RFC1: Motifs and phenotypes.

Violette Delforge, Celine Tard, Jean-Baptiste Davion, Kathy Dujardin, Anna Wissocq, Claire-Marie Dhaenens, Eugenie Mutez, Vincent Huin

Available online at
ScienceDirect
www.sciencedirect.com
Elsevier Masson France
EM|consulte
www.em-consulte.com

Updates in Neurogenetics

RFC1: Motifs and phenotypes

V. Delforge [a], C. Tard [a,b], J.-B. Davion [a,b], K. Dujardin [a,b], A. Wissocq [c], C.-M. Dhaenens [a,c], E. Mutez [a,b], V. Huin [a,c,*]

[a] Inserm, U1172 – LilNCog – Lille Neuroscience & Cognition, CHU de Lille, University Lille, 59000 Lille, France
[b] Department of Neurology and Movement disorders, CHU de Lille, 59000 Lille, France
[c] Department of Toxicology and Genopathies, UF Neurobiology, CHU de Lille, 59000 Lille, France

INFO ARTICLE

Article history:
Received 30 January 2024
Received in revised form
22 March 2024
Accepted 25 March 2024
Available online xxx

Keywords:
RFC1
CANVAS syndrome
Ataxia
DNA repeat expansion
Mutation
Phenotype

ABSTRACT

Biallelic intronic expansions $(AAGGG)_{exp}$ in intron 2 of the *RFC1* gene have been shown to be a common cause of late-onset ataxia. Since their first description, the phenotypes, neurological damage, and pathogenic variants associated with the *RFC1* gene have been frequently updated. Here, we review the various motifs, genetic variants, and phenotypes associated with the *RFC1* gene. We searched PubMed for scientific articles published between March 1st, 2019, and January 15th, 2024. The motifs and phenotypes associated with the *RFC1* gene are highly heterogeneous, making molecular diagnosis and clinical screening and investigation challenging. In this review we will provide clues to give a better understanding of *RFC1* disease. We briefly discuss new methods for molecular diagnosis, the origin of cough in *RFC1* disease, and research perspectives.

Abbreviations

ALS	amyotrophic lateral sclerosis
BVP	bilateral vestibulopathy
CANVAS	cerebellar ataxia, neuropathy, vestibular areflexia syndrome
CIAP	chronic idiopathic axonal polyneuropathy
CSF	cerebrospinal fluid
GER	gastroesophageal reflux
HSAN	hereditary sensory and autonomic neuropathy
MOCA	Montreal Cognitive Assessment
MRI	magnetic resonance imaging
MSA	multiple system atrophy
NCS	nerve conduction study
NfL	neurofilament light chain
PD	Parkinson's disease
RBD	rapid eye movement sleep behavior disorder
RCC	refractory chronic cough
RFC1	replication factor C subunit 1
SNAP	sensory nerve action potential

* *Corresponding author.*
E-mail address: vincent.huin@inserm.fr (V. Huin).
https://doi.org/10.1016/j.neurol.2024.03.006

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

1. Introduction

The *RFC1* gene, located on chromosome 4, encodes a ubiquitous protein, replication factor C subunit 1, which is the largest subunit of replication factor C, involved in replication and DNA repair [1–5]. In 2019, biallelic intronic $(AAGGG)_n$ expansions in intron 2 of the *RFC1* gene were shown to be a common cause of late-onset ataxia, in particular, most cases of cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) [6,7]. This disorder belongs to a vast and growing group of genetic diseases due to DNA repeat expansions. However, this genetic disease stands out due to the particular combination of large expansions and a change in the expanded nucleotide motif. Cortese et al. reported that, in the general population, the expanded locus generally consists of a stretch of ~11 (AAAAG) pentanucleotides, but this locus appears to be highly variable, as non-pathogenic $(AAAAG)_n$ and $(AAAGG)_n$ expansions can be frequently observed [6].

Since their first description, the phenotypes, neurological damage, and pathogenic variants associated with the *RFC1* gene have been frequently updated. This review focuses on: (i) the various pathological/non pathological expanded motifs and other genetic variants of the *RCF1* gene, which make molecular diagnosis increasingly difficult; and (ii) the phenotypic spectrum and related pathological conditions associated with the *RFC1* gene, which appear to be highly variable.

2. Materials and methods

We searched the literature published in English between March 1st, 2019, and January 15th, 2024, in the database of the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/pubmed) using the key words ''RFC1'', ''CANVAS'', and ''Replication Factor C1''. We manually screened bibliographies of the publications that met these criteria and each relevant review to ensure that we did not miss any potential articles. Exclusion criteria were papers addressing non-human studies or the RFC1 protein only or papers focusing on ''Reduced Folate Carrier 1'', i.e., the *SLC19A1* gene. We excluded animal or cellular studies, conference proceedings, authors' corrections, replies, and editorials. The complete screening strategy is shown in Online material Fig S1.

3. Results

3.1. Molecular aspects

The *RFC1* locus is highly dynamic, giving rise to a large number of pathogenic and non-pathogenic motifs. To date, the pathophysiological mechanism of *RFC1* expansions are still uncertain, without subsequent functional testing. Most of the elements that define the pathogenicity of the motifs and other described variants depend on: (i) their frequency in patients; (ii) their presence in the homozygous state in healthy controls; and/or (iii) their association with another pathogenic variant *in trans*. The various motifs observed thus far are presented in Fig. 1.

3.1.1. Non-pathogenic motifs

The expanded locus in intron 2 of the *RFC1* gene is precisely located at position chr4:39,348,425-39,348,485 in the reference genome hg38. It consists of the 3′ end of a short tandem repeat, AluSx3, a subtype of the Alu element. Various motifs can be observed in the general population. The most frequent allele corresponds to ~11 repeats of the pentanucleotide (AAAAG). The allele frequency in Caucasians of $(AAAAG)_{11}$ is ~0.75. Other common non-pathogenic motifs are the expanded $(AAAAG)_n$ and expanded $(AAAGG)_n$ motifs (respective allele frequencies: ~0.13 and ~0.08) [8]. Recently, Scriba et al. analyzed the *RFC1* locus in 19,241 ethnically diverse samples from the gnomAD v3 database [9]. This bioinformatic analysis of whole genome sequencing data retrieved 34,768/38,482 alleles with the $(AAAAG)_n$ motif and 1,914/38,482 alleles with the $(AAAGG)_n$ motif (respective allele frequencies: ~0.9 and ~0.05). These two motifs have been observed in the homozygous state in healthy individuals and should thus be considered benign. However, recent studies reported a pathogenic role for certain highly expanded alleles with > 500 500 $(AAAGG)_n$ (see below, ''3.1.3. Pathogenic motifs'').

In large cohorts of 1,069 amyotrophic lateral sclerosis (ALS) patients and 853 controls, Abramzon et al. did not discover any carriers of the homozygous $(AAGGG)_n$ expansion. However, after Sanger sequencing of the samples suspected to carry biallelic expansions, they observed that certain individuals carried complex motifs with different repeat units. In total, they reported 18 different combinations of expansions in ALS patients and/or controls. None was observed in the homozygous state in the ALS patients. The $(AAAGGG)_n$ motif alone or associated with another motif on the same allele has been observed in the homozygous state in healthy controls. In total, the allele frequencies of this rare and likely benign allele $(AAAGGG)_n$ in Caucasians (mixed ALS patients and controls) were 12/1,706 (0.007) in the ALS cohort and 13/2,138 (0.006) in the controls [10].

3.1.2. Motifs of unknown significance

Since the first report in 2019, the existence of other non-pathogenic allelic conformations was suspected, with 18/608 (0.03) uncharacterized expansions in healthy individuals [6]. Further studies revealed the highly dynamic nature of the *RFC1* locus [9,11–15]. Scriba et al. reported 20 different motifs in the gnomAD v3 database, among them 16 with a frequency < 0.01. Most of these motifs have been observed in the gnomAD v3 database or in various cohorts of patients with various neurological disorders and controls. However, these motifs were only found in the heterozygous state, which makes it difficult to define their pathogenicity [9]. In the same study, the authors showed that the motif $(AAGAG)_{exp}$ has a frequency of 474/38,482 (0.012). This motif has been previously reported in the heterozygous state in Brazilian and Canadian cohorts of adult-onset ataxia and in healthy Canadian controls [11]. Finally, Scriba et al. reported a high proportion of complex interrupted patterns in their cohort of 242 Australasian patients with neurological diseases. They showed that some alleles comprised more than one repeat motif [9]. Such

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

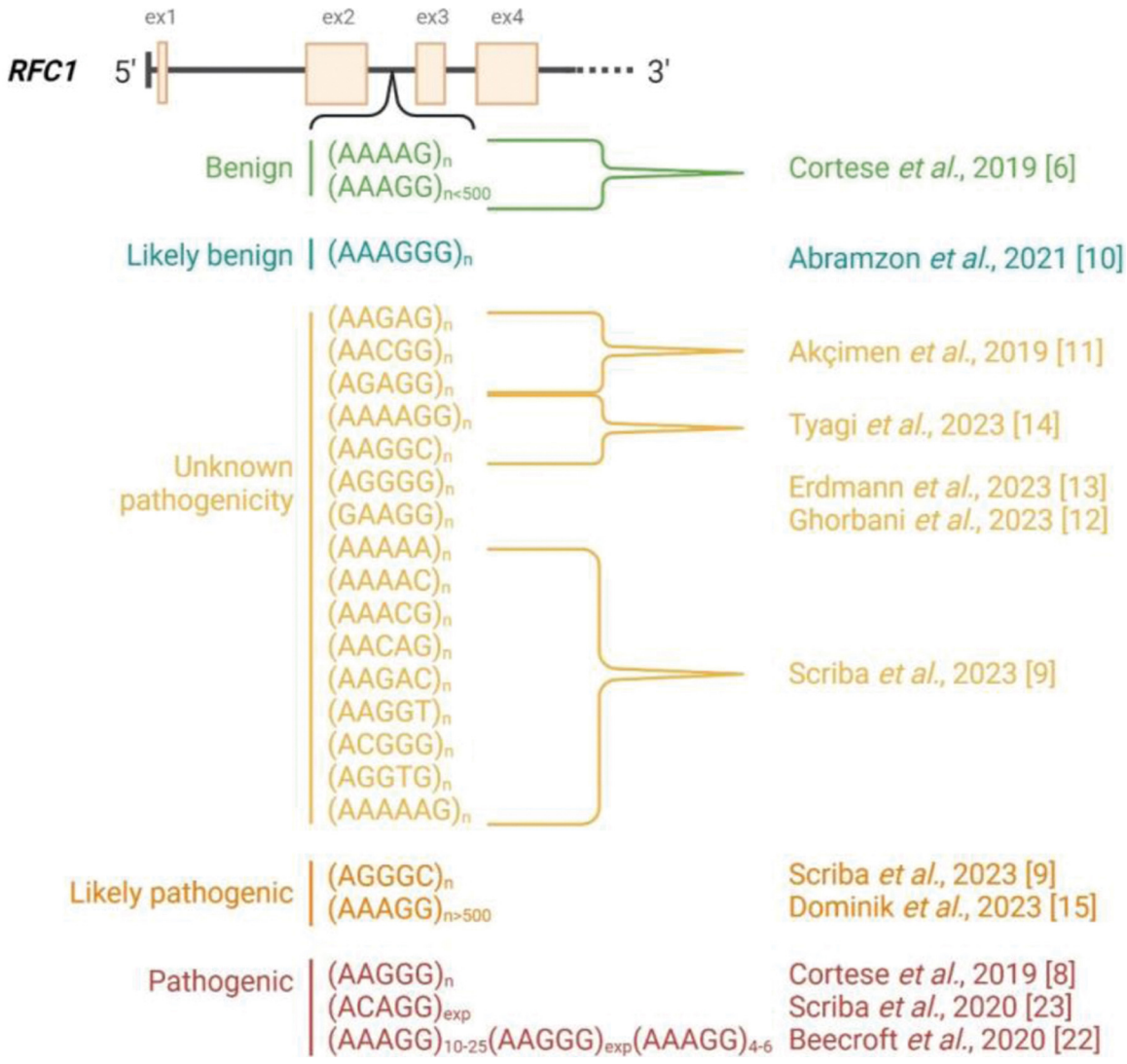

Fig. 1 – Motifs of *RFC1* repeat expansions.

variability and complex interrupted patterns at the *RFC1* locus were confirmed by two other studies in the same year [14,15].

In a large Indian cohort, Tyagi et al. screened for *RFC1* expansions and found 27 different repeat motifs, sometimes in the homozygous state or in complex alleles with different repeat motif units in the same allele. However, the phenotype of the carriers of these repeat motif arrangements is not known [14].

Dominik et al. reported complex alleles in two CANVAS families. The $(AAGGG)_n(AAGGC)_n$ motif was carried in the homozygous state in two affected siblings, whereas the $(AAAGG)_n(AGAGG)_n$ motif was in a compound heterozygous state with the common pathogenic $(AAGGG)_n$ motif in one sporadic CANVAS patient [15]. *In silico* predictions favor the formation of G-quadruplexes, which may be involved in the pathophysiology [16]. However, the low number of affected patients and the association with large repeats of the common pathogenic $(AAGGG)_n$ motif on the same allele as the $(AAGGG)_n(AAGGC)_n$ motif makes it impossible to say whether or not the $(AGAGG)_n$ and $(AAGGC)_n$ motifs are pathogenic or not.

