Neurology Asia 2022; 27(2) : 515 – 520

Rare homozygous *PRKN* exon 7 duplication in a Ibanese patient from Northwestern Borneo with young onset Parkinson's disease

*[1,2]Siaw Cheng Wong, *[2]Zhun Foo Tan, *[3]Yi-Wen Tay, [1]Wan Chung Law, [3]Azlina Ahmad-Annuar, [4,5]Ai Huey Tan, [4,5]Shen-Yang Lim
*These authors contributed equally to this work and are co-first authors

[1]Neurology Unit, Department of Internal Medicine, Sarawak General Hospital, Ministry of Health, Malaysia; [2]Medical Department, Sri Aman Hospital, Ministry of Health, Malaysia; [3]Department of Biomedical Science, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; [4]Division of Neurology, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; [5]The Mah Pooi Soo & Tan Chin Nam Centre for Parkinson's & Related Disorders, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

Abstract

We describe the clinical features of a Sarawakian man of Ibanese ethnicity with young-onset Parkinson's disease (PD), who carried a very rare homozygous *PRKN* exon 7 duplication. Truncal dystonia was a prominent feature on presentation, in addition to classical parkinsonian motor features. This report adds to the very limited literature on monogenic causes of PD in Southeast Asia and specifically the indigenous group in the Borneo region.

Keywords: Parkinson's disease, genetics, monogenic, Parkin, *PRKN*, Asia, indigenous

INTRODUCTION

The cause of Parkinson's disease (PD) is complex and involves a combination of both genetic and environmental factors in most cases.[1] A small but significant proportion of PD cases can be attributed to a single genetic Mendelian cause – so called monogenic PD.[2-4] Monogenic autosomal recessive PD (AR-PD) typically presents with early onset of motor manifestations before the age of 40. As studies in PD genetics have mainly focused on Caucasians in Europe and North America, knowledge about PD genetics in other parts of the world, such as Southeast Asia, is significantly lacking.[1,5-8] This deficit is even more pronounced among the indigenous populations of Southeast Asia.[9,10]

Island Southeast Asia (ISEA), including the island of Borneo, is home to hundreds of different ethnic groups, with rich cultural, linguistic, and genetic diversities.[11,12] Although previous studies have suggested a common origin of all Southeast Asian populations through a single north-to-south migration wave, more recent research revealed a multi-layered population structure in the ISEA, with at least three major ancestral components in association with Papuan-, Austroasiatic- and Austronesian-speaking populations.[11] Many of these indigenous groups in ISEA remain geographically isolated, and have been largely uncharacterized in population genetics.[13]

Here, we report on an indigenous person of Ibanese descent from Sarawak (Northwestern Borneo) with young onset PD associated with a rare homozygous mutation (exon 7 duplication) in *PRKN*. As an additional point of interest, our patient had an unusual clinical presentation with truncal dystonia being a prominent feature.

CASE REPORT

A 39-year-old man of Sarawak Ibanese ethnicity presented with symptoms of tremor, muscle stiffness, change in posture, and limping gait, resulting in difficulties carrying out his daily farming activities. Onset of symptoms was two years prior, and his condition was slowly progressive. There was no apparent diurnal variation in the symptoms. Neurological examination showed that he had dystonia in

Address correspondence to: Shen-Yang Lim MD FRACP FASc, Neurology Laboratory, Level 6 South Block, University of Malaya Medical Centre, 50603 Kuala Lumpur, Malaysia. Email: limshenyang@gmail.com

Date of Submission: 16 April 2022; Date of Acceptance: 22 April 2022

https://doi.org/10.54029/2022frs

the trunk with phasic mild-to-moderate truncal forward flexion movements most apparent when walking (forwards as well as backwards) (Video 1). There was also bilateral striatal hand, with 'swan neck' deformity of mild-to-moderate severity (Video 2), and mild foot dystonia with striatal toe. Additionally, there were signs of parkinsonism with rigidity and bradykinesia in the upper and lower limbs (including moderate slowness and reduced amplitude of repetitive finger tapping movements) (Video 2), and slight upper limb postural tremor. Eye movements were normal and there were no signs of cerebellar dysfunction. Tendon reflexes were normal with flexor plantar responses.

