Cognitive impairment is not uncommon in patients with biallelic RFC1 AAGGG repeat expansion, but the expansion is rare in patients with cognitive disease

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Short communication

Cognitive impairment is not uncommon in patients with biallelic *RFC1* AAGGG repeat expansion, but the expansion is rare in patients with cognitive disease

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ABSTRACT

Introduction: The biallelic repeat expansion $(AAGGG)_{exp}$ in *RFC1* causes cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS). Recently, cognitive impairment has been reported in patients with CANVAS and a broader neurodegenerative process associated with *RFC1* has been suggested. Furthermore, rare cases of multiple system atrophy, Parkinson's disease, amyotrophic lateral sclerosis or CANVAS with features of dementia with Lewy bodies have been found.
Objective: We hypothesized that the biallelic $(AAGGG)_{exp}$ is associated with neurodegeneration manifested as cognitive symptoms and that atypical RFC1 disease may be found among patients with cognitive disorder.
Methods: Clinical data on nine patients with biallelic $(AAGGG)_{exp}$ were reviewed and 564 patients with Alzheimer's disease or frontotemporal dementia (FTD) were investigated for biallelic *RFC1* $(AAGGG)_{exp}$.
Results: Five patients with biallelic $(AAGGG)_{exp}$ were found with a cognitive impairment and in four of them the phenotype resembled FTD. However, biallelic $(AAGGG)_{exp}$ was not detected among patients with Alzheimer's disease or FTD.
Conclusion: Cognitive impairment is a feature in patients with the biallelic $(AAGGG)_{exp}$, but the pathogenic expansion seems to be rare in patients with dementia. Studies on patients with diverse phenotypes would be useful to further explore the involvement of *RFC1* in neuronal degeneration and to identify atypical phenotypes, which should be taken into account in clinical practice.

1. Introduction

The biallelic repeat expansion $(AAGGG)_{exp}$ in the intronic region of the *RFC1* gene has been shown to cause cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) and late-onset ataxia [1, 2]. The phenotype implies that cerebellum, dorsal root ganglia, and vestibular system are affected, but *RFC1* appears to be associated with a multisystemic involvement [3,4]. A recent study on four CANVAS patients has suggested that *RFC1*-related ataxia is a multisystemic

neurodegenerative disorder affecting also cognitive domains and pyramidal tract neurons [5]. Furthermore, the expansion has been found in patients with other phenotypes, such as multiple system atrophy (MSA), clinically confirmed Parkinson's disease (PD) and motor neuron pathology [6–9]. We have previously identified nine patients with biallelic $(AAGGG)_{exp}$ in a population-based screening of patients with inherited ataxia or polyneuropathy [10]. We hypothesized that the repeat expansion could be associated with a wider spectrum of neurodegenerative diseases. Therefore, we reviewed the clinical phenotypes of the

nine patients and then examined 564 Finnish patients with cognitive disorders for biallelic (AAGGG)$_{exp}$ in *RFC1*.

2. Methods

We screened the biallelic *RFC1* (AAGGG)$_{exp}$ in 564 Finnish patients with the cognitive disorders Alzheimer's disease (AD) or frontotemporal dementia (FTD) (Table 1). The patients have been examined by experienced neurologists at the University Hospital of Oulu or Kuopio, including screening laboratory tests, a neuropsychological examination, and magnetic resonance imaging or computed tomography of the brain. If needed, electrodiagnostic examination, cerebrospinal fluid analyses of Aβ42, tau and phospho-tau or functional neuroimaging by fluorodeoxyglucose positron emission tomography were carried out. The diagnoses have been made according to the prevailing diagnostic criteria, and all diagnoses were re-evaluated for the purpose of this study.

Clinical data on nine patients with the biallelic *RFC1* (AAGGG)$_{exp}$ [10] were reviewed in order to evaluate possible multisystemic disorder. The patients had been identified in a population-based screening for inherited ataxia or polyneuropathy. Five patients were detected among patients with sporadic or familial ataxia with unknown etiology, and four were identified in a cohort of patients with polyneuropathy [10]. Patient records were systematically reassessed with respect to information on cognitive disorders, motor neuron disease and parkinsonism. The most recent neurologic examination was reviewed. Findings from routine brain MRI were aggregated when available.

