Received: 22 December 2022 | Accepted: 23 January 2023

DOI: 10.1111/ene.15717

european journal of neurology
the official journal of the european academy of neurology

ORIGINAL ARTICLE

Association of biallelic *RFC1* expansion with early-onset Parkinson's disease

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Funding information
Sigrid Jusélius Foundation; Terttu Foundation; Yrjö Jahnsson Foundation; Finnish Parkinson Foundation; Japan Agency for Medical Research and Development (AMED), Grant/Award Number: JP22ek0109348, JP22ek0109549 and JP22ek0109486; JSPS Kakenhi, Grant/Award Number: JP 21K0786 and JP20K07907; Medical Research Center Oulu; Oulu University Hospital

Abstract

Background and Purpose: The biallelic repeat expansion $(AAGGG)_{exp}$ in the replication factor C subunit 1 gene (*RFC1*) is a frequent cause of cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) as well as late-onset ataxia. The clinical spectrum of RFC1 disease has expanded since the first identification of biallelic $(AAGGG)_{exp}$ and includes now various nonclassical phenotypes. Biallelic $(AAGGG)_{exp}$ in *RFC1* in patients with clinically confirmed Parkinson's disease (PD) has recently been found.

Methods: A nationwide cohort of 273 Finnish patients with early-onset PD was examined for the biallelic intronic expansion in *RFC1*. The expansion $(AAGGG)_{exp}$ was first screened using extra long polymerase chain reactions (Extra Large-PCRs) and flanking multiplex PCR. The presence of biallelic $(AAGGG)_{exp}$ was then confirmed by repeat-primed PCR and, finally, the repeat length was determined by long-read sequencing.

Results: Three patients were found with the biallelic $(AAGGG)_{exp}$ in *RFC1* giving a frequency of 1.10% (0.23%–3.18%; 95% confidence interval). The three patients fulfilled the diagnostic criteria of PD, none of them had ataxia or neuropathy, and only one patient had a mild vestibular dysfunction. The age at onset of PD symptoms was 40–48 years and their disease course had been unremarkable apart from the early onset.

Conclusions: Our results suggest that $(AAGGG)_{exp}$ in *RFC1* is a rare cause of early-onset PD. Other populations should be examined in order to determine whether our findings are specific to the Finnish population.

KEYWORDS
Parkinson's disease, repeat expansion diseases, RFC1 disease

INTRODUCTION

Several genes have been linked to monogenic Parkinson's disease (PD) [1]. Biallelic repeat expansion $(AAGGG)_{exp}$ in the replication factor C subunit 1 gene (*RFC1*) has recently been shown to be a frequent cause of cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS), as well as late-onset ataxia [2, 3]. The phenotype of *RFC1* $(AAGGG)_{exp}$ is variable and may also include autonomic, extrapyramidal and cognitive signs [4, 5]. Biallelic *RFC1* $(AAGGG)_{exp}$ has also been found in some patients with parkinsonism [4, 6]. Furthermore, nonclassical phenotypes, such as multiple system atrophy or amyotrophic lateral sclerosis, have been described [7, 8].

The biallelic *RFC1* $(AAGGG)_{exp}$ has recently been found in three Finnish patients with PD indicating that the phenotypic spectrum of *RFC1* expansion includes rare cases of PD [9]. A conspicuous feature of genetic PD is an earlier onset of symptoms [1]. Here a nationwide cohort of Finnish patients with clinically diagnosed early-onset PD (EOPD) was investigated for the *RFC1* expansion.

METHODS

A nationwide cohort of 441 Finnish patients with medicated PD who were abstracted from the medication reimbursement registry maintained by the Social Insurance Institution of Finland (Kela) has been characterized previously [10]. Permanent residents in Finland are entitled to full reimbursement for prescription medication in PD that is granted on the basis of a written statement by a general neurologist confirming that the diagnosis fulfils international criteria for PD. Patients <55 years of age at the time of the PD diagnosis were screened and the 273 patients <50 years of age at the time of first symptoms were defined as EOPD. Patients with secondary parkinsonism and Parkinson's plus syndromes were excluded. Patients with *RFC1* $(AAGGG)_{exp}$ were examined clinically to verify that their phenotype conformed to the Movement Disorder Society (MDS) criteria of PD and to verify possible signs of CANVAS.