3.1.3. *Pathogenic motifs*

Among the various pathogenic motifs, biallelic $(AAGGG)_n$ expansions in intron 2 of the *RFC1* gene are the most frequent pathogenic variants. The allele frequencies of $(AAGGG)_n$ expansions in the general population is ~0.031 in the general population [9] and up to 0.045 in Caucasians [7]. They were initially reported in CANVAS [6] and then shown to be associated with various phenotypes, from isolated sensory neuropathy to more complex clinical presentations grouped under the name of *RFC1* CANVAS/spectrum disorders [8]. The size for the pathogenic $(AAGGG)_{exp}$ alleles generally range from > 250 to > 2000 repeats (maximum number of reported repeats = ~4000) [6,15,17,18]. However, smaller expansions (~100–160 repeats) have been shown to be associated with a neurodegenerative disorder similar to multiple system atrophy [19]. The allele frequency in Caucasians of such $(AAGGG)_{exp}$ alleles ranges from 4/608 (0.007) to 21/466 (0.045) in the literature [6,7]. This pathogenic variant arose from a common haplotype shared by Caucasian and Asian populations [7] and has subsequently been observed in Asian

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

populations [20,21]. More recently, Scriba et al. analyzed the *RFC1* locus in 19,241 ethnically diverse samples from the gnomAD v3 database [9]. This bioinformatic analysis of whole genome sequencing data retrieved 1202 alleles with the $(AAGGG)_n$ motif, corresponding to an allele frequency of 0.031.

In a recent study, Currò et al. reported an inverse correlation between the repeat size of both smaller and larger alleles with $(AAGGG)_n$ expansions and an earlier age or severity of the disease. Larger repeat size, especially of the smaller allele, were shown to be associated with an earlier age at: (i) neurological onset; (ii) dysarthria; (iii) dysphagia; and (iv) first walking aid. A comparison of patients with either isolated sensory neuropathy, complex sensory neuropathy, or full-blown CANVAS showed the more complex phenotypes to be associated with larger repeat size. Larger repeat size in the smaller allele only was associated with more pronounced cerebellar vermis atrophy. Finally, the authors did not show meiotic instability nor significant variation of repeat size between tissues [22].

Other pathogenic expansions showing variations in the pentanucleotide repeat sequence have been reported in various populations. In Māori, Beecroft et al. observed an alternate pathogenic allele configuration $(AAAGG)_{10–25}(AAGGG)_{exp}(AAAGG)_{4–6}$ [23]. Patients carrying such biallelic expansions had a CANVAS phenotype, with similar clinical impairment as CANVAS patients with European ancestry. The authors showed that their patients shared a common disease haplotype, suggestive of a founder effect in this population. Moreover, the authors showed the same core haplotype as previously described by Cortese et al. [6], supporting a single origin of the pathogenic $(AAGGG)_n$ expansions [23]. In Asian and Asia-Pacific CANVAS families, several studies showed an $(ACAGG)_n$ expansion in the homozygous state or in a compound heterozygous state with $(AAGGG)_{exp}$ [21,24–27]. This $(ACAGG)_{exp}$ shares a common haplotype, suggesting a single origin, but the same expansion with a different haplotype has been found in Dutch patients [28]. The CANVAS feature in these patients was similar, with perhaps more frequent lower neuron involvement that needs to be confirmed in a larger cohort of patients [25].

In 2023, Dominik et al. reported new motifs associated with CANVAS [15]. They found $(AGGGC)_n$ expansion in a compound heterozygous state with the common pathogenic $(AAGGG)_n$ expansion in six patients from five unrelated families with a CANVAS or sensory neuropathy phenotype. In the same study, they found a higher percentage of compound heterozygous $(AAGGG)_{exp}/(AAAGG)_{exp}$ carriers among ataxia cases and showed that the patients with CANVAS/spectrum disorder ($n = 5$) and compound heterozygous $(AAGGG)_{exp}/(AAAGG)_{exp}$ carriers had larger $(AAAGG)_n$ expansions than healthy controls with a similar genotype ($n = 8$). Large expansions of > 500 repeats units of the $(AAAGG)_n$ motif should thus be considered as likely pathogenic.

3.1.4. *Other pathogenic variants*

In 2022, five studies identified patients with a CANVAS phenotype and exhibiting compound heterozygosity for the pathogenic $(AAGGG)_{exp}$ and a conventional pathogenic variant (i.e. single nucleotide variant or small insertion/deletion) in the *RFC1* gene on the other allele [29–33]. All of these reported variants were nonsense, frameshift, or splice variants, supporting a loss-of-function of *RFC1* in CANVAS patients. Ronco et al. suggested that such compound heterozygous patients may have a more severe phenotype, with frequent dysautonomia, an earlier need for walking aids, and an earlier onset of neurological impairment [31]. We compiled all the published patients with compound heterozygosity (i.e. $(AAGGG)_{exp}$/conventional variant) and compared them to 94 patients with extensive phenotyping [34]. There was no difference in the frequency of dysautonomia (6/14 versus 39/63, $P = 0.31$) (Wilcoxon rank sum test). However, there was, indeed, an earlier need for walking aids (median age: 58.5 years, interquartile range: 54.5–59 years, $n = 8$, $P = 0.006$) and an earlier onset of neurological impairment (median age at onset: 44.5 years, interquartile range: 37.75–45.75 years, *n*: 14, $P = 3 \times 10^{-5}$). The conventional variants were associated with lower *RFC1* mRNA levels in peripheral whole blood [29,32] or fibroblasts [31].

To date, the pathophysiological mechanism of *RFC1* pathogenic expansions is not understood and the loss-of-function of the *RFC1* gene remains a hypothesis. The pathogenicity of these conventional variants is thus not certain and we propose considering them as being likely pathogenic because of: (i) their low frequency in the databases of controls; (ii) their detection *in trans* with the pathogenic $(AAGGG)_{exp}$; (iii) their *in-silico* prediction and the intolerance of the *RFC1* gene to haploinsufficiency; and (iv) multiple publications reporting similar variants. These other pathogenic variants in the *RFC1* gene are presented in Fig. 2.

3.2. *Clinical aspects*

RFC1 expansions were initially found to be a common cause of late onset ataxia and, in particular, CANVAS, a clinical syndrome first described in 1991 [36] and clinically defined in 2011. It is characterized by cerebellar ataxia, sensory neuropathy, and bilateral vestibular areflexia [37]. The family history is generally consistent with autosomal recessive inheritance (affected siblings or parental consanguinity) or a sporadic presentation. However, the high frequency of the *RFC1* $(AAGGG)_n$ pathogenic expansion in the general population (~0.031 in the general population and up to 0.045 in Caucasians) is compatible with some cases of pseudodominant inheritance. Since then, the phenotypes and clinical impairment associated with *RFC1* pathogenic expansions have been regularly updated to include more restricted or more complex phenotypes. The various clinical impairments associated with *RFC1* mutations and their possible investigations are presented in Fig. 3 and in Online material Table S1.

3.2.1. *The CANVAS core phenotype*

Biallelic *RFC1* pathogenic expansions were initially described in CANVAS patients [6,7] or those affected by a disorder that always included sensory neuropathy associated with cerebellar ataxia and/or vestibular areflexia [6,17]. Since then, the spectrum of manifestations associated with *RFC1* has been shown to range from isolated neuropathy [18] to more complex phenotypes [34].

RFC1 CANVAS and spectrum disorders [8] frequently start with a dry spasmodic cough, but the first recognized

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

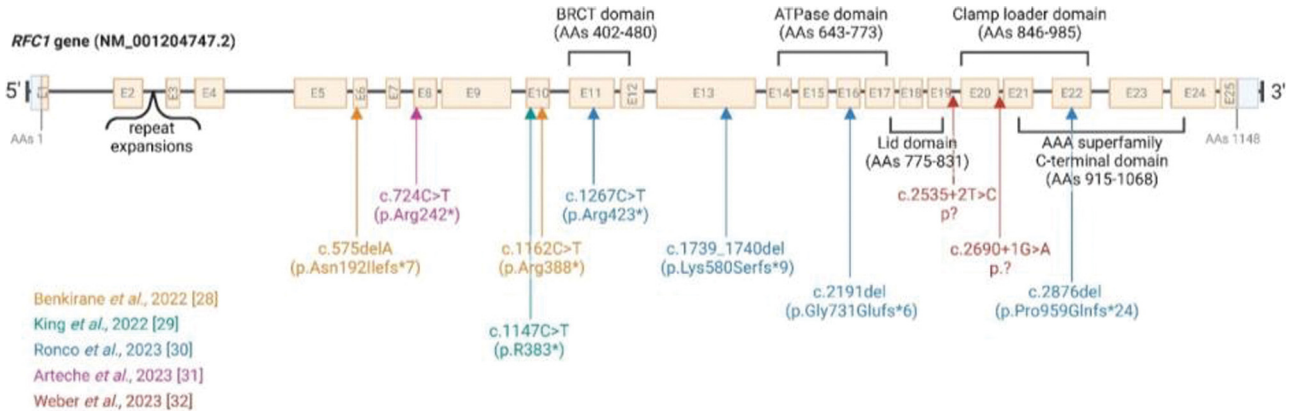

Fig. 2 – Conventional pathogenic variants in *RFC1*. Localisation of the reported conventional pathogenic variants in the *RFC1* gene compared to the domain of the RFC1 protein. Exons and introns are shown as boxes and horizontal lines, respectively. Untranslated regions are indicated as grey boxes and coding exons are marked in orange. Domains were investigated via InterProScan [35]. BRCT domain: breast cancer type 1 susceptibility C Terminus domain, AA: amino acid.

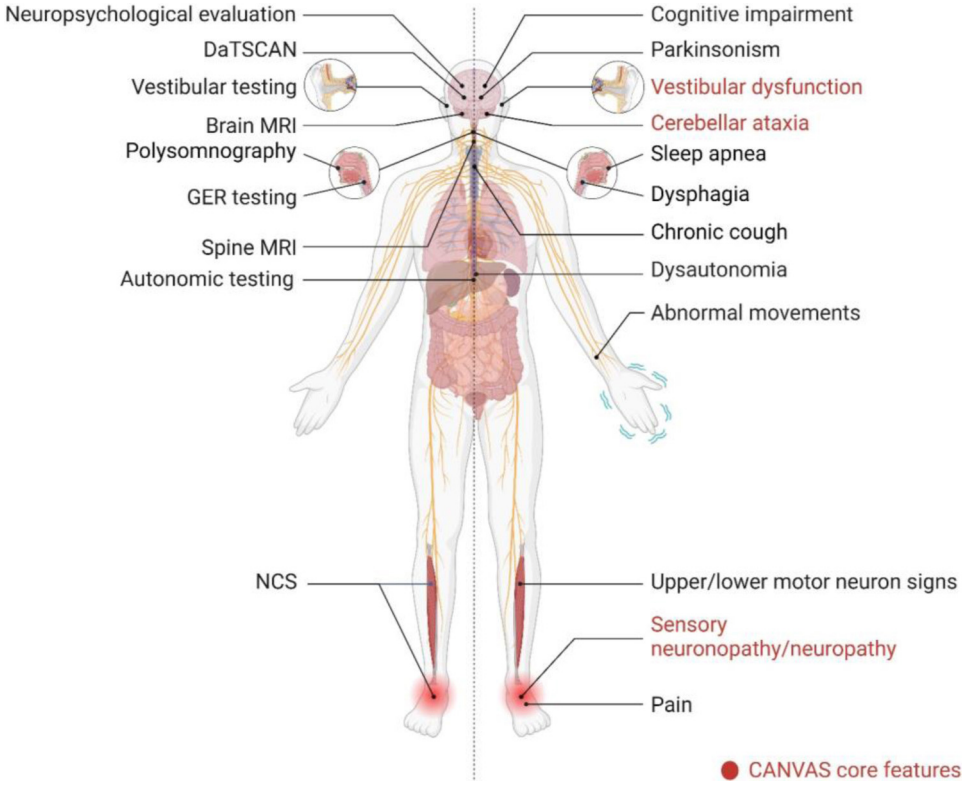

Fig. 3 – Phenotype and investigations associated with *RFC1* pathogenic expansion. Investigations (left) that can be performed to show *RFC1*-associated clinical features (right), which consist of a spectrum of clinical impairments including or not the CANVAS core features (red). GER: gastroesophageal reflux; MRI: magnetic resonance imaging; NCS: nerve conduction study.

symptoms are usually features of sensory neuropathy and/or gait disturbances during the sixth decade. Patients experience sensory neuropathy consisting frequently of sensory neuronopathy, but other patterns can be found during clinical examination and nerve conduction studies (NCS). Related symptoms include unsteadiness, altered sensations (pinprick, vibration, position sense, and nociception), numbness, paresthesia (pins and needles), sensory ataxia, positive Romberg sign, and/or neuropathic pain. Cerebellar ataxia is frequently observed and can be associated with mild to severe cerebellar atrophy, which may help to distinguish it from late-onset Friedreich ataxia, in which cerebellar atrophy is mild or absent. Oculomotor recording may show abnormal eye movements, including gaze-evoked, downbeat, and horizon-

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

tal nystagmus, saccadic pursuit, and dysmetric saccades. Bilateral vestibular areflexia can be found during vestibular testing using the video head-impulse test, caloric response (video-oculography system and an irrigation unit), or rotatory chair. Bilateral vestibulopathy leads to oscillopsia, an illusion of unstable vision in which still objects appear to oscillate.