Written informed consents were given by the patients or their legal caregivers. The research protocol was approved by the Ethical Committees of the Northern Ostrobothnia Hospital District and of the Northern Savo Hospital District.

Biallelic expansion in *RFC1* was investigated using PCR for large and complex amplicons (XL-PCR) and flanking multiplex PCR for *RFC1* and *FBN1* [8]. XL-PCR was carried out as previously [8]. Reaction conditions in flanking multiplex PCR were modified for PhireII Hot Start DNA polymerase (Thermo Fisher Scientific, Waltham, MA, U.S.A.) using previously reported primers [1,10]. *RFC1* and *FBN1* primers were used with the final concentrations of 0.5 μM and 0.75 μM, respectively. DMSO was added to the reaction mixture with the final concentration of 3%. Other reaction components were used as in the provided protocol. Reactions were performed in Piko™ thermal cycler (Thermo Fisher Scientific) according to the recommended three-step cycling protocol and annealing temperature of 62 °C. In the case of the biallelic expansion (AAGGG)$_{exp}$, no specific product for *RFC1* is obtained. Therefore, biallelic (AAGGG)$_{exp}$ was excluded based on normal PCR products in both analyses.

3. Results

Review of nine patients with the biallelic expansion (AAGGG)$_{exp}$ in *RFC1* revealed five patients with cognitive dysfunction (Table 2). Frontal or frontotemporal cortical atrophy was detected in MRI of three patients, while cerebellar atrophy was found in each of the five patients

Table 1
Demographic characteristics of the 564 patients in the study.

Diagnosis	Patients (N)	Age at onset (years)	Genetic diagnosis (N)
Alzheimer's disease	301	58.4 (43–80)	2[a]
Frontotemporal dementia			
bvFTD	185	60.2 (36–84)	42[b]
svPPA	15	59.3 (55–65)	1[b]
nfvPPA	30	66.8 (47–80)	5[b]
FTD-ALS	33	61.3 (49–73)	16[b]

[a] *APP* duplication.
[b] *C9orf72* expansion. Age at onset; mean (range). Abbreviations: ALS, amyotrophic lateral sclerosis; bv, behavioral variant; FTD, frontotemporal dementia; nfv, nonfluent variant; PPA, primary progressive aphasia; sv, semantic variant.

with ataxia. None of the nine patients presented with symptoms of parkinsonism or motor neuron disease.

Four patients were found with dementia, and one patient had mild cognitive impairment (Table 2), but no specific diagnosis of cognitive disorder could be entered for the five patients. Neuropsychological examination suggested FTD, however, as four of the patients had variable features including behavioral changes, impairment in executive functions and language, and two patients had a psychiatric diagnosis. Among the seven patients that had had brain MRI, there were six patients with cerebellar atrophy and four patients (57%) with cerebral cortical atrophy.

Such high proportions of patients with cognitive impairment suggested that biallelic (AAGGG)$_{exp}$ in *RFC1* may be found among patients with various cognitive disorders. Therefore, we examined 564 patients with a previously diagnosed cognitive disorder for the biallelic (AAGGG)$_{exp}$ in *RFC1*. The expansion was not found in Alzheimer's disease (95% confidence interval: 0–1.0%) or in FTD (95% confidence interval: 0–1.1%).

4. Discussion

We found that five out of nine patients with biallelic (AAGGG)$_{exp}$ had cognitive dysfunction. The proportion of patients with cognitive dysfunction varies in patients with RFC1 disease, however. Mild cognitive impairment has been reported in 25% of the patients with biallelic (AAGGG)$_{exp}$ [3], and 10.5% of the patients had moderate impairment in another study [11]. Even though these patients had not received any specific diagnosis for the cognitive disorder, their neuropsychological test results or screening test scores indicated cognitive impairment [3,5,11]. We found that four patients presented with a neuropsychological profile suggesting FTD and that two of them had a psychiatric diagnosis. Intriguingly, behavioral-psychiatric symptoms have been reported as the first manifestation of RFC1 disease in an Italian family [12].