Molecular genetics

Screening of the biallelic $(AAGGG)_{exp}$ was carried out using extra long polymerase chain reactions (Extra Large-PCRs) and flanking multiplex PCR for the intronic expansion region, as previously (Figure 1) [5, 9]. The core haplotype defined by four single nucleotide polymorphisms (SNPs) (4-39364970-T-C (rs6844176), 4-39363236-T-C [rs17584703]), 4-39327482-GA (rs11096992) and 4-39317086-A-G (rs2066790) (GRCh38) was determined as previously [9]. A more detailed haplotype was constructed from the exome sequencing data [11]. Repeat-primed PCR was used to confirm the presence of the biallelic $(AAGGG)_{exp}$ and the repeat length was determined for the three patients by long-read sequencing (Oxford Nanopore Technologies) [9]. Additional southern blotting was performed for patients P1 and P2 but could not be carried out for patient P3 because of the low DNA concentration. Pathogenic variants in genes causing PD has previously been excluded [11].

Ethics

The study has been approved by the Ethics Committees of Turku University Hospital and Yokohama City University Faculty of Medicine. Patients or their legal caregivers gave their written informed consent and patient P2 gave her written informed consent to publish identifiable material. All clinical examinations were done according to the Declaration of Helsinki (2013).

RESULTS

Three out of the 273 patients with EOPD were found to harbour biallelic $(AAGGG)_{exp}$ giving a genotype frequency of 1.10% (95% confidence interval 0.23%–3.18%). The number of repeated units varied from 141 to 831 (Figure 1). The three patients fulfilled the MDS diagnostic criteria for PD, none of them had symptoms or signs of ataxia or neuropathy, or reported chronic cough, and only one patient had mild vestibular dysfunction (Table 1). The median age at onset of

FIGURE 1 Investigation of biallelic *RFC1* expansion $(AAGGG)_{exp}$ in three patients with early-onset Parkinson's disease. (a) XL-PCR: lanes 1 and 12, GeneRuler 100 bp Plus DNA ladder; lanes 2–5 and 10, samples showing normal variation of *RFC1* in the intronic region of interest; lanes 6–8, patients P1–P3; lane 9, positive control; lane 11, no template control. (b) Flanking multiplex PCR for *RFC1* and *FBN1*; the order of the samples is as in figure (a). (c) Repeat-primed PCR for $(AAGGG)_{exp}$: patients P1–P3; NC, negative control; NTC, no template control; PC, positive control. (d) Waterfall plots of long-read sequencing showing consensus reads in patients P1–P3.