His family history (Figure 1) was remarkable for an affected elder brother (II:11) who was said to have had early-onset parkinsonism in his early 30s. This brother subsequently became disabled and died in his early 40s; further details were unavailable. Other siblings were unavailable for examination, but were reported to be clinically unaffected. There was apparently no parental consanguinity.

The evaluation was directed at investigating for causes of dystonia-parkinsonism syndromes. His blood work, including caeruloplasmin, full blood count, and renal and liver profiles, was normal, as was a 24-hour urine copper collection. Brain MRI (1.5Tesla) with standard T1-weighted, T2-weighted, FLAIR, and SWI sequences was normal, and in particular there were no signs of abnormal metal accumulation in the brain.

Treatment with levodopa-benserazide (200/50mg) half tablet three times daily was initiated, resulting in significant improvement of symptoms and functional ability, and he was able to carry out his farming activities. At the time of writing (current age 42 years, 5 years after motor symptom onset), his medications consisted of levodopa-benserazide (200/50mg) half tablet four times daily and pramipexole sustained release 0.375mg daily. Mild truncal flexion dystonic movements were still apparent, but had improved (Video 3), as had limb bradykinesia (Video 4) and rigidity. There was no postural instability, but mild choreiform dyskinesias were observed. Cognitive function was intact with Mini-Mental State Examination (MMSE) score of 29/30. His Clinical Impression of Severity Index (CISI-PD) score[14-16] was 6 (Motor signs: 2, Disability: 2, Motor complications: 2, Cognitive status: 0), indicating mild global severity of PD. A checklist of his PD features, summarized in Table 1, indicated a relatively low burden of PD-associated non-motor symptoms.

Genetic testing

Twenty millilitres of peripheral blood was collected from the patient in EDTA tubes and DNA extracted using the FavorPrep™ Genomic DNA Maxi kit (Favorgen). Multiplex ligation-dependent probe amplification (MLPA) was performed on the sample using the SALSA® MLPA® Probemix P051 Parkinson mix 1 (MRC Holland) to look for copy number variations in the *PRKN, PINK1, DJ-1*, and *SNCA* genes. Both positive and negative controls were included in the run as references. Subsequently, fragment separation was carried out

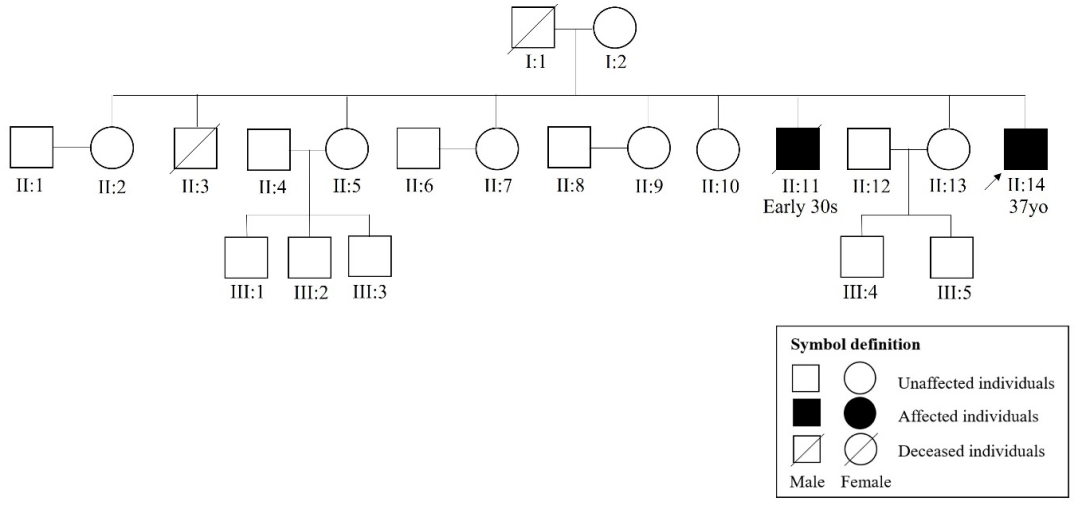

Figure 1. Family pedigree. The index case (II:14) is indicated by an arrow and age of onset is written below each affected individual.

