

# Genetic Paradoxes in an Italian Family with *PARK2* Multiexon Duplication

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Biallelic mutations in the *PARK2* gene, which encodes Parkin, are the most common cause of autosomal recessive, early onset Parkinson disease (EOPD), with frequency inversely correlated with age at onset and complete penetrance. Single heterozygous *PARK2* mutations are occasionally detected in patients with PD and in healthy individuals and are considered minor susceptibility factors toward the risk of sporadic later onset PD.<sup>1</sup>

## Case Report

We report on a 41-year-old male patient with EOPD (Fig. 1A). Motor signs and symptoms started at age 36 years with slowness and rigidity of the left upper limb. A dopamine (L-dopa) transporter (DAT) scan revealed bilaterally reduced presynaptic dopaminergic captation with right prevalence. Clinical progression of symptoms was slow, and motor signs greatly improved with dopaminergic medications (ropinirole 8 mg daily and L-dopa/carbidopa 300/75 mg daily in 3 doses). The latest examination showed mild bradykinesia and rigidity of the upper limbs with left predominance, reduced arm movements during walking, and mild hypomimia (Fig. 2A, C, Video S1). Psychiatric assessment revealed depression and mystic delusions, which started soon after the onset of motor symptoms and required treatment with atypical antipsychotics. The patient denied olfaction disturbances, constipation, or sleep abnormalities such as rapid eye movement sleep behavior disorder.

Because of the early age at onset and clinical presentation, the patient underwent genetic testing for EOPD genes. Multiplex ligation-dependent probe analysis (MLPA) (SALSA kit P051; MRC Holland, Amsterdam, the Netherlands) detected a heterozygous duplication of Exons 2 and 3 of the *PARK2*

gene, which was subsequently confirmed by quantitative real time polymerase chain reaction (qRT-PCR) on genomic DNA (Fig. 1C). No other pathogenetic variants emerged from Sanger sequencing of all exons or exon-intron junctions of the genes *PARK2*, phosphatase and tensin homolog-induced kinase 1 (*PINK1*), Daisuke-Junko-1 (*DJ-1*), guanosine triphosphate cyclohydrolase1; (*GCH1*), and glucocerebrosidase (*GBA*).

To properly phase the exon duplications, segregation analysis was performed. However, the patient's mother and brother did not carry any *PARK2* exon duplication, suggesting that the proband was heterozygous for a duplication of Exons 2 and 3 *in cis* on the same allele. To further confirm this observation, we obtained a blood sample from the 71-year-old paternal aunt, because the patient's father was deceased. Surprisingly, by MLPA and qRT-PCR analysis, this lady was identified as homozygous for Exon 2 and 3 duplication, because she carried 4 copies of *PARK2* Exons 2 and 3 (Fig. 1B). Clinical examination failed to identify any parkinsonian signs but disclosed a postural-action tremor and right-hand dystonic posturing during writing, which started since her 40s. The patient denied olfaction disturbances, constipation, or sleep abnormalities such as rapid eye movement sleep behavior disorder. Qualitative data revealed a mild decrease in DAT in the left caudate and putamen (Fig. 2B, Video S2).

This family is an interesting example of a paradox of Mendelian genetics: on 1 hand, the single heterozygous proband presents with typical EOPD, a phenotype that is usually seen in carriers of biallelic mutations; on the other hand, the homozygous 71-year-old aunt is nearly asymptomatic, although *PARK2* biallelic mutations are usually considered fully penetrant by the fifth decade of life.

