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A Peruvian family with a novel *PARK2* mutation: Clinical and Pathological Characteristics

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Abstract

Background—Mutations in *PARK2* result in autosomal recessive young onset Parkinson's disease (YOPD). Although there have been a number of reports on the clinical characteristics of *PARK2*-related PD, there is limited information available on the associated neuropathologic changes.

Design—We describe the clinical and pathological characteristics of a Peruvian family with YOPD. The proband and one unaffected sibling were screened for *PARK2* dosage and point mutations. One affected sibling had detailed neuropathologic examination.

Setting—*Instituto Nacional de Ciencias Neurológicas* (INCN) in Lima, Peru

Results—The proband and two of her four siblings developed YOPD and both parents were unaffected. The clinical course has been characterized by akinetic-rigid parkinsonism predominantly affecting the lower limbs and dyskinesias. Analysis of *PARK2* showed that the proband is compound heterozygous for a novel acceptor splice site mutation in intron 5 (IVS5-1G>A) and an exon 7 deletion. Neuropathologic assessment of an affected sibling revealed severe neuronal loss in the substantia nigra (SN) and loss of tyrosine hydroxylase immunopositive

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fibers in the striatum. No Lewy body pathology was observed using standard histology or immunohistochemistry for α -synuclein.

Conclusions—Consistent with most neuropathologic reports of patients with *PARK2* mutations, we did not observe Lewy body inclusions, despite marked SN degeneration and severe dopaminergic denervation of the striatum. These data describe a novel splice site mutation and further extend the clinicopathological characterization of *PARK2*-associated PD.

Keywords

familial parkinsonism; parkin; mutations; pathology

Introduction

Mutations of the gene encoding the parkin protein, *PARK2*, are the most common cause of autosomal recessive young-onset Parkinson's disease (YOPD).[1] Homozygous and compound heterozygous mutations of *PARK2* that lead to exon rearrangements and deletions are the most common genetic change linked to clinical parkinsonism. Although more than 100 point mutations in *PARK2* have also been reported, the pathogenicity of most is unclear.[2-5] Notably, a recent genetic population study found no compelling evidence for an association of simple (single) heterozygous *PARK2* mutations with PD.[4]

PARK2-associated PD is notable for an early age of onset, a long disease course with slow progression, and a positive family history.[3, 6, 7] Limb dystonia, choreic limb dyskinesia, early wearing-off phenomenon, and levodopa response are also described in these cases.[8, 9]

To the best of our knowledge, 17 cases of *PARK2*-related PD with both genetic and pathologic characterization have been published to date.[10-20] Common pathologic features have included severe neuronal loss in the substantia nigra (SN), variable neuronal loss in the locus coeruleus (LC), and relatively rare Lewy body pathology.[20]

We herein describe the genetic, clinical, and pathological characteristics of a Peruvian YOPD family with a newly discovered *PARK2* mutation.

Materials and Methods

Subjects

Over the past six decades, we have followed and treated a Peruvian family with early onset parkinsonism at the *Instituto Nacional de Ciencias Neurológicas* (INCN) in Lima, Peru. The INCN was opened as a shelter for homeless people with chronic disease in 1701. Between 1937 and 1984 approximately 30 patients, including three siblings from this family (Figure 1), were hospitalized, and later abandoned by family members, and became permanent residents ("living at the hospital"; frequently assisting, if able with hospital activities). Informed consents were obtained for use of clinical material and autopsy. Informed consent and Institutional Review Board approval was obtained for the genetic studies.

Neuropathology

An autopsy was performed on one member of this family (case II-2, Figure 1). Histological evaluations included hematoxylin-eosin (H&E) and modified Bielschowsky methods.

