



Pseudodominance in RFC1-Spectrum Disorder

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Abstract

Cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS) and disease spectrum is an autosomal recessive disorder associated with biallelic repeat expansion (RE) in the *RFC1* gene. A high carrier frequency in the healthy population determines the possibility of having affected members in two consecutive generations. We describe pseudodominance in two families affected with *RFC1* disorder (10 affected, 5 oligo/asymptomatic individuals). In Family A, after the 75-year-old index case was diagnosed with CANVAS, the 73-year-old wife decided to undergo screening for carrier testing. Although she did not report any symptoms, she resulted positive for the biallelic AAGGG RE thus leading to a diagnosis in the asymptomatic offspring as well and revealing a pseudodominant pattern of inheritance. In Family B pseudodominance was suspected after the identification of the *RFC1* RE in the proband affected by sensitive neuropathy because of a positive family history for undetermined polyneuropathy in the mother. The post-mortem identification of the *RFC1* RE in a sample specimen from the deceased mother, who had been under our care, allowed the solution of a “cold case”. Our report suggests that pseudodominance is a confounding phenomenon to consider in *RFC1*-spectrum disorder and genetic counselling is instrumental in families with affected individuals.

Keywords Pseudodominance · RFC1-spectrum disorder · CANVAS · High carrier frequency · Sensory ataxia

Introduction

Cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS; OMIM: 614575) is an autosomal recessive adult onset neurological disease characterised by motor incoordination due to concomitant impairment of sensory neurons,

cerebellum and vestibular system. Biallelic AAGGG RE in intron 2 of *RFC1* gene were firstly identified in 2019 in CANVAS and the disease spectrum (from now on shortened as *RFC1*-spectrum) that includes less common features such as dysautonomia, movement disorders, motor neuron affection and cognitive impairment [1–3].

The estimated high allele frequency in the healthy population determines the possibility of having affected members in two consecutive generations in a family with no consanguinity [1, 2, 4] We report two families with *RFC1*-spectrum disorder in which pseudodominance is observed.

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Methods

Two families (10 affected and 5 oligo/asymptomatic individuals) were examined by a standardised rating system in a tertiary neurology referral centre.

RFC1 genetic test was performed as previously described [1]. Samples with no amplifiable products on flanking PCR and positive repeat-primed PCR for the AAGGG repeat were considered likely positive for biallelic AAGGG *RFC1*

expansions, after the exclusion of the non-pathogenic AAAGG and AAAAG expansions on the other allele. Direct sequencing confirmed the AAGGG repeat conformation.

The study was approved by the local institutional ethics committee and complied with all relevant ethical regulations. All patients gave informed consent prior to their inclusion in the study.

Results

Family A (Fig. 1, A)

Pt II-5, the 75-year-old proband, complained of slowly progressive gait imbalance from the age of 45 years. At that time brain MRI and vestibular tests were normal. He also reported a chronic cough since a young age. The following years he complained of difficulties in swallowing and speaking fluently. Acquired causes of cerebellar ataxias and common CAG repeat ataxias (SCA 1, 2, 3, 6, 7, 12, 17) had been previously excluded. When first examined in our Unit at age 70 we observed a severe ataxic gait, impaired smooth pursuits, dysarthria and dysmetria. Vibration sense and deep tendon reflexes (DTRs) were diminished distally and head-impulse test revealed bilateral vestibular hypofunction. Sensory peripheral neuropathy was detected on nerve conduction studies (NCS); brain MRI showed diffuse cerebellar atrophy. He reported that two of his siblings presented gait difficulties as well. The patient tested positive for *RFC1* biallelic AAGGG expansions. His siblings were offered genetic counselling after he was diagnosed with CANVAS.

Pt II-6, the 70-year-old sister of the index case, experienced gait unsteadiness since 60 years of age and reported chronic cough, early satiety and constipation. At examination she presented gait ataxia, choreo-dystonic movements of both hands mainly triggered by speaking and distal sensory loss.

Pt II-8, the 67-year-old brother of the index case, reported having difficulties in walking in darker environments and a feeling of cold feet for 4 years. He presented sensory ataxia, gaze evoked nystagmus and dysarthria. Head impulse test showed bilateral vestibular areflexia.

