Ann Acad Med Singap 2021;50:353-5
https://doi.org/10.47102/annals-acadmed.sg.2020508

Rare homozygous *PRKN* exon 8 and 9 deletion in Malay familial early-onset Parkinson's disease

Dear Editor,

Little is known about the genetics of Parkinson's disease (PD) in Southeast Asian populations.[1] We extended knowledge of the Southeast Asian monogenic PD landscape by describing a Malaysian Malay family with early-onset PD (EOPD), defined as onset at <50 years of age[2] and a rarely reported homozygous *PRKN* exon 8 and 9 deletion.

The index patient III:9, aged 29 years, was referred to us with a diagnosis of dopa-responsive dystonia (DRD), onset of which occurred at the age of 26 years with leg cramps and generalised tremor. Two older sisters were also affected (Fig. 1A). The patient had stopped taking levodopa-benserazide and baclofen 2 months prior to a planned pregnancy that had miscarried. During the pregnancy, her symptoms worsened with leg cramps, toe curling and backward falls. After the miscarriage, a quarter tablet levodopa (200mg)/benserazide (50mg) and baclofen 10mg 4 times a day were resumed, with medication effect lasting 4–5 hours. There was no diurnal variation of symptoms, but sleep benefit was reported. "On" condition examination revealed mild upper limb postural and action tremors, and mild-to-moderate bradykinesia of upper and lower limb movements, with no dystonic posturing. Posture and gait were normal, with negative pull test. There were no upper motor neuron signs. Magnetic resonance imaging of the brain was normal. The patient returned several months later with worsening "off" periods and troublesome dyskinesias. "Off" state examination revealed dystonic ankle inversion and toe clawing. Foot tapping was moderate to severely bradykinetic with 1-person assistance needed for walking, and Unified Parkinson's Disease Rating Scale motor score was 38 (indicating moderate to severe parkinsonism). In view of the prominent motor response complications, we considered the diagnosis of EOPD to be more likely than that of DRD. Trihexyphenidyl 2mg/day caused faintness without motoric benefit.

The patient returned at the age of 31 years, 2 months pregnant with significant motoric worsening, despite continuation with a quarter tablet levodopa/benserazide 4 times a day. Her Montreal Cognitive Assessment and Sniffin Sticks (olfactory function) scores were normal (27/30 and 10/12, respectively). Her condition subsequently stabilised with levodopa/benserazide 4–5 times a day (treatment was uninterrupted during pregnancy). The baby was delivered vaginally 2 weeks premature but otherwise normal. She had another successful pregnancy (during which she took a quarter tablet levodopa/benserazide 5–8 times a day) at the age of 34 years. When she was last reviewed at the age of 35 years, her levodopa/benserazide intake had escalated to a quarter tablet 10–12 times a day (levodopa-equivalent daily dosage 500–600mg/day), with "on" periods lasting only 2–2.5 hours, but with preserved magnitude of levodopa response. Dopamine agonists were unavailable because of her financial constraints. As of the year 2020, her children (aged 8, 5 and 3 years) have developed normally.

Patient III:4 was seen at the age of 44 years, with symptom onset in her mid-30s. She had right arm tremor and slow movements; right leg cramping; and toe clawing. Gait was slow with imbalance, but there were no falls. She experienced sleep benefit. Her motor Unified Parkinson's Disease Rating Scale score (untreated) was 30 (indicating moderate parkinsonism). Response to a quarter tablet levodopa (200mg)/benserazide (50mg) 1–2 times a day was excellent. Her Montreal Cognitive Assessment and Sniffin Sticks test scores were 24/30 and 10/12, respectively. Last reviewed at the age of 50 years, she was taking a quarter tablet 3 times a day (levodopa-equivalent daily dosage 150mg/day) and continued to have a good levodopa response lasting 5 hours. During "off" periods, she had difficulty walking and performing house chores.

Subject III:8 reportedly had parkinsonian features but was not a patient of ours. Currently 41 years old, she developed motor symptoms at the age of 29 years and apparently had fairly severe manifestations, but responded well to low-dose levodopa.

The current study received institutional ethical approval for genetic analysis. Multiplex ligation-dependent probe amplification was performed using the SALSA MLPA Probemix P051 Parkinson mix 1 (MRC Holland, Netherlands) to detect *PRKN*, *PINK1*, *DJ-1* and *SNCA* copy number variations. Mutation screening of *PRKN* (NM_004562.3), *PINK1* (NM_032409.3), *GCH1* (the usual cause of DRD, NM_000161.3) coding exons was performed by Sanger sequencing. Both III:4 and III:9 carried a homozygous *PRKN* exon 8 and 9 deletion: c.(871+1_872-1)_

(1083+1_1084-1)del (Fig. 1B). No pathogenic coding variants were found in *GCH1*, *PINK1*, *DJ-1* and *SNCA*. DNA was unavailable from unaffected family members; however, the deletion was not present in 25 controls of Malay ethnicity of mean age 61.2 years (standard deviation 9.9). In silico analysis had predicted a frameshift that resulted in a truncated PRKN protein with only 290 wildtype amino acids compared with the normal 465 (Fig. 1C). Proteomic analysis on PRKN from patient tissues was not performed.