Table 1: Demographic and clinical features of our patient with a homozygous exon 7 duplication *PRKN* mutation

Gender	Male
Age at PD motor symptom onset/Disease duration(yrs)	37 / 5 years
Ancestry/Geographic location	Iban/Sarawak
Family history	✓
Parental consanguinity	×
Met QSBB clinical diagnostic criteria for PD	✓
Bradykinesia	✓
Rigidity	✓
Tremor	✓
Gait difficulty	✓
Gait freezing	×
Postural instability / Falls	×
Dystonia	✓
Sleep benefit	✓
Upper motor neuron signs (hyperreflexia and extensor plantar response or sustained clonus)	×
Clear favourable response to dopaminergic medication	✓
Motor fluctuations	✓
Levodopa-induced dyskinesias	✓
Rapid Eye Movement(REM) sleep behavioural disorder(RBD)	×
Insomnia	×
Excessive daytime sleepiness	×
Depression	×
Anxiety	×
Mild cognitive impairment	×
Dementia	×
Visual hallucinations	×
Impulsive-compulsive behaviours	×
Constipation	×
Urinary dysfunction	×
Orthostatic giddiness or hypotension	×
Pain	×
Hyposmia	×
Underweight (body mass index [BMI]<18.5kg/m^2)	×
Atypical features more commonly seen in Parkinson-plus syndromes (e.g., eye movement abnormalities such as nystagmus or vertical gaze palsy)	×

This standardized reporting checklist is based in part on MDSGene recommendations (https://www.mdsgene.org).[5,17]
✓ = present; × = Absent.; QSBB= Queen Square Brain Bank.

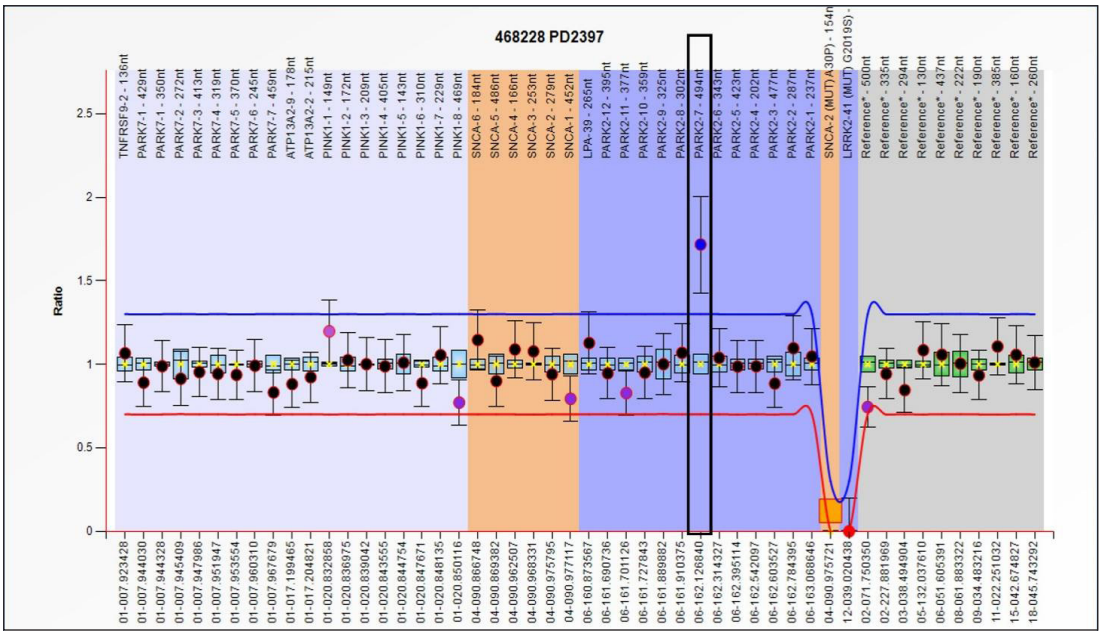

Figure 2. Electropherogram from the Coffalyser software. A homozygous duplication of PARK2/*PRKN* exon 7 is highlighted by the black box.

