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Clinical phenotype of Parkinson's disease with a homozygous *PRKN* p.Cys441Arg mutation

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Abstract

We describe the clinical features of a Malaysian woman with Parkinson's disease (PD) who carried the *PRKN* p.Cys441Arg mutation in the homozygous state. She had a fairly "classic" clinical presentation for PARK-*Parkin*, including juvenile onset and a clear response to dopaminergic medication complicated by motor fluctuations and dyskinesias. She had a substantial benefit with apomorphine infusion treatment, which to our knowledge has not been reported for PARK-*Parkin*. Our report contributes to the very scarce literature on monogenic causes of PD in the Malaysian population, and highlights an alternative treatment option to oral dopaminergic medication or deep brain stimulation surgery.

Keywords: Parkinson's disease, parkin, genetics, Asian, apomorphine, pregnancy.

INTRODUCTION

Around 2-3% of PD patients worldwide are estimated to have a monogenic cause for their PD, although this proportion can be substantially higher in selected populations.[1,2] PD caused by *PRKN* mutations (i.e., PARK-*Parkin*) is the most common form of young-onset PD across all ethnic groups studied so far[2] and displays an autosomal recessive pattern of inheritance. According to the MDSGene database, which collates all English reports of monogenic PD with individual-level data, the median age at PD onset for PARK-*Parkin* (*n*=1,000) is 31 years, although the range is very wide, spanning from 3 to 81 years.[3,4]

To date, there have been very few published studies of monogenic PD in the Malaysian population.[5-8] In this report, we describe the clinical phenotype of a patient with juvenile-onset familial PD who carried a homozygous p.Cys441Arg (c.1321T>C) *PRKN* mutation. As an additional point of historical interest, she was to our knowledge the first patient in Malaysia (and, indeed, in Southeast Asia) to receive subcutaneous apomorphine infusion therapy for severe motor response complications (personal communication, Stadpharm), and experienced substantial benefit from this treatment.

CASE REPORT

This 47-year-old woman of Chinese ancestry was referred to our Centre in December 2009, aged 36 years, for consideration of either apomorphine infusion or deep brain stimulation (DBS). She had started to have gradual onset of leg tremor and body stiffness in her early teens, followed by difficulty walking. PD was diagnosed three years after initial symptom onset, around age 16 years.

Initially, for the first few years, the patient responded well to levodopa-carbidopa (250/25 mg), ½ tablet tid, which made her feel "almost normal again". At the age of 22 years, she gave birth to a healthy son (levodopa treatment was continued throughout the pregnancy). At the age of 26 years, she became pregnant again, but because of concerns regarding possible birth defects from PD medications, and the risk of the child inheriting the same illness, she and her husband opted for an abortion. Around this time, motor fluctuations and dyskinesias had become a significant problem, and for the next ten years

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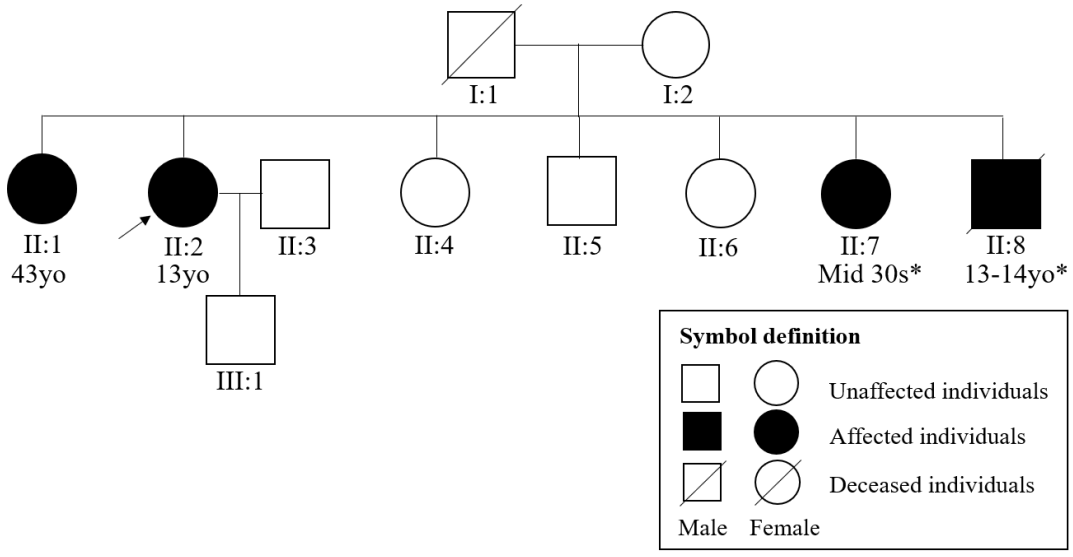
she was mostly housebound due to this. Over time, her medications were gradually increased and in 2009, she was taking levodopa-carbidopa (250/25) ½ tablet morning, followed by levodopa-carbidopa (100/25 mg) ½ tablet every two hours (doses higher than ½ tablet would cause severe dyskinesias), pramipexole (0.125 mg) 1 tablet tid, selegiline 5 mg/d, benzhexol 2 mg/d, and amantadine 100 mg/d. Despite adjustments to her PD medications, she suffered from disabling motor response complications. During her OFF-periods, she had severe gait freezing and was observed in clinic not to be able to even take one step forward. Her gait freezing would sometimes lead to falls, and sometimes she had to crawl on the floor to move about. During ON periods, which would last around two hours, she could ambulate fairly well without falls, but had severe and disabling dyskinesias. Her Montreal Cognitive Assessment Score was 21/30. The patient's global clinical severity measured using the CISI-PD[7,9-12] was 15/24 (Motor signs 4, Disability 4, Motor complications 5, Cognitive status 2; applied retrospectively based on the patient's medical chart).

