Clinical Genetics

WILEY

CLINICAL GENETICS

RESEARCH LETTER OPEN ACCESS

Biallelic *RFC1* Expansions Are a Rare Cause of Early-Onset and Familial Parkinson's Disease

Anja Kovanda[1,2] | Lara Šušmelj[1] | Helena Jaklič[1,2] | Tadeja Lukežič[1,2] | Aleš Maver[1,2] | Igor Petrovic[3] | Natasa Dragasevic Miskovic[3] | Marina Svetel[3] | Valentino Rački[4] | Vladimira Vuletič[4] | Ivana Novakovic[5] | Borut Peterlin[1,2]

[1]Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia | [2]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia | [3]Neurology Clinic, Faculty of Medicine, University of Belgrade, Belgrade, Serbia | [4]Department of Neurology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia | [5]Institute of Human Genetics and Neurology Clinic, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Correspondence: Borut Peterlin (borut.peterlin@kclj.si; borut.peterlin@guest.arnes.si)

Received: 9 September 2025 | **Revised:** 14 October 2025 | **Accepted:** 15 October 2025

Funding: This work was supported by the Slovenian Research and Innovation Agency (ARIS) (P3-0326, J3-4517).

Parkinson's disease (PD) is a progressive neurodegenerative disease with an average onset around 65 years of age. Onset before age 50 is considered early-onset PD (EOPD), and about 15% of patients have hereditary or familial PD (FPD). Several genes are known to cause monogenic PD; however, ~90% of patients remain undiagnosed, even in FPD and EOPD. The motor and non-motor symptoms, as well as molecular pathways of PD, show overlap with other neurological disorders. The cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) is an autosomal recessive (AR) late-onset neurodegenerative disorder that is, in most cases, caused by biallelic $(AAGGG)_{exp}$ in the *RFC1* gene; although, compound heterozygosity with pathogenic single nucleotide variants has also been reported [1]. Recent studies show only two-thirds of patients present with the complete CANVAS phenotype and that biallelic $(AAGGG)_{exp}$ may be present in patients suspected of having other disorders. Indeed, biallelic $(AAGGG)_{exp}$ was identified in patients with medicated parkinsonism and EOPD [2, 3] in the Finnish population, and also, much more rarely, even in sporadic PD patients [4]. Therefore, it appears that biallelic $(AAGGG)_{exp}$ may represent a rare cause of PD with no or minimal additional clinical signs of CANVAS, and thus determining the prevalence of *RFC1* expansions in FPD is of particular interest in the field of PD genetics.

We tested 135 patients with FPD (66, 48.9%) and/or EOPD (69, 51.1%) who were part of a previously established cohort and provided written informed consent for genetic testing [5]. All were negative for monogenic PD by exome sequencing (ES) and MLPA testing [5]. CANVAS testing was performed by using a two-step fragment analysis PCR and ES testing approach, recently developed to facilitate routine CANVAS testing [6].

In our EOPD and FPD cohort, we found biallelic $(AAGGG)_{exp}$ in *RFC1* in 2/135 patients (1.5%) (Table 1).

Both positive patients had EOPD, with one patient reporting a relative with EOPD. Similarly to recent studies, our PD patients did not present with additional distinct CANVAS presentations [2, 3].

We additionally found four heterozygous pathogenic expansion carriers (Table 1, 4/135, 2.6%), in whom no pathogenic SNV in *RFC1* was identified; therefore, in whom the heterozygous state does not currently explain the clinical phenotype.

Previous Finnish population study reports the prevalence of biallelic $(AAGGG)_{exp}$ in patients with EOPD and medicated parkinsonism to be between 1.1% and 1.9% [2, 3], which is similar to our observation of 1.5%. Interestingly, recent research on sporadic PD patients from the Parkinson's Progression Markers Initiative study found the estimated frequency of biallelic pathogenic *RFC1* expansions in PD to be 0.43% [4].

While the original research of CANVAS suggested the allelic frequency of the $(AAGGG)_{exp}$ to be between 0.7% and 4.0% in different populations, Finnish researchers found an estimated

TABLE 1 | Results of two-step PCR and ES testing in patients with PD.