on the ABI 3730XL DNA Analyzer, and data were analyzed using the Coffalyser.Net data analysis software (www.coffalyser.net) to determine the relative peak height of each exon. Any positive results were confirmed by another two independent MLPA analyses. This study was approved by the Medical Research Ethics Committees, University of Malaya Medical Centre (UMMC) and Ministry of Health Malaysia, and the patient provided written informed consent.

Based on the results, the patient was found to carry a homozygous *PRKN* exon 7 duplication [c.(734+1_735-1) (871+1_872-1)dup] (Figure 2). No structural variations were found in *PINK1*, *DJ-1*, and *SNCA*. To understand the effect of this mutation, *in silico* analysis was carried out using the online ExPASy (http://www.expasy.org) tool. The homozygous duplication of coding sequences in exon 7 is postulated to cause a frameshift, and subsequently a premature stop codon, resulting in an approximate 35% truncation of the Parkin protein with only 303 amino acids as compared to 465 amino acids in the wildtype protein, and loss of part of the RING1, and the IBR, REP, and RING2, domains of Parkin (Figure 3).

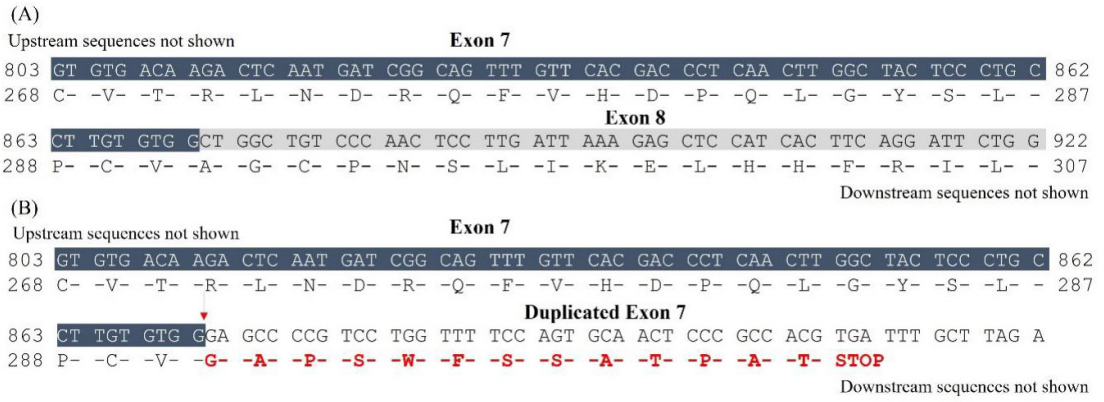

Figure 3. Wildtype amino acid sequence encoded by *PRKN* and predicted effect on translation due to exon 7 duplication. (A) Partial wildtype mRNA sequences of *PRKN* exons 7 and 8 with corresponding amino acids encoded are shown (NM_004562.3). (B) After intronic splicing, the duplicated exon 7 is assumed to lie immediately after the original exon 7 in the mutant mRNA transcript, and has been translated as such below to predict the frameshift that leads to a premature stop codon. This stop codon truncates the normal Parkin protein with 465 amino acids to a shorter protein with 303 amino acids during translation.