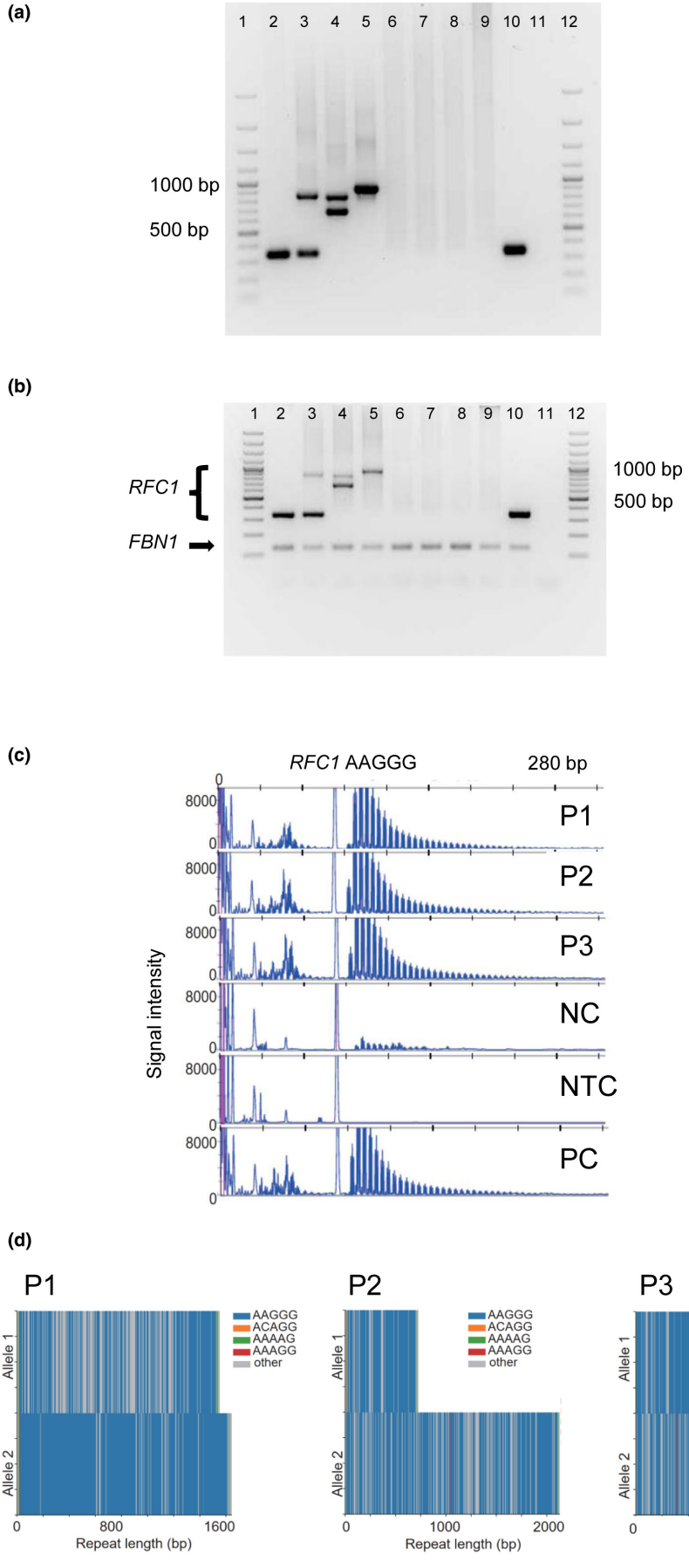
(a)
1 2 3 4 5 6 7 8 9 10 11 12
1000 bp
500 bp
(b)
1 2 3 4 5 6 7 8 9 10 11 12
RFC1
FBN1
1000 bp
500 bp
(c)
RFC1 AAGGG
280 bp
Signal intensity
P1
P2
P3
NC
NTC
PC
(d)
P1
P2
P3
AAGGG
ACAGG
AAAAG
AAAGG
other
Allele 1
Allele 2
Repeat length (bp)

TABLE 1 Clinical characteristics of the three patients with early-onset Parkinson's disease and biallelic $(AAGGG)_{exp}$ in *RFC1*

Patient	P1	P2	P3
Sex	Male	Female	Female
Age[a] (years)	74[b]	61	69
Age at onset (years)	47	40	48
Repeat units (*N*)	311/321	141/410	228/831
Family history	Negative	Positive	Negative
Phenotype	Tremor-dominant	Tremor-dominant	Akinetic-rigid
Tremor	Yes	Yes	No
Rigidity	Yes	No	Yes
On–off variation	Yes	Yes	No
Dyskinesia	Yes	Yes	Yes
Balance impairment	Yes	Yes	Yes
Dementia	Yes	No	No
MMSE	8	NA	29
UPDRS (total)	107	22	NA
UPDRS (III, motor)	68	13	NA
Modified Hoehn and Yahr	4	3	NA
Ataxia	No	No	No
Polyneuropathy	No	No	No
Hearing impairment	No	No	No
HIT	No	Yes	NA
Bilateral vestibular dysfunction	No	Yes	NA
Autonomic dysfunction	Postural hypotension	No	No
Chronic cough	No	No	No
Cerebellar atrophy, MRI	No	No	No

Abbreviations: HIT, Head Impulse Test; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; NA, not available; UPDRS, Unified Parkinson's Disease Rating Scale.

[a]Age, current age and age at last clinical examination are the same.