Based on two major prospective cohorts [17,34], the median age at onset, excluding cough, is 54 years (interquartile range: 47–60.75 years, $n = 190$). Cough is frequently the first symptom, but sensory neuropathy is, to date, the most studied feature and is frequently the only clinical impairment. Subsequently, neurodegeneration expands following a pattern of spatial progression to other neurological systems in later stages of the disease. However, progression of the disease is variable, as the onset of the disease can consist of broad multisystemic neurodegeneration for certain patients [38]. The progression of *RFC1* CANVAS/spectrum disorders is slow, with an average progression of 1.3 points per year using the scale for the assessment and rating of ataxia (mainly evaluating the cerebellar part of the symptoms). However, a small subset of patients may experience sudden phases of rapid progression. The median age at first walking aid is 63.5 years (interquartile range: 60–70 years, $n = 26$) [34]. Half of the patients need a cane after 10 years from disease onset and one quarter are wheelchair dependent after 15 years [17]. Premature death related to *RFC1* disease has been reported and was due to severe dysphagia with cachexia, cough, and immobility/being bedridden after 13 to 22 years of the disease [34]. It is possible that falls, dysphagia, and severe dysautonomia could also cause the death of some patients. Nevertheless, *RFC1*-related disorders do not appear to lead to a significant reduction in life expectancy, as the median age of death is 77 years (range: 59–87) [17].

3.2.1.1. *The full-blown CANVAS phenotype*. According to the studies and after the exclusion of case-reports, the patients who presented with the classical triad of CANVAS were associated with a high diagnostic yield of *RFC1* biallelic $(AAGGG)_n$ expansion, for a total of 179/222 (80.6%) positive CANVAS patients [6,7,12,17,34,39–41]. However, electrophysiological data on all subjects was not always available and the diagnosis of CANVAS did not always fulfill the criteria [42]. When focusing on the patients with an NCS examination and the presence of sensory neuronopathy, the diagnostic yield increased to 137/155 (88.4%) [6,7,17,34,41]. *RFC1* biallelic pathogenic expansions are thus the major cause of CANVAS. Other known genetic causes consist of mutations in the genes *ATXN3* (spinocerebellar ataxia-3, Machado-Joseph disease; OMIM #109150), *FXN* (Friedreich's ataxia; OMIM #229300), *RNF170* (ataxia, sensory, 1, autosomal dominant; OMIM #608984), and *POLG* (sensory ataxic neuropathy, dysarthria, and ophthalmoparesis; OMIM #607459) and other single large-scale mitochondrial DNA deletion syndromes. Rafehi et al. also reported one CANVAS patient harboring two variants (one pathogenic variant and one variant of unknown significance *in trans*) in the *SACS* gene (spastic ataxia, Charlevoix-Saguenay type; OMIM #270550) [7].

3.2.1.2. *CANVAS spectrum*. Some *RFC1* patients may have initially only one or two features of the classical triad of CANVAS ± cough, but do not fulfil the diagnostic criteria of CANVAS. Until recent studies on patients with chronic unexplained cough [43], it was expected that all patients would be affected by clinical sensory neuropathy, or at least subclinical sensory neuropathy assessed by NCS [17]. Sensory neuropathy is thus a major feature of *RFC1*-related diseases and may consist of sensory neuronopathy, sensory neuropathy, sensorimotor neuropathy [12,26,44], or small fiber neuropathy [45]. The presence of sensory neuronopathy appears to increase the diagnostic yield [44,46,47]. Proprioceptive ataxia, sensory symptoms, and abnormal NCS with a diminished sensory nerve action potential (SNAP), an electrophysiological sign related to sensory neuronopathy, are discriminating features for positive *RFC1* testing [34]. This also highlights the interest of searching for different signs of sensory neuropathy and performing NCS on patients suspected of having inherited cerebellar ataxia and/or vestibular areflexia.

After the first report in which the authors observed biallelic *RFC1* expansion in 33/150 (22%) of CANVAS or late-onset ataxia (onset > 35 years) patients [6], a number of studies focused on late-onset and non-dominant cerebellar ataxia. These studies reported a diagnostic yield of *RFC1* biallelic $(AAGGG)_n$ expansion of between 1.5% and 15% of patients [21,25,34,39,40,48–53]. The inclusion/exclusion of multiple system atrophy (MSA), previous genetic testing, the notion of familial history/sporadic cases, the frequencies of consanguinity, and ethnicity in the different cohorts could explain the differences between studies. In a prospective study of 205 patients with sporadic late-onset (> 40 years) cerebellar ataxia, *RFC1* was the second genetic cause (after *SPG7* mutations), with 3/205 (1.5%) $(AAGGG)_{exp}$ biallelic carriers [54]. In a monocentric study, the authors screened the *RFC1* gene in 20 patients with at least two of the following features: progressive ataxia, sensory neuropathy/neuronopathy, vestibulopathy, and chronic cough. They identified biallelic $(AAGGG)_{exp}$ in 13/20 (65%) patients [46].

3.2.1.3. *Isolated sensory neuropathy*. In 2021, Currò et al. screened 225 patients diagnosed with chronic idiopathic axonal polyneuropathy (CIAP) (125 with sensory neuropathy, 100 with sensory-motor neuropathy) for *RFC1* expansions and found 43/125 (34.4%) sensory neuropathy patients with biallelic $(AAGGG)_{exp}$ [18]. They did not find *RFC1* expansions in patients with sensorimotor neuropathy [18]. Among the *RFC1*-positive patients, 10/43 (23.3%) had isolated sensory neuropathy and 8/43 (18.6%) presented with sensory neuropathy and cough. Vestibular and/or cerebellar involvement were identified in 25/43 (58.1%). The distribution of sensory involvement was length-dependent in 20/29 (69%) cases. Motor nerve conduction studies were normal for all but two patients. In a cohort of 234 patients with CIAP, Tagliapietra et al. showed biallelic $(AAGGG)_{exp}$ in 21/40 (52.5%) pure sensory CIAP, 10/56 (17.8%) predominantly sensory, and 3/138 (2.2%) sensorimotor neuropathy cases. Sensory ataxia, dysautonomia, retained deep tendon reflexes, and axonal neuropathy were discriminative features for positive *RFC1* testing. Most of the 34 *RFC1* patients had no cerebellar signs and no cough and none had vestibulopathy [55]. We also reported one *RFC1* patient with cramp-fasciculation syndrome at onset and a

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

history of chronic cough, leading to a diagnosis of mixed motor and sensory neuronopathy, preceding cerebellar ataxia by 15 years [56]. After the exclusion of other molecular diagnoses, Ando et al. screened for *RFC1* expansions in 1475 Japanese cases with inherited peripheral neuropathy and identified biallelic pathogenic expansions in 18/1475 (1.2%) patients. Cerebellar ataxia and/or vestibular dysfunction were observed in only 5/16 (31.3%). The most common phenotype of the *RFC1* patients in this study was sensory-motor neuropathy [27].

3.2.1.4. *Isolated vestibular areflexia*. Oscillopsia, a common sequela of bilaterally vestibular areflexia, was reported as the first symptom for 6/100 *RFC1* patients, and it was the only initial symptom for 3/100 (3%) [17]. Isolated vestibular areflexia was then shown to be an informative clinical impairment for *RFC1* genetic testing. Borsche et al. reported three *RFC1* patients with vestibular impairment assessed by an abnormal vestibulo-ocular reflex on video-oculography of the horizontal head impulse test. These three patients had neither cerebellar ataxia nor neuropathy at the first visit. Some later developed cerebellar ataxia found during clinical follow-up visits [57]. Traschütz et al. screened 168 patients with idiopathic bilateral vestibulopathy (BVP), 41 with a probable diagnosis and 127 meeting the full diagnostic criteria of BVP, and found 10/127 (8%) and 1/41 (2%) patients with biallelic *RFC1* $(AAGGG)_n$ expansions, respectively. Most *RFC1* patients had at least associated sensory neuropathy and only one had isolated BVP. None had BVP associated only with cerebellar features. This study identified *RFC1* mutations as the first monogenic cause of BVP [58].

3.2.1.5. *Isolated cerebellar ataxia*. A few studies have reported *RFC1* patients affected with isolated cerebellar ataxia. Two reported eight patients, but their phenotypes and the investigations performed on them (especially NCS) were not detailed [39,40]. Montaut et al. described one patient with isolated ataxia, without sensory neuronopathy, bilateral vestibular areflexia, or chronic cough [50]. However, the patient carried two small expansions of 32 and 155 $(AAGGG)_n$, which are below the threshold of 250 [8]. In a recent study that included a small cohort of 54 patients with pure cerebellar ataxia without evidence of sensory neuronopathy, the authors did not find any *RFC1* biallelic expansions [57]. They concluded that *RFC1* expansions are highly unlikely in cases with isolated cerebellar ataxia. To date, there have not been enough studies to confirm or refute previously reported associations and involvement of the *RFC1* gene in isolated cerebellar ataxia is still a subject of debate.

3.2.2. *Other clinical impairments*

3.2.2.1. *Cough*. Although not part of the ''CANVAS'' acronym, cough is frequently associated with *RFC1* mutations and is sometimes included in the core CANVAS phenotype [34]. A cough can precede the onset of neurological symptoms [17,58]. Traschütz et al. showed that in a cohort of *RFC1* patients, 46/64 (72%) had chronic cough with an onset at a median age of 35 years (range: 16–69 years, interquartile range: 30–42 years) [34]. Cortese et al. reported chronic cough in 64/100 (64%) *RFC1* patients and it was the initial symptom for one third [17]. When studying a cohort selected based on the presence of sensory neuronopathy using clinical and electrophysiological criteria from the Camdessanché score [59], we observed chronic cough in 33/34 (97.1%) of *RFC1* patients [44]. Interestingly, Traschütz et al. also reported that among patients, some with chronic cough, the onset of gait ataxia was earlier for those with chronic cough (*n*: 46, age 50.3 ± 7.3 years) than those without (*n*: 19, age 61.7 ± 7.4 years, *t*-test, $P < 0.001$). Chronic cough could thus be a marker of the sensitivity of patients to nerve damage [34]. Conversely, some authors have reported a decrease in the frequency of cough episodes after the onset of ataxia [58,60].

The presence of a chronic cough is a discriminating feature for positive *RFC1* testing and its presence in different cohorts increases the diagnostic yield [34,46,61]. Tatineni et al. characterized the cough of 13 *RFC1* patients using questionnaires, an esophagram, a modified barium swallow study, esophageal manometry, and video laryngostroboscopy. The cough was dry (67%), disturbed sleep (75%), and triggered by various factors, including talking, eating, and dry/spicy foods. They showed frequent associated laryngeal alterations, including vocal fold lesions or atrophy. Most treatments, especially standard reflux therapy, were ineffective. Neuromodulating medications (e.g., amitriptyline, gabapentin, and pregabalin) were useful for only 3/8 patients. The patients inconsistently responded to superior laryngeal nerve injections [60]. Cough in *RFC1* patients may also represent a major source of impairment and significantly alter their quality of life [60,62,63].

A recent study on a cohort of patients with refractory chronic cough (RCC) and no diagnosis of CANVAS showed 11/68 (16.2%) to have *RFC1* biallelic pathogenic expansions without ataxia or vestibular syndrome, and only a small subset to have neuropathy [43]. Thus, genetic testing of patients with RCC could allow the diagnosis of *RFC1* disease at the earliest stages. The authors also showed 6/68 (8.8%) of patients with RCC to be heterozygous for pathogenic $(AAGGG)_n$ expansions. To date, it is not clear whether only one pathogenic mutation is sufficient to favor the appearance of chronic cough.

3.2.2.2. *Dysautonomia*. Dysautonomia was already known to be a common feature associated with CANVAS before the description of *RFC1* expansions and is thought to arise from neuronopathy involving the autonomic nervous system [64]. According to the studies and depending on the criteria for patient inclusion and the investigations on them, the presence of dysautonomia is highly variable and ranges from to 21% [18] to 75% [23] of *RFC1*-positive cases. In the two studies that reported on the most patients, the authors reported dysautonomia in 30/46 (65.2%) [17] and 39/63 (61.9%) [34]. It occurred ~5 years (interquartile range: 1–11 years) after the onset of gait ataxia [34]. When focusing on isolated phenotypes, dysautonomia was less frequently observed [18,65]. Dysautonomia is a discriminative feature for *RFC1* expansions when associated with CIAP [55].

