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Early Peripheral Nerve Involvement at the Time of Coughing in Patients With *RFC1* Intronic Expansion

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Abstract

Objectives

Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome results from variations in *RFC1* and is mostly caused by intronic biallelic pathogenic expansions (RE-*RFC1*). Refractory chronic cough (RCC) is frequently observed for years to decades preceding ataxia onset. Whether peripheral nerves are involved in the presymptomatic phase characterized by RCC is uncertain.

Methods

Here, patients previously screened for RCC and identified as having at least one RE-*RFC1* intronic expansion underwent a comprehensive clinical and neurophysiologic assessment and were screened for additional exonic variations.

Results

Fourteen patients with RCC and RE-*RFC1* were investigated. Seven patients presented with biallelic RE-*RFC1* (Bi-RE-*RFC1*) while 7 presented with monoallelic RE-*RFC1* (Mono-RE-*RFC1*). In patients with Mono-RE-*RFC1*, no additional exonic variation was identified, and clinical examinations were normal. Most of the patients with Bi-RE-*RFC1* presented with subtle neurologic impairment, mainly exhibiting decreased lower limb vibration sense (85.7%). Nerve conduction studies revealed that all patients with Bi-RE-*RFC1* exhibited lower sensory sum scores than patients with Mono-RE-*RFC1* (median 20.2 μV vs 84.9 μV, $p = 0.0012$). In addition, the radial-to-sural sensory ratios were null or inverted (>0.5) in all patients but one with Bi-RE-*RFC1*, which is consistent with sensory neuronopathy.

Discussion

Patients with Bi-RE-RFC1 already exhibit widespread sensory neuron involvement at the time of apparently isolated RCC.

Introduction

Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) is a hereditary condition with autosomal recessive inheritance that is clinically characterized by progressive cerebellar ataxia, constantly associated with sensory neuronopathy.[1] The clinical spectrum of the disorder has dramatically expanded, and CANVAS is now considered a multisystem disease.[2] The recently identified genetic cause of CANVAS is predominantly biallelic intronic repeat expansion of a pentanucleotide in the replication factor C subunit 1 (RE-*RFC1*).[3] However, a few patients carry a heterozygous expansion associated with a truncating variant in the other allele of *RFC1*.[4,5]

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Of interest, many patients with CANVAS present with a chronic, unexplained cough.[6] Cough in patients with CANVAS is supposedly of a neurogenic origin[7] and fulfills the definition of refractory chronic cough (RCC).[8] As RCC usually precedes the development of other core CANVAS symptoms by years or even decades, we recently investigated the presence of *RFC1* intronic expansion in a series of patients affected by RCC who had not yet been diagnosed with CANVAS. We previously found that 17 (25%) patients in a series of 68 had at least one pathologic intronic expansion, of which 11 (16.2%) carried a biallelic expansion.[9]

To date, evidence of peripheral nervous system involvement at a very early stage of CANVAS (i.e., when patients complain of isolated RCC) has not been reported. In this study, we investigated whether patients from this series showed clinical evidence of ataxia and/or neuronopathy using clinical examinations and electrodiagnostic studies.

Methods

Study Protocol and Participants

Patients with RCC and at least one RE-*RFC1* intronic expansion, previously studied for their cough characteristics, were included in the current neurologic study after screening for the presence of *RFC1* exonic variations.[9]

These patients underwent standardized clinical and neurophysiologic examinations to identify the classical features of CANVAS. Electrodiagnostic studies were performed unilaterally on the nondominant side. For the motor conduction study, the fibular and median nerves were examined by recording the extensor digitorum brevis and abductor pollicis brevis, respectively. For the sensory conduction studies, the sural, median, ulnar, and radial nerves were examined antidromically. A sensory sum score and a motor sum score were calculated by summing all sensory nerve action potentials (SNAPs) and compound muscle action potentials (CMAPs), respectively. The sensory ratio was calculated by dividing the sural by the radial SNAP amplitude.

Standard Protocol Approvals, Registrations, and Patient Consents

This study was conducted in accordance with the Declaration of Helsinki, the French ethics requirements (RnIPH 2021-145), and the National Commission for Data Protection and Liberties (CNIL number 2206723v0). Written informed consent was obtained from all patients.

Statistical Analyses

Clinical and electrodiagnostic data were compared between patients with RCC and biallelic RE-*RFC1* (Bi-RE-*RFC1*) and those with RCC and Mono-RE-*RFC1*, the latter serving as the control group. Proportions and quantitative variables were compared using the Fisher exact test and the Mann-Whitney *U* test, respectively. Statistical analyses were two-tailed with a significance level of 0.05.

Data Availability

Data will be made available on reasonable request.

