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RFC1 expansions can mimic hereditary sensory neuropathy with cough and Sjogren's syndrome

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Letter

Sir,

We read with great interest the article by Cortese and colleagues (Cortese *et al.*, 2020) describing 100 carriers of the *RFC1* expansion. This study explores the phenotypic spectrum of *RFC1* expansions, identified as a cause of cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) and late onset ataxia (Cortese *et al.*, 2019; Rafehi *et al.*, 2019). They concluded that *RFC1* should be considered in all cases of sensory ataxic neuropathy, especially if there are manifestations of cerebellar impairment, vestibular dysfunction, and cough.

In collaboration with this group, we assessed for *RFC1* expansions in a cohort from Sydney, Australia, to further explore the phenotypic spectrum. We recruited probands without a genetic diagnosis and with a phenotype of hereditary cerebellar ataxia (HCA), hereditary peripheral neuropathy, or CANVAS including archival cases. Participants were recruited through the Molecular Medicine Laboratory, Concord Repatriation General Hospital. Subjects' consent was obtained according to the Declaration of Helsinki. The study has been approved by the Sydney Local Health District Human Ethics Committee (HREC/17/CRGH/8).

DNA was extracted using standard techniques and forwarded for *RFC1* analysis to UCL Queen Square Institute of Neurology and The National Hospital for Neurology, London, UK. Individuals had repeat primed PCR to detect the pathogenic (AAGGG) and non-pathogenic (AAAGG or AAAAG) repeat expansions, as described (Cortese *et al.*, 2019). Confirmation of the repeat expansion and determination of the size was performed using Southern Blot analysis.

We investigated 17 probands with a variety of initial diagnoses including HCA without sensory neuropathy (n = 5), HCA with sensory neuropathy (n = 2), hereditary sensory neuropathy (HSN, n = 4), CANVAS (n = 2), Sjögren's ataxic sensory neuropathy (n = 1), paraneoplastic neuropathy (n = 1), inherited spastic ataxia with neuropathy (n = 1), and sensory ataxic neuropathy (n = 1). The age onset was 56.5 ±- 11.6, age at examination was 66.1 ± 10.3 (mean ± standard deviation in years). The sample included 8 males and 9 females. There was a positive family history in 10/17, sensory neuronopathy on clinical examination in 11/17, sensory neuronopathy on nerve conduction studies in 11/17 (11/14

tested), documented abolished vestibuloocular reflex on head impulse test (HIT) in 7/17, documented bilateral vestibular areflexia on vestibular testing in 4/17, cerebellar dysfunction on clinical examination in 13/17, cerebellar atrophy on MRI in 6/17, cough documented in 8/17 and sural biopsies performed in 2/17. An affected relative was available for study in 1 subject only (proband R190103).

We identified a biallelic pathogenic AAGGG expansion in 5 of the 17 individuals using repeat primed PCR, confirmed on Southern Blot analysis (Figure).

The 5 individuals with biallelic expansions had several diagnoses at presentation, highlighting that *RFC1* expansions can mimic a variety of disorders (Table 1, Figure). Despite different diagnoses at presentation, 4/5 patients had their diagnosis revised to CANVAS and fulfilled proposed criteria (Szmulewicz *et al.*, 2016) for clinically possible, probable or definite CANVAS on retrospective review. Notably, all probands with biallelic *RFC1* expansions had evidence of sensory neuropathy, as well as a positive HIT and cough when tested (4/5 cases). In comparison, those probands without biallelic *RFC1* expansions frequently did not have a HIT performed (8/12) or it was negative (1/12) and were rarely questioned on cough (9/12), reflecting limitations in data collection. Biallelic *RFC1* expansions were not found in HCA although the majority (5/7) did not have sensory neuropathy.

Highlighting the diversity of presentations of *RFC1*, we identified biallelic expansions in 2/4 families who were initially diagnosed with HSN (although not in a family with HSN with cough linked to chromosome 3p22-p24 (Kok *et al.*, 2003; Spring *et al.*, 2005)).

This included a large Lebanese family in which the proband (R110358) presented at the age of 60 years with a 10 year history of recurrent cough, preceding a 5 year history of burning feet. He had severe coughing fits associated in which her face would go red as well as a positive HIT. He had 2 affected sisters; he also had a possibly affected mother and maternal grandmother with a cough, but these family members were deceased and so unavailable for further assessment.

A 62 year old man diagnosed with HSN with cough and gastroesophageal reflux was also found to have biallelic *RFC1* expansions (R60355). He had a cough which started 20 years prior to assessment and his sensory symptoms only started 4-5 years prior; the cough was attributed to reflux and was treated with a Nissen Fundoplication.