In hereditary sensory and autonomic neuropathy (HSAN) associated with chronic cough, Beijer et al. found biallelic *RFC1* expansions in 9/12 (75%) families [61]. In a less restricted study, Yuan et al. molecularly diagnosed *RFC1* disease in 20/79 (25.3%) patients in a cohort of 79 Japanese patients with HSAN,

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

making *RFC1* the major cause of HSAN [66]. In *RFC1* HSAN patients, dysautonomia spans across multiple domains, involves both the sympathetic and parasympathetic nervous systems, and is characterized by small-fiber autonomic axonopathy [67].

The enteric nervous system is also affected. Indeed, numerous *RFC1* patients experience constipation and/or gastroesophageal reflux (GER). Because of the chronic cough, which may precede gait ataxia by decades, some *RFC1* patients have been previously diagnosed with GER, a major cause of chronic cough with normal thoracic imaging. However, GER is a common feature in *RFC1* disease and was reported in 31% of *RFC1* patients but was not associated with cough [34]. Using an esophageal pH test, high-resolution esophageal manometry, or an esophagram or modified barium swallow study, two studies reported frequent GER and esophageal dysmotility in *RFC1* CANVAS patients [60,62].

3.2.2.3. *Upper and lower motor neuron signs*. Motor nerve conduction is normal for most *RFC1* patients [6]. However, recent studies showed that the phenotype of *RFC1* pathogenic expansions should be extended to include motor neuron involvement. Initially, motor neuron involvement was described by Scriba et al. in patients with expansions of the $(ACAGG)_n$ motif and experiencing muscle waiting, fasciculations, elevated creatine kinase levels, and sensorimotor peripheral neuropathy [24]. These results were later confirmed in other patients with the same repeat expansion motif [25–27]. Several studies also hypothesized that motor involvement was more common for patients with biallelic $(ACAGG)_n$ pathogenic expansions than those with heterozygous $(AAGGG)_n/(ACAGG)_n$ or biallelic $(AAGGG)_n$, especially for involvement of the lower motor neurons [25–27]. Given the small number of reported patients with $(ACAGG)_{exp}$, it is not possible to draw any conclusions. Further studies are warranted to confirm (or not) whether the different motifs of *RFC1* pathogenic expansions have an influence on the frequency of motor neuron damage. The proportion of motor neuron signs in *RFC1* patients ranges from 12/67 (17.9%) [58] to 24/38 (63.2%) [44]. It is important to note that patients in these cohorts had biallelic expansions of the $(AAGGG)_{exp}$ motif.

Evocative of lower motor neuron impairment, Traschütz et al. reported muscle atrophy in 14/68 (20.6%) patients. They also reported abnormal conduction study of motor nerves, generally mild, for 28/59 (47.5%) patients and muscle weakness for 7/68 (10.3%) [34]. Reduced or abnormal compound muscle action potentials have been reported for 2/94 (2.1%) patients in the upper limbs and 10/93 (10.8%) in the lower limbs [17] to 2/8 (25%) in the upper limbs and 3/9 (33.3%) in the lower limbs [48]. As for sensory neuropathy, the lower limbs appear to be more frequently affected by motor neuropathy than the upper limbs. In a cohort of patients selected based on the presence of sensory neuronopathy, we observed lower motor neuron signs (fasciculations, wasting, weakness) or a neurogenic pattern by NCS in 16/38 (42.1%) *RFC1* patients [44].

Because decreased deep tendon reflexes due to sensory neuropathy are frequently observed in *RFC1* disease, upper motor neuron involvement (brisk reflexes, extensor plantar responses, and/or spasticity) is less frequently investigated. It is thus difficult to know the proportion of patients with this impairment. Cortese et al. observed retained or even brisk ankle jerks in 45/100 (45%) cases, but all patients had a normal plantar reflex. One patient, however, had a previous diagnosis of spastic paraplegia [17]. We observed upper motor neuron signs in 18/38 (47.4%) patients [44].

We also performed a neuropathological examination of a patient with biallelic pathogenic expansions and motor neuron involvement that included cramps in the lower limbs, extensor plantar reflexes, diffuse fasciculations, brisk reflexes, and an extensor response on plantar reflex. Although the lower motor neuron in the anterior horn of the spinal cord was preserved, there was an astrogliosis pattern in contact with the motor neuron dendrites and axonal swelling of the synapse between the upper and lower motor neuron. These data suggest synaptic dysfunction between the motor neurons [44]. Another neuropathological examination of one *RFC1* patient reported a moderate reduction in the lower motor neurons [68].

3.2.2.4. *Cognitive and psychiatric impairments*. Several groups have reported cognitive impairment in *RFC1* patients [26,34,44,52,69,70]. In a large cohort, Traschütz et al. showed mild cognitive impairment in 13/52 (25%) patients, but the cognitive assessment method was not detailed. In this study, the authors showed that the age and duration of disease of patients with cognitive impairment were no different than those without [34]. Herrmann et al. reported two *RFC1* CANVAS patients with a Mini-Mental State Exam score of 24 or 25, but the formal education duration was not recorded. Neuropsychological evaluation of three patients showed impaired memory for one patient and deficits in memory, attention, executive function, and conceptualization for another [69]. Watanabe et al. reported 4/6 (66.7%) *RFC1* patients from one Japanese family with cognitive decline assessed by the frontal assessment battery versus the Mini-Mental State Exam. These results suggest a predominance of frontal lobe dysfunction in this family [26]. Using the Montreal Cognitive Assessment (MOCA), we reported four *RFC1* patients with mild to moderate cognitive impairment, but did not test all the patients from our cohort [44]. Using the Mini-Mental State Exam and revised Hasegawa dementia scale, Ando et al. observed 2/13 (15.4%) patients with pathogenic *RFC1* expansions and cognitive decline [52].

Korpioja et al. showed that 4/9 (44.4%) *RFC1* patients had cognitive impairment. The authors also showed no *RFC1* biallelic pathogenic expansions in 564 Finnish patients with Alzheimer's disease or frontotemporal dementia. Pathogenic expansion in the *RFC1* gene appears thus to be rare in dementia. Interestingly, in their cohort, 2/9 patients with *RFC1* expansions were followed for diverse psychiatric disorders [70].

All four patients in the study of Hermann et al. also showed depressive symptoms [69], whereas only 3/76 (3.9%) patients in a study of Traschütz et al. had depression [34]. The link between these psychiatric disorders and *RFC1* pathogenic expansions is thus not certain. In an Italian family with five siblings carrying biallelic pathogenic expansions, all patients had behavioral and/or psychiatric disorders, such as depressive-anxious traits, panic attacks, and alcohol abuse. The minor signs of parkinsonism in three *RFC1* patients from the same Italian family may link alteration of the basal ganglia

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

and/or the dopaminergic system with the observed behavioral and psychiatric disorders [71].

In a recent monocentric study, we showed that 71% of *RFC1* patients had an abnormal score at the MOCA and 71% met the criteria for definite cerebellar cognitive affective/Schmahmann syndrome. Among them, three patients had dementia and seven mild cognitive impairment. The severity of cognitive impairment did not correlate with the severity of clinical manifestations. We concluded that cognitive manifestations in *RFC1* disease are highly underestimated and recommend routine cognitive screening for these patients [72].

3.2.2.5. *Parkinsonism*. The phenotype associated with *RFC1* pathogenic biallelic expansions has recently been extended to include parkinsonism. One 82-year-old Japanese *RFC1* patient showed impaired olfactory function and abnormal ^{123}I-ioflupane (DaTSCAN) single-photon emission imaging, but no signs of parkinsonism [20]. In 2020, da Silva Schmitt et al. reported the first case of parkinsonism in a *RFC1* patient. The symptoms of his disease started with a chronic cough, followed by dopa-responsive parkinsonism with an abnormal dopamine transporter scan, and then the other signs of the classical triad of CANVAS [73]. Following this first description, several research groups reported *RFC1* CANVAS/spectrum disorder cases associated with parkinsonism [26,44,52,71,74,75]. Most patients were dopa-responsive, but only slight or no responses to levodopa were reported [71,75]. Traschütz et al. reported features of parkinsonism with bradykinesia and postural instability in 22/75 (29.3%) and 32/61 (52.5%) *RFC1* patients, respectively. One patient was treated with levodopa, with a relatively good response of bradykinesia. The authors noted the overlap with MSA, cerebellar subtype and progressive supranuclear palsy, two neurodegenerative causes of parkinsonism [34]. We reported 4/38 (10.5%) *RFC1* patients with parkinsonism, a rate 10-fold higher than that expected in a matched healthy population of similar age [44]. In the same study, we reported one *RFC1* CANVAS patient with dopa-responsive parkinsonism and a neuropathological examination consistent with a neuropathological diagnosis of Parkinson's disease (PD). In a small cohort of 13 *RFC1* patients, Azevedo et al. showed that 9/13 (69%) patients had an abnormal DaTSCAN and this result did not correlate with the duration of the disease or ataxia. Among these nine patients, only three had parkinsonism [76]. These studies all show that parkinsonism is an additional feature of *RFC1* expansions.

3.2.2.6. *Sleep disorders*. Sleep disorders are not rare and are probably not sufficiently investigated in *RFC1* disease. Chronic cough affects the quality of life and disturbs sleep for 75% of patients [60]. Other sleep disorders include sleep apnea, which may have an influence on the onset of cognitive impairment, and rapid eye movement sleep behavior disorder (RBD), which can suggest alpha-synuclein neurodegeneration and may be related to parkinsonism. Tsuchiya et al. first reported sleep apnea in one case [21]. Other research groups then reported this clinical feature too [28,34,58,62]. Traschütz et al. described sleep apnea in 8/78 (10.3%) [34] patients and Malaquias et al. in 4/67 (6%) [58]. Beecroft et al. first reported RBD in *RFC1* patients [23]. It was then observed in *RFC1* patients with parkinsonism [15,44,75,77,78] or those without [34,58].

3.2.2.7. *Dysphagia*. Dysphagia frequently complicates the disease course in later stages and is classically explained by cerebellar degeneration. However, other neurological features, such as parkinsonism, may cause oropharyngeal dysphagia. Moreover, it should be noted that there is a high frequency (71%) of esophageal dysmotility in the *RFC1* CANVAS/Spectrum [60]. This condition could be secondary to dysautonomia and be the cause or consequence of GER, but it may also be a cause of esophageal dysphagia in *RFC1* patients. In a small study of 11 patients, Casanueva et al. reported a degree of impairment of swallowing for all patients and associated alterations in the quality of life using dedicated questionnaires [79].

3.2.2.8. *Pain*. Sensory neuropathy concerns most *RFC1* patients. Thus, loss of the sensation of pain, as well as neuropathic pain, both related to sensory neuropathy, are to be expected. It has been reported that between 16/83 (19.3%) [34] and 42/105 (40%) [17] *RFC1* patients experience neuropathic pain. The painful sensations are similar to other types of neuropathic pain and are described as a stabbing pain or electric shock sensations by the patients. They often originate in the legs and appear to be worse at night [63]. Between 16/63 (25.7%) [17] and 14/43 (32.6%) [18] *RFC1* patients have also reported frequent paresthesia. Currò et al. reported that 10/43 (23.3%) patients experienced pain at the onset of the disease and this proportion increased at a later evaluation to 17/43 (39.5%) [18]. Magy et al. reported sudden attacks of neuropathic pain for 3/8 (37.5%) patients [63]. In a retrospective study of a cohort of 40 *RFC1* patients with CANVAS, complex neuropathy, or isolated neuropathy, Tagliapietra et al. reported a non-length-dependent impairment in nociception in both clinical and paraclinical investigations. Mild to moderate neuropathic pain showed a weak correlation with disease duration and no correlation with disease severity or age at evaluation [45]. Neuropathic pain is thus a frequent feature of *RFC1* disease and may significantly alter the quality of life of patients. *RFC1* pathogenic expansions have also been reported to cause a reduction in the sensation of pain due to the damaged sensory nerves [66]. Such reduction in the sensation of pain was observed in a cohort of patients diagnosed with HSAN and concerned 17/20 (85%) *RFC1* patients. The loss of nociception was also observed by Tagliapietra et al. but was remarkably patchy [45].

3.2.2.9. *Hyperkinetic movements*. Hyperkinetic movement disorders are rarely observed in *RFC1* disease. In the study of Traschütz et al., they consisted of orofacial dyskinesia (5%), orofacial dystonia (5%), or limb chorea (2%) [34]. Basal ganglia atrophy found in *RFC1* patients [74] could explain such abnormal movement and parkinsonism. A few studies have reported myoclonus, sometimes as the first symptom [28,34,80].