Despite the large growth in PD incidence in the Asia Pacific,[1] including the Southeast Asian region with

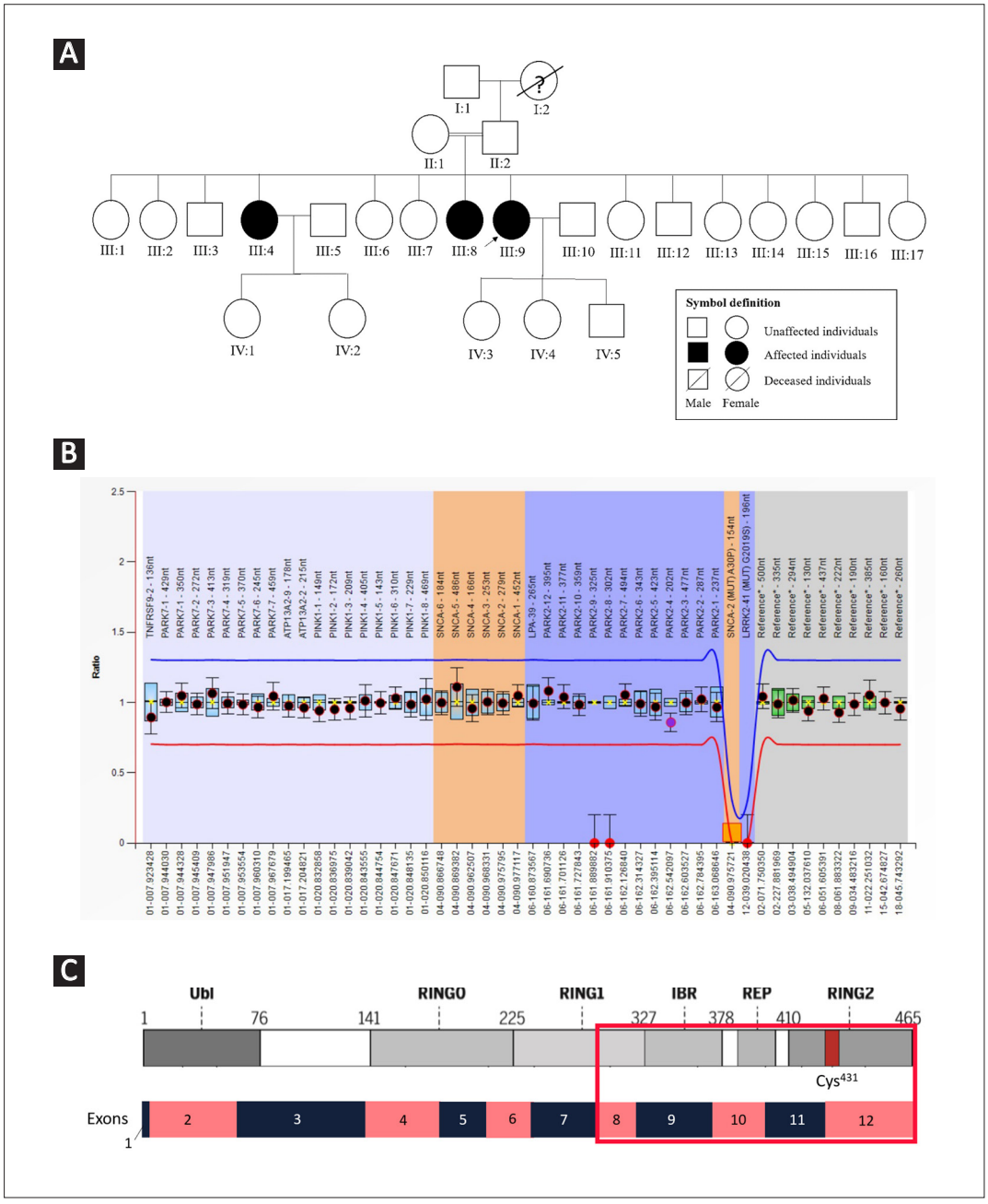

Fig. 1. (A) Family pedigree. Proband (III:9) indicated by arrow. Genetic testing performed for III:4 and III:9. The paternal grandmother (I:2) reported parkinsonian features, with symptom onset in her 40s, but was not formally diagnosed. (B) Multiplex ligation-dependent probe amplification showed homozygous *PRKN* exon 8 and 9 deletion. (C) Domains of PRKN protein (top) aligned with *PRKN* exons (bottom). The upper segment indicates the 5 domains within PRKN (Ubl, RING0, RING1, IBR and RING2) with their corresponding amino acids. The red box shows the predicted loss of the RING1, IBR and RING2 domains due to the coding frameshift caused by homozygous exon 8 and 9 deletion.

