LETTER

Diagnostic yield of testing for *RFC1* repeat expansions in patients with unexplained adult-onset cerebellar ataxia

INTRODUCTION

Cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS) is an adult-onset, slowly progressive neurodegenerative disorder characterised in its full form by cerebellar ataxia, sensory neuropathy and bilateral vestibulopathy. Autonomic dysfunction and dry cough are also frequent features. CANVAS is usually sporadic, but occasionally occurs in siblings. Recently, a biallelic intronic AAGGG repeat expansion (AAGGG)_{exp} in *RFC1* was identified as the cause in almost all tested familial cases with full CANVAS syndrome.^{1,2} Interestingly, biallelic (AAGGG)_{exp} in *RFC1* were also found in 22% of sporadic, previously idiopathic late-onset ataxia cases.¹ The percentage of positive cases increased to 63% if only sporadic patients with combined late-onset cerebellar ataxia and sensory neuropathy were considered, and to 92% in sporadic cases with full CANVAS.¹ In a cross-sectional description of 100 patients with biallelic *RFC1* (AAGGG)_{exp}, all cases had sensory neuropathy on nerve conduction studies (NCS), while only 79% of cases had cerebellar involvement.³ Whether biallelic *RFC1* (AAGGG)_{exp} can

cause cerebellar ataxia without sensory neuropathy, remains uncertain. Here, we determined the diagnostic yield of testing for *RFC1* repeat expansions in a single-centre cohort of unexplained adult-onset ataxia cases.

PATIENTS AND METHODS

We included all index patients with ataxia who were evaluated at University Hospitals Leuven in the Movement Disorders Clinic between March 2005 and September 2019 or the Neuromuscular Clinic between January 2000 and December 2018 and fulfilled the following criteria: (1) onset age 18 years or older (onset as early as 19 years has been reported in an *RFC1* case³); (2) ataxia as prominent clinical feature; (3) clinical evidence for cerebellar origin of the ataxia, that is, cerebellar oculomotor disorder (broken pursuit, dysmetric saccades) and/or cerebellar dysarthria; (4) no family history suggesting dominant inheritance; (5) no identified genetic or acquired cause and (6) no rapid progression typical of multiple system atrophy. We performed short-range flanking PCR and repeat-primed PCR for the (AAAAG)_{exp}, (AAAGG)_{exp} and (AAGGG)_{exp} *RFC1* alleles on genomic DNA using described primers.¹

RESULTS

We included 85 non-related index patients with unexplained adult-onset cerebellar ataxia (onset age: 52±15.1 years

(mean±SD); 41 female). Nine of the 85 patients had an affected sibling, but these siblings were not included in the current analysis. All 85 probands had previously tested negative for spinocerebellar ataxia (SCA) type 1, 2, 3, 6 and 7. A negative genetic test had been obtained for SCA10, SCA12, SCA17 and dentatorubral-pallidoluysian atrophy in 62/85 patients, for Friedreich's ataxia in 61/85 and for Fragile X-associated tremor ataxia syndrome in 37/85.

We identified biallelic (AAGGG)_{exp} in *RFC1* in nine patients (10.6%; five female; six sporadic). Clinical features are summarised in table 1.

Onset age was 56±9.4 years. At the time of this study, five of nine patients had died (age at death 77±5.9 years; mean survival from onset: 20±5.5 years).

All nine positive cases had a neuropathy on NCS with predominant or exclusive sensory involvement. In eight of nine cases, this was associated with clinical symptoms and signs of sensory neuropathy. In three cases, NCS also showed motor involvement, although less prominent than the sensory component. Vestibular testing using video-oculography, caloric stimulation and a rotatory chair was performed in three of nine cases. All three had bilateral vestibular hyporeflexia or areflexia and therefore full CANVAS syndrome (two sporadic, one familial). Six of nine cases had autonomic dysfunction and five of nine had chronic cough.

When we restricted our cohort to patients with both cerebellar ataxia and

Table 1 Clinical characteristics of patients with biallelic pathogenic *RFC1* expansions