Screening PCR	No. of patients (%)	Confirmatory RP-PCR result			ES re-analysis of *RFC1* SNV	No. of patients (%)	Biallelic pathogenic *RFC1* variants	No. of patients (%)
		$(AAAAG)_{exp}$	$(AAAGG)_{exp}$	$(AAGGG)_{exp}$				
Both alleles $n < 250$	36 (26.7)	/	/	/	/	36 (26.7)	**Negative**	**133 (98.5)**
One allele below $n < 250$	95 (70.3)	Yes	No	No	/	83 (61.5)		
		Yes	Yes	No	/	7 (5.2)		
		No	Yes	No	/	2 (1.5)		
		Yes	No	Yes	Negative	1 (0.7)		
		No	No	Yes	Negative	2 (1.5)		
No alleles below $n < 250$	4 (3.0)	Yes	No	No	/	1 (0.7)		
		Yes	No	Yes	Negative	1 (0.7)		
		No	No	Yes	/	2 (1.5)	**Positive**	**2 (1.5)**
							Total	**135 (100)**

allele frequency of 0.5% [2, 3] in their general population. Interestingly, in the Parkinson's Progression Markers Initiative study, which used short-read sequencing analysis for *RFC1* biallelic expansion screening and long-read sequencing using Oxford Nanopore Technology for validation, no carriers were identified among the controls [4]. This suggests that perhaps the short-read screening and long-read sequencing confirmation approach may underestimate the true prevalence of the pathogenic $(AAGGG)_{exp}$ in carrier and biallelic states, while the original research has perhaps initially overestimated the prevalence of the $(AAGGG)_{exp}$. This highlights both the potential genetic heterogeneity of the expansion across different populations and the difficulties and discrepancies associated with existing laboratory methods of detection.

While the mechanisms of pathogenicity remain to be explained, G-quadruplex-mediated toxicity has also been suggested to play a role independently of the loss-of-function mechanism of *RFC1*. The disease mechanisms in PD remain to be determined, in particular whether, in addition to the *RFC1* expansion, similar pathogenic expansions will be identified in PD-associated genes with AR inheritance in the future and whether such discoveries will help explain the missing genetic contribution in FPD and EOPD.

Indeed, in our well-characterized FPD and EOPD cohort, the pathogenic biallelic *RFC1* expansion was more common than previously identified compound heterozygous *PARK2* pathogenic variants [5], suggesting *RFC1* expansion testing is merited in FPD and EOPD patients alike.

The results of our study represent an independent confirmation that pathogenic biallelic *RFC1* expansions contribute to the genetic etiology of PD and can be found in FPD and EOPD with a frequency similar to that of established AR PD genes. While currently *RFC1*-positive PD is not clearly distinguishable from *RFC1-negative* PD, such genetic background may play a role in future therapies or other interventions.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. A. Cortese, M. M. Reilly, and H. Houlden, *RFC1 CANVAS/Spectrum Disorder* (University of Washington, Seattle, 2025), http://www.ncbi.nlm.nih.gov/books/NBK564656/.

2. P. Ylikotila, J. Sipilä, T. Alapirtti, et al., "Association of Biallelic RFC1 Expansion With Early-Onset Parkinson's Disease," *European Journal of Neurology* 30 (2023): 1256–1261.

3. L. Kytövuori, J. Sipilä, H. Doi, et al., "Biallelic Expansion in RFC1 as a Rare Cause of Parkinson's Disease," *NPJ Parkinsons Disease* 8, no. 1 (2022): 6.

4. P. Alvarez Jerez, K. Daida, A. Miano-Burkhardt, et al., "Profiling Complex Repeat Expansions in RFC1 in Parkinson's Disease," *NPJ Parkinsons Disease* 10, no. 1 (2024): 108.

5. A. Kovanda, V. Rački, G. Bergant, et al., "A Multicenter Study of Genetic Testing for Parkinson's Disease in the Clinical Setting," *NPJ Parkinsons Disease* 8, no. 1 (2022): 149.

6. H. Jaklič, I. B. Božović, B. Peterlin, and A. Kovanda, "Streamlined Two-Step Fragment Analysis PCR and Exome Sequencing of RFC1 for Diagnostic Testing of Suspected CANVAS Patients," *Clinical Genetics* 106 (2024): 632–637.