3.2.3. *Neuroimaging*

3.2.3.1. *Brain MRI*. Cerebellar atrophy is the most prominent neuroradiological finding in *RFC1* CANVAS/Spectrum disor-

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

ders and was reported in 35/42 (83.3%) *RFC1* cases in the first description of the causative gene [6]. In 56 *RFC1*-positive patients, Traschütz et al. more precisely described the neuroimaging findings. Cerebral atrophy was mild to moderate for most patients but was severe or absent for some, highlighting the variability of this feature. The cerebellar atrophy affected the vermis more than the hemisphere, and its presence was not associated with a longer duration of disease. Cerebral atrophy was detected in 42% of patients. Brainstem atrophy was detected in 13% of reviewed MRIs and associated with disease duration and clinical features, including dysphagia, urinary urge, and oculomotor impairment. Signal abnormalities of the basal ganglia in two patients was later confirmed as a characteristic feature of *RFC1* disease [34]. Using 3T MRI, Matos et al. found atrophy of the cerebellum, brainstem, and basal ganglia. They noted deep cerebral white matter damage, whereas cerebral cortical damage was relatively restricted [74]. Usually evocative of MSA, a ''hot cross bun'' sign on T2-weighted imaging has been reported for *RFC1* patients [77]. Two cases of *RFC1* CANVAS also showed mild cerebellar atrophy, with greater involvement of the vermis and a (pseudo-)eye-of-the-tiger sign corresponding to hyperintensity of the central region surrounded by a ring of hypointensity on coronal and transverse T2-weighted images of the globus pallidus [81].

3.2.3.2. *Spine MRI*. Cortese et al. reported that 19/42 (45.2%) *RFC1* patients in their study had spinal cord atrophy and 4/34 (11.8) showed T2 hyperintensities in the posterior column [17]. Using 3T MRI on 17 *RFC1* patients, Rezende et al. observed spinal cord atrophy at all cervical levels, anteroposterior flattening in the lower cervical/upper thoracic regions, and diffusivity abnormalities in the posterior columns. The authors also found atrophy of the grey matter in the cervical spinal cord [82]. El Houjeiry et al. published the case-report of one *RFC1* patient initially presenting with slowly progressive ataxia, paresthesia, and an isolated spinal cord lesion that was hyperintense in T2-weighted images and mimicked dysimmune myelitis [83].

3.2.4. *Neuropathological examination*

3.2.4.1. *Brain*. To date, seven studies have described neuropathological examinations of six patients with biallelic $(AAGGG)_{exp}$ and two patients with biallelic $(ACAGG)_{exp}$ [6,25,44,50,68,84,85]. The most notable finding in all studied cases was cerebellar atrophy, which appeared to primarily affect the vermis. It was accompanied by widespread depletion of Purkinje cells and Bergmann gliosis in all patients. The granule-cell layer showed neuronal loss in two cases [50,85] and the cerebellar dentate nucleus showed signs of gliosis and neuronal loss in two patients [50,84]. In three patients, there was a pallor of the *substantia nigra* and a depletion of neurons in this region [44,84,85]. Among these three patients, one had parkinsonism [44], one had impaired olfactory function and an abnormal DaTSCAN, but no signs of parkinsonism [20,85] and the last showed no signs of parkinsonism [84]. The two patients with features of parkinsonism also had Lewy bodies in the *substantia nigra* and *locus coeruleus*. These data appear to be consistent with the already discussed findings suggesting that impairment in the dopaminergic system could lead to parkinsonism in some *RFC1* patients. The other brain regions that were inconstantly altered were the frontal cortex and the *medulla oblongata* (vestibular, hypoglossal and inferior olivary nuclei).

There were neurofibrillary tangles in all neuropathological examinations, which could have been age-related in most cases. Khurana et al. reported multisystem tauopathy in their examination. Three studies also reported neuropathological changes consistent with Alzheimer's disease of varying severity [6,68,85]. Immunostaining for p62 and TDP-43 were negative in all studies.

In addition, Cortese et al. performed fluorescence *in situ* hybridization using oligonucleotides that recognize the $(AAGGG)_n$ repeat expansions in the vermis of an *RFC1* patient. They did not find any evidence of RNA foci formation in their samples [6]. This result contradicts those of another study that showed RNA foci in two patients with biallelic $(AAGGG)_n$ or $(ACAGG)_n$ repeat expansions [85].

3.2.4.2. *Spinal*. Spinal cord examinations have revealed severe degeneration of the posterior column [25,44,50,68,85]. A moderate loss of motor neurons was observed for four patients in the anterior horn of the spinal cord [68,84,85]. We did not note any loss of motor neurons but we have observed axonal swelling of cells in contact with the motor neurons. TDP-43 and p62 immunostaining of the motor neurons in the anterior horn did not reveal any inclusions [44].

3.2.4.3. *Nerves*. The main observation in sural nerve biopsies from *RFC1* patients has been the depletion of myelinated fibers [6,17,55,63,84,86,87]. Disorganization around Schwann cells was observed in radial and sural nerves of *RFC1* patients [63]. Hirano et al. observed damage in myelinating Schwann cells, with cytoplasmic inclusions. They hypothesized that *RFC1* patients occasionally develop demyelination neuropathy or Schwann-cell damage [87]. The loss of small myelinated fibers could be related to the neuropathic pain experienced by *RFC1* patients [63]. However, both large and small myelinated and unmyelinated fibers appear to be affected [55]. The presence of multiple collagen pockets has also been observed, reflecting unmyelinated fiber damage [63,87]. In addition, Tagliapietra et al. noted that the involvement of unmyelinated fibers was greater in *RFC1* patients (85%) than in those with other CIAP (41%). Signs of active degeneration and regeneration clusters are uncommon in nerve biopsies of *RFC1* patients [17,55,63]. Moreover, regeneration clusters were significantly less represented in *RFC1* patients than in those with other CIAP [55]. Muscle and skin biopsies confirmed chronic denervation [6,55,58,63,84]. Intraepidermal nerve fiber density was similar at the thigh and leg, consistent with non-length-dependent axonal degeneration and suggestive of sensory neuronopathy [55].

3.2.5. *Other associations and overlapping phenotypes*

3.2.5.1. *MSA*. Because of frequent multisystem involvement and similar clinical symptoms, such as cerebellar ataxia (which can rapidly progress in some patients), dysautonomia, parkinsonism, and cognitive impairment, sporadic presentation of *RFC1* disease overlaps with MSA, especially the cerebellar subtype [34]. However, in the previous guidelines

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

for the diagnosis of MSA, the presence of neuropathy, a highly frequent feature in *RFC1* disease, was a red flag against a diagnosis of MSA [88]. Moreover, given the speed of progression and severity of MSA, which has a very poor prognosis, with a life expectancy limited to ~10 years after the onset of the disease [89], it is thus difficult not to consider these pathologies as two different entities.

These similarities, at least at presentation, led several teams to study *RFC1* expansions in cohorts of MSA patients. Several studies did not find any *RFC1* biallelic pathogenic expansions in cohorts of patients with ''probable'' or ''possible'' MSA [50,90]. In a cohort of 336 pathologically confirmed MSA patients, Sullivan et al. found no biallelic pathogenic expansions [91]. Further studies reported 3/282 (1.1%) [19], 3/207 (1.4%) [77], and 3/44 (6.8%) [80] patients with *RFC1* biallelic pathogenic expansions in cohorts of ''probable'' or ''possible'' MSA. Among these nine patients, 5/9 had parkinsonism non-responsive to levodopa, 3/4 had vestibular impairment, and one had history of chronic cough [19,77]. An abnormal DaTscan does not appear to contribute to the differential diagnosis between CANVAS and MSA [80]. NCS was not performed on all patients. The authors concluded that *RFC1* pathogenic expansions can cause symptoms similar to MSA, which can be confusing during diagnosis. In these studies, the diagnosis of ''probable'' or ''possible'' MSA relies purely on clinical observations and was based on the previous diagnostic criteria. None of these nine patients had a neuropathological examination.

In summary, the overlap of symptoms between MSA and CANVAS complicates their differential diagnosis. The association between *RFC1* pathogenic expansions and MSA is still a subject of debate, given that the diagnoses of MSA in the cohorts reported in the literature were not confirmed by neuropathological examination.

3.2.5.2. *PD*. Rare cases of pathogenic expansions have been reported in cohorts of patients with isolated or prominent parkinsonism. In a cohort of 559 Finnish patients with parkinsonism, Kytövuori et al. found 3/559 (0.5%) biallelic pathogenic expansions [78]. These three patients fulfilled the criteria of PD, but they also showed comorbidity unusual for PD patients, such as chronic cough, sensory neuropathy, vestibulopathy, abnormal deep tendon reflexes, and/or abnormal eye movements. The same research group reported 3/273 (1.1%) patients with *RFC1* pathogenic expansions in a cohort of patients diagnosed with early-onset PD [92]. One patient had mild vestibular dysfunction but the other two did not show signs of CANVAS. In these two studies, the $(AAGGG)_n$ repeat units were relatively small (range: 141–831), sometimes below the threshold of 250 [8]. Further studies are warranted to confirm the association of *RFC1* biallelic pathogenic expansions and the occurrence of PD.

3.2.5.3. *ALS*. *RFC1* patients may have lower and/or upper motor neuron signs. Neuropathological examinations are also suggestive of motor neuron alterations [44,68]. However, it is still difficult to compare CANVAS and classic ALS, given the rapid progression and very poor prognosis of ALS. Abramzon et al. found no biallelic pathogenic expansion in a cohort of 1069 American sporadic ALS patients, indicating that *RFC1* is not a common cause of sporadic ALS [10]. Schoeberl et al. reported the case of one ALS patient with associated subclinical sensory neuronopathy, bilateral vestibulopathy, and biallelic $(AAGGG)_n$ expansions [93]. To date, it is impossible to state whether *RFC1* can cause rare inherited ALS, whether $(AAGGG)_n$ expansions could be a genetic risk factor, or whether *RFC1* disease can convert to ALS, as described in some cases of spastic paraplegia [94].

4. Discussion

The *RFC1* locus is a highly dynamic region of the human genome and numerous motifs have thus far been described. Various motifs and loss-of-function variants can be associated with certain phenotypic differences, but they do not appear to cause disorders with dramatically different phenotypes. The steady increase in the identification of pathogenic motifs or motifs of uncertain significance makes the molecular diagnosis of this disease complex. These difficulties in molecular diagnosis are also a limitation of this review, as many of the studies we report here concerned a small number of cases and did not always confirm their analyses by Southern-blotting, a similar technique, or long-read sequencing. New molecular methods, such as optical genome mapping [95] or Nanopore DNA long-read sequencing [9], have been used for the diagnosis of *RFC1* disease. In the future, it is highly likely that the use of these new methods, allowing a more precise analysis of these expansions, along with the study of the motifs that compose them, will provide predictions on the associated phenotypes and/or prognosis.

As recently shown by Curro et al., a larger repeat size of both pathogenic alleles correlate with an earlier age at neurological onset. However, the effect is very small and explains only up to 6% of the variability. The authors suggested that additional environmental or genetic modifiers may influence the disease [22]. Indeed, considering the late-onset of the disease and the variability in phenotypes, it is possible that aging and environmental exposure could have a more important role than in other genetic diseases with a pediatric or neonatal onset. For example, alcohol consumption might favor neuropathy and cerebellar ataxia, whereas exposure to other neurotoxic agents, such as pesticides, could favor parkinsonism in *RFC1* patients. Epidemiological studies on the role of the environment and, in particular, exposure to toxic substances or comorbidities, would therefore be necessary.

Given the higher frequency of the *RFC1* allele with $(AAGGG)_n$ expansions (i.e. 0.045) among Caucasians [7], it is possible to estimate the proportion of carriers of biallelic $(AAGGG)_{exp}$ to a maximum of 2/1000 in the Caucasian population. This is ~40-fold greater than initially described. *RFC1* disease may thus be a non-rare genetic disorder. The high variability in phenotype and the late-onset of *RFC1* CANVAS/spectrum disorders raises the question of whether such biallelic expansions are fully penetrant or not. Further studies on large populations of older healthy individuals are warranted to assess the penetrance of this genetic disorder. Another unresolved question is the impact of heterozygous pathogenic expansion on health in the general population. Fan et al. reported no differences in $(AAGGG)_n$ allele frequencies

