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**Twinkle-associated familial parkinsonism with Lewy pathology: Cause or predisposition?**

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## INTRODUCTION

Mitochondrial dysfunction is a recognised cause of autosomal recessive Parkinson's disease (PD) and may contribute to idiopathic disease.<sup>1</sup> Twinkle protein is a DNA helicase coded by the *C10orf2* gene which, along with polymerase gamma and other proteins, is responsible for regulating mitochondrial replication. Heterozygous *C10orf2* mutations are a recognised cause of chronic progressive ophthalmoplegia (CPEO) and other neurological manifestations, but their relationship with parkinsonism is unclear. Here, we report a case (along with postmortem examination findings) of familial parkinsonism associated with a heterozygous mutation in *C10orf2*, alongside reviewing previously published cases.

## CASE REPORT

A 61 year-old man was referred to our clinic with an 18-month history of left leg dragging and left arm motor dysfunction (e.g. difficulty putting hand in pocket). Family members commented that he had a softer voice and reduced facial expression. Non-fatigable bilateral eyelid ptosis was present on his driving licence three years earlier. His mother had been diagnosed with Parkinson's disease (PD): she presented with shuffling gait and poor balance in her early 60s, responded well to levodopa but developed peak-dose dyskinesias, and died aged 79.

Examination confirmed bilateral ptosis (palpebral fissures 8mm vertically and normal levator excursion) and a mild complex ophthalmoplegia. There was evidence of



parkinsonism (predominantly affecting the left hemibody), which improved with levodopa (**Video**,<http://links.lww.com/WNL/B196>).

MR brain scan showed patchy small vessel ischaemic changes in the pons but no other abnormalities. Single fibre electromyogram was abnormal with mean jitter duration 47 microseconds (normal <36). Acetylcholine receptor antibodies were negative. *POLG* testing revealed no pathogenic mutations. Muscle biopsy was booked but the patient did not attend. A provisional diagnosis of PD with CPEO was made, although the possibility of a unifying aetiology related to mitochondrial dysfunction was considered.

Over the next few years, additional medications (selegiline, entacapone and pramipexole) were sequentially added due to the development of motor fluctuations including mild generalised dyskinesias. Around seven years after diagnosis, he began to develop non-motor complications including cognitive decline, falls (ultimately requiring a walking frame), neuropsychiatric symptoms (visual hallucinations and paranoia) and swallowing difficulties. These progressively worsened despite medication alterations (pramipexole and selegiline stopped, rivastigmine and quetiapine started). He was eventually admitted to a nursing home and died approximately 10 years after diagnosis due to a presumed aspiration pneumonia.

Just prior to his death, further genetic testing revealed a heterozygous variant in the *C10orf2* gene on chromosome 10 (c.908G>A, p.[Arg303Gln]). Bioinformatic analysis predicted that this variant was pathogenic and had a very low allele frequency on



gnomAD (0.0012%). PD gene panel testing identified no other clinically relevant variants.

Postmortem examination revealed severe, patchy neuronal cell loss in the substantia nigra with evidence of limbic (transitional) stage Lewy body disease according to the Montine classification (**Figure**).<sup>2</sup> In the substantia nigra, alpha-synuclein immunostains labelled Lewy bodies and Lewy neurites, as well as diffusely filled perikarya. Lewy bodies were also present in the locus coeruleus, periaqueductal gray matter, basal temporal neocortex and cingulate gyrus (Figures C,D,H); but sparse in other regions of the neocortex. There were no abnormalities in the cerebellum. There was no significant deposition of tau, beta-amyloid or TDP-43 proteins.

## DISCUSSION

We propose that heterozygous *C10orf2* mutations may be a rare cause of parkinsonism and should be considered in patients with a positive family history and/or other features of a mitochondrial disorder (e.g. CPEO). We searched the literature and found eight previously reported cases of parkinsonism associated with heterozygous *C10orf2* mutations (**Table**).<sup>3-7</sup> They are unlikely to be a major contributor to overall PD heritable risk; indeed, *C10orf2* does not appear as a risk loci on the most recent meta-analysis of genome-wide association studies.<sup>8</sup>

We cannot exclude the possibility that the association may be a coincidence, especially since we were unable to perform segregation analysis. Unfortunately, only fixed brain tissue was available for the post-mortem examination which precluded



molecular analysis (such as mtDNA deletion load). In the single reported autopsy case of a patient with heterozygous *C10orf2* mutation, there was also significant loss of substantia nigra neurons (even though the patient did not have clinical evidence of parkinsonism), but no Lewy bodies were present (unlike our case).<sup>9</sup> We hope that neuropathological analysis of future cases will help to determine the precise pathological underpinnings of parkinsonism in heterozygous *C10orf2* mutation carriers.