Pt II-4, the 73-year-old wife of II-8 decided to undergo screening for carrier testing after her non-consanguineous husband (Pt II-1) had received a diagnosis of CANVAS. She unexpectedly resulted positive for the biallelic AAGGG RE but had never reported any neurological symptoms. After genetic diagnosis, we examined her and observed reduced DTRs and subtle bilateral intention tremor at nose-finger test. She declined further neurophysiological testing.

Pt III-1 and III-2 were offered genetic counselling after their father was diagnosed with CANVAS and their mother

Fig. 1 (A) Pedigrees of the two families. AA: wild type members; Aa: heterozygous AAGGG repeat expansion carriers; aa: biallelic AAGGG repeat expansion carriers. Oligo/asymptomatic patients are shown in grey. **B^I, B^{II}, B^{III}**. **Morphological findings in the skin biopsy from Pt III 1 Fam (B)** Confocal analysis of skin innervation (x40) disclose an almost complete denervation of the epidermal layer in the leg (B^I) and thigh (B^{II}) compared with an age-matched healthy control (HC) presenting several nerve fibers that cross the dermal-epidermal basement membrane and run free as epidermal fibers in the thigh (B^{III}). Bar = 50 micron; **C, D, E - Morphological findings in the muscle and nerve biopsy from Pt II 4 Fam B.** (C) Quadriceps muscle biopsy stained with haematoxylin and eosin (20X) shows increased muscle fibers variability with angulated atrophic and hypertrophic fibers; (D) Quadriceps muscle biopsy stained with ATPase pH 4,3 shows fiber type grouping with many type 2c fibers (10X); (E) Sural nerve biopsy, semi-thin resin section shows a severe reduction in myelinated fiber density (20X)