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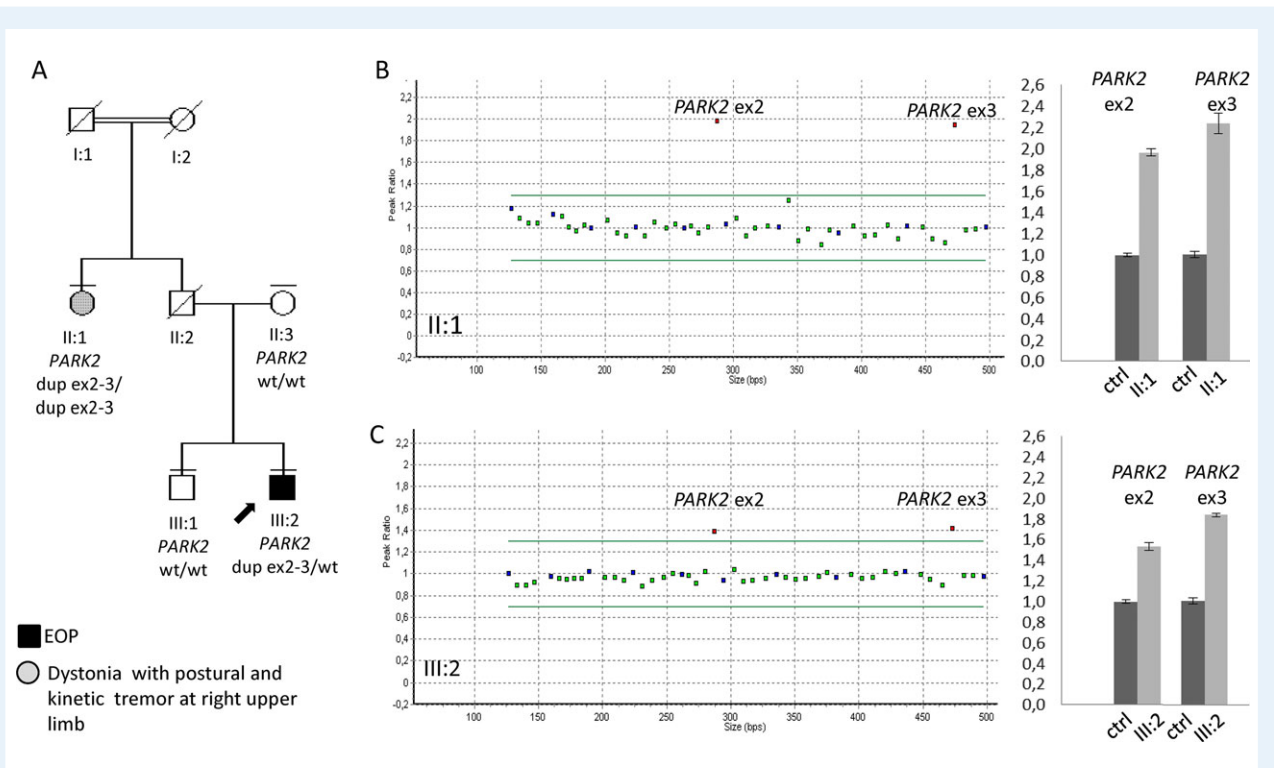
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Relevant disclosures and conflicts of interest are listed at the end of this article.

Supporting information may be found in the online version of this article.

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**FIG. 1.** **A:** Pedigree of the Italian family. Males and females are represented by squares and circles, respectively. Dead family members are marked with a diagonal bar. Double lines indicate a consanguineous marriage; horizontal bars above symbols denote individuals who underwent genetic testing, the black symbol denotes early onset Parkinson disease (EOP), and the gray symbol refers to tremor and dystonia without parkinsonism. Dup indicates duplicate; wt, wild type. **B,C:** Multiplex ligation-dependent probe analysis (*left*) and quantitative real-time polymerase chain reaction (*right*) indicate **(B)** 4 copies of Exons 2 and 3 (ex2 and ex3, respectively) in the aunt (II:1) and **(C)** three copies of the same exons in the proband (III:2). Quantitative real-time polymerase chain reaction was performed on genomic DNA by amplification of parkin 2 (*PARK2*) Exons 2 and 3. Normalization was obtained using *telomerase* as a housekeeping gene. Ctrl indicates wild-type control.