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

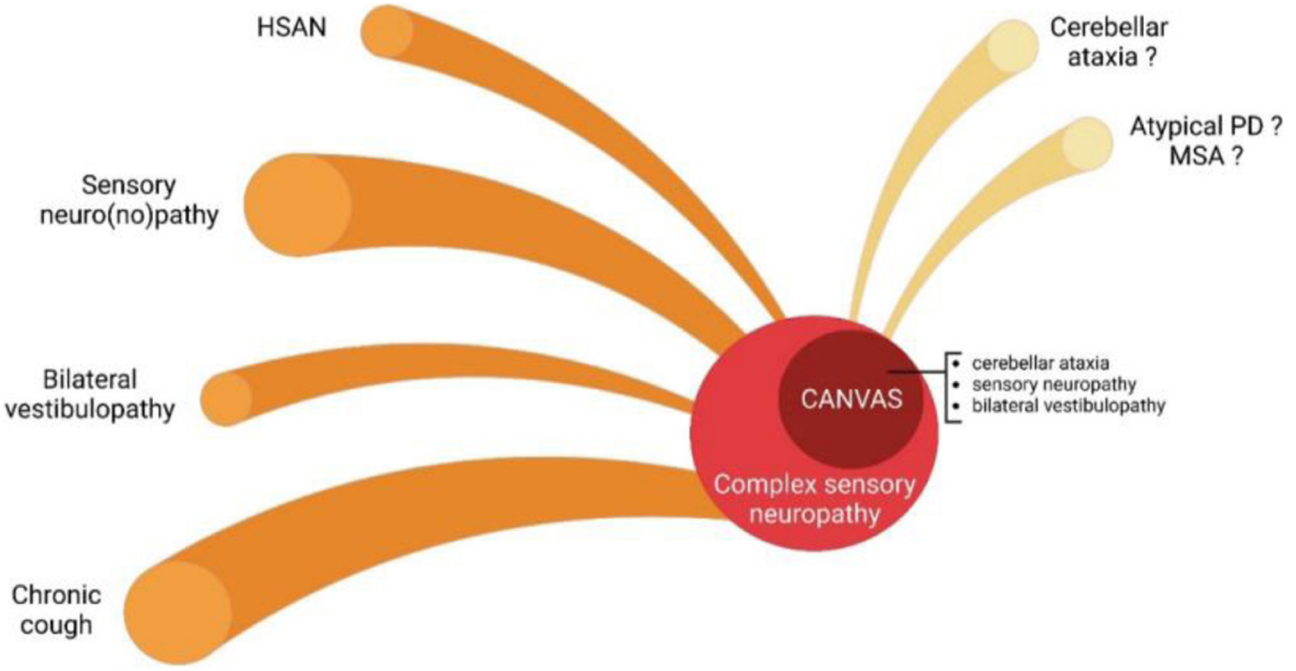

Fig. 4 – Different entryways into *RFC1* pathology. The first signs presented by *RFC1*-positive patients (orange) can lead to a wider complex sensory neuropathy (red) or CANVAS (dark red), with clinical features such as those described in paragraph 3.2.2. Other reported entryways have not yet been confirmed (yellow) due to the small number of cases described. CANVAS: cerebellar ataxia, neuropathy, and vestibular areflexia syndrome; PD: Parkinson's disease; MSA: multiple-system atrophy; HSAN: hereditary sensory and autonomic neuropathy.

between MSA, late-onset ataxia and healthy controls [90]. However, in some studies, the authors reported a high allele frequency, up to 28/136 (~0.21) in the refractory chronic cough cohort in a study of Guilleminault et al. [43]. It may therefore be informative to assess the frequency of heterozygous $(AAGGG)_n$ carriers in large cohorts of patients with neurological diseases with a late-onset (for example: Alzheimer's disease, PD, dementia with Lewy bodies) to determine whether heterozygous $(AAGGG)_n$ expansion could constitute a genetic risk factor for these diseases.

RFC1 disease can encompass a large number of clinical features and initial presentations, leading to complex phenotypes with frequent multisystemic involvement (Fig. 4). Neuroradiological findings, as clinical impairments, have provided additional evidence to the heterogeneous and multisystemic nature of *RFC1* disease [96]. *RFC1* disease should be considered as continuous spectrum of phenotypes. In incomplete CANVAS or isolated phenotypes, the diagnosis may be challenging, leading to frequent molecular screening. There are still no guidelines for the prescription of genetic testing for these patients and biomarkers of *RFC1* disease are yet to be found. A few studies have provided clues for the development of such biomarkers. Indeed, taupathology and neurofibrillary tangles in the brain have been observed in every neuropathological examination to date [6,20,25,44,50,68,84,85]. Although likely related to aging or associated with Alzheimer's disease, Traschütz et al. reported elevated levels of tau protein in the CSF of a patient with sensory neuropathy and chronic cough. They also reported elevated levels of neurofilament light (NfL) chain in the CSF [38]. Serum NfL levels of *RFC1* patients have been measured and were significantly higher in *RFC1* patients than healthy controls and correlated with cerebellar involvement [97]. Another putative plasma biomarker could be the elevated creatine kinase previously shown in patients with $(ACAGG)_n$ or $(AAGGG)_n$ expansions [24,25,27].

Beyond the classic clinical presentations, *RFC1*-related diseases have been found in patients previously diagnosed with a wide variety of conditions, including other genetic diseases and those related to metabolic or toxic causes or various auto-immune pathologies, such as Sjögren's syndrome [17,18,57,63,83,87,98–101]. Other diagnostic corrections are awaited, especially for diseases with cough as the main clinical feature. The origin of cough in *RFC1*-related disorders is not known. Chronic cough is frequently hypothesized to be a consequence of GER, but it was not associated with cough in a cohort of *RFC1* patients [34]. It is highly probable that chronic cough is the first neurological sign of the disease. Various neurocentric hypotheses can be proposed. First, neuronal loss in the vagal nucleus could explain the cough, as we showed in a neuro-examination of a CANVAS patient [44]. Second, such alterations of the vagal nucleus may be secondary to vagal neuronopathy or damage to the afferent sensory nerves, of which the terminals are located in the major airways or lung parenchyma. Therefore, it is possible that damage of the vagal nerve could be the first event in *RFC1* disease. Third, other brain or cerebellar regions involved in the control of the cough reflex could also be involved.

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

The mechanism leading to *RFC1* CANVAS/spectrum disorders is still unknown. Although Cortese et al. did not find any RNA foci formation [6], Wada et al. showed the presence of some in the brain of a patient without the presence of RNA-binding proteins [85]. This could indicate that the pathogenic expansions lead to a toxic RNA gain-of-function mechanism. However, there are also reports of compound heterozygous patients with pathogenic variants in the *RFC1* gene (missense, nonsense, and frameshift) and pathogenic expansion. These patients have the same symptoms as other *RFC1* CANVAS patients [29–33] and showed reduced *RFC1* mRNA levels. In addition, recent studies have shown the formation of G-quadruplexes and triplexes by pathogenic $(AAGGG)_n$ expansions [15,16,102]. This would suggest a loss-of-function mechanism, as observed in Friedreich's ataxia [103].

In conclusion, *RFC1* disease is a recently discovered genetic disorder that explains many neurological conditions. The most frequent pathogenic variants are biallelic $(AAGGG)_n$ expansions in intron 2 of the gene, but there are numerous pathogenic/non-pathogenic motifs. *RFC1*-related disease is highly heterogeneous and ranges from various isolated neurological conditions, mostly sensory neuropathy, to complex neurological pathologies with multisystemic involvement, such as CANVAS. New phenotypes and clinical presentations are likely to be discovered over the next few years. Much effort is still needed to completely understand the clinical picture. Future studies using cellular or animal models will likely improve our understanding of the pathophysiological mechanisms and perhaps aid the development of treatment for this disease.

Funding

This work was supported by the University of Lille and the Lille University Hospital (CHU Lille). This work was also supported by grants from the ''Programme d'Investissements d'Avenir LabEx (excellence laboratory) DISTALZ (Development of Innovative Strategies for a Transdisciplinary approach to ALZheimer's disease)''.

Authors contribution

VD: acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted. CT: acquisition of data, revising it critically for important intellectual content, final approval of the version to be submitted. J.-B. D: acquisition of data, revising it critically for important intellectual content, final approval of the version to be submitted. KD: acquisition of data, revising it critically for important intellectual content, final approval of the version to be submitted. AW: acquisition of data, revising it critically for important intellectual content, final approval of the version to be submitted. C.-M.D: acquisition of data, revising it critically for important intellectual content, final approval of the version to be submitted. EM: acquisition of data, revising it critically for important intellectual content, final approval of the version to be submitted. VH: conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be submitted.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgement

Figures were created by the authors with BioRender software (biorender.com).

Online supplement. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurol.2024.03.006.

REFERENCES

[1] Bunz F, Kobayashit R, Stillmant B. Biochemistry cDNAs encoding the large subunit of human replication factor C (simian virus 40/DNA replication/DNA binding/ATPase/DNA polymerase accessory protein); 90. 1993 [Online. Available: https://www.pnas.org].
[2] Pennaneach V., Salles-Passador I., Munshi A., Brickner H., Regazzoni K., Dick F., et al. The large subunit of replication factor C promotes cell survival after DNA damage in an LxCxE motif-and Rb-dependent manner Culescu et al., 1998). Rb binds to E2F family of transcription factors and represses transcriptional activation by E2F. Additionally, the Rb-E2F complex at the promoter actively represses transcription of genes important for cell-cycle progression. Successive phosphorylation of volume 7, 2001.
[3] Majka J, Burgers PMJ. The PCNA-RFC families of DNA clamps and clamp loaders; 2004.
[4] Tomida J, Masuda Y, Hiroaki H, Ishikawa T, Song I, Tsurimoto T, et al. DNA damage-induced ubiquitylation of RFC2 subunit of replication factor C complex. J Biol Chem 2008;283(14):9071–9. http://dx.doi.org/10.1074/jbc.M709835200.
[5] Overmeer RM, Gourdin AM, Giglia-Mari A, Kool H, Houtsmuller AB, Siegal G, et al. Replication factor C recruits DNA polymerase δ to sites of nucleotide excision repair but is not required for PCNA recruitment. Mol Cell Biol 2010;30(20):4828–39. http://dx.doi.org/10.1128/mcb.00285-10.
[6] Cortese A, Simone R, Sullivan R, Vandrovcova J, Tariq H, Yan YW, et al. Biallelic expansion of an intronic repeat in RFC1 is a common cause of late-onset ataxia. Nat Genet 2019;51(4):649–58. http://dx.doi.org/10.1038/s41588-019-0372-4.
[7] Rafehi H, Szmulewicz DJ, Bennett MF, Sobreira NLM, Pope K, Smith KR, et al. Bioinformatics-based identification of expanded repeats: a non-reference intronic pentamer expansion in RFC1 causes CANVAS. Am J Hum Genet 2019;105(1):151–65. http://dx.doi.org/10.1016/j.ajhg.2019.05.016.

