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Parkinson's disease with homozygous *PINK1* p.Leu489Pro mutations in two Indian sisters

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Abstract

We describe the clinical features of two sisters with Parkinson's disease (PD) of Indian descent living in Malaysia. Both were homozygous for the known *PINK1* mutation p.Leu489Pro (c.1466T>C). The proband, who has been followed up by us over a span of 35 years, had a fairly "classic" clinical presentation for PARK-*PINK1*, including young onset, a clear response to dopamine replacement therapy, and development of troublesome motor fluctuations and dyskinesias. Her dyskinesias improved substantially with clozapine treatment, which to our knowledge has not specifically been reported for PARK-*PINK1*; this treatment has been sustained for nearly a decade. The clinical phenotype of the older sister was more akin to later-onset "idiopathic" PD; however, her brain MRI showed abnormal signal in the posterior limb of the internal capsules and the hypothalamus. Our report contributes to the scarce literature on monogenic PD in the Malaysian / Indian population, and further supports the pathogenicity of the *PINK1* p.Leu489Pro variant.

Keywords: Parkinson's disease, *PINK1,* genetics, Asian, Indian, clozapine, MRI, corticospinal tract

INTRODUCTION

From an ethno-cultural perspective, China and India, the two most populous nations on earth (with populations of approximately 1.4 billion each) are strongly represented in the Malaysian population. In Malaysia, people of Chinese and Indian descent, ≈6.7 million and ≈2.0 million in number, make up the second and third largest ethnic groups corresponding, respectively, to ≈21% and 6% of a total population of 33 million.[1] Findings from research conducted in diasporic groups could have significant relevance at a global level. Moreover, in the coming decades, the prevalence of PD is projected to continue to grow substantially[2] particularly in developing countries, where there is accelerating growth of the ageing segment of the population; this includes China, India and many other Asia-Pacific nations.[3]

In the vast majority of cases, the aetiology of PD is complex and involves polygenic and environmental factors acting in concert with age-related processes.[4] However, a small but significant proportion of cases can be attributed to a single Mendelian genetic cause - so-called monogenic PD.[5,6] Numerically speaking, monogenic PD cases are relatively fewer, but the biological insights yielded from the study of these patients and their families have transformed the scientific understanding of PD development and progression.[4-8] It is also envisaged that in the not-too-distant future, therapies may become available that are targeted according to the patient's underlying genetic and molecular pathophysiology.[9,10]

One monogenic form of PD is PARK-*PINK1*, first described in consanguineous Italian and Spanish families in the early 2000s.[11,12] *PINK1*

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gene mutations are the second most frequent cause of autosomal recessive early-onset PD (AR-EOPD), after *PRKN* gene mutations.[6] To date, 151 cases of PARK-*PINK1* have been recorded in the MDSGene database,[13,14] which collates all English language reports of monogenic PD with individual-level data. However, among patients of Indian ancestry, we were able to find only one case of PARK-*PINK1* in the literature.[13,15] This patient from northern India with young-onset PD harboured a homozygous p.Gln267* (c.799C>T) mutation[15] that was classified as "probably pathogenic".[13] Additionally, based on personal communication (Prof. Anthony Lang), a patient previously reported from Canada[16,17] was also likely to be of Indian or Sri Lankan Tamil ancestry. A systematic study screening 250 PD patients in India found no case of homozygous or compound heterozygous *PINK1* mutations.[18]

Herein, we describe the phenotypic details of two sisters of Indian Tamil descent harbouring the *PINK1* NM 032409.3:c.1466T>C (p.Leu489Pro), rs1553146903 mutation in homozygous state. The proband has been seen by us over a span of 35 years, since her initial diagnosis with young-onset PD; she had severe dyskinesias that responded to low-dose clozapine treatment. The clinical presentation of the older sister was that of later-onset "idiopathic" PD; however, her brain MRI showed signal abnormalities that were reported only very recently, in a cohort of Malay patients harbouring a different *PINK1* (p.Leu347Pro) mutation.[19]