Results

Fourteen patients with RCC harboring at least one RE-*RFC1* gene were included in this study. Among these patients, 7 harbored Bi-RE-*RFC1* while 7 harbored Mono-RE-*RFC1*. No additional exonic variations were identified by NGS in the 7 patients with RCC harboring Mono-RE-*RFC1*.

The clinical characteristics of the patients are shown in the Table. Patients with either Bi-RE-*RFC1* or Mono-RE-*RFC1* had a comparable age distribution ($p = 0.87$), with a median age of 61 years in both groups. Decreased sense of vibration in the lower limbs was the most frequent abnormality observed on neurologic examination among patients with Bi-RE-*RFC1* compared with those with Mono-RE-*RFC1* (85.7% vs 0%; $p = 0.0047$). Ataxia, paresthesia, neuropathic pain, abnormal stretch reflex, and tactile and needle hypoesthesia were also more common in patients with Bi-RE-*RFC1*, although the difference between groups was not significant. None of the patients reported oscillopsia, and only one patient in each group reported mild vertigo.

The key grouped electrodiagnostic data are shown in the Figure. Every patient with Bi-RE-*RFC1* exhibited a lower sensory sum score than those with Mono-RE-*RFC1* (median 20.2 μV vs 84.9 μV respectively, $p = 0.0012$). All but one patient with Bi-RE-*RFC1* displayed either null (abolished sural SNAP) or inverted (>0.5) radial/sural sensory ratios. In patients with Mono-RE-*RFC1*, the sural/radial sensory ratios were consistently <0.5 without exception. All SNAPs were of normal amplitude in patients with Mono-RE-*RFC1* except for a patient in their 80s who had decreased sural SNAP. Parameters of motor conduction studies were normal according to local normative values in all patients. Notably, motor sum scores were comparable between the Bi-RE-*RFC1* and Mono-RE-*RFC1* groups (median 12.2 mV vs 12.8 mV, $p = 0.71$). Detailed electrodiagnostic data for each patient are provided in eTables 1 and 2.

Discussion

In this study, we present the clinical and electrodiagnostic characteristics of patients with RE-*RFC1* that were screened for RCC without any known neurologic symptoms. Of interest, most patients with Bi-RE-*RFC1*, that is, early-stage patients with CANVAS, presented with subtle neurologic signs on fine examination, notably vibration hypoesthesia of the lower limbs (85.7%), in contrast with prominent sensory conduction abnormalities in all.

Despite the design of our previous study, which theoretically would have allowed us to detect CANVAS at a very early stage, some patients we currently studied had been coughing for

Table Clinical Characteristics of Patients With RCC and 1 or 2 *RFC1* Repeat Intronic Expansion Alleles

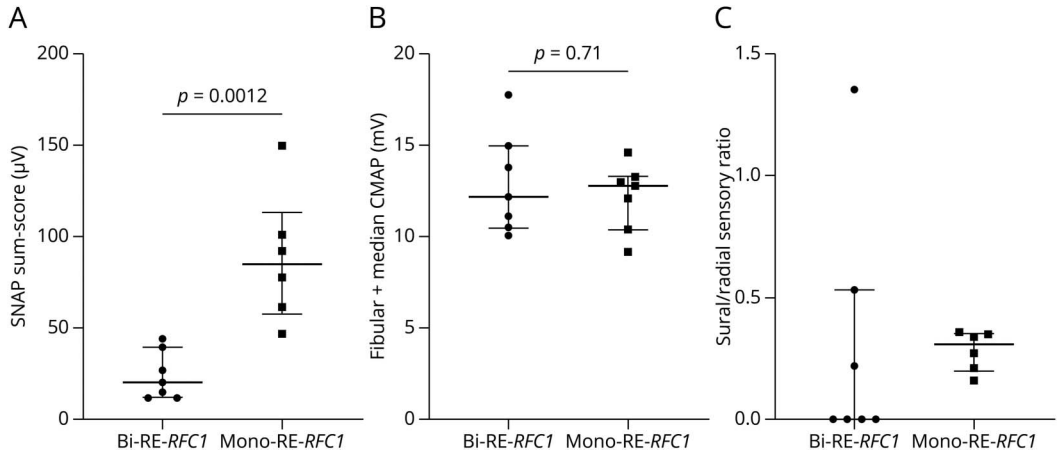
RFC1 variation	Mono-RE-*RFC1*							Bi-RE-*RFC1*							*p* Value
Patient #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Decade at current evaluation	80s	50s	40s	60s	40s	60s	50s	60s	50s	40s	40s	60s	70s	70s	0.87
Decade at RCC onset	60s	30s	30s	60s	40s	20s	40s	40s	30s	30s	30s	30s	50s	50s	0.97
Vertigo	-	-	-	-	-	-	Mild	Mild	-	-	-	-	-	-	NA
Oscillopsia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA
Ataxia	-	-	-	Mild	-	-	-	-	Moderate	-	-	Mild	Mild	Mild	0.27
Dysarthria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA
Dysphagia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA
Paresthesia	-	-	-	-	-	-	-	-	LL	-	-	4L	-	-	0.46
Neuropathic pain	-	-	-	-	-	-	-	-	LL	-	-	4L	-	-	0.46
Tactile hypoesthesia	-	-	-	-	-	-	-	-	LL	-	-	-	-	-	1
Needle hypoesthesia	-	-	-	-	-	-	-	LL	-	-	-	-	-	-	1
Vibration hypoesthesia	-	-	-	-	-	-	-	LL	LL	-	LL	LL	LL	LL	0.0047
Abolish stretch reflex	-	-	4L	-	-	Ankles	-	-	-	-	LL	Ankles	Ankles	-	1