Evaluation for Lewy body pathology utilized two antibodies for α -synuclein (antibodies LB509 and syn 303, generous gifts J.Q. Trojanowski, 1:400) using methodology previously described in detail.[21] In addition, immunostaining was performed for A β (6E10, Signet Labs, 1:400), tau (Tau-2, Sigma, 1:500, 1:500; PHF-1, generous gift P. Davies, 1:10; AT8, Endogen, 1:250; RD3 and RD4, Upstate Cell Signaling Solutions, 1:800 and 1:80 respectively), and TDP-43 (Proteintech, 1:2000), also as previously described.[22]

Genetics

DNA extracted from peripheral white blood cells was available for the proband and her unaffected sister through the Latin American Research Consortium on the Genetics of Parkinson's Disease (LARGE-PD). We also attempted to obtain DNA for an affected brother (II-2) using brain tissue that had been fixed for over 20 years, but were unsuccessful. All 12 coding exons as well as corresponding intron/exon boundaries were studied by direct sequencing (primers and PCR protocols available upon request). Exon dosage analysis was conducted using multiplex ligation-dependent probe amplification (MLPA; MRC-Holland, Amsterdam: <http://www.mrc-holland.com>). There are 2 commercial probe kits available for *PARK2*: SALSA P051 and P052. We used both kits, P052 for primary analysis and P051 for replication, as there is little overlap in the probes contained in each kit. Assays and analysis were performed according to the manufacturer's protocol, using a minimum of 100 ng of genomic DNA per sample. We verified all MLPA abnormal exon dosage results using real-time qPCR as previously described. [4]

Results

Three affected siblings were brought to INCN by their family; one left after a short stay (II-1), while the other two continued to live at the hospital for decades. The proband (II-3) continues to reside at the INCN and was evaluated clinically and a blood sample was taken for molecular analysis. One sibling (II-2) died at age 60 after 44 years at the INCN and underwent autopsy. One sibling (II-5) is followed by her primary neurologist (LT) and continues to show no symptoms or signs of neurologic disease (Figure 1).

II-3 (Proband)

She was born in the central highlands of Peru and is currently 74 year-old. She initially developed parkinsonism at 15 years of age with prominent rigidity, global bradykinesia, and mild bilateral resting tremor that was most prominent in her left lower extremity. She was hospitalized at age 16 years at the *INCN*, where she has lived continuously since that admission. The clinical course has been characterized by prominent lower versus upper extremity involvement leading to wheelchair dependence after 10 years of disease. Initial treatment used trihexiphenidyl with limited therapeutic responsiveness. She was switched to biperiden and continues on that therapy. A trial of high-dose levodopa therapy (in the 1970s, without carbidopa) was discontinued due to severe gastrointestinal side effects and she

refused all subsequent offers of levodopa therapy. She currently is also on a pramipexole trial, but this has been associated with the development of generalized moderate dyskinesias. Currently she is severely dysarthric, confined to a wheelchair, and fully dependent for activities of daily living, both basic and instrumental. On mental status examination she is alert and oriented to person and place. However, she scores poorly on both the mini-mental status examination (9 out of 30 points) and Montreal Cognitive Assessment (4 out of 30 points). Notably, she has had limited education (3 years of formal education) and has significant verbal communication difficulties. On motor examination she has a mild bilateral postural tremor, no resting tremor, moderate and persistent dyskinesias in both limbs and trunk, and left lower extremity dystonic posturing. Unified Parkinson's Disease Rating Scale Part III motor score was 62 [23] and Hoehn & Yahr scale was a V. MRI brain imaging at age 74 years revealed moderate to severe cortical atrophy and imaging changes in the right frontal lobe consistent with an old infarct.

Molecular analysis for *PARK2* dosage and point mutations identified an exon 7 deletion (ratio 0.5) in one chromosome (Figure 2A) and a novel heterozygous point mutation (not previously reported in public databases) within the acceptor splice site of intron 5 (IVS5-1G>A) (Figure 2B) in the other. Several splice prediction tools (SplicePort [<http://spliceport.cs.umd.edu/>], NetGene2 [<http://www.cbs.dtu.dk/services/NetGene2/>], NNSPLICE 0.9 [http://www.fruitfly.org/seq_tools/splice.html], and Human Splicing Finder HSF [<http://www.umd.be/HSF/>]) predicted that this variant generates an alternative splice site one base pair downstream from the normal acceptor splice site (CAG/GAA [HSF score= -30.32] to CAAG/AA [HSF score=+52]). This causes a frameshift which results in a truncated protein.