[b]Age at death.

parkinsonism was 47 years (range 40–48 years) and the median disease duration was 21 years (range 21–27 years). The mother of patient P2 was considered to have had PD, but pathogenic variants in PD-causing genes have previously been excluded in patient P2.

Patient P1 was a 74-year-old man with onset of PD symptoms at age 47 years. PD was diagnosed at age 51 years and the phenotype was tremor-dominant. Dyskinesias, on–off phenomenon and dementia developed after age 65 years. The symptoms progressed gradually so that rigidity, motor and non-motor symptoms were present in the last clinical examination at age 74 years, 2 months before his death. He had not been independent in activities of daily living, and he lived in a nursing home because of PD-related dementia. There were no signs of CANVAS in the clinical examination. Magnetic resonance imaging (MRI) at age 67 years showed mild cerebral atrophy in parietal lobe and grade 1 hippocampal atrophy, but not cerebellar atrophy or white matter lesions (Figure S1). His daily dose of levodopa was 625 mg. He had had hypertension and hyperlipidemia. Patient P1 died from COVID-19 pneumonia.

Patient P2 is a 61-year-old woman, who had experienced first PD symptoms at age 40 years and who was diagnosed with PD 1 year later (Video S1). B-CIT-SPECT (2 beta-carboxymethoxy-3 beta-(4-iodophenyl)tropane single photon emission computed tomography) at age 42 years revealed depletion of dopamine transporter in the right putamen. Brain MRI was normal (Figure S1). In the beginning the phenotype was asymmetrical tremor-dominant, but tremor disappeared completely after deep brain stimulation surgery at age 53 years. The patient has had on–off phenomena and dyskinesias since the age of 50 years. PD has been quite well controlled with deep brain stimulation and medication during the last few years. The patient had a mild vestibular areflexia, but no other signs of CANVAS. Spinal stenosis is a significant comorbidity. She is independent in activities of daily living. She reported relatives with PD.

Patient P3 is a 69-year-old woman with onset of PD symptoms at age 48 years and later the same year the diagnosis of PD was confirmed with B-CIT-SPECT. Dyskinesia has been the dominant motor complication during the last few years. The patient has had occasional visual hallucinations, but she is still independent in activities of daily living. In addition to PD, the patient has atrial fibrillation and has had a hip replacement. The last clinical examination at age 69 years revealed moderate rigidity. The Mini-Mental State

Examination score was 29/30 and brain MRI showed grade 2/4 medial temporal lobe atrophy, enlarged ventricles due to central atrophy and no signs of vascular degeneration at age 69 years (Figure S1).

The three patients were homozygous with respect to the four-SNP core haplotype that is universally associated with $(AAGGG)_{exp}$. Detailed haplotyping of patients P1 and P2 revealed two distinct haplotypes defined by SNPs located in the neighbouring genes *WRD19* and *KLB* (Table S1). The three previously identified patients in the PD-NEF (PD North and East of Finland) cohort [9] also shared these two haplotypes.

DISCUSSION

The genotype frequency of biallelic $(AAGGG)_{exp}$ was 1.10% amongst patients with EOPD being twice as high as the frequency of 0.53% in Finnish patients with PD [9] who had not been selected according to the age of onset. The current cohort includes patients nationwide, whereas our previous PD-NEF cohort was ascertained from a defined geographic region. The three patients with biallelic $(AAGGG)_{exp}$ identified in PD-NEF and, interestingly, the three current patients were born in a region in Finland that has been inhabited only since the 16th century by a small founding population [12]. Patient P2 reported two affected family members, but a diagnostic algorithm based on family history information yielded the diagnosis of definite PD only for the mother, whereas the diagnosis could not be confirmed for the maternal uncle [13].

Clinical examination revealed that the three patients with biallelic $(AAGGG)_{exp}$ had PD with good levodopa response. Interestingly, two of the core features of CANVAS, ataxia or neuropathy, were not found. It should be noted, however, that electrodiagnostic examinations were not performed to exclude subclinical neuropathy or to verify vestibular dysfunction. In a detailed clinical examination, none of the three patients had symptoms or signs of neuropathy and only patient P2 had a mild vestibular dysfunction. Two patients had tremor-dominant PD and one had an akinetic-rigid phenotype. All patients had dyskinesias and balance impairment in clinical examination. Two patients with $(AAGGG)_{exp}$ in PD-NEF had chronic cough, which is a frequent manifestation of CANVAS, but none of the current patients reported cough.