Findings in brain MRI also supported the concept of a broader RFC1 disease. Cerebellar atrophy has been reported in 22 out of 30 patients (73%), while diffuse cortical and subcortical atrophy was found in five cases (17%) [11]. Analysis of brain MRI in 56 *RFC1*-positive patients has revealed a predominantly parietal cerebral atrophy that was detected in 42% of MRIs [3]. Furthermore, a significant increase in markers of neuronal damage in CSF, protein tau and neurofilament light chain, has recently been associated with RFC1 disease [4].

We have recently reported that the frequency of biallelic (AAGGG)$_{exp}$ is 0.5% in Finnish patients with a clinical diagnosis of PD [8]. No or minimal signs of CANVAS were found in the three patients identified in that study and, indeed, an increasing number of studies reporting atypical phenotypes suggests that RFC1 disease may include various neurodegenerative disorders. In addition to PD, three patients with MSA-P or MSA-C have been found among 282 patients [6] and another three patients among 207 patients with possible or probable MSA [7], while none were found in a cohort of patients with neuropathologically confirmed MSA [13]. In addition, the expansion has been detected in a patient with CANVAS and features resembling dementia with Lewy bodies [14]. Upper or lower motor neuron involvement has been found in 24 of 38 patients [11], and a patient with *RFC1*-associated ALS has recently been reported [9], but the expansion has not been discovered in a large cohort of patients with sporadic ALS [15]. Altogether, studies on patients with diverse phenotypes are needed to further explore the involvement of *RFC1* in multisystemic degeneration manifested as heterogeneous neurological symptoms.

The RFC1 disease seems to affect primarily neurons in the cerebellum, dorsal root ganglia, and vestibular system, but the clinical data suggest that also basal ganglia and cerebral cortical neurons are affected. Neurodegenerative diseases share common mechanisms that lead to loss of specific neurons or synapses in the brain [16]. Clinical data on patients with RFC1 disease suggest that the *RFC1* gene is

Table 2
Patients with biallelic (AAGGG)$_{exp}$ in *RFC1*. The most recent examination was reviewed.

Patient[a]	Gender	Age	NCD[b]	CI AAO	MRI	Neurocognitive profile
P1	F	64	minor	63	mild central atrophy, mild frontal cortical atrophy	deficits in executive functions, impaired verbal fluency
P2	F	83	major	60	mild cerebellar atrophy, mild vascular degenerative changes	memory problems that progressed to dementia
P3	F	72[c]	major	67	cerebellar atrophy	behavioral changes, major deficits in executive functions, difficulties in sustained attention and concentration, memory problems, delusions
P4	M	64	major	59	cerebellar atrophy, frontotemporal cortical atrophy	behavioral changes, major deficits in executive functions, progressive aphasia leading to near-mutism, severe difficulties in working memory and attention
P5	F	80	major	75	cerebellar atrophy, frontal cortical atrophy	severe deficits in executive functions and language, wide cognitive difficulties, lack of initiative
P6	M	74	none	n.a.	cerebellar atrophy, mild parietal cortical atrophy	n.d.
P7	F	71	none	n.a.	cerebellar atrophy	n.d.
P8	F	74	none	n.a.	not available	n.d.
P9	M	82	none	n.a.	not available	n.d.

[a] Patients with polyneuropathy (P1, P2, P8, P9) or patients with ataxia (P3-P7).
[b] Neurocognitive disorder, as defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5).
[c] Age at death. Abbreviations: AAO, age at onset; CI, cognitive impairment; n.a., not applicable; n.d., no data; MMSE, mini-mental state examination; MRI, magnetic resonance imaging.

involved in a cell process that takes place in various cell populations in the brain. Our findings support the idea that RFC1 disease is a multi-systemic neurodegenerative disorder affecting not only vestibular, cerebellar and sensory functions but also cognitive domains. Indeed, cognitive impairment may have remained undiagnosed, as a detailed neuropsychological examination is required to reveal the deficits [3,5]. To conclude, cognitive impairment is not uncommon in patients with RFC1 disease and, clearly, detailed neuropsychological analyses are needed in order to further characterize this phenotype.

Author contributions

Declaration of competing interest

None.

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