II-2 (Affected sibling/autopsy case)

He had symptom onset at age 14 years with rigidity and bradykinesia, more prominent in the left lower limb with gait disturbance and resting tremor. His clinical course was characterized by slow progression, predominantly affecting the lower limbs, leading to wheelchair dependence after 10 years of disease. Notably, his upper limbs were mildly affected. He died at age 60 after a stroke and respiratory complications.

The autopsy showed that the brain weighed 1250 g and had signs of atherosclerosis, infarcts in the left internal capsule and left parietal cortex, and depigmentation of the substantia nigra. Microscopic examination revealed severe loss of pigmented neurons in the SN without evidence of significant gliosis (Figure 3A). No Lewy bodies or other inclusions were noted with H&E staining. Neuronal number appeared to be preserved in the locus coeruleus (Figure 3B). Examination of the remainder of the brainstem, limbic system, and neocortex failed to reveal significant pathology, except for changes consistent with infarction. The medulla, locus coeruleus, substantia nigra, amygdala, hippocampus, cingulate gyrus, and frontal cortex were negative for α -synuclein immunopositive pathology (LB509 and syn303). Staining with ubiquitin, TDP-43, A β , and tau antibodies also failed to reveal pathologic changes except for Braak stage II [24] neurofibrillary tangle pathology (Figure 3E). There was marked loss of tyrosine hydroxylase immunostained fibers in the striatum (Figure 3C and 3D).

We attempted to obtain DNA using his brain tissue but were unsuccessful probably due to the fact that had been fixed for over 20 years,

II-5 (Unaffected sibling)

Screening for *PARK2* dosage and point mutations in this unaffected sibling showed the presence of the same novel heterozygous point mutation within the acceptor splice site of intron 5 (IVS5-1G>A) identified in the proband. However she did not carry the exon 7 deletion or any other dosage or point mutations.

II-1 (Affected sibling)

He had an age of onset (15 years) and initial clinical course very similar to the other affected siblings with lower extremity predominance of symptoms. He died at age 26 years due to an automobile accident.

Discussion

We identified a Peruvian family with YOPD in which the proband (II-3) was compound heterozygous for two *PARK2* mutations *in trans*, a deletion of exon 7 and a novel point mutation in the acceptor splice site of intron 5 (IVS5-1G>A). Deletions of exon 7 are a well-established cause of *PARK2*-related PD based on data from humans and animal models.[2, 25] We believe that IVS5-1G>A is also pathogenic based on *in silico* predictions that suggest this variant causes a frameshift and introduction of a premature stop codon at amino acid position 223 which results in a truncated protein product that lacks several functional domains including RING1, IBR, and RING2. However, an important caveat is that we could not unequivocally demonstrate segregation of IVS5-1G>A with disease since we were only able to genotype two members of the pedigree.

The neuropathologic changes observed in our autopsied patient are consistent with many, but not all, reports of genetically confirmed cases of *PARK2*-associated YOPD. In our case, we observed severe neuronal loss in the SN, but without evidence of any associated Lewy body, α -synuclein, or tau pathology. We confirmed severe loss of tyrosine hydroxylase immunopositive fibers in the striatum, consistent with the substantial loss of SN neurons. Of the seventeen previously reported *PARK2*-associated cases with neuropathologic characterization, five showed the presence of Lewy body pathology [10, 15, 20, 26], 2 lacked classic Lewy bodies but had α -synuclein pathology in the form of Lewy neurites [20] or atypical α -synuclein positive inclusion bodies [17] and ten lacked abnormal α -synuclein deposition of any kind.[11-14, 18-20, 27] Farrer et al were the first to report a potential *PARK2*-associated case with classic PD-like Lewy body pathology in a patient with an exon 3 deletion and a point mutation (p.R275W) located in the RING1 domain.[10] Sasaki and colleagues [17] reported on a case with a homozygous exon 3 deletion and with α -synuclein pathology restricted to the pedunculopontine nucleus. Here, the authors observed round, α -synuclein positive inclusions that differed in appearance from Lewy bodies in that they were basophilic.[17] Pramstaller and colleagues [15] reported a compound heterozygote with exon 7 and 9 deletions and Lewy body pathologic changes. Miyakawa and colleagues reported a case of late onset PD (61 years old) with a homozygous exon 2-4 deletion with