CASE REPORT

Patient 1

This 75-year-old retired teacher was diagnosed with PD at age 40 years, in 1985. The initial symptoms were tremors of the hands and feet at age 35 years. Over the next few years, she experienced weakness of the right leg with a tendency to fall, and increased perspiration. Propranolol was given with no benefit. When first seen by us in 1985 (CT Tan), she had resting more than postural tremors of the fingers and toes, with cogwheel rigidity at the wrists and mild slowing of repetitive left arm movements. She walked dragging her legs. The family history at this time was unremarkable (her older sister, Patient 2, described below, developed PD later, as did two paternal cousins, although further details about this were unavailable). Her functional status was good, and treatment was deferred. When she returned 1.5 years later, she was noted to be very slow generally and gave a history of backward falls. Levodopa-benserazide 200/50 mg half tablet bid was commenced. She improved very significantly with PD medication treatment and in 1993 was coping well (on levodopa-benserazide 200/50 mg half tablet tid, selegiline bid and benzhexol PRN), except for complaints of ongoing leg weakness and swelling of the knees which were thought to be rheumatological rather than neurological in origin (subsequently confirmed to be osteoarthritis). Her condition maintained well over the next few years and follow-up at our Centre was irregular as she lived interstate. In 2003, a brain MRI was reported to show mild cerebral and cerebellar atrophy (the images were subsequently lost and unavailable for review).

In 2011, the patient was referred back to our centre for management of very troublesome and unpredictable OFFs and severe dyskinesias. The patient had become largely wheelchair-confined (probably due in large part to knee osteoarthritis, which she declined surgery for), and she had developed severe hand contractures (attributed to a period of stopping her Madopar). She had been taking levodopa-benserazide 200/50 mg up to eight times daily with pramipexole 1.5 mg tid, amantadine 100 mg tid and clonazepam 0.5 mg at night. Over the preceding year, she had also been experiencing mild hallucinations, auditory more than visual.

She was considered for apomorphine infusion therapy, but she and her family opted instead for clozapine treatment for the dyskinesias, gradually uptitrated from 6.25 mg at night. The dyskinesias reduced significantly (by ≈30%) at a dosage of 50 mg daily; increasing the dosage to 75 or 100 mg daily resulted in further improvement (≈50% reduction) but caused troublesome drooling; on the other hand, dyskinesias were not controlled when attempts were made to reduce the dosage to 37.5 mg daily. The improvement in dyskinesias was confirmed by direct observation during regular clinic follow-ups, every few months. She has continued on clozapine for almost nine years, with no adverse effects. At times, her dyskinesias were still observed to be moderate in severity, e.g, such that they would make her slide down her chair.

She was last reviewed in clinic in January 2020. She did not complain of OFF periods. Physically, she was significantly limited by the osteoarticular problems affecting her knees and hand contractures, and required assistance with all personal activities of daily living (eating,

walking, toileting, showering and dressing). Her medications consisted of levodopa-benserazide (200/50 mg), ¼ tab qid, pramipexole extended-release 1.5 mg bid, amantadine 100 mg tid and clozapine 50 mg at night. She also took domperidone 1 mg tid, dexlansoprazole, lactulose and bisacodyl. There were no cognitive complaints. Examination (1 hour 15 minutes post-levodopa intake) revealed a bright woman, conversing appropriately. She was orientated to place and time and scored 3/3 for short-term recall. There was no obvious speech impairment. She had generalized dyskinesias, without facial involvement, that were mild at rest but became more pronounced (moderate in amplitude) when performing physical and especially mental tasks. Her fists were clenched. She could only stand and walk, with hips and knees flexed, with moderate one-person assistance. Her Clinical Impression of Severity Index (CISI-PD) score,[20-22] done blinded to genetic status, was 14 (Motor signs 5, Disability 5, Motor complications 3, Cognitive status 1).[23,24] A checklist of her PD features is given in Table 1.