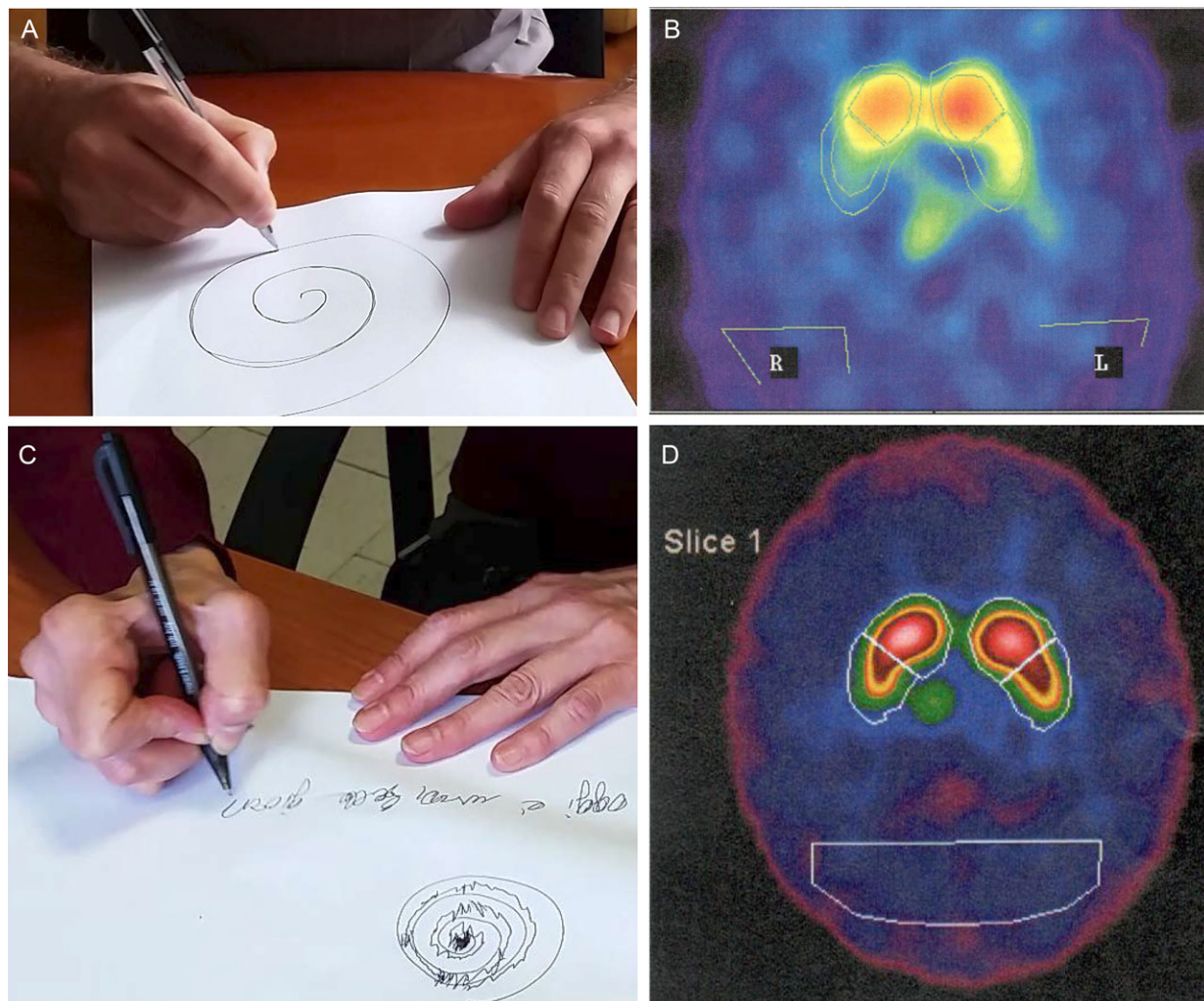
In the proband, molecular testing of other EOPD genes failed to detect additional mutations, and obvious environmental or acquired causes of PD were excluded. However, it must be noted that single heterozygous *PARK2* mutations have occasionally been reported in patients with EOPD.<sup>2</sup> Even the same heterozygous Exon 2 and 3 duplication was identified in 4 patients with sporadic PD (of whom 1 had EOPD) and 3 asymptomatic relatives.<sup>3–6</sup> We can speculate that, along with the heterozygous *PARK2* duplication, other yet unidentified factors may have contributed to the development of EOPD in this patient.