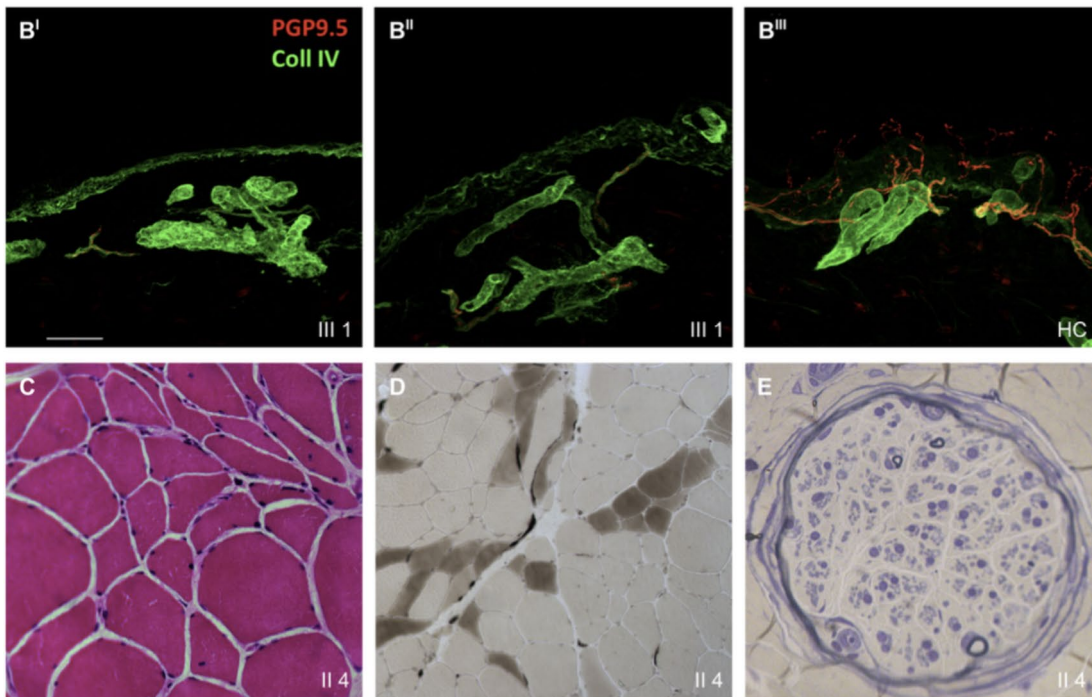
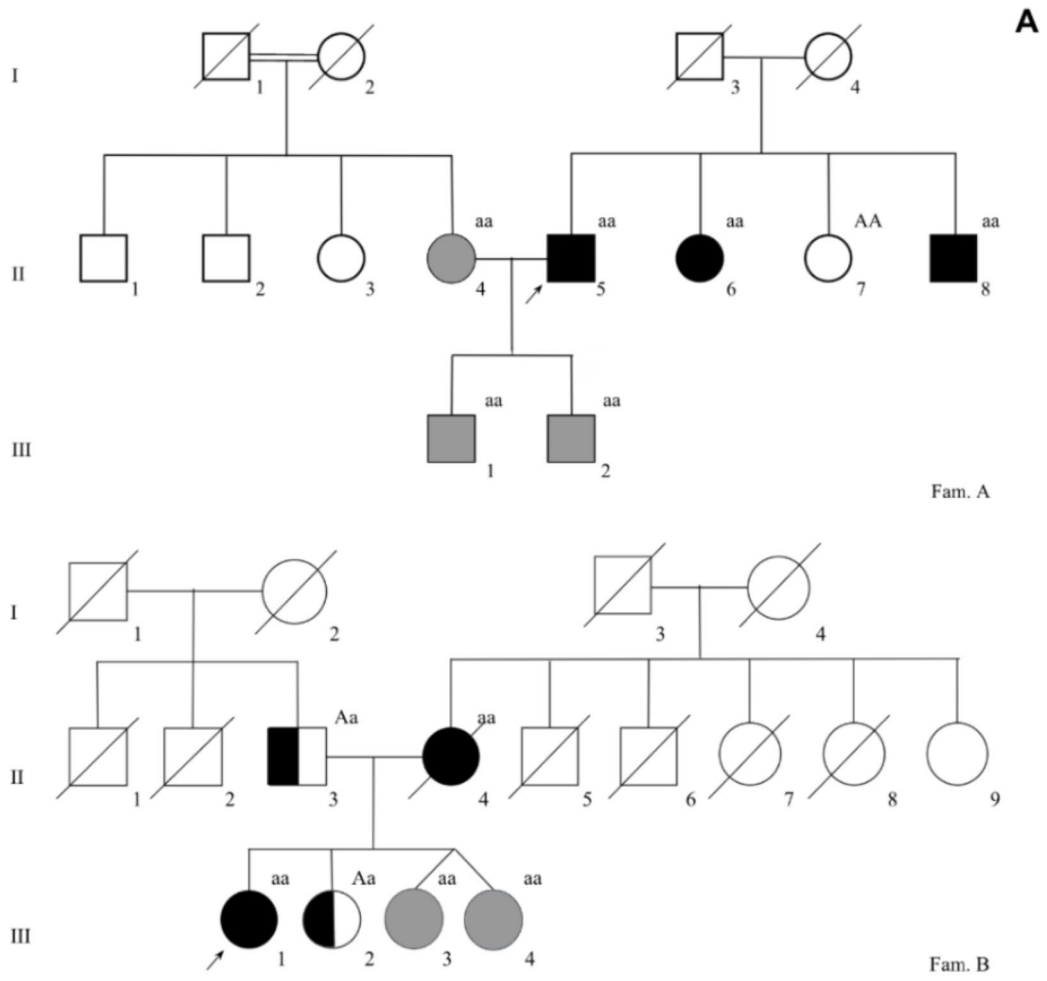
harboured the biallelic expansion in *RFC1*. Neurological examination in III-1 was normal whereas III-2 complained of occasional blurry vision, presented difficulties in standing on one foot but tandem gait was normal and showed a subtle symmetrical intention tremor at finger to nose test.

Family B (Fig. 1, A)

Pt III-1, the index case in family B, is a 50-year-old woman who was referred to us after having received a diagnosis of Fabry disease. She had dilated cardiomyopathy, joint pain and acroparesthesias and carried the pathogenic heterozygous variant c.337T>C p.(Phe 113Leu) in the *GLA* gene. The concentration of Lyso-Gb3 was 1,6 ng/mL (normal values < 1,8 ng/mL).

Family history was significant for her mother who had been under our care and diagnosed 10 years before with sensorymotor neuropathy. During her first clinical evaluation with us, she reported cramps and persistent cough. She presented distal sensory loss and absent DTRs. NCS revealed a sensory neuropathy. Skin biopsy showed a severe small fiber neuropathy with almost complete somatic denervation in the analyzed sites (i.e. thigh and leg) and without Gb3 deposits (Fig. 1, B^I-B^{II}). Brain MRI was normal. Given her clinical and paraclinical features not fully explained by her previous diagnosis of Fabry disease and in the suspicion of a double-trouble we decided to rule out CANVAS. Genetic analysis identified biallelic AAGGG RE in *RFC1*. At one year follow-up she presented missteps at tandem gait and oral dyskinesias.