Patient 2

Fewer details were available for this patient who was primarily followed up in a peripheral hospital. PD was diagnosed at age 60 years in 1997 when she presented with non-specific upper and lower limb pain, difficulty turning, facial hypomimia and stooped posture. A Mini Mental State Examination (MMSE) done in a geriatrics clinic in 2007 was scored 29/30. She was seen at our Centre in 2011 aged 74 years. She remained active and was caring for her physically disabled husband, but was experiencing episodes of festination resulting in near-falls forwards. The patient was satisfied with her treatment consisting of levodopa-benserazide 200/50 mg ½ tablet taken together with piribedil 50 mg, both tid; and sustained-release levodopa-benserazide (100/25 mg) 1 capsule at night. Clonazepam 1 mg was taken at night and helped significantly to improve sleep. Onset of mediation effect was 20 minutes after medication intake. She would sometimes experience morning foot dystonia and was advised that the sustained-release levodopa-benserazide could be increased to two capsules at night. She was in the ON-medication state when examined 2.5 hours post-dose. There were generalized dyskinesias, moderate-to-severe in amplitude (which was apparently unusual for her and worse than her typical day-to-day condition). Gait was mildly slowed and a bit unsteady spontaneously; pull test was positive with retropulsion, but she was able to correct herself and avoided falling. Her CISI-PD,[20-22] done retrospectively and unblinded to genetic status, was scored 9 (Motor signs 3, Disability 3, Motor complications 3, Cognitive status 0). Brain MRI scan was done in 2012, shown in Figure 1. In August 2019, she was taking levodopa-benserazide 200/50 mg ¼ tablet 6x daily. She complained of painful off-period dystonia and entacapone 200 mg 1 tablet qid was added.

Genetic testing

In both patients, genomic DNA was extracted from peripheral blood samples using standard techniques, and tested using a next generation-sequencing (NGS)-based PD gene panel (Parkinson panel at Centogene AG, Rostock, Germany). This included all exons of *PRKN*, *PINK1*, *DJ-1*, *SNCA*, *LRRK2*, *GBA*, *VPS35*, *PLA2G6*, *RAB39B*, and exons 11, 37, 43, 61 and 69 of *VPS13C*.[6,19,23] The local ethics committee approved the protocol, and written informed consent was obtained. A known pathogenic mutation NM 032409.3:c.1466T>C (p.Leu489Pro), rs155314690[13,14,16,17,25] was detected in exon 7 of the *PINK1* gene in homozygous state. They did not carry any other known pathogenic protein-changing variant in the genes targeted by the gene panel. In addition, multiplex ligation-dependent probe amplification (MLPA) analysis was performed to look for gene dosage alterations; no exon multiplications or deletions were found.

DISCUSSION

To our knowledge, PARK-*PINK1* due to a homozygous p.Leu489Pro mutation has not been described previously, although there is one case in the literature[13,16,17] due to *compound heterozygous* p.Leu489Pro/Glu240Lys mutations. This patient from Canada had PD onset at age 30 years and long disease duration (living at least until age 74 years).[16,17] Therefore, this current report which specifically links PD in two patients with the p.Leu489Pro mutation *per se* provides further clinical evidence for the pathogenicity of the p.Leu489Pro variant. Previous functional work also provided support for pathogenicity of this variant as analysis in fibroblasts derived from a patient with the compound heterozygous p.Leu489Pro/p.Glu240Lys mutations showed that the PINK1 protein was expressed but the cells showed a higher basal apoptotic rate.[17] Importantly, as this is relevant to our patients with the p.Leu489Pro mutation, the authors showed

Table 1: Demographic and clinical features of our 2 sisters with homozygous *PINK1* p.Leu489Pro mutations

	Patient 1	Patient 2
Gender	F	F
Age at PD onset / Disease duration (yrs)	35 (motor symptom onset) / 40 (still alive)	60 (diagnosis) / 23 (still alive)
Ancestry / Geographic location	Tamil Indian / Malaysia	Tamil Indian / Malaysia
Family history	✓	✓
Met QSBB clinical diagnostic criteria for PD	✓	✓
Bradykinesia	✓	✓
Rigidity	✓	✓
Tremor	✓	×
Gait difficulty	✓	✓
Gait freezing	×	✓
Postural instability / Falls	✓	✓
Dystonia	✓ (hands)	✓ (foot dystonia)
Obvious asymmetry	×	×
Diurnal variation	×	
Sleep benefit	×	
UMN signs	×	
Clear favourable response to dopaminergic medication	✓	✓
Motor fluctuations	✓ (moderate-to-severe in mid-disease stage, but became relatively mild / disappeared over time with advancing disease)	✓ (moderate severity)
LD-induced dyskinesias	✓ (severe at its worst, but improved moderately with clozapine)	✓ (moderate-to-severe)
RBD	×	
Insomnia	✓	✓
Excessive daytime sleepiness	×	✓
Depression	×	×
Anxiety	×	×
Mild cognitive impairment	×	
Dementia	×	×
Visual hallucinations	✓ (mild only)	×
Impulsive-compulsive behaviours	×	×
Constipation	✓	✓
Urinary dysfunction	×	×
Orthostatic giddiness or hypotension	×	✓
Pain	×	✓
Hyposmia	✓	
Underweight	×	✓
Atypical features more commonly seen in Parkinson-plus syndromes	×	×

This standardized reporting checklist is based in part on MDSGene recommendations (https://www.mdsgene.org). ✓=Present; X=Absent; Blank tabs indicate that data were unavailable. LD=Levodopa; QSBB=Queen Square Brain Bank; RBD=Rapid eye movement (REM) sleep behaviour disorder; UMN signs=Upper motor neuron signs.