[8] Cortese A, Reilly MM, Houlden H. RFC1 CANVAS/spectrum disorder. GeneReviews® [Internet], Seattle (WA): University of Washington, Seattle; 1993–2023. Accessed: Jan. 18, 2024. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/.
[9] Scriba CK, Stevanovski I, Chintalaphani SR, Gamaarachchi H, Ghaoui R, Ghia D, et al. RFC1 in an Australasian neurological disease cohort: extending the genetic heterogeneity and implications for diagnostics. Brain Commun 2023;5(4). http://dx.doi.org/10.1093/braincomms/fcad208.
[10] Abramzon Y, Dewan R, Cortese A, Resnick S, Ferrucci L, Houlden H, et al. Investigating RFC1 expansions in sporadic amyotrophic lateral sclerosis. J Neurol Sci 2021;430. http://dx.doi.org/10.1016/j.jns.2021.118061.
[11] Akçimen F, Ross JP, Bourassa CV, Liao C, Rochefort D, Gama MTD, et al. Investigation of the RFC1 repeat expansion in a Canadian and a Brazilian ataxia cohort: identification of novel conformations. Front Genet 2019;10. http://dx.doi.org/10.3389/fgene.2019.01219.
[12] Ghorbani F, de Boer-Bergsma J, Verschuuren-Bemelmans CC, Pennings M, de Boer EN, Kremer B, et al. Prevalence of intronic repeat expansions in RFC1 in Dutch patients with CANVAS and adult-onset ataxia. J Neurol 2022;269(11):6086–93. http://dx.doi.org/10.1007/s00415-022-11275-9.
[13] Erdmann H, Schöberl F, Giurgiu M, Leal Silva RM, Scholz V, Scharf F, et al. Parallel in-depth analysis of repeat expansions in ataxia patients by long-read sequencing. Brain 2023;146(5):1831–43. http://dx.doi.org/10.1093/brain/awac377.
[14] Tyagi N., Uppili B., Sharma P., Parveen S., Saifi S., Jain A., et al. Investigation of RFC1 tandem nucleotide repeat locus in diverse neurodegenerative outcomes in an Indian cohort. doi: 10.1101/2023.06.05.23290839.
[15] Dominik N, Magri S, Currò R, Abati E, Facchini S, Corbetta M, et al. Normal and pathogenic variation of RFC1 repeat expansions: implications for clinical diagnosis. Brain 2023;146(12):5060–9. http://dx.doi.org/10.1093/brain/awad240.
[16] Abdi MH, Zamiri B, Pazuki G, Sardari S, Pearson CE. Pathogenic CANVAS-causing but not non-pathogenic RFC1 DNA/RNA repeat motifs form quadruplex or triplex structures. J Biol Chem 2023;299(10). http://dx.doi.org/10.1016/j.jbc.2023.105202.
[17] Cortese A, Tozza S, Yan Yau W, Rossi S, Beecroft SJ, Jaunmuktane Z, et al. Cerebellar ataxia, neuropathy, vestibular areflexia syndrome due to RFC1 repeat expansion. Brain 2020;143(2):386–90. http://dx.doi.org/10.1093/brain/awaa015.
[18] Currò R, Salvalaggio A, Tozza S, Gemelli C, Dominik N, Galassi Deforie V, et al. RFC1 expansions are a common cause of idiopathic sensory neuropathy. Brain 2021;144(5):1542–50. http://dx.doi.org/10.1093/brain/awab072.
[19] Wan L, Chen Z, Wan N, Liu M, Xue J, Chen H, et al. Biallelic intronic AAGGG expansion of RFC1 is related to multiple system atrophy. Ann Neurol 2020;88(6):1132–43. http://dx.doi.org/10.1002/ana.25902.
[20] Nakamura H, Doi H, Mitsuhashi S, Miyatake S, Katoh K, Frith MC, et al. Long-read sequencing identifies the pathogenic nucleotide repeat expansion in RFC1 in a Japanese case of CANVAS. J Hum Genet 2020;65(5):475–80. http://dx.doi.org/10.1038/s10038-020-0733-y.
[21] Tsuchiya M, Nan H, Koh K, Ichinose Y, Gao L, Shimozono K, et al. RFC1 repeat expansion in Japanese patients with late-onset cerebellar ataxia. J Hum Genet 2020;65(12):1143–7. http://dx.doi.org/10.1038/s10038-020-0807-x.
[22] Currò R, Dominik N, Facchini S, Vegezzi E, Sullivan R, Galassi Deforie V, et al. Role of the repeat expansion size in predicting age of onset and severity in RFC1 disease. Brain 2024;32. http://dx.doi.org/10.1093/brain/awad436/7513227.
[23] Beecroft SJ, Cortese A, Sullivan R, Yau WY, Dyer Z, Wu TY, et al. A Maori – specific RFC1 pathogenic repeat configuration in CANVAS, likely due to a founder allele. Brain 2020;143(9):2673–80. http://dx.doi.org/10.1093/brain/awaa203.
[24] Scriba CK, Beecroft SJ, Clayton JS, Cortese A, Sullivan R, Yau WY, et al. A novel RFC1 repeat motif (ACAGG) in two Asia-Pacific CANVAS families. Brain 2020;143(10):2904–10. http://dx.doi.org/10.1093/brain/awaa263.
[25] Miyatake S, Yoshida K, Koshimizu E, Doi H, Yamada M, Miyaji Y, et al. Repeat conformation heterogeneity in cerebellar ataxia, neuropathy, vestibular areflexia syndrome. Brain 2022;145(3):1139–50. http://dx.doi.org/10.1093/brain/awab363.
[26] Watanabe K, Nakashima M, Wakatsuki R, Bunai T, Ouchi Y, Nakamura T, et al. Cognitive impairment in a complex family with AAGGG and ACAGG repeat expansions in RFC1 detected by ExpansionHunter Denovo. Neurol Genet 2022;8(3). http://dx.doi.org/10.1212/NXG.0000000000000682.
[27] Ando M, Higuchi Y, Yuan J, Yoshimura A, Taniguchi T, Kojima F, et al. Comprehensive genetic analyses of inherited peripheral neuropathies in Japan: making early diagnosis possible. Biomedicines 2022;10(7). http://dx.doi.org/10.3390/biomedicines10071546.
[28] van de Pol M, O'Gorman L, Corominas-Galbany J, Cliteur M, Derks R, Verbeek NE, et al. Detection of the ACAGG repeat motif in RFC1 in two Dutch Ataxia families. Mov Disord 2023;38(8). http://dx.doi.org/10.1002/mds.29441. John Wiley and Sons Inc:1555–1556.
[29] Benkirane M, Da Cunha D, Marelli C, Larrieu L, Renaud M, Varilh J, et al. RFC1 nonsense and frameshift variants cause CANVAS: clues for an unsolved pathophysiology. Brain 2022;145(11):3770–5. http://dx.doi.org/10.1093/brain/awac280.
[30] King KA, Wegner DJ, Bucelli RC, Shapiro J, Paul AJ, Dickson PI, et al. Whole-genome and long-read sequencing identify a novel mechanism in RFC1 resulting in CANVAS syndrome. Neurol Genet 2022;8(6). http://dx.doi.org/10.1212/NXG.0000000000200036.
[31] Ronco R, Perini C, Currò R, Dominik N, Facchini S, Gennari A, et al. Truncating variants in RFC1 in cerebellar ataxia, neuropathy, and vestibular areflexia syndrome. Neurology 2023;100(5):E543–54. http://dx.doi.org/10.1212/WNL.0000000000201486.
[32] Arteche-López A, Avila-Fernandez A, Damian A, Soengas-Gonda E, de la Fuente RP, Gómez PR, et al. New cerebellar ataxia, neuropathy, vestibular areflexia syndrome cases are caused by the presence of a nonsense variant in compound heterozygosity with the pathogenic repeat expansion in the RFC1 gene. Clin Genet 2023;103(2):236–41. http://dx.doi.org/10.1111/cge.14249.
[33] Weber S, Coarelli G, Heinzmann A, Monin M-L, Gerard M, Durr A, et al. Two RFC1 splicing variants in CANVAS. Brain 2023;146:e14–6. http://dx.doi.org/10.1093/brain/awac466/6881570.
[34] Traschütz A, Cortese A, Reich S, Dominik N, Faber J, Jacobi H, et al. Natural history, phenotypic spectrum, and discriminative features of multisystemic RFC1 disease. Neurology 2021;96(9):E1369–82. http://dx.doi.org/10.1212/WNL.0000000000011528.
[35] Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, et al. InterProScan 5: genome-scale protein function

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

classification. Bioinformatics 2014;30(9):1236–40. http://dx.doi.org/10.1093/bioinformatics/btu031.
[36] Bronstein AM, Mossman S, Luxon LM. The neck-eye reflex in patients with reduced vestibular and optokinetic function; 1991.
[37] Szmulewicz D, Waterston MJ, Halmagyi G, Mossman S, Chancellor FA, McLean FC, et al. Sensory neuropathy as part of the cerebellar ataxia neuropathy vestibular areflexia syndrome; 2011 [Online. Available: www.neurology.org].
[38] Traschutz A, Wilke C, Haack TB, Bender B, Synofzik M. Sensory axonal neuropathy in RFC1-disease: tip of the iceberg of broad subclinical multisystemic neurodegeneration. Brain 2022;145(3):E6–9. http://dx.doi.org/10.1093/brain/awac003. Oxford University Press.
[39] Aboud Syriani D, Wong D, Andani S, De Gusmao CM, Mao Y, Sanyoura M, et al. Prevalence of RFC1-mediated spinocerebellar ataxia in a North American ataxia cohort. Neurol Genet 2020;6(3). http://dx.doi.org/10.1212/NXG.0000000000000440.
[40] Gisatulin M, Dobricic V, Zühlke C, Hellenbroich Y, Tadic V, Münchau A, et al. Clinical spectrum of the pentanucleotide repeat expansion in the RFC1 gene in ataxia syndromes. Neurology 2020;95(21):E2912–23. http://dx.doi.org/10.1212/WNL.0000000000010744.
[41] Costales M, Casanueva R, Suárez V, Asensi JM, Cifuentes GA, Diñeiro M, et al. CANVAS: a new genetic entity in the otorhinolaryngologist's differential diagnosis. Otolaryngol Head Neck Surg 2022;166(1):74–9. http://dx.doi.org/10.1177/01945998211008398.
[42] Szmulewicz DJ, Roberts L, Mclean CA, Macdougall HG, Michael Halmagyi G, Storey E. Neurology® clinical practice proposed diagnostic criteria for cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS); 2016.
[43] Guilleminault L, Chazelas P, Melloni B, Magdelaine C, Villeneuve T, Brouqières D, et al. Repeat expansions of RFC1 in refractory chronic cough a missing piece of the puzzle?; 2023. http://dx.doi.org/10.1016/j.chest.2022.11.014.
[44] Huin V, et al. Motor neuron pathology in CANVAS due to RFC1 expansions. Brain 2022;145(6):2121–32. http://dx.doi.org/10.1093/brain/awab449.
[45] Tagliapietra M, Incensi A, Ferrarini M, Mesiano N, Furia A, Rizzo G, et al. Clinical and pathology characterization of small nerve fiber neuro(no)pathy in cerebellar ataxia with neuropathy and vestibular areflexia syndrome. Eur J Neurol 2023;30(12):3834–41. http://dx.doi.org/10.1111/ene.16018.
[46] Sánchez-Tejerina D, Alvarez PF, Laínez E, Martinez VG, Santa-Cruz DI, Verdaguer L, et al. RFC1 repeat expansions and cerebellar ataxia, neuropathy and vestibular areflexia syndrome: experience and perspectives from a neuromuscular disorders unit. J Neurol Sci 2023;446. http://dx.doi.org/10.1016/j.jns.2023.120565.
[47] Méreaux JL, Grangeon L, Bédat-Millet AL, Guyant-Maréchal L. CANVAS, a sensory neuronopathy to look for in ataxia. Rev Neurol 2023;179(8):910–3. http://dx.doi.org/10.1016/j.neurol.2022.12.014.
[48] Van Daele SH, Vermeer S, Van Eesbeeck A, Lannoo L, Race V, Van Damme P, et al. Diagnostic yield of testing for RFC1 repeat expansions in patients with unexplained adult-onset cerebellar ataxia. J Neurol Neurosurg Psychiatry 2020;91(11):1233–4. http://dx.doi.org/10.1136/jnnp-2020-323998. BMJ Publishing Group.
[49] Kontogeorgiou Z, Kartanou C, Tsirligkani C, Anagnostou E, Rentzos M, Stefanis L, et al. Biallelic RFC1 pentanucleotide repeat expansions in Greek patients with late-onset ataxia. Clin Genet 2021;100(1):90–4. http://dx.doi.org/10.1111/cge.13960.
[50] Montaut S, Diedhiou N, Fahrer P, Marelli C, Lhermitte B, Robelin L, et al. Biallelic RFC1-expansion in a French multicentric sporadic ataxia cohort. J Neurol 2021;268(9):3337–43. http://dx.doi.org/10.1007/s00415-021-10499-5.
[51] Lipponen J, Helisalmi S, Raivo J, Siitonen A, Doi H, Rusanen H, et al. Molecular epidemiology of hereditary ataxia in Finland. BMC Neurol 2021;21(1). http://dx.doi.org/10.1186/s12883-021-02409-z.
[52] Ando M, Higuchi Y, Yuan JH, Yoshimura A, Higashi S, Takeuchi M, et al. Genetic and clinical features of cerebellar ataxia with RFC1 biallelic repeat expansions in Japan. Front Neurol 2022;13(952493). http://dx.doi.org/10.3389/fneur.2022.952493.
[53] Gama MTD, Braga-Neto P, Rangel DM, Godeiro C, Alencar R, Embiruçu EK, et al. Autosomal recessive cerebellar ataxias in South America: a multicenter study of 1,338 patients. Mov Disord 2022;37(8):1773–4. http://dx.doi.org/10.1002/mds.29046. John Wiley and Sons Inc..
[54] Bogdan T, Wirth T, Iosif A, Schalk A, Montaut S, Bonnard C, et al. Unravelling the etiology of sporadic late-onset cerebellar ataxia in a cohort of 205 patients: a prospective study. J Neurol 2022;269(12):6354–65. http://dx.doi.org/10.1007/s00415-022-11253-1.
[55] Tagliapietra M, Cardellini D, Ferrarini M, Testi S, Ferrari S, Monaco S, et al. RFC1 AAGGG repeat expansion masquerading as chronic idiopathic axonal polyneuropathy. J Neurol 2021;268(11):4280–90. http://dx.doi.org/10.1007/s00415-021-10552-3.
[56] Kermorvant H, Debs R, Maisonobe T, Huin V, Stojkovic T, Lenglet T. Cramp-fasciculation syndrome phenotype of cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) due to RFC1 repeat expansion. Clin Neurophysiol 2022;134:34–6. http://dx.doi.org/10.1016/j.clinph.2021.11.005. Elsevier Ireland Ltd.
[57] Hadjivassiliou M, Currò R, Beauchamp N, Dominik N, Grunewald RA, Shanmugarajah P, et al. Can CANVAS due to RFC1 biallelic expansions present with pure ataxia? J Neurol Neurosurg Psychiatry 2023. http://dx.doi.org/10.1136/jnnp-2023-331381.
[58] Malaquias MJ, Braz L, Santos Silva C, Damásio J, Jorge A, Lemos JM, et al. Multisystemic RFC1 – related disorder. Neurol Clin Pract 2023;13(5). http://dx.doi.org/10.1212/CPJ.0000000000200190.
[59] Camdessanché JP, Jousserand G, Ferraud K, Vial C, Petiot P, Honnorat J, et al. The pattern and diagnostic criteria of sensory neuronopathy: a case-control study. Brain 2009;132(7):1723–33. http://dx.doi.org/10.1093/brain/awp136.
[60] Tatineni S, Jarrouj G, Gomez CM, Baird BJ. Characterization of a chronic cough in cerebellar ataxia, neuropathy, vestibular areflexia syndrome. Laryngosc Investig Otolaryngol 2023;8(3):730–8. http://dx.doi.org/10.1002/lio2.1067.
[61] Beijer D, Dohrn MF, De Winter J, Fazal S, Cortese A, Stojkovic T, et al. RFC1 repeat expansions: a recurrent cause of sensory and autonomic neuropathy with cough and ataxia. Eur J Neurol 2022;29(7):2156–61. http://dx.doi.org/10.1111/ene.15310.
[62] Palones E, Curto E, Plaza V, Gonzalez-Quereda L, Segarra-Casas A, Querol L, et al. Clinical and functional characteristics, possible causes, and impact of chronic cough in patients with cerebellar ataxia, neuropathy, and bilateral vestibular areflexia syndrome (CANVAS). J Neurol 2023. http://dx.doi.org/10.1007/s00415-023-12001-9.
[63] Magy L, Chazelas P, Richard L, Deschamps N, Frachet S, Vallat JM, et al. Early diagnosis in Cerebellar Ataxia,