Pt II-4, the deceased mother of III-1, had suffered from cramps, unsteadiness and persistent cough since age 35. Her last neurological examination at 70 years old documented severe ataxia, dysarthria, gaze evoked nystagmus, distal sensory loss, diminished vibration sense at lower limbs, absent DTRs, orofacial dyskinesias and involuntary irregular movements of the hands. Electrophysiological studies were consistent with a severe sensory neuropathy. She had



undergone muscle biopsy with findings of diffuse denervation and sural nerve biopsy showing a severe loss of myelinated fibres (Fig. 1C-E). She died six years ago at 72 years of age. A retrospective diagnosis of *RFC1*-spectrum disorder was reached upon genetic testing in skeletal muscle biopsy DNA.

Pt III-3 and Pt III-4 were diagnosed after genetic counselling. Both reported mild chronic cough and sporadic sensory symptoms at lower limbs.

II-3, was genetically tested and resulted a carrier.

Clinical and paraclinical features in the two families are summarised in Table 1.

Discussion

Since the identification of a biallelic AAGGG RE responsible for CANVAS the complexity of the genetics and the clinical heterogeneity of the disease have gradually emerged [1, 3, 5].

We report two unrelated Italian families in which *RFC1* spectrum disorder presented a pseudodominant pattern of inheritance. Despite a low occurrence of the disease in population polymorphic databases, the heterozygous occurrence of *RFC1* pathological RE in healthy controls is high. In the work by Cortese et al., the allelic carrier frequency of the AAGGG RE in the 304 healthy controls screened was 0,7% and the prevalence at birth of the recessive AAGGG expansion in *RFC1* was estimated approximately 1:20,000 [1]. Conversely, Rafehi et al. estimated a frequency of heterozygous carriers of 4,5% and other groups have presented a variable heterozygous carrier frequency in healthy controls depending on ethnicity (namely, 3–4% in Caucasians, 1–2,24% in Chinese and 7,8% in Japanese) [2, 5–9]. Moreover, a recent paper analysing 19,241 genomes from GnomAD v3 reported a frequency of AAGGG RE carriers of 6,25% (1202/19241) [10]. Taken together, it seems safe to estimate an allele frequency of 0.7–4% in the healthy population, thus supporting the potential occurrence of vertical transmission in *RFC1*-spectrum disorder as in our kindred. This element should be considered to understand the intra-familial variability and the spectrum of the disorder, in fact it has been demonstrated that the RE size functions as a disease modifier and that remains stable across generations [11] but it is still unclear if the RE remains unchanged also in the instance of pseudodominance.

However the genetic complexity of the locus is also characterised by heterogeneity in the repeat configuration and conformation and less frequent repeat motifs in the homozygous or compound heterozygous state with AAGGG expansions have been discovered to be responsible for CANVAS

and can also play a significant role in phenotypic variability [12–22].

A study analysing gnomAD v3 found that the pathogenic RE ACAGG initially discovered in individuals from Niue, Indonesia and Japan had an estimated prevalence of carriers of 0,26% in South Asian populations and was not found in Europeans but more recently three Dutch patients were reported to carry this expansion motif [12, 15]. The configuration [(AAAGG)10–25(AAGGG)exp] originally found in Māori tribes was later found in other cohorts [10, 16, 17]. Moreover, a relatively more severe form of the disease has been reported in patients with AAGGG expansions in trans with heterozygous nonsense and frameshift variants [18, 22]. Finally, another pathogenic repeat motif AGGGC in compound heterozygous state with AAGGG expansions was recently found in Caucasians and mixed ethnicity patients from the 100,000 Genome Project [13]. Hence it is reasonable to hypothesise the prevalence of the disease could be higher due to an overall higher prevalence of carriers of pathogenic REs or truncating variant in the *RFC1* locus.

In a previous report pseudodominance was suspected in three patients with *RFC1*-spectrum disorder but was not genetically confirmed [4]. In a consanguineous family with an apparent vertical transmission three members were found to harbour biallelic ACAGG expansions and three had ACAGG and AAGGG RE in a compound heterozygous state [14].

More recently four Portuguese families from a cohort of 67 *RFC1*-positive patients were reported to have a pseudodominant type of inheritance [23].