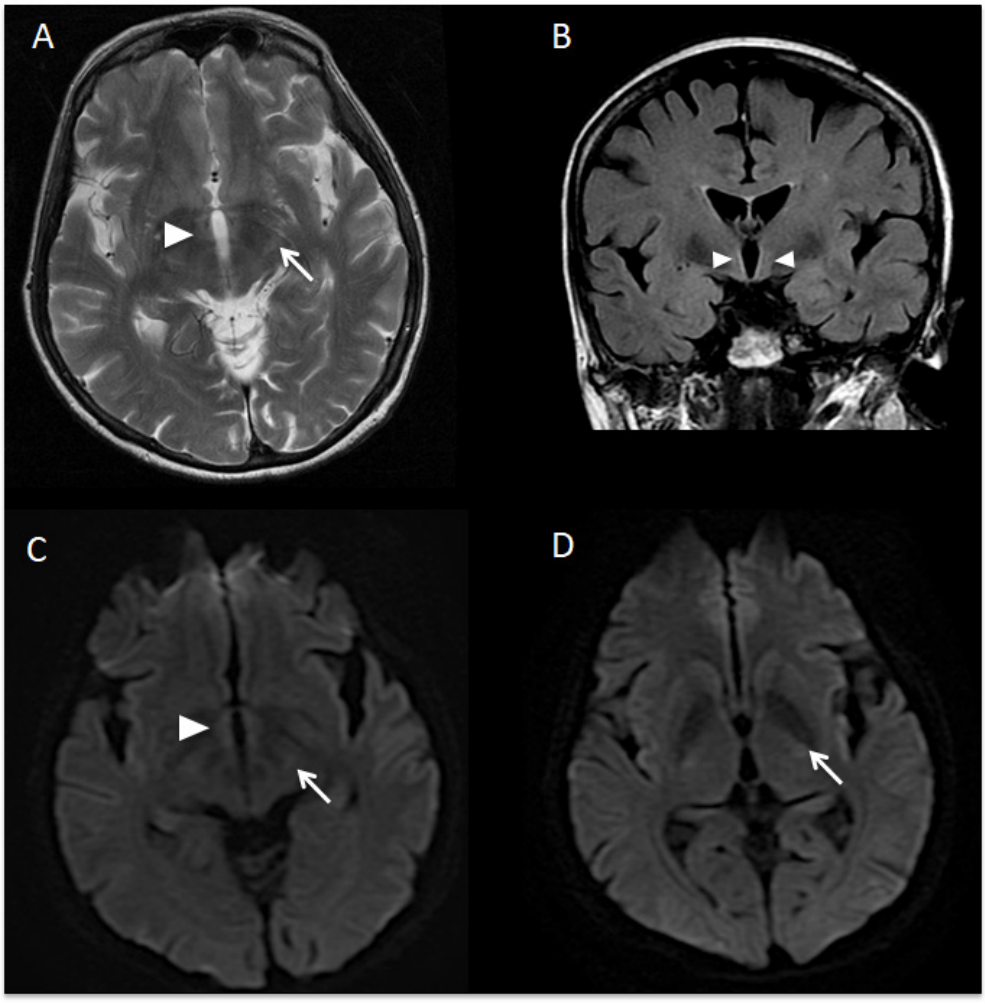

Figure 1. Brain MRI of Patient 2. There are bilateral symmetrical T2-weighted hyperintensities affecting the corticospinal tracts in the posterior limb of the internal capsules (PLIC) (arrow) and the hypothalamus (arrowhead) (A and B); these regions are isointense on T1 (not shown), bright on DWI (C, D) and isointense on apparent diffusion coefficient (ADC) map (not shown), indicating mild restricted diffusion. There are no signal abnormalities affecting the corticospinal tracts in the posterior centrum semiovale (not shown).

