Genetics and clinical features of RFC1 CANVAS

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Abstract

Since its identification the frequency and phenotype spectrum of *RFC1* disease has expanded, ranging from typical CANVAS, where it explains over 90% of cases, to site-restricted variants affecting prominently or exclusively the sensory nerves, the cerebellum or the vestibular system. Given the carrier frequency of the AAGGG expansion in 1-6% of healthy chromosomes and across different ethnicities, *RFC1* disease due to biallelic AAGGG expansions likely represents the most common cause of recessive ataxia. Given the wide phenotype spectrum, the differential diagnosis of RFC1 is broad. Thorough clinical examination assessing the three affected systems, as well as the presence of chronic cough, are key to suspect the disease and prompt genetic testing.

Bullet points:

- Biallelic AAGGG expansions in RFC1 are identified as a frequent cause of late onset ataxia across different ethnicities
- RFC1 disease spectrum ranges from full CANVAS to complex neuropathy with cerebellar or vestibular dysfunction, to isolated sensory neuropathy with or without cough
- Although slowly progressive, RFC1 disease leads to significant disability in half of cases
- Enquiring about the presence of cough and routine assessment of eye movements (nystagmus, broken pursuits) and bedside head impulse test in all cases of sensory neuropathy is key to suspect RFC1 and prompt genetic testing

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Main text

First descriptions of the CANVAS clinical syndrome

The first report of the clinical picture now referred to as CANVAS (Cerebellar Ataxia Neuropathy Vestibular Areflexia Syndrome) dates back to 1991 when a syndrome with combined vestibular, neuropathic and cerebellar dysfunction was described with absent vestibular function and reduced smooth pursuit-optokinetic eye movements (1) –

In 2004 Migliaccio et al reported four patients with the syndrome of cerebellar ataxia with bilateral vestibulopathy (2). The term CANVAS was coined in 2011 when the same group reported 23 additional patients with the syndrome and showed that a length-dependent sensory deficit is an integral feature of this syndrome(3). Autonomic dysfunction and a dry spasmodic cough were also later identified in some patients with this condition(4,5).

Notably, pathological studies in typical CANVAS cases showed loss of Purkinje cells, predominantly in the vermis of the cerebellum, and a marked loss of sensory neurons in the dorsal root and V, VII, and VIII cranial nerve ganglia(6,7). Albeit conducted on small number of patients these post-mortem studies were very useful as they localised the origin of sensory and vestibular impairment of CANVAS to the spinal and cranial sensory and vestibular ganglia, respectively.

Identification *RFC1* expansion causing CANVAS, late-onset ataxia and sensory neuro(no)pathy

Through a combined recessive linkage and whole-genome sequencing approach we identified biallelic AAGGG expansions in the second intron of Replication factor complex subunit 1 (*RFC1)* in familial and sporadic CANVAS and late-onset ataxia cases (8).

Following its identification, the novel repeat expansion associated with CANVAS has been confirmed by multiple groups and in different ethnicities (9,8,10–14). The phenotype spectrum of RFC1 CANVAS and disease spectrum is expanding and, importantly, in our series up to 15% cases have isolated sensory neuropathy without cerebellar of vestibular involvement.

More recently, novel pathogenic $(AAAGG)_{10\text{-}25}(AAGGG)_{exp}(AAAGG)_{4\text{-}6}$ and $(ACAGG)_{exp}$ configurations were identified individual from Oceania and East Asia (15–17).

Despite the clinical similarities between CANVAS and multi system atrophy, *RFC1* expansion were absent or very rare in MSA cases of Caucasian or Chinese Han origin, further supporting its specificity to CANVAS and disease spectrum (18–21).

The mechanism underlying neurodegeneration in *RFC1* CANVAS is still unknown. *RFC1* is a gene implicated in DNA replication and repair. Notably, preliminary studies did not show a reduced expression nor an overt loss of function of RFC1 protein(8,11)

Genetic testing for RFC1 expansions

The molecular diagnosis of RFC1 CANVAS relies on a bespoke testing entailing flanking and repeat-primed PCR. Given the large size of the AAGGG expansions,

ranging from several hundreds to several thousand repeated units, confirmation of the presence of biallelic repeat expansions and their size can be obtained only by laborious and time-consuming Southern Blotting.