Apart from early onset, the three patients had clinically unremarkable PD. The median age at onset of parkinsonism was 47 years (range 40–48 years), whereas that in the three patients in PD-NEF was 59 years (range 51–65 years) [9]. In the Kela registry, the nationwide modal interval for new medication reimbursements for PD is 70–79 years. Rather interestingly, however, the allele sizes were correlated so that the median allele size amongst the early-onset patients was 316 repeat units, whilst that in the later-onset patients in PD-NEF was 743 units. Significantly larger allele sizes have been reported in CANVAS, where the median size is 1044 repeat units amongst 34 patients (range 400–2000) [2]. The allele size has not been found to correlate with disease severity or onset of symptoms amongst 51 *RFC1*-positive patients with CANVAS or ataxic disorder [14]. Results on four patients with MSA are conflicting. The allele sizes are 100–160 units in two patients with onset of symptoms at 57 and 60 years [7] or 887–1495 units in two patients with onset at 62 years [15]. The relationship between allele size and age of onset in PD is intriguing, as a larger expansion is associated with more severe phenotype in some repeat expansion diseases [16]. However, the number of patients with PD studied so far is small and the allele size-related phenotypic variation is not straightforward.

Our study suggests that the biallelic $(AAGGG)_{exp}$ in *RFC1* is a rare cause of EOPD. Our present and previous data show that the expansion can be found in Finnish patients with clinically confirmed PD and with no or minimal signs of CANVAS. It is suggested that non-Finnish patients with PD should be screened for *RFC1* in order to determine whether the association of *RFC1* with PD is population-specific or not.

AUTHOR CONTRIBUTIONS

Pauli Ylikotila: Conceptualization; investigation; writing – original draft. **Jussi Sipilä:** Investigation; writing – review and editing. **Tiina Alapirtti:** Investigation; writing – review and editing. **Riitta Ahmasalo:** Investigation; writing – review and editing. **Eriko Koshimizu:** Methodology; writing – review and editing. **Satoko Miyatake:** Methodology; writing – review and editing. **Anri Hurme-Niiranen:** Methodology; writing – review and editing. **Ari Siitonen:** Methodology; writing – review and editing. **Hiroshi Doi:** Methodology; writing – review and editing. **Fumiaki Tanaka:** Methodology; writing – review and editing. **Naomichi Matsumoto:** Methodology; writing – review and editing; supervision. **Kari Majamaa:** Conceptualization; investigation; funding acquisition; writing – original draft; supervision; resources. **Laura Kytövuori:** Conceptualization; investigation; funding acquisition; writing – review and editing; methodology; supervision; resources.

FUNDING INFORMATION

This study was funded in part by grants from the Sigrid Jusélius Foundation, the Terttu Foundation, the Yrjö Jahnsson Foundation and the Finnish Parkinson Foundation and from the Japan Agency for Medical Research and Development (AMED) (grant numbers JP22ek0109486, JP22ek0109549, JP22ek0109348) and JSPS Kakenhi (grant numbers JP20K07907, JP 21K0786). The study has received funding from the Medical Research Center Oulu and state research funding from Oulu University Hospital.

CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

Sequence data cannot be made publicly available because of restrictions imposed by the EU and Finnish General Data Protection Regulation (GDPR). Access to sequence data can be applied from the Innovation Agent of the University of Oulu (innovationcentre@oulu.fi). Qualified researchers will be required to complete 'Material and data transfer agreement for the transfer of human

materials (personal data)'. Genetic variation data have been submitted to ClinVar (SCV002600060). Other data are available within the article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ylikotila P, Sipilä J, Alapirtti T, et al. Association of biallelic *RFC1* expansion with early-onset Parkinson's disease. *Eur J Neurol*. 2023;00:1-6. doi:10.1111/ene.15717