that either mutation could cause the loss of a protective effect of the PINK1 protein against neuronal apoptosis induced by staurosporine in transfected SHSY-5Y cells.[17] The p.Leu489Pro mutation within the catalytic kinase domain of PINK1 is predicted to affect PINK1 function through loss of the kinase activity and/or instability of the mutant protein.[17] PINK1 plays a critical role in mitochondrial homeostasis, and mitochondrial dysfunction is linked to oxidative stress, accumulation of misfolded α-synuclein, and inflammation, all key events in the pathophysiology of PD.[6,26,27]

In general, PARK-*PINK1* and PARK-*Parkin* cases are characterized by slow motor progression, good dopa-responsiveness but with frequent development of motor complications, and infrequency of dementia.[6,28,29] A possible greater burden of neuropsychiatric features has been suggested in PARK-*PINK1* compared to PARK-*Parkin*,[6] although the literature supporting this contention appears to be quite limited.[29] Members of the original Italian *PINK1* families showed evidence of frontal lobe (executive) dysfunction, and impulsive-compulsive behaviours (ICBs) were commonly observed.[30] It is difficult to know the frequencies of these problems due to large gaps in the available phenotypic data. For example, in the MDSGene database, whether cognitive decline was present was "unknown" in 69/151

(46%) of the PARK-*PINK1* patients collated.[13,14] Dementia appears to be uncommon, for example being absent in several series of PARK-*PINK1* patients.[19,28,31] Although limited conclusions can be made based on only two patients, the findings of our study were consistent with the existing literature - the patients did not display a heavy burden of non-motor symptomatology (Table 1), including neuropsychiatric complications, despite their advanced age and long duration of PD.

An additional point of clinical interest was the response of Patient 1's severe dyskinesias to clozapine. Although clozapine treatment has been used for neuropsychiatric indications including psychosis in PARK-*PINK1* cases,[28] to our knowledge there have been no reports of treatment success for dyskinesias. Clozapine has level I evidence for treating levodopa-induced dyskinesias in PD,[32,33] but its routine use has been hampered by the need for regular peripheral white blood cell count monitoring, due to a risk of agranulocytosis.[34] Besides amantadine, which is usually first-line treatment, few other oral pharmacological treatment options currently exist for levodopa-induced dyskinesias. Thus, clinicians and patients often have to resort to more invasive device-aided therapies for treatment of disabling motor complications.[35,36] Fortunately, our patient has continued to have a satisfactory response to clozapine and did not experience any serious treatment-related adverse events over almost a decade of use.

Finally, it is notable that brain MRI abnormalities were observed in Patient 2. Although MRI scans are typically normal in PD, including AR-EOPD,[29] we recently reported similar findings in PARK-*PINK1* patients with homozygous p.Leu347Pro mutations (three with corticospinal tract involvement, one of whom also had hypothalamic involvement).[19] The MRI signal changes are relatively subtle, and it is possible that some prior cases may have been missed without careful examination. The significance of these changes is unclear. Although a minority of PARK-*Parkin* and PARK-*PINK1* patients have been noted to have upper motor neuron signs, typically hyperreflexia,[13,14] our patients did not demonstrate such features. There are also rare case reports of corticospinal tract involvement detected by brain MRI and neurophysiological studies of PARK-*Parkin* patients;[37,38] since PINK1 is known to act upstream of Parkin[6,8] it is perhaps unsurprising that manifestations described to occur with dysfunction of the Parkin pathway might also be observed with PINK1 deficiency. The hypothalamus plays a critical role in neuroendocrine and autonomic functions, and is known to be affected by the neuropathological process in PD.[39,40] Hypothalamic involvement in PD has been linked to sleep dysfunction, dysautonomia and weight loss,[39-42] which are very common and important problems in PD[39-43] and (in the case of orthostatic hypotension and weight loss) associated with poorer survival.[43-45]

In conclusion, our report contributes to the scarce literature on monogenic PD in the Malaysian / Indian population. It provides insights into the clinico-radiological phenotype of PARK-*PINK1*, and further supports the pathogenicity of the *PINK1* p.Leu489Pro variant. We also documented a beneficial and well-tolerated response to clozapine treatment for severe dyskinesias, with caveats that this was open label and involved only a single case.