Pseudodominance has been described in other autosomal recessive neurological diseases. Friedreich Ataxia, Wilson's disease, Charlevoix-Saguenay ataxia and oculomotor apraxia type 1 and 2 are those in which ataxia is part of the phenotype [24].

A few clinical messages emerge from this report. First, our findings support the impression that many cases are not diagnosed due to the heterogeneous spectrum with asymptomatic or mildly affected patients even in old age, a further element strengthening the importance of extensive genetic counselling in families where there are affected individuals. In our series, sensory neuropathy could be detected with NCS in three oligo/asymptomatic patients, whereas cerebellar atrophy was evident with longer disease duration.

Second, clinical observation should orient genetic analysis despite the presence of different disorders and an apparent dominant transmission. In family 2, the diagnosis was delayed because the sensitive neuropathy in Pt III-1 was considered to be a clinical manifestation of Fabry disease overlooking features like the chronic cough and the severe somatic small fibre neuropathy detected in the skin biopsy,

Table 1 Clinical features of the patients

	Family A					Family B			
	Pt II 4	Pt II 5	Pt II 6	Pt II 8	Pt III 1	Pt II 2	Pt III 1	Pt III 3	Pt III 4
Age at onset	-	45	60	63	-	35	51	49	49
Disease duration	-	30	10	4	-	37	1	1	-
First symptom	-	Unsteadiness	Unsteadiness	Unsteadiness	-	Cramps	Cramps	Sporadic LL paresthesia	Sporadic LL numbness
Cerebellar ataxia	+	+	+	+	-	+	-	-	-
Sensory symptoms	-	-	+	+	-	+	+	+	+
Vestibular dysfunction	-	+	+	+	-	-	-	-	-
Autonomic dysfunction	-	-	+	+	-	-	-	-	-
Chronic cough	-	+	+	+	-	+	+	+	+
Additional features	-	Dysphagia	Mild bilateral sensorineural hearing loss	Moderate bilateral sensorineural hearing loss	-	Orofacial dyskinesias, choreic movements of the hands	Orofacial dyskinesias	-	-
SARA at baseline	0,5	18	7	8	0	N.A.	2,5	0	0
SARA at one year follow up	1	19	7	8	0	N.A.	-	-	-
EMNG	Reduced median nerve SAPs, absent sural nerve SAPs	Globally absent SAPs, with reduced median nerve SAPs	Absent SAPs at four limbs	Absent SAPs at four limbs	N.P.	Absent SAPs at four limbs	Absent SAPs at four limbs	Absent SAPs at superficial peroneal nerves, reduced sural nerve SAPs	Reduced median and sural nerves SAPs.
Motor Evoked Potentials	N.P.	Prolonged CMCT at four limbs	Normal	Normal	N.P.	N.P.	N.P.	N.P.	N.P.
Brainstem Auditory Evoked Potentials	N.P.	Reduced amplitude of wave V	Normal	Normal	N.P.	N.P.	N.P.	N.P.	N.P.
Brain and spine MRI	N.P.	Cerebellar atrophy	Normal	Normal	N.P.	Cerebellar atrophy	Normal	N.P.	N.P.
Sudosecan (Electrochemical skin conductance)	N.P.	N.P.	UP 65 μ s, asymmetry 8%; LL 52 μ s, asymmetry 1%	UP 53 μ s, asymmetry 5%; LL 78 μ s, asymmetry 5%	N.P.	N.P.	UL 81 μ s, asymmetry 1%; LL 81 μ s, asymmetry 1%	UL 86 μ s, asymmetry 1%; LL 85 μ s, asymmetry 1%	UL 78 μ s, asymmetry 1%; LL 81 μ s, asymmetry 1%

EMNG= Electromyoneurography; LL lower limbs; SAPs= sensory action potentials; CMCT= central motor conduction time. N.A. Not Available. N.P. Not Performed; abnormal values in electrochemical skin conductance detected by Sudosecan are underlined

suggestive of a ganglionopathy as described in CANVAS [23, 25].

Conclusion

Our reports suggest that pseudodominance can be a frequent and confounding phenomenon to consider in *RFC1*-spectrum disorder. Genetic counselling is instrumental in families with affected individuals.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical Approval This study was approved by the ethics committee of Policlinico G. Martino, Messina and was performed in line with the principles of the Declaration of Helsinki.

Consent to Participate All included patients or their deputies provided written informed consent. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

Competing Interests The authors declare no competing interests.

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