It is worth noting that RFC1 expansions are not detected by the currently available NGS gene panels. Other techniques, including long-read sequencing (22), may have the potential to reliably assess the presence, size and sequence of repeat expansions in *RFC1* and may represent a valuable alternative to the current multi-step algorithm.

Clinical features of RFC1 disease spectrum

We recently described the clinical features in a 100 genetically confirmed cases with biallelic *RFC1* expansions (23). Cases were enrolled from multiple centres from the UK, Europe, South America, Australia and New Zealand. Detailed findings at history and examination are summarized in **Figure 1**.

Mean age of onset of neurological manifestations was 52 years, ranging from 19 to 76 years, and mean age at the time of the study was 72 years.

Progressive imbalance was the most common complaint and represented the first symptom in half of the cases. Notably, imbalance was often reported as worse in the dark indicating a prominent peripheral large fiber sensory component.

Over two thirds of individuals complained of sensory symptoms, including loss of feeling, pins and needles and neuropathic pain. Neurological examination showed impaired sensation to pinprick, vibration and joint position, more typically in a length-dependent distribution. Reflexes were reduced/absent, retained or even brisk. Power was always normal.

Oscillopsia, defined as a visual disturbance in which objects appear to oscillate during head movements, a common sequela of a bilaterally impaired vestibulo-ocular reflex, was reported by one third of cases. Conversely, vertigo, defined as an abnormal sensation of motion in which the individual or the individual's surroundings seem to whirl dizzily, stemming from an acute/subacute and generally unilateral impairment of the vestibular system, was rare in RFC1 CANVAS and, when reported, probably unrelated. Bedside head impulse test revealed bilateral vestibular hypofunction in up to 90% of cases. Previous studies have also found that an impaired visually enhanced vestibulo-ocular reflexes is common and indicates the co-existence of vestibular and cerebellar pathology(2).

Dysarthria and dysphagia, which are likely attributable to cerebellar dysfunction, frequently complicated the disease course in later stages. However abnormal eye movements of putative cerebellar origin, including gaze-evoked, downbeat and horizontal nystagmus, broken pursuits and dysmetric saccades, were frequent and present earlier in the disease course.

Notably, a chronic spasmodic dry cough was reported in two thirds of the cases and as early as in the 2nd decade of life, up to three decades before any neurological symptom develops.

Autonomic dysfunction, including postural hypotension, erectile dysfunction, chronic constipation, urinary dysfunction and altered sweating were reported in half of the cases but rarely disabling.

Brain MRI showed cerebellar atrophy, and particularly of the vermis, in ~60% of the cases; vestibular testing was abnormal in over 90% of cases tested, while nerve conduction studies universally showed a sensory neuropathy in all cases tested. The sensory action potentials were reduced or absent and motor studies were normal in 90% of patients with minor reduced motor action potentials and / or distal denervation in 10%. Blink and masseter reflexes can be impaired as a result of the widespread sensory neuronopathy also involving the trigeminal nerves(5). The T reflex, whose integrity implies a normal function of both peripheral motor fibres and muscle spindle afferent fibres. was assessed by a different group in a small series of CANVAS patients and was normal, explaining the frequent occurrence of normo- or hyperreflexia (5). Autonomic testing showed the presence of a parasympathetic and/or sympathetic dysfunction in half of our series of genetically confirmed cases and in up to 80% in a previous study(4). Finally, a role of nerve ultrasound was suggested by a single group, showing in CANVAS patients a reduced cross-sectional area of upper and lower limb nerves(24,25).

The disease had a slowly progressive course. Half of individuals needed a stick after 10 years of disease duration and one fourth were wheelchair-dependent five years later. However, life expectancy did not seem to be affected.

Two-thirds of genetically confirmed cases had full CANVAS after clinical examination and investigations. None had isolated cerebellar syndrome or bilateral vestibular failure, but a sensory neuropathy was the only clinically detectable manifestation of the disease in 15 cases. Although the natural history of the disease still needs to be fully investigated, our retrospective data suggest a pattern of spatial progression from the early involvement of sensory neurons to the vestibular system and later the cerebellum.

An illustrative case of RFC1 CANVAS is represented in **table 1**.
Interrogating patients for an history of unexplained cough and performing a thorough clinical examination including bedside head impulse testing looking for bilateral vestibular areflexia should be part of the routine evaluation of any patient with ataxia and/or sensory neuro(no)pathy.