REFERENCES

1. Department of Statistics Malaysia. http://pqi.stats.gov.my/searchBI.php?tahun=2019&kodData=2&kodJadual=1&kodCiri=4&kodNegeri=00. Accessed 18 April 2020.
2. Dorsey ER, Sherer T, Okun MS, Bloem BR. The emerging evidence of the Parkinson pandemic. *J Parkinsons Dis* 2018;8(s1):S3-S8.
3. Lim SY, Tan AH, Ahmad-Annuar A, *et al*. Parkinson's disease in the Western Pacific Region. *Lancet Neurol* 2019;18:865-79.
4. Poewe W, Seppi K, Tanner CM, *et al*. Parkinson disease. *Nat Rev Dis Primers* 2017;3:17013.
5. Blauwendraat C, Nalls MA, Singleton AB. The genetic architecture of Parkinson's disease. *Lancet Neurol* 2020;19:170-8.
6. Domingo A, Klein C. Genetics of Parkinson disease. *Handb Clin Neurol* 2018;147:211-27.
7. Polymeropoulos MH, Lavedan C, Leroy E, *et al*. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 1997;276(5321):2045-7.
8. Park JS, Davis RL, Sue CM. Mitochondrial dysfunction in Parkinson's disease: New mechanistic insights and therapeutic perspectives. *Curr Neurol Neurosci Rep* 2018;18:21.
9. Sardi SP, Cedarbaum JM, Brundin P. Targeted therapies for Parkinson's disease: From genetics to the clinic. *Mov Disord* 2018;33:684-96.
10. Miller S, Muqit MMK. Therapeutic approaches to enhance PINK1/Parkin mediated mitophagy for the treatment of Parkinson's disease. *Neurosci Lett* 2019;705:7-13.
11. Valente EM, Abou-Sleiman PM, Caputo V, *et al*. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 2004;304:1158-60.
12. Valente EM, Bentivoglio AR, Dixon PH, *et al*. Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on