>650 million inhabitants, we were unable to find any published reports of *PRKN* mutations (the commonest cause of autosomal recessive EOPD globally).[3,4]

Globally, *PRKN* exon 3 deletions are most commonly reported. The homozygous exon 8 and 9 deletion in our patients has been reported in 2 families originating from Algeria and India.[5-7] Since our patients had ancestral links to the Middle East, they may share common ancestors with some of these patients. However, other studies from the Middle East, including a study on 25 Saudi patients,[8] did not find the same exon 8 and 9 deletion.

PRKN functions as an E3 ubiquitin ligase in post-translational ubiquitination of protein substrates, mediating their turnover and proteasomal degradation. Currently, there are no functional data specifically documenting the pathogenicity of the exon 8 and 9 deletion. However, in silico analyses have predicted frameshifts leading to a premature stop codon, truncating PRKN by approximately 38%, and lacking the RING1, IBR and RING2 domains (Fig. 1C). The loss of these domains—or conceivably loss of protein expression due to protein instability—would likely have an impact on the ubiquitin-proteasomal system, leading to impaired autophagic degradation of mitochondria and oxidative stress.

The clinical presentation of our patients was similar to that previously described for PARK-PRKN,[3] with young onset or prominence of dystonia or both. Interestingly, the motoric symptoms of the index patient deteriorated during pregnancy despite levodopa continuation; however, on the positive side, she had 3 normal offspring. This case is similar to a report of successful pregnancy in PARK-PRKN, with motor worsening and healthy children.[9,10] In the literature, pregnancy appears to have variable effects on PD symptoms (worsened, unchanged or improved), and have been proposed to be mediated by hormonal or levodopa-pharmacokinetic changes or both.

In conclusion, our study contributes to the limited literature regarding monogenic PD in an underrepresented population. This finding may have implications for EOPD families of Malay ancestry in Singapore, Indonesia, Brunei, southern Thailand and beyond.

Acknowledgements

This work was supported by a grant awarded to SYL from the Ministry of Higher Education Malaysia (FRGS/1/2020/SKK0/UM/01/2). AAA is supported by a grant from the Ministry of Higher Education Malaysia (FRGS/1/2018/SKK08/UM/02/3). SYL and AHT were supported by the University of Malaya Parkinson's Disease and Movement Disorder Research Program (PV035-2017). The authors thank the family of the patients for participating in this study.

REFERENCES

1. Lim SY, Tan AH, Ahmad-Annuar A, et al. Parkinson's disease in the Western Pacific Region. Lancet Neurol 2019;18:865-79.
2. de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol 2006;5:525-35.
3. 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.
4. Puschmann A. Monogenic Parkinson's disease and parkinsonism: Clinical phenotypes and frequencies of known mutations. Parkinsonism Relat Disord 2013;19:407-15.
5. Lucking CB, Abbas N, Durr A, et al. Homozygous deletions in parkin gene in European and North African families with autosomal recessive juvenile parkinsonism. The European Consortium on Genetic Susceptibility in Parkinson's Disease and the French Parkinson's Disease Genetics Study Group. Lancet 1998;352:1355-6.
6. Thobois S, Ribeiro MJ, Lohmann E, et al. Young-onset Parkinson disease with and without parkin gene mutations: A fluorodopa F 18 positron emission tomography study. Arch Neurol 2003;60:713-8.
7. Madegowda RH, Kishore A, Anand A. Mutational screening of the parkin gene among South Indians with early onset Parkinson's disease. J Neurol Neurosurg Psychiatry 2005;76:1588-90.
8. Al-Mubarak BR, Bohlega SA, Alkhairallah TS, et al. Parkinson's disease in Saudi patients: A genetic study. PLoS One 2015; 10:e0135950.
9. Brüggemann N, Klein C. Parkin type of early-onset Parkinson disease. In: Adam MP, Ardinger HH, Pagon RA, et al. (eds). GeneReviews®. Seattle, US: University of Washington; 1993-2020. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1478/. Accessed on 31 August 2020.
10. Olivola S, Xodo S, Olivola E, et al. Parkinson's disease in pregnancy: A case report and review of the literature. Front Neurol 2020;10:1349.

Yi Wen Tay, [1]*BBMedSc*, Jia Lun Lim, [1,2]*BBMedSc*, Ai Huey Tan, [2,3]*MD*, Azlina Ahmad-Annuar, [1]*PhD*, Shen-Yang Lim, [2,3]*MD*

[1] Department of Biomedical Science, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[2] The Mah Pooi Soo and Tan Chin Nam Centre for Parkinson's and Related Disorders, University of Malaya, Kuala Lumpur, Malaysia
[3] Division of Neurology, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Correspondence: Prof Shen-Yang Lim, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.
Email: sylim@ummc.edu.my