Pt	Age at onset (years), gender	Age at examination (years)	Age at death (years)	Cerebellar atrophy on MRI	Downbeat nystagmus	Sensory symptoms / signs	SNAP	CMAP	Vestibular investigation	Autonomic	Chronic cough	Familial
1	73, M	84	87	–	+	+	UL: ↓/absent LL: absent	UL: ↓ LL: ↓	NP	U	–	–
2	56, F	71	75	+	–	–	UL: ↓/absent LL: NP	UL: ↓ LL: ↓	NP	–	+	–
3	39, F	46	–	+	+	+	UL: ↓/absent LL: absent	UL: normal LL: normal	Bilateral vestibular hyporeflexia	U, OH	+	–
4	53, M	69	79	+	–	+	UL: ↓ LL: absent	UL: normal LL: normal	NP	–	+	–
5	49, M	59	74	+	–	+	UL: ↓ LL: absent	UL: normal LL: ↓	NP	U	–	Brother with similar symptoms (NA)
6	57, F	67	72	–	+	+	UL: ↓ LL: absent	UL: normal LL: normal	NP	U	–	–
7	64, F	69	–	+	+	+	UL: ↓ LL: absent	UL: NP LL: normal	NP	–	+	Brother with similar symptoms (NA)
8	53, F	70	–	+	–	+	UL: ↓ LL: absent	UL: normal LL: normal	Bilateral vestibular areflexia	Dry mouth	–	–
9	56, M	66	–	+	+	+	UL: absent LL: ↓/absent	UL: normal LL: normal	Bilateral vestibular areflexia	OH, I, C	+	Brother with similar symptoms

C, constipation; CMAP, compound muscle action potential; F, female; I, impotence; LL, lower limbs; M, male; NA, not available for evaluation; NP, not performed; OH, orthostatic hypotension; PT, patient ID; SNAP, sensory nerve action potential; U, urinary dysfunction; UL, upper limbs.

sensory neuropathy, the diagnostic yield of *RFC1* testing increased to 28.1% (9/32). If we only considered patients with ataxia onset after 35 years (similar to the cut-off for late-onset ataxia in a previous *RFC1* study¹), the yield was 12.7% (9/71). If we only included patients with onset after 35 years and both cerebellar ataxia and sensory neuropathy, the yield was 30.0% (9/30).

Three of the nine positive cases had a sibling with reportedly similar symptoms. One of these (brother of patient 9) was available for evaluation and was found to also have full CANVAS and a biallelic (AAGGG)_{exp} in *RFC1*. His clinical features were described in a recent case report⁴ and he was not included in the determination of diagnostic yield, which was restricted to the 85 index patients.

Six of 85 patients (7.1%) were heterozygous for (AAGGG)_{exp}, which is considered non-pathogenic carrier status.¹ Three of them carried the reference (AAAAG)₁₁, two the (AAAAG)_{exp} and one the (AAAGG)_{exp} configuration on the other allele. The (AAGGG)_{exp}/(AAAAG)_{exp} carrier had ataxia onset at 58 years and sensory neuropathy.

Eight patients carried two repeat expansions of repeat units other than (AAGGG): biallelic (AAAAG)_{exp} in four, biallelic (AAAGG)_{exp} in one and (AAAAG)_{exp}/(AAAGG)_{exp} in three.

DISCUSSION

Biallelic (AAGGG)_{exp} in *RFC1* were a relatively frequent cause of adult-onset cerebellar ataxia in our cohort. Importantly, all nine positive cases also had a sensory or sensorimotor neuropathy, although this was subclinical in one. None had pure cerebellar ataxia with normal findings on NCS. Based on these data, we recommend performing NCS in all cases of unexplained adult-onset cerebellar ataxia to detect a subclinical or confirm a clinically suspected sensory neuropathy. If NCS shows sensory neuropathy, *RFC1* testing is highly recommended. If NCS shows no sensory nerve impairment, *RFC1* mutations seem very unlikely. Consistent with this, a recent description of 100 patients with biallelic *RFC1* (AAGGG)_{exp} identified

sensory neuropathy as a common feature in all cases, while cerebellar ataxia was less frequent, leading the authors to suggest progression from sensory neuropathy early on to later cerebellar dysfunction.³

The frequency of positive cases in our adult-onset ataxia cohort was lower than in a London-based cohort, even if we used the same onset age cut-off of 35 years (12.7% in our study vs 22%),¹ but was higher than recently reported for adult-onset ataxia cohorts from Canada (4.3%) and Brazil (0.6%).⁵ This may be due to differences in ethnic composition of the cohorts or different inclusion criteria for adult-onset ataxia.

We found one patient with cerebellar ataxia and subclinical sensory neuropathy and a (AAGGG)_{exp}/(AAAGG)_{exp} state. This constitution was previously reported in a CANVAS patient,² but also in a healthy control.¹ Although we cannot exclude that the (AAGGG)_{exp}/(AAAGG)_{exp} configuration might cause disease, evidence for its pathogenicity is currently insufficient. The (AAGGG)_{exp}/(AAAAG)_{exp} configuration is also considered non-pathogenic.¹

Limitations of our study were the lack of vestibular investigations in six of nine cases, the retrospective nature of clinical descriptions and the fact that we did not determine exact repeat expansion lengths. Longitudinal studies of biallelic *RFC1* (AAGGG)_{exp} carriers from early to advanced stages will be required to fully characterise the clinical phenotype.

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