Twinkle protein is important for maintaining mtDNA integrity. In a mouse model expressing mutant Twinkle, there was accelerated accumulation of mtDNA deletions and loss of TH-positive neurons (leading to motor impairment).<sup>10</sup> Patients with biallelic *C10orf2* mutations typically present with severe and complex neurological phenotypes (e.g. infantile-onset spinocerebellar ataxia, epilepsy, sensory polyneuropathy, Perrault syndrome, adult-onset mitochondrial myopathy) alongside systemic features, but not parkinsonism. The classical pathology in these patients includes severe neuronal loss in the substantia nigra in the absence of Lewy bodies or alpha-synuclein deposition, often accompanied by degeneration of the cerebellar-dentato-olivary system. Further studies are required to explain why most patients with biallelic *C10orf2* mutations do not exhibit parkinsonism despite demonstrating severe substantia nigra neuronal loss (which also occurs with biallelic *POLG* mutations).<sup>9</sup>

Heterogeneous neuropathology is a recognised feature of genetic PD associated with mitochondrial dysfunction. A review of autopsy findings in 18 homozygous or compound heterozygous *Parkin* cases found Lewy bodies in only 6 patients, despite evidence of neuronal loss in the substantia nigra in all cases.<sup>11</sup> In the two postmortem



cases of biallelic *PINK1* mutations, Lewy bodies were present in one<sup>12</sup> and absent in the other.<sup>13</sup> The role of heterozygous *Parkin* and *PINK1* mutations is controversial, however it is intriguing that the limited number of autopsy studies have shown diffuse Lewy bodies in both groups. This may support the concept of genetic predisposition to PD by the mitochondrial dysfunction caused by these heterozygous states, which is in line with a recent report showing greater Lewy body pathology in older patients with mitochondrial dysfunction due to a range of nuclear and mtDNA genetic defects.<sup>14</sup>

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We are grateful to Prof. Robert Taylor, Dr. Nichola Lax and Dr. Grainne Gorman (Wellcome Centre for Mitochondrial Research, Newcastle University, UK) for their advice on assessment of mitochondrial pathology in postmortem brain samples during preparation of this manuscript. D. P. Breen is supported by a Wellcome Clinical Research Career Development Fellowship (214571/Z/18/Z).

## APPENDIX 1: AUTHORS

Name	Location	Contribution
David P Breen, MBChB, PhD	University of Edinburgh, Edinburgh, Scotland	Wrote the paper
David G Munoz, MD, MSc	St Michael's Hospital, Toronto, Canada	Interpreted the post-mortem brain examination and wrote the paper
Anthony E Lang, MD	Toronto Western Hospital, Toronto, Canada	Managed the patient throughout his life and wrote the paper





**Video-<http://links.lww.com/WNL/B196>**

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## **FIGURE TITLE: Neuropathology of Twinkle-associated parkinsonism**

### **FIGURE LEGEND**

A. Low power view of the substantia nigra showing patchy loss of neurons, with areas of complete loss (green circle) contrasting with others of partial preservation (yellow circle), bar 800  $\mu\text{m}$ . Luxol Fast Blue (LFB) staining on hematoxylin and eosin.

B. Substantia nigra immunostained for alpha synuclein showing Lewy bodies (blue arrows), Lewy neurites (red arrows), and neurons with cytoplasm diffusely filled with alpha synuclein (green arrows), bar 200  $\mu\text{m}$ . The inset shows Lewy bodies (blue arrows) in two neurons on LFB staining, bar 100  $\mu\text{m}$  (applies to all insets).

C. Locus coeruleus immunostained for alpha synuclein, bar 200  $\mu\text{m}$ . The inset shows Lewy bodies on LFB staining (blue arrow).

D. Periaqueductal gray (aqueduct at center bottom) immunostained for alpha synuclein showing labelled neurons with Lewy bodies (inset, blue arrow), bar 2 mm.