We further describe biallelic *RFC1* expansions in a woman (R19977) initially diagnosed as Sjogren's syndrome. She presented at 60 years of age with a 6 month history of gait imbalance with recurrent falls and paraesthesias in the feet and a tendency to inadvertently burn her hands, and a cough attributed to gastro-esophageal reflux treated with a Nissen Fundoplication. Examination revealed evidence of cerebellar signs, distal sensory neuropathy, ataxic gait, and positive Romberg's sign. Nerve conduction studies showed absent sensory responses, and a sural biopsy showed severe axonal loss (Figure). She was diagnosed with Sjogren's sensory ataxic neuropathy, although there was uncertainty given the absence of sicca features and a negative ENA. She was treated with azathioprine, mycophenolate, and intravenous immunoglobulin with no improvement. Further assessment 3 years after presentation revealed a bilaterally abnormal HIT consistent with CANVAS. Of potential interest, she was diagnosed with sensorineural hearing loss (Table).

Additionally, we identified 3 individuals who carried a single pathogenic *RFC1* expansion (R19955, R19954 and R190103). This included a 73 year old man (R19955) with peripheral neuropathy and a persistent cough for over 6 years with an unremarkable CT chest, in whom the diagnosis of paraneoplastic neuropathy was considered but with negative antineuronal antibodies. He was later found to have bilateral vestibular dysfunction consistent with CANVAS (Table, Video). This individual was heterozygous for both the pathogenic AAGGG and non-pathogenic AAAGG expansion. For proband R190103, a heterozygous pathogenic expanded allele did not segregate in her affected brother (R190104). Proband R19954 had a heterozygous pathogenic expansion and had sporadic cerebellar ataxia without sensory neuropathy.

Our study highlights that *RFC1* expansions can present with a variety of disorders, such as HSN with cough and Sjorgen's ataxic neuropathy, consistent with the findings of Cortese and colleagues (Cortese *et al.*, 2020).

We note that in many instances the cough may precede the observation of peripheral neuropathy by many years, in keeping with findings of Cortese and colleagues. Individuals with *RFC1* expansions may have severe, paroxysmal bouts of coughing (proband R110358), and in other cases may describe a dry, tickly cough occurring at night and disturbing sleep (R60355). The cough may be brought to the attention of a range of medical specialties who may not immediately draw the association with peripheral neuropathy, leading to

investigation for primary lung pathology or attribution to gastro-oesphageal reflux pathology (R60355 and R19977).

Furthermore, although vestibular dysfunction was universally present in biallelic *RFC1* expansion carriers in this cohort, there was often a delay before a HIT and/or vestibular testing were performed and vestibular impairment was detected, highlighted by the finding that the majority of probands (52.9%, 9/17) did not have a HIT recorded.

We note that in at least one proband, an early diagnosis of CANVAS may have prevented treatments with potential serious adverse effects such as immunotherapy for Sjogren's syndrome. Furthermore, 2 of the 6 *RFC1* expansion carriers had sural nerve biopsies, a test which may have serious complications that in the future may be avoided with greater awareness of this condition.

Additionally, 3 families with *RFC1* expansions had a multigenerational family history suggestive of autosomal dominant inheritance (R110358, R60355 and R19975). In these cases, the children, parents or grandparents of the proband had some features suggestive of CANVAS (e.g. cough or gait abnormalities) but were not available for assessment. We suspect that in some instances RFC1 may show a pseudodominant pattern of inheritance given the relatively high population frequency of the pathogenic expanded allele (Cortese *et al.*, 2019) and this may complicate interpretation of the mode of inheritance.

Furthermore, one individual (R19955) had a definite diagnosis of CANVAS and was heterozygous for both the AAGGG pathogenic expansion as well as the non-pathogenic AAAGG expansion. A possible explanation is that there is a may be another mutation type on the other allele (e.g. a sequencing variant), but we acknowledge the difficulty of definitive confirmation of *RFC1* expansion testing which requires sequencing of the repeats, as highlighted by a recent study (Akcimen *et al.*, 2019).

In summary, clinicians should be aware that *RFC1* expansions may masquerade as a variety of different disorders, and that early detection of vestibular impairment and genetic testing may be critical to the diagnosis and have management implications. Biallelic pathogenic *RFC1* expansions were identified for a relatively high percentage of cases (29.4%, 5/17), and we agree that *RFC1*-related disorders are frequently under recognised or misdiagnosed. Cortese and colleagues highlight the importance of a thorough clinical examination; consistent with this we recommend routinely assessing for the HIT and cough in individuals

with neuropathy, findings which may provide a clue to the presence of an *RFC1* expansion-related disorder.

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Data available on request from the authors.