Lewy body pathology detected in many brain areas in a pattern consistent with sporadic PD. [26] Most recently, Doherty and colleagues reported 3 PD cases with α -synuclein pathology. Two had Lewy bodies located in brainstem and/or transentorhinal cortex and one lacked Lewy bodies but had sparse Lewy neurites in the SN.[20] Thus, including our case, the majority of *PARK2* -associated PD cases examined to date (11 of 18), fail to show Lewy body or any α -synuclein pathology.

Interestingly, these α -synuclein negative cases had an earlier onset (mean age of onset of 26.0 years), compared to the seven reported cases with convincing pathogenic *PARK2* mutations and α -synuclein pathology which had a mean age of onset of 42.1 years. [10, 15, 17, 20, 26] Also notable is the fact that three out of the seven cases with α -synuclein pathology were heterozygous for the p.R275W mutation. [10, 20] This mutation has been previously reported to retain a functional RING domain, preserving the E3 ligase activity and the ability to produce aggresomes. [28] Thus, it appears that a subset of *PARK2* mutation-associated YOPD cases can develop Lewy body/ α -synuclein pathologic changes and the limited data to date suggest that this might be associated with mutations that cause a partial loss of function such as p.R275W. This may also explain the lower age at onset observed in α -synuclein negative cases in which the mutations frequently cause a total loss of protein function.

The clinical syndrome observed in our family is generally consistent with that reported in other cases of *PARK2*-associated YOPD.[29] Age of onset was less than 16 years for all affected individuals with a very prolonged clinical course in cases II-2 and II-3. Other commonly reported features, including dyskinesias and dystonias, were also been observed in the proband. Response to anticholinergics, seen in our proband, has also been reported in *PARK2*-associated YOPD.[30] However, all three affected siblings displayed prominent involvement of the lower extremities leading to relatively early wheelchair dependence, which is not typical of *PARK2*-related PD. [29, 30]

In conclusion, we report a novel *PARK2* mutation in a Peruvian family with YOPD. Consistent with most, but not all, neuropathologic reports of patients with *PARK2*-related PD, we did not observe Lewy bodies or abnormal α -synuclein deposition, perhaps due to the more deleterious nature of the mutations in our family. This is despite marked SN degeneration and severe dopaminergic denervation of the striatum.

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Highlights

- We are describing a Peruvian family with early onset parkinsonism followed and treated for more than 6 decades at the Instituto Nacional de Ciencias Neurológicas (INCN) in Lima, Peru
- We identified in our family two *PARK2* mutations in trans, a deletion of exon 7 and a novel point mutation in the acceptor splice site of intron 5 (IVS5-1G>A)
- The neuropathologic changes observed in one autopsied patient from this family are similar to most reports of genetically confirmed cases of *PARK2*-associated YOPD, including severe neuronal loss in the SN, but without evidence of any associated Lewy body, α -synuclein, or tau pathology

Figure 1.
Family pedigree. Blackened symbols denote subjects with PD.

Figure 2.

A) Proband's MLPA (P052 kit) ratio chart showing *PARK2* heterozygous deletion of exon 7. Orange lines highlight upper and lower normal dosage range. B) IVS5-1G>A Sequence and predicted change in the splice site.

Figure 3.

Neuropathologic findings in Case II-2 revealed severe neuronal loss in the substantia nigra (A), but relative preservation of neuronal number in the locus coeruleus (B). Tyrosine hydroxylase immunohistochemistry revealed severe loss of immunopositive fibers in the striatum (C) in comparison to a normal striatum (D). Tau immunopositive (AT8) inclusion and neuritis in the entorhinal cortex, consistent with Braak stage II (E). Bar = 150µm (A) and 75µm (B-E)