18]. Although no signs of cognitive decline were observed, the onset of PD in her lower extremities and the better quality of life on levodopa treatment were more consistent with *LRRK2* mutation-related Parkinsonism than idiopathic PD[1]. The other patient reported from India[9] harbouring the same pathogenic variant presented with similar clinical features including age of onset (35 vs. 32 yr) except for tremor and sleep disorder.

In conclusion, p.Gly2019Ser mutation in a patient with classical PD was observed from eastern parts of India. However, this variant was not detected in other forms of Parkinsonian patients. The female patient with this mutation exhibited both tremor and postural instability phenotypes with no disturbances in cognition. The overall low frequency of the p.Gly2019Ser variant restricts the identification of population-specific genetic modifiers in Indian patients with PD and suggests its limited role in PD manifestation in Indians.

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***Conflicts of Interest*:** None.

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