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Competing interests

The authors report no competing interests

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Figure Legends

Figure. Left panel. Southern blot results confirming *RCF1* biallelic AAGGG expansions. The number of repeats for each allele are shown in parentheses. R19975 (821, 1015), R19982 (1070, 1070), R110358 (292, 1192), R60355 (526, 821), R200002 (1128, 1128). Patient R19955 was heterozygous for the AAGGG expansion (1325). Patient R40143 was positive for the repeat primed (AAAAG) PCR which are non-pathogenic expansions. We considered pathogenic repeat expansions greater than 250. Kb – Kilobase. Centre panel. Pedigrees of families with biallelic pathogenic *RCF1* expansions. Black filled symbols indicate affected individuals. Grey filled symbols indicate family members whose affected status is uncertain. Arrow indicates proband. Diagonal line indicates deceased individuals. 'M' indicates allele with pathogenic RFC1 expansion, '-' indicates allele without pathogenic RFC1 expansion. Right panel. Right sural nerve biopsy (toluidine blue) from R19977 showing a severe reduction in myelinated fibre density with only a few large myelinated fibres remaining in most fascicle. There were no inflammatory cell infiltrates. On the teased fibre preparation most fibres were of small diameter and thinly remyelinated. 10% of fibres show evidence of previous demyelination/remyelination. Immunofluorescence stains for immunoglobulin, complement, fibrinogen and the Congo red stain for amyloid were negative. The findings are consistent with severe axonal loss consistent with a neuropathy or ganglionopathy.

Video legend

Video. Examination of individual R19955 demonstrating an ataxic gait required a unilateral walking aid and a bilaterally positive head impulse test (assessed by author KRK). Although his phenotype is consistent with CANVAS he has only a single heterozygous pathogenic expansion in the *RFC1* gene.

Table. Clinical features of individuals with pathogenic *RFC1* expansions

ID	Gender	Age at onset	Age at examination	Ethnicity	Family history	Sensory neuronopathy (clinical)	Sensory neuronopathy (NCS)	Bilateral vestibular areflexia (clinical - abolished VOR at HIT)	Bilateral vestibular areflexia (vestibular testing)	Cerebellar dysfunction (clinical)	Cerebellar Atrophy on MRI	Cough	Sural biopsy	Additional features	Previous genetic testing	Initial diagnosis considered	Revised diagnosis
Biallelic Pathogenic expansions																	
R110358	M	50	60	Lebanese	Yes (2 sisters, mother and maternal grandmother)	Yes	Yes	Yes	Not available	Yes	Not available	Yes	No			Hereditary sensory neuropathy with cough	CANVAS
R60355	M	41	62	Caucasian	Yes (son)	Yes	Yes	Not available	Not available	No	Not available	Yes	No	Gastroesophageal reflux, oesophageal manometry studies not performed/not available.	*SPTLC1* exon 5 and 6 sequencing negative.	Hereditary sensory neuropathy with cough and gastroesophageal reflux	
R19975	F	60	63	Caucasian	Yes, brother, father, paternal grandmother (not available	Yes	Yes	Yes	Yes, cVEMP not obtainable, ocular VEMPs not obtainable on the right side	Yes	No	Not available	Yes, severe axonal loss	Dysarthria. She was also diagnosed with mild to moderate sensorineural hearing loss with symptom	SCA1, SCA2, SCA3, SCA6, SCA7, SCA12 and SCA17 negative.	Sjogren's ganglionopathy	CANVAS

					to be studied)									onset in her early 60's. There was no family history of hearing impairment and no known contributing environmental factors. Gastroesophageal reflux, oesophageal manometry studies not performed/not available.			
R19982	F	54	67	Caucasian	Yes (sister diagnosed SCA1, lives in Netherland)	Yes	Yes	Yes	Not available (ordered)	Yes	Yes	Yes (49/70 for the Hull Cough Hypersensitivity Questionnaire, 43 for the Leicester cough	No	Spasticity, dysarthria	Hereditary spastic paraplegia panel on WES negative. Hereditary ataxia panel on WES negative. SCA1, 2, 3, 6, 7, 12, 17 and Friedreich's	Inherited spastic ataxia with neuropathy	CANVAS

												questionnaire)			ataxia negative.		
R200002	M	49	55	Caucasian	No	Yes	Yes	Yes	VEMPs absent	Yes	Not available	Yes	No		Yes, SCA1, SCA2, SCA3, SCA6, SCA7, SCA12 and SCA17 negative.	CANVAS	
Heterozygous pathogenic expansions																	
R19955	M	58	73	Caucasian	No	Yes	Yes	Yes	VEMPs, caloric testing responses absent bilaterally	Yes	Yes	Yes	No		No	Neuropathy related to insulin resistance, paraneoplastic neuropathy	CANVAS
R19954	F	63	79	Caucasian	No	No	No	Not available	VEMPs normal	Yes	No	Not available	No		Yes, *FMR1* expansion negative	Hereditary cerebellar ataxia	
R190103	F	41	60	Caucasian	Yes (dominant, 2 affected brothers and affected father)	No	Not available	Not available	Not available	Yes	Yes	Not available	No	Dysarthria, dysphonia, dysphagia and sialorrhoea	Not available	Hereditary cerebellar ataxia	

M, male; F, female, HIT – head impulse test, NCS – nerve conduction study, VEMP - vestibular evoked myogenic potential, cVEMP - vestibular evoked myogenic potentials elicited from the sternocleidomastoid muscle (cervical), VOR - vestibulo-ocular reflex.