Her younger brother also had PD with symptoms beginning age 13-14 years. In subsequent years, two sisters developed PD with onset of symptoms in their fourth and fifth decades (Figure 1). These siblings were unavailable for examination/testing. Their father died from a motor vehicle accident aged around 70 years with no signs of PD prior, and the mother was healthy in her 70s; their union was reported to be non-consanguineous.

She was considered a suitable candidate for device-aided therapy, but the patient was reluctant to undergo DBS surgery because of perceived risks. Apomorphine infusion was therefore commenced in April 2011. Just prior to this, her ON periods lasted only 1-2 hours. There was significant improvement with the apomorphine infusion, and with a low dose of 1.0 mg/h in May 2011 her oral PD medications could be reduced with reduction in dyskinesias. She described the treatment as "giving her a new life". Because she lived far away, she was reviewed in person at our Centre only intermittently. When last seen in January 2013, she had continued to use a low dose of apomorphine (1.75mg/h=0.35mL/hour). She reported having no OFF periods at all (but symptoms would return within half an hour upon stopping the infusion), and dyskinesias occurred very occasionally only. She mobilized well without falls and could do all house chores and was able to easily venture out of the house. Remarkably, levodopa-carbidopa and pramipexole could be stopped; she took only a night-time dose of piribedil 50 mg, amantadine 100 mg, benzhexol 2 mg and selegiline 5 mg, as well as diazepam 5 mg bid.

She was maintained on apomorphine infusion



The index case (II:2) is indicated by an arrow. Age of onset is written below each affected family member and asterisk (*) indicates the estimate given by the index case.

Figure 1. Family pedigree.

treatment with ongoing but reducing benefit (with reemergence of troublesome motor response complications) until January 2020 when, unfortunately, the infusion had to be stopped due to logistical difficulties (unavailability of pump-related disposable equipment from the manufacturer). This resulted in significant worsening of the patient's mobility and recurrence of falls. At the time of writing, a new supplier and funding by the health authorities were being sought. Other clinical features as of June 2020 (34 years after motor symptom onset) are summarized in Table 1.

The patient's DNA was tested as part of an ongoing multicentre collaborative study of Malaysian PD genetics, using a next-generation sequencing (NGS)-based PD gene panel (Parkinson panel at Centogene AG, Rostock, Germany).[2,7,8] 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. The panel included all exons of *PRKN*, *PINK1*, *DJ-1*, *SNCA*, *LRRK2*, *GBA*, *VPS35*, *PLA2G6*, *RAB39B*, and exons 11, 37, 43, 61 and 69 of *VPS13C*.

A known pathogenic mutation in *PRKN* was detected in homozygous state (p.Cys441Arg, c.1321T>C, exon 12).[4,13-18] The patient also carried a single heterozygous *PINK1* Val350Leu variant (gnomAD minor allele frequency [MAF] in East Asians: 0.0014, 0.0001 overall; https://gnomad.broadinstitute.org/variant/1-20972141-G-C?dataset=gnomad_r2_1). This was classified as a "variant of uncertain significance (VUS)", although it was predicted to be probably damaging/deleterious by PolyPhen (Polymorphism Phenotyping) and SIFT (Sorting Intolerant From Tolerant) tools. She did not carry any other rare MAF <0.01 variant in the PD genes targeted by the gene panel. We also excluded exon rearrangements in *PRKN*, *PINK1*, *DJ-1*, and *SNCA* by multiplex ligation-dependent probe amplification (MLPA) analysis (P051 kit, MRC Holland).

DISCUSSION

PRKN, encoding the E3 ubiquitin protein ligase Parkin, is involved in the clearance of dysfunctional mitochondria (mitophagy).[2,19,20] Impaired mitochondrial homeostasis resulting from Parkin deficiency is linked to oxidative stress, accumulation of misfolded α-synuclein, and inflammation, all key events in the pathophysiology of PD.[2,19-21] Around 180 different *PRKN* mutations have been reported in the literature so far, occurring in homozygous or compound heterozygous forms.[2,3] Structural variants are the most common mutation type, in 43.2%; followed by missense mutations in 22.3% and frameshift mutations in 16.5% among all reported mutation carriers.[2,3]