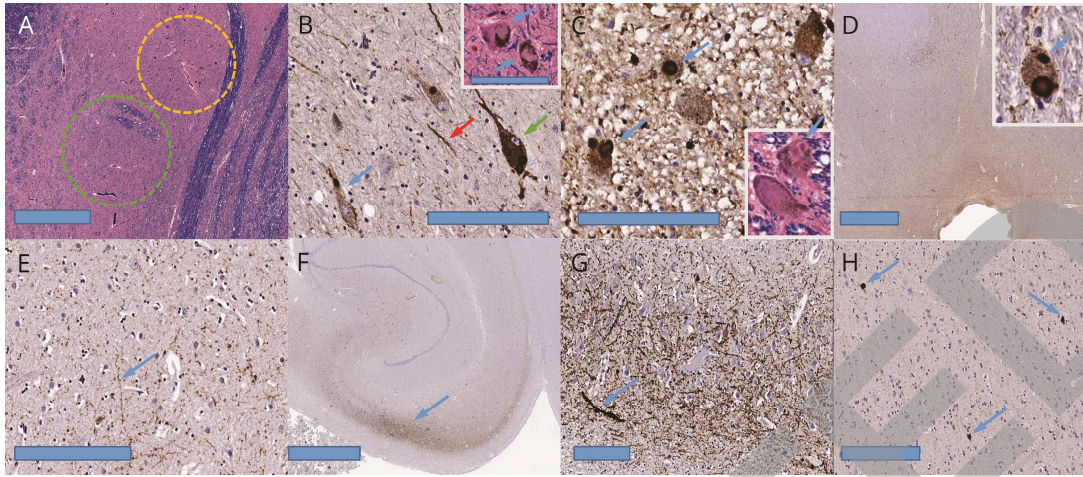
E. Putamen immunostained for alpha synuclein showing Lewy neurites (blue arrow), bar 200  $\mu\text{m}$ .

F. Hippocampus immunostained for alpha synuclein showing labelling in the CA2 sector (blue arrow), bar 2.5 mm

G. Higher power view of the alpha synuclein immunostained CA2 area of the hippocampus showing predominantly horizontally oriented Lewy neurites, bar 200  $\mu\text{m}$ .



H. Temporal neocortex immunostained for alpha synuclein showing scattered cortical Lewy bodies (blue arrows), bar 200  $\mu\text{m}$ .



**TABLE: Previously reported cases of parkinsonism associated with heterozygous *C10orf2* mutations**

Ref/case	Sex	Onset <sup>a</sup>	Family history	MR scan	DAT scan	Muscle biopsy	Levodopa response	Mutation
3/III-3	F	Early 50s	Yes	NR	NR	NR	NR	R374Q
3/III-2	F	41	Yes	Normal	NR	Abnormal	Yes	R374Q
3/III-4	F	Early 40s	Yes	NR	NR	NR	Yes	R374Q
4/	F	57	Yes	Lacunar infarct in basal ganglia	NR	NR	No	A475T
5/III-1	M	50	Yes	Normal	Abnormal	Normal	Not treated	R334Q
6/	M	70	No	Non-specific white matter lesions	Abnormal	Abnormal	Not treated	A303T
7/II-2	F	Early 80s	Yes	Mild white matter changes	Abnormal	NR	Yes	G1750A
7/II-3	F	77	Yes	NR	Abnormal	Abnormal	Not treated	G1750A

<sup>a</sup>Age of onset of parkinsonism, <sup>b</sup>One of more first-degree family member. MR=magnetic resonance, DAT=dopamine transporter, M=male, F=female, NR=not reported. All cases had CPEO which began 1-2 decades prior to their parkinsonism (three cases underwent corrective eye surgery), with one exception where the patient had childhood-onset ptosis. Similar numbers of males and females were affected and the majority had a positive family history. Dopamine transporter imaging was universally abnormal when it was performed, whilst 3 out of 5 MR brain scans showed white matter abnormalities. Muscle biopsy tended to be abnormal when it was performed (with mitochondrial changes including COX negative fibres and mtDNA deletions) but one was normal. Most patients responded to levodopa when it was initiated, although limited information was available on progression of symptoms. Brain postmortem assessment was not performed in any of these cases.

## **VIDEO LEGEND**

Examination took place a few years after he presented to our clinic. He was taking levodopa (total daily dose 800mg) and his parkinsonian signs were less marked. The video shows evidence of bilateral ptosis with mild restriction of eye movements (particularly upgaze). There was no improvement on vestibulo-ocular reflex testing (not shown). There was very mild bradykinesia in the limbs, along with mild choreiform movements. Gait was fairly normal apart from a slight lean to the right and mild impairment on tandem walking.

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