human chromosome 1p35-p36. *Am J Hum Genet* 2001;68:895-900.
13. MDSGene website (https://www.mdsgene.org), accessed 20 April 2020.
14. Kasten M, Hartmann C, Hampf J, *et al*. Genotype-phenotype relations for the Parkinson's disease genes Parkin, PINK1, DJ1: MDSGene systematic review. *Mov Disord* 2018;33:730-41.
15. Halder T, Raj J, Sharma V, Das P. Novel P-TEN-induced putative kinase 1 (PINK1) variant in Indian Parkinson's disease patient. *Neurosci Lett* 2015;605:29-33.
16. Rogaeva E, Johnson J, Lang AE, *et al*. Analysis of the PINK1 gene in a large cohort of cases with Parkinson disease. *Arch Neurol* 2004;61:1898-904.
17. Petit A, Kawarai T, Paitel E, *et al*. Wild-type PINK1 prevents basal and induced neuronal apoptosis, a protective effect abrogated by Parkinson disease-related mutations. *J Biol Chem* 2005;280:34025-32.
18. Biswas A, Sadhukhan T, Majumder S, *et al*. Evaluation of PINK1 variants in Indian Parkinson's disease patients. *Parkinsonism Relat Disord* 2010;16:167-71.
19. Tan AH, Lohmann K, Tay YW, *et al*. PINK1 p.Leu347Pro Mutations in Malays: Prevalence and Illustrative Cases. *Parkinsonism Relat Disord* 2020;79:34-39.
20. Martínez-Martín P, Forjaz MJ, Cubo E, Frades B, de Pedro Cuesta J; ELEP Project Members. Global versus factor-related impression of severity in Parkinson's disease: A new clinimetric index (CISI-PD). *Mov Disord* 2006;21:208-14.
21. Martínez-Martín P, Rojo-Abuin JM, Rodríguez-Violante M, *et al*. Analysis of four scales for global severity evaluation in Parkinson's disease. *NPJ Parkinsons Dis* 2016;2:16007.
22. Martínez-Martín P, Rodríguez-Blázquez C, Mario Alvarez, *et al*. Parkinson's disease severity levels and MDS-Unified Parkinson's Disease Rating Scale. *Parkinsonism Relat Disord* 2015;21:50-4.
23. Lim SY, Lim JL, Ahmad-Annuar A, *et al*. Clinical phenotype of LRRK2 R1441C in two Chinese sisters. *Neurodegen Dis* 2020;20:39-45.
24. Lim SY, Lim KB, Hor JW, Tan AH. Brief clinical rating scales should not be overlooked. *Mov Disord* 2020;35(10):1884-5.
25. Choi HK, Choi Y, Kang H, *et al*. PINK1 positively regulates HDAC3 to suppress dopaminergic neuronal cell death. *Hum Mol Genet* 2015;24:1127-41.
26. Grünewald A, Kumar KR, Sue CM. New insights into the complex role of mitochondria in Parkinson's disease. *Prog Neurobiol* 2019;177:73-93.
27. Sliter DA, Martinez J, Hao L, *et al*. Parkin and PINK1 mitigate STING-induced inflammation. *Nature* 2018;561:258-62.
28. Bonifati V, Rohé CF, Breedveld GJ, *et al*. Early-onset parkinsonism associated with PINK1 mutations: frequency, genotypes, and phenotypes. *Neurology* 2005;65:87-95.
29. Niemann N, Jankovic J. Juvenile parkinsonism: Differential diagnosis, genetics, and treatment. *Parkinsonism Relat Disord* 2019;67:74-89.
30. Ricciardi L, Petrucci S, Guidubaldi A, *et al*. Phenotypic variability of PINK1 expression: 12 Years' clinical follow-up of two Italian families. *Mov Disord* 2014;29:1561-6.
31. Marongiu R, Ferraris A, Ialongo T, *et al*. PINK1 heterozygous rare variants: prevalence, significance and phenotypic spectrum. *Hum Mutat* 2008;29(4):565.
32. Durif F, Debilly B, Galitzky M, *et al*. Clozapine improves dyskinesias in Parkinson disease: a double-blind, placebo-controlled study. *Neurology* 2004;62:381-8.
33. Fox SH, Katzenschlager R, Lim SY, *et al*. International Parkinson and movement disorder society evidence-based medicine review: Update on treatments for the motor symptoms of Parkinson's disease. *Mov Disord* 2018;33:1248-66.
34. Yaw TK, Fox SH, Lang AE. Clozapine in parkinsonian rest tremor: A review of outcomes, adverse reactions, and possible mechanisms of action. *Mov Disord Clin Pract* 2015;3:116-24.
35. Antonini A, Moro E, Godeiro C, Reichmann H. Medical and surgical management of advanced Parkinson's disease. *Mov Disord* 2018;33:900-8.
36. Katzenschlager R, Poewe W, Rascol O, *et al*. Apomorphine subcutaneous infusion in patients with Parkinson's disease with persistent motor fluctuations (TOLEDO): a multicentre, double-blind, randomised, placebo-controlled trial. *Lancet Neurol* 2018;17:749-59.
37. De Rosa A, Volpe G, Marcantonio L, *et al*. Neurophysiological evidence of corticospinal tract abnormality in patients with Parkin mutations. *J Neurol* 2006;253:275-9.
38. Kuroda Y, Mitsui T, Akaike M, Azuma H, Matsumoto T. Homozygous deletion mutation of the parkin gene in patients with atypical parkinsonism. *J Neurol Neurosurg Psychiatry* 2001;71:231-4.
39. De Pablo-Fernández E, Breen DP, Bouloux PM, Barker RA, Foltynie T, Warner TT. Neuroendocrine abnormalities in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 2017;88:176-85.
40. Lim SY, Fox SH, Lang AE. Overview of the extranigral aspects of Parkinson disease. *Arch Neurol* 2009;66:167-72.
41. De Pablo-Fernandez E, Courtney R, Holton JL, Warner TT. Hypothalamic α-synuclein and its relation to weight loss and autonomic symptoms in Parkinson's disease. *Mov Disord* 2017;32:296-8.
42. Dayan E, Sklerov M, Browner N. Disrupted hypothalamic functional connectivity in patients with PD and autonomic dysfunction. *Neurology* 2018;90:e2051-8.
43. Tan AH, Hew YC, Lim SY, *et al*. Altered body composition, sarcopenia, frailty, and their clinico-biological correlates, in Parkinson's disease. *Parkinsonism Relat Disord* 2018;56:58-64.
44. Ma K, Xiong N, Shen Y, *et al*. Weight loss and malnutrition in patients with Parkinson's disease: Current knowledge and future prospects. *Front Aging Neurosci* 2018;10:1.
45. Goldstein DS, Holmes C, Sharabi Y, Wu T. Survival in synucleinopathies: A prospective cohort study. *Neurology* 2015;85:1554-61.