Abbreviations: Bi-RE-*RFC1*, biallelic repeat expansion of *RFC1*; LL, lower limbs; Mono-RE-*RFC1*, monoallelic repeat expansion of *RFC1*; RCC, refractory chronic cough; -, normal; 4L, four limbs.

several years. The diagnosis of RCC with no identified cause necessitates numerous investigations which frequently allow for an accurate diagnosis after years. In contrast to limited neurologic symptoms and signs, all patients harboring Bi-RE-*RFC1* exhibited a significant, even severe, impairment in sensory nerve conduction studies. This discrepancy is common in inherited neuropathies, suggesting that the neurologic damage may have begun many years before the current assessment.

The non–length-dependent pattern of sensory involvement in some patients, together with no motor abnormality in all patients, was consistent with sensory neuronopathy. We did not include videonystagmography and brain MRI in this exploratory study because abnormalities revealed by these tests are known to be inconsistent and late, respectively. Our results demonstrate that widespread sensory neuropathy is already present in all patients during the so-called presymptomatic

Figure 1 Main Neurophysiologic Characteristics of Patients With RCC and 1 or 2 *RFC1* Intronic Repeat Expansions



(A) Comparison of SNAP sum score between Bi-RE-*RFC1* and Mono-RE-*RFC1*. (B) Comparison of motor sum score between Bi-RE-*RFC1* and Mono-RE-*RFC1*. (C) Comparison of sensory ratio between Bi-RE-*RFC1* and Mono-RE-*RFC1*, showing a bimodal distribution in Bi-RE-*RFC1* suggesting a non–length-dependent pattern of sensory involvement. Plot: bar includes the 25th–75th percentiles and median line. Bi-RE-*RFC1* = biallelic repeat expansion of *RFC1*; CMAP = compound muscle action potential; Mono-RE-*RFC1* = monoallelic repeat expansion of *RFC1*; SNAP = sensory nerve action potential.

phase of the disease. This is well illustrated by the condition of a patient in their 40s (#11) who presented with a significantly reduced sensory sum score of 12 μV and no ataxia on fine neurologic examination, 5 years after the onset of RCC.

Patients with Mono-RE-*RFC1* were deemed unaffected by CANVAS. Our previous study found that 8.8% of patients with RCC had Mono-RE-*RFC1*, which is higher than expected, considering the anticipated 0.7% estimate within the European population.[3] We verified that no additional exonic variations were present in the patients with mono-RE-*RFC1*. No neurologic abnormalities resulting from large fiber involvement were observed in these patients. This could be explained by the tendency to develop cough hypersensitivity in Mono-RE-*RFC1* without overt widespread neurologic involvement. Indeed, small fibers (not formally evaluated in this study) may contribute to cough hypersensitivity in patients with Mono-RE-*RFC1*. Alternatively, this could be due to selection bias, considering the small number of patients we previously reported. Furthermore, a subsequent study suggested a higher allele frequency of RE-*RFC1* at 0.023,[10] which does not appear to be significantly lower than the Mono-RE-*RFC1* rate we reported among patients with RCC. Collectively, these data are insufficient to warrant the suspicion of a link between Mono-RE-*RFC1* and RCC.

In summary, we show that at the time of apparently isolated RCC in the presymptomatic phase of CANVAS, all patients with Bi-RE-*RFC1* already exhibit widespread peripheral sensory neuron involvement. These findings are likely to be confirmed in the future as the association between RCC and CANVAS becomes more widely known in the pulmonary community, allowing earlier diagnosis. Moreover, this finding reinforces the hypothesis that RCC in CANVAS is of neurogenic origin.

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