DISCUSSION

The *PRKN* gene, also known as PARK2, encodes Parkin, an autoinhibited E3 ubiquitin ligase that is neuroprotective against cellular insults such as alpha-synuclein toxicity and proteasome dysfunction.[2,4] *PRKN* mutations can be point mutations and/or deletions or duplications.[2,4] Mutations in the *PRKN* gene represent the most common cause of AR-PD worldwide, but has been understudied in the Southeast Asian region, with only a few families with PARK-*PRKN* reported to date.[6,7,9]

Our study reports on a homozygous duplication of exon 7, which appears to be very rare, as most mutations involving exon 7 were found to be deletions. There is uncertainty about the only other possible homozygous exon 7 duplication in the literature[18], concerning a man with unusually late onset of PARK-*PRKN*, having tremor at the age of 73 years and unclear dopa response. Information presented in the paper implied that the duplication was in homozygous state, but this was not explicitly stated. Other cases with exon 7 duplications were reported to be compound heterozygous with different *PRKN* mutations (exon deletions) in the other allele.[19] *PRKN* deletions and duplications are understood to be frameshift mutations that lead to a premature stop codon several positions downstream, resulting in loss of Parkin function.[19]

PARK-*PRKN* is characterized by slow progression of motor features, which are typically responsive to dopaminergic medication treatment, and infrequency of dementia.[2,4] The clinical features of our patient are in keeping with this general pattern. Although dystonia, particularly involving the foot, is common in AR-PD cases (18%, 21%, and 46% of *PRKN*, *PINK1*, and *DJ-1* cases, respectively, according to the MDSGene database[17]), truncal involvement in PARK-*PRKN* was only highlighted in one report involving three patients.[20] These patients were either stooped forward (as in our case) or laterally flexed.[20] However, truncal dystonia may not be specific to *PRKN*, since this feature has also recently been observed, for example, in PD cases associated with *Glucocerebrosidase* (*GBA*) gene mutations.[10] The collection and analysis of large genotype-phenotype datasets (for example, as part of the ongoing Global Parkinson's Genetics Project or GP2; https://gp2.org) will provide clarity on the potential for this, as well as other features, to be useful differentiating signs in clinical practice.

The Iban, also known as the Sea Dayak, is one of the largest indigenous groups in the state of Sarawak in East Malaysia.[12] The Ibanese are indigenous to Borneo and, besides Sarawak, are concentrated in the Indonesian province of West Kalimantan and Brunei. There has been a lack of medical research in this population and, to our knowledge, nothing published on PD to date. A large scale genome-wide single-nucleotide polymorphism (SNP) study in diverse East and Southeast Asian populations suggested that the Iban exhibit greatest genetic similarity to Indonesian and mainland Southeast Asian populations.[12] Interestingly, significant contributions from people currently inhabiting Borneo into the genetic architecture of Malayo-Polynesians in the Near and Remote Oceania regions has also been reported.[11] Taken together, PD genetic research in indigenous populations in Borneo may represent a new frontier in understanding the genetic landscape of the broader Asian-Oceanian region.

In conclusion, our report contributes to the very limited literature on monogenic PD in the Southeast Asian and specifically the indigenous context, and further supports the pathogenicity of *PRKN* exon 7 duplication as a cause for young-onset PD.

ACKNOWLEDGMENTS

We thank the patient for his permission for publication of this report and the videos. We would like to thank the Director General of Health Malaysia for his permission to publish this article.

DISCLOSURE

Financial support: This work was supported by a grant awarded to SYL from the Ministry of Higher Education Malaysia (FRGS/1/2020/SKK0/UM/01/2) and the University of Malaya Parkinson's Disease and Movement Disorders Research Program (PV035-2017) awarded to SYL and AHT.

Video 1. Showing truncal dystonia. (https://neurology-asia.org/content/27/2/neuroasia-2021-27(2)-515-v1.mp4)
Video 2. Showing striatal hand and upper limb bradykinesia. (https://neurology-asia.org/content/27/2/neuroasia-2021-27(2)-515-v2.mp4)
Video 3. Showing truncal dystonia improved post-treatment. (https://neurology-asia.org/content/27/2/neuroasia-2021-27(2)-515-v3.mp4)

Video 4. Showing upper limb bradykinesia improved post-treatment. (https://neurology-asia.org/content/27/2/neuroasia-2021-27(2)-515-v4.mp4)

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