Table 1. Illustrative case of RFC1 CANVAS

CASE STUDY: Progressive imbalance and distal pins&needls in a 50 yo man
A man presented at the age of 50 with a 3-year history of dizziness and imbalance, particularly when walking on uneven surfaces and in the dark, along with loss of feeling and pins and needles in his feet. He did not complain of weakness, muscle cramps or symptoms of dysautonomia. His symptoms have been slowly progressive, and had worsened in the last year. His past medical history revealed a dry chronic spasmodic since his twenties. Family history for neurological diseases was negative including in his parents, now both deceased, and two younger brothers. Neurological examination showed a slightly broad-based ataxic gait. Tandem walking was impossible, and Romberg was positive. There was gaze-evoked horizontal nystagmus and slow pursuit eye movements were broken. Head impulse test showed an impaired vestibular ocular reflex bilaterally as in bilateral vestibular areflexia and visually enhanced VOR, pointing to a combined vestibular and cerebellar impairment,

was also altered. Sensory examination revealed reduced pinprick to the knees and the wrists, reduced vibration to the costal margin but normal position sense. Reflexes were all present and plantars were down going. Muscle tone, bulk and strength were normal. There were no extrapyramidal signs and no orthostatic hypotension on bedside examination. Nerve conduction studies showed absent sensory action potentials in the upper and lower limbs with normal motor studies. Brain MRI revealed mild cerebellar vermis atrophy (**Figure 2A**) , while spine MRI showed atrophy and T2 hyperintensity of the posterior columns. Video head-impulse test confirmed the presence of bilateral vestibular areflexia (**Figure 2B**). Relevant blood tests including B12, folate, HbA1c, immunofixation, paraneoplastic autoantibodies were negative. RFC1 testing confirmed the presence of a biallelic AAGGG expansion.
SUGGESTIVE SIGNS OF RFC1 CANVAS
progressive sensory ataxic neuropathy* chronic cough altered vestibular ocular reflex and visually enhanced VOR gaze evoked nystagmus, broken pursuits dysarthria and dysphagia (more advanced stages of the disease) * consider RFC1 testing in any unexplained sensory neuropathy after negative lab screening for acquired causes.
ATYPICAL SIGNS, MAKING RFC1 CANVAS LESS LIKELY
Absence of sensory neuropathy Presence of motor involvement Neurological onset in the 1st decade of life Rapid progression Prominent dysautonomia

Differential diagnosis

Given the complex phenotype of *RFC1* CANVAS the differential diagnosis includes genetic and acquired causes of neuropathy, ataxia and vestibular disease. The key differential diagnoses are summarized in **Table 2** (26–42).

FRDA shows remarkable similarities with CANVAS. Both diseases are caused by recessive repeat expansions and lead to progressive degeneration of sensory neurons, accompanied by cerebellar dysfunction. Vestibular areflexia can also be observed in FRDA. As opposed to CANVAS, onset of FRDA is typically before 25 years of age. Also, muscle atrophy, spasticity and skeletal deformities (pes cavus, scoliosis) are frequent in FRDA but absent in CANVAS. Cardiomyopathy, diabetes, vision and hearing involvement also often complicate the disease course of FRDA (30–32).

Among other differential genetic causes, we have recently shown that the phenotype spectrum of the dominant R199C mutation in *RNF170*, formerly identified in Eastern Canadian patients with posterior column ataxia (26), encompasses autosomal dominant sensory ganglionopathy and vestibular areflexia and should be considered in *RFC1* negative CANVAS cases, particularly with dominant family history and normal cerebellar function (27) .

The main differential diagnosis in patients with late-onset cerebellar ataxia is Multisystem atrophy (MSA), a rapidly progressive neurodegenerative disease. The aggressive disease course of MSA (28), the early and severe autonomic involvement, the absence of sensory neuropathy and vestibular dysfunction, the association with rapid eye movement sleep behaviour disorder, parkinsonism, and typical MRI pattern with putaminal, pontine, and middle cerebellar peduncle atrophy and 'hot cross-bun' signs cruciform T2 hyperintensity in the pons(29), help to distinguish MSA from RFC1 disease (20).