There have been six reports involving a total of 11 patients carrying the p.Cys441Arg mutation as a compound heterozygote, in combination with a different point mutation or a deletion.[4,13-18] These patients originated from France (*n*=4),[13,14] China (*n*=4)[15,16], Taiwan (*n*=2),[17] and Iran (*n*=1),[18] with a median age at PD onset of 23 (range 18-33) years. To our knowledge, a homozygous p.Cys441Arg mutation has yet not been reported. The *PRKN* p.Cys441Arg mutation is classified as "definitely pathogenic" based on well-defined pathogenicity scoring criteria.[3] Studies with fibroblasts from a patient with the *PRKN* p.Cys441Arg mutation in the compound heterozygous state revealed a complete loss of Parkin expression.[22] Other studies showed associated reduction of Parkin expression and loss of E3 ubiquitin protein ligase activity.[23,24]

A notable feature of our patient was her very early age at PD onset, with motor symptoms beginning around 13 years of age (the earliest among all PD patients in our Centre's database of ≈2,000 subjects, unpublished data). It has been said that "proper diagnosis is the first step in treating the whole person".[25] Her initial diagnosis of PD was delayed for several years, which was perhaps unsurprising given the rarity of juvenile-onset PD. Indeed, studies found that diagnostic delays were more pronounced the earlier the age at PD onset,[26] and 44% of PARK-*Parkin* patients had delayed diagnosis of ≥10 years (these patients had a mean age at onset of 19 years).[27] In developing countries, lack of awareness of PD among the general public, people living with PD, and healthcare professionals can further contribute to delays in obtaining proper diagnosis and care.[5,28-30]

This case also illustrates the value of a specific *genetic* diagnosis, which when combined with appropriate genetic counseling could potentially have averted an unnecessary abortion, since the risk for maternal-to-child transmission of PD in the setting of an autosomal recessive genetic mutation is rather low. Genetic testing of the father (husband of the patient) could even have excluded an increased risk for a genetic form of PD. We note that although the clinical presentation (juvenile onset, severe motor complications, affected siblings but unaffected parents) might already

Table 1: Demographic and clinical features of our patient with a homozygous p.Cys441Arg (c.1321T>C) *PRKN* mutation

Gender	F
Age at PD motor symptom onset /Disease duration (yrs)	13 / 34 (still alive)
Ancestry / Geographic location	Chinese / Malaysia
Family history	✓
Met QSBB clinical diagnostic criteria for PD	✓
Bradykinesia	✓
Rigidity	✓
Tremor	✓
Gait difficulty	✓
Gait freezing	✓
Postural instability / Falls	✓
Dystonia	✓
Obvious asymmetry	✓
Diurnal variation	✓
Sleep benefit	✓
UMN signs	×
Clear favourable response to dopaminergic medication	✓
Motor fluctuations	✓
LD-induced dyskinesias	✓
RBD	×
Insomnia	✓
Excessive daytime sleepiness	×
Depression	✓ (mild)
Anxiety	✓
Mild cognitive impairment	✓
Dementia	×
Visual hallucinations	×
Impulsive-compulsive behaviours	×
Constipation	×
Urinary dysfunction	×
Orthostatic giddiness or hypotension	×
Pain	×
Hyposmia	×
Underweight	× (weight gain)
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.

have suggested a high likelihood of autosomal recessive young-onset PD (AR-YOPD) on clinical grounds alone, we recently encountered a pair of Malaysian siblings of Chinese ancestry with phenotype suggestive of AR-YOPD but ultimately turned out to have (autosomal dominant) *LRRK2* R1441C mutations instead.[7] Levodopa is now generally considered to be safe to be used during pregnancy, and does not appear to be associated with increased rates of miscarriage, birth complications or teratogenicity.[31]

AR-YOPD caused by *PRKN* and *PINK1* is generally regarded as having a "benign" clinical course with excellent dopa-response.[32] This is likely due to the relatively limited extent of extra-nigral pathology.[33,34] Even so, motor response complications can be very severe and cause major disability, sometimes resulting in worse global clinical disease severity compared to other patients with similar disease duration, as evidenced by this case[12] and a PARK-*PINK1* patient we have reported with video.[8] Whether a levodopa-sparing strategy can modify the development and progression of these complications continues to be debated.[35-37] In any case, once troublesome motor complications develop, device-aided therapies are beneficial in PARK-*Parkin*, with stronger evidence currently available for DBS[38] than for dopaminergic infusions.[39] We found case reports of PARK-*PRKN* patients treated with levodopa-carbidopa intestinal gel,[39] but not with apomorphine infusion. Despite its long-standing clinical use (mainly in Europe), a formal evidence base for apomorphine infusion was only recently demonstrated in a double-blind randomized placebo-controlled trial.[40]

In conclusion, our report adds to the very scarce literature on monogenic causes of PD in the Malaysian population, and provides further supporting evidence for the pathogenic impact of the *PRKN* p.Cys441Arg mutation. It also highlights the clinical and prognostic relevance of achieving a specific genetic diagnosis for patients. We documented a substantial benefit with apomorphine infusion therapy; future research stratifying patients according to genetic status will undoubtedly become increasingly important when studying the impact of symptomatic as well as disease-modifying treatment approaches.[41]

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