Neuropathy, Vestibular Areflexia Syndrome (CANVAS) by focusing on major clinical clues: beyond ataxia and vestibular impairment. Biomedicines 2022;10(8). http://dx.doi.org/10.3390/biomedicines10082046.
[64] Wu TY, Taylor JM, Kilfoyle DH, Smith AD, McGuinness BJ, Simpson MP, et al. Autonomic dysfunction is a major feature of cerebellar ataxia, neuropathy, vestibular areflexia 'CANVAS' syndrome. Brain 2014;137(10):2625–6. http://dx.doi.org/10.1093/brain/awu220.
[65] Traschütz A, Heindl F, Bilal M, Hartmann AM, Dufke C, Riess O, et al. Frequency and phenotype of RFC1 repeat expansions in bilateral vestibulopathy. Neurology 2023;101(10):E1001–13. http://dx.doi.org/10.1212/WNL.0000000000207553.
[66] Yuan JH, Higuchi Y, Ando M, Matsuura E, Hashiguchi A, Yoshimura A, et al. Multi-type RFC1 repeat expansions as the most common cause of hereditary sensory and autonomic neuropathy. Front Neurol 2022;13:986504. http://dx.doi.org/10.3389/fneur.2022.986504.
[67] Schmitt GS, Lima FD de, Matos PCAAP, Martinez ARM, González-Salazar C, et al. Dysautonomia in RFC1-related disorder: clinical and neurophysiological evaluation. Clin Neurophysiol 2022;142:68–74. http://dx.doi.org/10.1016/j.clinph.2022.07.501.
[68] Reyes-Leiva D, Aldecoa I, Gelpi E, Rojas-García R. Motor neuron involvement expands the neuropathological phenotype of late-onset ataxia in RFC1 mutation (CANVAS). Brain Pathol 2022;32(4). http://dx.doi.org/10.1111/bpa.13051. John Wiley and Sons Inc.
[69] Herrmann L, Gelderblom M, Bester M, Deininger N, Schütze T, Hidding U, et al. Multisystemic neurodegeneration caused by biallelic pentanucleotide expansions in RFC1. Parkinsonism Relat Disord 2022;95:54–6. http://dx.doi.org/10.1016/j.parkreldis.2022.01.001. Elsevier Ltd.
[70] Korpioja A, Krüger J, Hurme-Niiranen A, Solje E, Katisko K, Lipponen J, et al. Cognitive impairment is not uncommon in patients with biallelic RFC1 AAGGG repeat expansion, but the expansion is rare in patients with cognitive disease. Parkinsonism Relat Disord 2022;103:98–101. http://dx.doi.org/10.1016/j.parkreldis.2022.08.034.
[71] Colucci F, Di Bella D, Pisciotta C, Sarto E, Gualandi F, Neri M, et al. Beyond canvas: behavioral onset of rfc1-expansion disease in an Italian family – causal or casual? Neurol Sci 2022;43(8):5095–8. http://dx.doi.org/10.1007/s10072-022-06137-1.
[72] Dujardin K, Tard C, Diglé E, Herlin V, Mutez E, Davion J, et al. Cognitive impairment is part of the phenotype of Cerebellar Ataxia, Neuropathy, Vestibular Areflexia Syndrome (CANVAS). Mov Disord 2024. http://dx.doi.org/10.1002/mds.29750.
[73] da Silva Schmitt G, Martinez ARM, da Graça FF, de Lima FD, Bonadia LC, Amorim BJ, et al. Dopa-responsive Parkinsonism in a patient with homozygous RFC1 expansions. Mov Disord 2020;35(10):1889–90. http://dx.doi.org/10.1002/mds.28286. John Wiley and Sons Inc.
[74] Matos PCAAP, Rezende TJR, Schmitt GS, Bonadia LC, Reis F, Martinez ARM, et al. Brain structural signature of RFC1-related disorder. Mov Disord 2021;36(11):2634–41. http://dx.doi.org/10.1002/mds.28711.
[75] Record CJ, Alsukhni RA, Curro R, Kaski D, Rubin JS, Morris HR, et al. Severe distinct dysautonomia in RFC1-related disease associated with Parkinsonism. J Peripheral Nerv Syst 2022;27(4):311–5. http://dx.doi.org/10.1111/jns.12515.
[76] Azevedo MPC, Lobo CC, Schmitt GS, Matos PCAAP, Barsottini OGP, Pedroso JL, et al. Nigrostriatal dysfunction in RFC1-related disorder/CANVAS. Parkinsonism Relat Disord 2023;115. http://dx.doi.org/10.1016/j.parkreldis.2023.105854.
[77] Sullivan R, Yau WY, Chelban V, Rossi S, Dominik N, O'Connor E, et al. RFC1-related ataxia is a mimic of early multiple system atrophy. J Neurol Neurosurg Psychiatry 2021;92(4):444–6. http://dx.doi.org/10.1136/jnnp-2020-325092. BMJ Publishing Group.
[78] Kytövuori L, Sipilä J, Doi H, Hurme-Niiranen A, Siitonen A, Koshimizu E, et al. Biallelic expansion in RFC1 as a rare cause of Parkinson's disease. NPJ Parkinsons Dis 2022;8(1). http://dx.doi.org/10.1038/s41531-021-00275-7.
[79] Casanueva R, López F, Costales M, Ordas A, Villanueva E, Llorente JL, et al. The presence of dysphagia in patients with cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS): a subjective and objective study. Eur Arch Otorhinolaryngol 2021;278(7):2585–92. http://dx.doi.org/10.1007/s00405-020-06534-2.
[80] Barghigiani M, De Michele G, Tessa A, Fico T, Natale G, Saccà F, et al. Screening for RFC-1 pathological expansion in late-onset ataxias: a contribution to the differential diagnosis. J Neurol 2022. http://dx.doi.org/10.1007/s00415-022-11192-x.
[81] Abreu VS, Silva JS, Igreja L, Malaquias MJ, Pinto CM. Pseudo-eye-of-the-tiger sign in cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS). Am J Med Genet A 2024;194(1):103–6. http://dx.doi.org/10.1002/ajmg.a.63419.
[82] Rezende TJR, Schmitt GS, de Lima FD, de Brito MR, Matos PCAAP, Bonadia LC, et al. RFC1-related disorder: in vivo evaluation of spinal cord damage. Mov Disord 2022;37(10):2122–8. http://dx.doi.org/10.1002/mds.29169.
[83] El Houjeiry E, Coudray S, Thouvenot E, Ion IM. Spinal cord lesion mimicking a dysimmune myelitis revealing CANVAS syndrome. J Spinal Cord Med 2023;46(2):332–6. http://dx.doi.org/10.1080/10790268.2022.2033936.
[84] Khurana V, de Gusmao CM, Glover M, Helgager J. Case 20-2021: a 69-year-old man with ataxia. N Engl J Med 2021;385(2):165–75. http://dx.doi.org/10.1056/nejmcpc2004992.
[85] Wada T, Doi H, Okubo M, Tada M, Ueda N, Suzuki H, et al. RNA Foci in two bi-Allelic RFC1 expansion carriers. Ann Neurol 2023. http://dx.doi.org/10.1002/ana.26848.
[86] Çakar A, Şahin E, Tezel S, Candayan A, Samancı B, Battaloğlu E, et al. Cerebellar ataxia, neuropathy and vestibular areflexia syndrome (canvas): an important cause of late-onset ataxia with unique clinical features. Acta Neurol Belg 2022;122(4):939–45. http://dx.doi.org/10.1007/s13760-021-01721-2.
[87] Hirano M, Kuwahara M, Yamagishi Y, Samukawa M, Fujii K, Yamashita S, et al. CANVAS-related RFC1 mutations in patients with immune-mediated neuropathy. Sci Rep 2023;13(1). http://dx.doi.org/10.1038/s41598-023-45011-8.
[88] Gilman S., Wenning FG, Low P, Brooks FD, Mathias FmC, Trojanowski FmJ, et al. Second consensus statement on the diagnosis of multiple system atrophy background: a consensus conference on multiple system atrophy (MSA) in 1998 established. 2008.
[89] Fanciulli A, Wenning GK. Multiple-system atrophy. N Engl J Med 2015;372(3):249–63. http://dx.doi.org/10.1056/NEJMra1311488.
[90] Fan Y, Zhang S, Yang J, Mao C yuan, Yang Z hua, Hu Z wei, et al. No biallelic intronic AAGGG repeat expansion in RFC1 was found in patients with late-onset ataxia and MSA. Parkinsonism Relat Disord 2020;73:1–2. http://dx.doi.org/10.1016/j.parkreldis.2020.02.017. Elsevier Ltd.
[91] Sullivan R, Yau WY, Chelban V, Rossi S, O'Connor E, Wood NW, et al. RFC1 intronic repeat expansions absent in pathologically confirmed multiple systems atrophy. Mov Disord 2020;35(7):1277–9. http://dx.doi.org/10.1002/mds.28074. John Wiley and Sons Inc.

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006

[92] Ylikotila P, Sipilä J, Alapirtti T, Ahmasalo R, Koshimizu E, Miyatake S, et al. Association of biallelic RFC1 expansion with early-onset Parkinson's disease. Eur J Neurol 2023;30(5):1256–61. http://dx.doi.org/10.1111/ene.15717.
[93] Schoeberl F, Abicht A, Kuepper C, Voelk S, Sonnenfeld S, Tonon M, et al. Sensory neuropathy due to RFC1 in a patient with ALS: more than a coincidence? J Neurol 2022;269(5):2774–7. http://dx.doi.org/10.1007/s00415-021-10835-9. Springer Science and Business Media Deutschland GmbH.
[94] Amador M-D-M, Muratet F, Teyssou E, Banneau G, Danel-Brunaud V, Allart E, et al. Spastic paraplegia due to recessive or dominant mutations in ERLIN2 can convert to ALS. Neurol Genet 2019;5(6). http://dx.doi.org/10.1212/nxg.0000000000000374.
[95] Facchini S, Dominik N, Manini A, Efthymiou S, Currò R, Rugginini B, et al. Optical genome mapping enables detection and accurate sizing of RFC1 repeat expansions. Biomolecules 2023;13(10). http://dx.doi.org/10.3390/biom13101546.
[96] Mascalchi M, Santorelli FM. The strange case of the multiple MRI phenotypes of RFC1 mutation. Cerebellum 2023;22(3):478–81. http://dx.doi.org/10.1007/s12311-022-01401-8. Springer.
[97] Quartesan I, Vegezzi E, Currò R, Heslegrave A, Pisciotta C, Iruzubieta P, et al. Serum neurofilament light chain in replication factor complex subunit 1 CANVAS and disease spectrum. Mov Disord 2023. http://dx.doi.org/10.1002/mds.29680.
[98] Budhram A, Mills JR, Shouman K, Dyck PJB, Hassan A, Zalewski NL. False-positive anti-neuronal nuclear antibody type 1 in a patient with RFC1 repeat expansion: preventing 'phenotype creep' in autoimmune neurology. J Neurol Sci 2020;416. http://dx.doi.org/10.1016/j.jns.2020.117018. Elsevier B.V.
[99] Kumar KR, Cortese A, Tomlinson SE, Efthymiou S, Ellis M, Zhu D, et al. RFC1 expansions can mimic hereditary sensory neuropathy with cough and Sjögren syndrome. Brain 2020;143(10.):82. http://dx.doi.org/10.1093/brain/awaa244. Oxford University Press.
[100] Fernández-Eulate G, Debs R, Maisonobe T, Latour P, Cohen-Aubart F, Saadoun D, et al. Sjögren syndrome and RFC1-CANVAS sensory ganglionopathy: co-occurrence or misdiagnosis? J Neurol 2023;270(1):460–5. http://dx.doi.org/10.1007/s00415-022-11382-7.
[101] Azzimonti M, Fazio R, Giordano A, Tagliapietra M, Ferrarini M, Rocca MA, et al. Association between inflammatory central nervous system lesions and Cerebellar Ataxia, Neuropathy and Vestibular Areflexia Syndrome (CANVAS): a case series. J Neurol 2022;269(10):5668–73. http://dx.doi.org/10.1007/s00415-022-11184-x. Springer Science and Business Media Deutschland GmbH.
[102] Hisey JA, Radchenko EA, Ceschi S, Rastokina A, Mandel NH, Mcginty RJ, et al. Pathogenic CANVAS (AAGGG)n repeats stall DNA replication due to the formation of alternative DNA structures''. DOI. 10.1101/2023.07.25.550509.
[103] Khristich AN, Mirkin SM. On the wrong DNA track: molecular mechanisms of repeat-mediated genome instability. J Biol Chem 2020;295(13):4134–70. http://dx.doi.org/10.1074/jbc.REV119.007678. American Society for Biochemistry and Molecular Biology Inc.

Please cite this article in press as: Delforge V, et al. RFC1: Motifs and phenotypes. Revue neurologique (2024), https://doi.org/10.1016/j.neurol.2024.03.006