Several mitochondrial diseases due to nuclear or mitochondrial genetic defects can manifest with ataxia, neuropathy and, more occasionally, bilateral vestibular areflexia. In particular, biallelic mutation in POLG associated with sensory ataxic neuropathy, dysarthria, and ophthalmoparesis (SANDO) should be considered in the diagnostic work-up of a patient with progressive sensory ataxic neuropathy. An earlier onset, the frequent association of chronic progressive external ophthalmoplegia and the multisystem involvement are clues to suspect a mitochondrial disease(42).

Conclusions

Since its identification the frequency and phenotype spectrum of *RFC1* disease has expanded, ranging from typical CANVAS, where it explains over 90% of cases, to site-restricted variants affecting prominently or exclusively the sensory nerves, the cerebellum or the vestibular system. Given the carrier frequency of the AAGGG expansion in up to 4% of healthy chromosomes and across different ethnicities, which is higher than *FXN* GAA expansion associated with FRDA (allele frequency ~1%), *RFC1* disease due to biallelic AAGGG expansions likely represents the most common cause of recessive ataxia. A thorough clinical examination assessing the three affected systems, as well as the presence of chronic cough, are key to suspect the disease and prompt genetic testing.

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TABLES

Table 2. Differential diagnoses of RFC1 CANVAS and disease spectrum

	Etiology	Key features
Sensory neuropathy/neuronopathy		
- Acquired causes	Paraneoplastic	History of neoplasms, subacute onset, antineuronal antibodies, CSF analysis
	Immune-mediated	Autoantibody screen systemic involvement
	Toxic/metabolic (diabetes mellitus type 2, B12 deficiency, copper deficiency)	Blood test
	Toxic	Platin-derived treatment, B6 intoxication
- Hereditary causes	HSN	Early onset, insensitivity to pain, painless ulcerations
	RNF170[22-23]	Dominant family history, normal cerebellar function
Late onset-cerebellar ataxia		
- Acquired causes	Multiple system atrophy[24-25]	Early and severe dysautonomia, parkinsonism, RBD, brain MRI findings (hot-cross bun sign)
	Paraneoplastic	Subacute onset, antineuronal antibodies, CSF analysis
	Toxic/metabolic	B1 deficiency/alcoholic abuse
	Vascular	Acute onset, cerebrovascular risk factors
	Inflammatory	Subacute onset, brain MRI findings, CSF analysis
	Idiopathic (ILOCA)	Presumed degenerative ataxia in which no other cause if found
- Hereditary causes	Friedreich ataxia[26-28]	Onset <25 years, pyramidal signs, scoliosis, pes cavus, systemic involvement (cardiac, endocrine, ocular)
	SCA3 (late-onset Machado-Joseph disease)[29]	AD, extrapyramidal features, S-M neuropathy, PEO
	Gerstmann-Straussler-Scheinker Syndrome[30-31]	Cognitive impairment, more rapid progression
Bilateral vestibular areflexia		
- Acquired causes	Ototoxic drugs, Meniere's syndrome, vestibular neuritis, tumors, infections, inflammation	More commonly unilateral, cochlear symptoms

- Hereditary causes	SCA (1,2,3,6)[32]	AD, mild vestibular impairment, additional signs
	CMT[33-35]	S-M neuropathy
	Usher syndrome type I, II[36-37]	Deafness, retinitis pigmentosa
Complex neurological diseases		
Mitochondrial diseases	POLG[38]	Ophtalmoplegia, multisystem involvement

HSN=hereditary sensory neuropathy; RBD=REM-sleep behaviour disorders; SCA=spinocerebellar ataxia; AD=autosomal dominant; S-M=sensory-motor; PEO=progressive external ophthalmoplegia

FIGURE LEGENDS

Figure 1. Clinical features of genetically confirmed RFC1 CANVAS and disease spectrum. A. Symptoms at onset and during disease progression B. Detailed neurological examination. HIT; Head impulse test

Figure 2. Investigations in a typical RFC1 CANVAS case. A. Brain MRI from a genetically confirmed RFC1 CANVAS patient showing parenchymal volume loss affecting the cerebellum, both within the cerebellar hemispheres and the cerebellar vermis. B. Video head impulse test. Head velocity (black lines) and eye velocity traces for head impulses rightward (top panel) and leftward (bottom panel). The eye traces show the presence of compensatory saccades (catch-up saccades, arrows) at the end of the head movement.