Even more puzzling is the extremely mild phenotype presented by the aunt, who was homozygous for *PARK2* Exon 2 and 3 duplication. In the literature, patients with the same homozygous duplication have never been reported, but this rearrangement was observed in compound heterozygosity with the p.(K211N) variant in 3 siblings with EOPD.<sup>7</sup> The only observed movement disorders in this lady were action tremor and dystonic posturing. Isolated dystonia is often the presenting symptom in patients who have *PARK2* mutations; however, dystonia usually involves the lower limbs and is always followed by the onset of parkinsonian signs.<sup>8</sup>

Only 2 asymptomatic (or mildly symptomatic) carriers of biallelic *PARK2* mutations have been reported to date, and

none of their heterozygous family members had PD. The first is the healthy 56-year-old sister of 4 patients with typical EOPD: All 5 of these siblings are compound heterozygotes for the p.(T240M) variant and the deletion of Exons 5 and 6.<sup>9</sup> The second is the mother of a Greek proband with typical EOPD because of compound heterozygous deletions of *PARK2* Exon 2 and Exons 5 through 7. The mother, a 71-year-old lady who presented only with slight rigidity, mildly reduced left arm swing, and a 20-year history of depression, was homozygous for *PARK2* Exon 2 deletion. Functional experiments on fibroblasts from this lady revealed a PINK1-Parkin-independent route of mitophagy activation, suggesting that the reduced penetrance rarely observed in *PARK2* mutation carriers could be caused by compensatory mechanisms at the cellular level.<sup>10</sup> Neither of these women underwent functional neuroimaging. In our patient, DAT scan examination was clearly abnormal, confirming an underlying dopaminergic denervation, which apparently was not sufficient to reach a threshold for the manifestation of a full parkinsonian phenotype.

The paradox presented here underlines the difficulties that clinicians may encounter in establishing genotype-phenotype correlates and providing adequate genetic counseling to patients and relatives, and the results confirm that monogenic disorders



**FIG. 2.** A,B: Spiral test and hand posture during writing were (A) normal in the proband but (B) showed tremor and dystonia in the aunt. C,D: A dopamine transporter scan of (C) the proband reveals reduced bilateral dopaminergic uptake at the basal ganglia, and (D) a scan of his aunt shows reduced dopaminergic uptake on the right side.

are more complex than previously thought. The identification of the genetic, epigenetic, and environmental landscape able to modulate the penetrance and expressivity of single gene mutations currently represents a major challenge of genetic research.

## Author Roles:

1. Research Project: A. Conception, B. Organization, C. Execution; 2. Statistical analysis: A. Design, B. Execution, C. Review and Critique; 3. Manuscript preparation: A. Writing the First Draft, B. Review and Critique.

S.P.: 1A, 1C, 2B, 3A, 3B

G.F.: 1B, 1C, 3B

M.G.: 1C, 2B, 3B

M.T.: 1C, 2B, 3B

I.B.: 1C, 3B

A.B.: 1A, 1B, 3B

G.F.: 1A, 1B, 3A, 3B

E.M.V.: 1B, 2C, 3B

## Disclosures

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## Supporting Information

**Video S1.** This video shows the proband displaying mild hypomimia with reduced spontaneous blinking, mild bradykinesia in the left limbs during the finger tapping and foot tapping tasks, no tremor, and normal writing.

**Video S2.** This video shows the proband’s aunt displaying dystonic posturing of the right hand with tremor.