Movement Disorders
CLINICAL PRACTICE

RESEARCH ARTICLE

Cranial Nerve Thinning Distinguishes RFC1-Related Disorder from Other Late-Onset Ataxias

Camila C. Lobo, MD,[1] Guilherme S.O. Wertheimer, MD,[2] Gabriel S. Schmitt, MD,[1] Paula C.A.A.P. Matos, MD,[3] Thiago J.R. Rezende, PhD,[1] Joyce M. Silva, MD,[1] Fabrício C. Borba, MD,[1] Fabrício D. Lima, MD, MSc,[1] Alberto R.M. Martinez, MD, PhD,[1] Orlando G.P. Barsottini, MD, PhD,[3] José Luiz Pedroso, MD, PhD,[3] Wilson Marques Jr MD, PhD,[4] and Marcondes C. França Jr MD, PhD[1,*]

Abstract: Background: RFC1-related disorder (RFC1/CANVAS) shares clinical features with other late-onset ataxias, such as spinocerebellar ataxias (SCA) and multiple system atrophy cerebellar type (MSA-C). Thinning of cranial nerves V (CNV) and VIII (CNVIII) has been reported in magnetic resonance imaging (MRI) scans of RFC1/CANVAS, but its specificity remains unclear.

Objectives: To assess the usefulness of CNV and CNVIII thinning to differentiate RFC1/CANVAS from SCA and MSA-C.

Methods: Seventeen individuals with RFC1/CANVAS, 57 with SCA (types 2, 3 and 6), 11 with MSA-C and 15 healthy controls were enrolled. The *Balanced Fast Field Echo* sequence was used for assessment of cranial nerves. Images were reviewed by a neuroradiologist, who classified these nerves as atrophic or normal, and subsequently the CNV was segmented manually by an experienced neurologist. Both assessments were blinded to patient and clinical data. Non-parametric tests were used to assess between-group comparisons.

Results: Atrophy of CNV and CNVIII, both alone and in combination, was significantly more frequent in the RFC1/CANVAS group than in healthy controls and all other ataxia groups. Atrophy of CNV had the highest sensitivity (82%) and combined CNV and CNVIII atrophy had the best specificity (92%) for diagnosing RFC1/CANVAS. In the quantitative analyses, CNV was significantly thinner in the RFC1/CANVAS group relative to all other groups. The cutoff CNV diameter that best identified RFC1/CANVAS was ·2.2 mm (AUC = 0.91; sensitivity 88.2%, specificity 95.6%).

Conclusion: MRI evaluation of CNV and CNVIII using a dedicated sequence is an easy-to-use tool that helps to distinguish RFC1/CANVAS from SCA and MSA-C.

Biallelic AAGGG repeat expansions in the second intron of replication factor complex subunit 1 (*RFC1*) gene were identified as the cause of cerebellar ataxia, (sensory) neuropathy and vestibular areflexia syndrome (RFC1/CANVAS) in 2019.[1] Since then, this genetic abnormality has been recognized as one of the leading causes of cerebellar ataxia worldwide, accounting for up to 22% of cases in some series.[2–7] Many affected patients present as sporadic late-onset ataxia and the classic CANVAS triad is often absent.[8] In parallel, additional non-ataxic features—such as chronic cough—have been lately reported.[9,10] Altogether, such phenotypic variability contributes to underdiagnosis and especially misdiagnosis of RFC1/CANVAS.

Spinocerebellar ataxias (SCA) and multiple system atrophy of the cerebellar type (MSA-C) account for a significant proportion of late-onset ataxias in clinical practice. These disorders share many clinical features with RFC1/CANVAS, in such a way that the differential diagnosis may become challenging. For instance, the hallmarks of MSA-C—parkinsonism, dysautonomia and

[1]*Department of Neurology, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, Brazil;* [2]*Department of Radiology, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, Brazil;* [3]*Department of Neurology and Neurosurgery, School of Medicine, Federal University of São Paulo (UNIFESP), São Paulo, Brazil;* [4]*Department of Neurosciences, School of Medicine, University of São Paulo at Ribeirão Preto (USP-RP), Ribeirão Preto, Brazil*

***Correspondence to:** Dr. Marcondes C. França Jr, Department of Neurology, University of Campinas (UNICAMP), Rua Tessália Vieira de Camargo, 126. Cidade Universitária "Zeferino Vaz," Campinas, Brazil; E-mail: mcfrancajr@uol.com.br; mcfjr@unicamp.br
Keywords: ataxia, cranial nerves, MRI, RFC1 expansion, CANVAS.
Received 22 June 2023; revised 11 October 2023; accepted 4 November 2023.
Published online 29 November 2023 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mdc3.13930

REM sleep behavior disorder—have lately been described in a significant proportion of patients with *RFC1* expansions.[10–14] Distinction between RFC1/CANVAS and some SCAs may be also difficult, particularly considering those SCA subtypes that present prominent peripheral sensory abnormalities (SCA2 and SCA3) and/or vestibular dysfunction (SCA3).[15–19] Moreover, autosomal dominant inheritance is not always obvious in many patients with SCA, either due to lack of reliable family information or de novo variants.[20] In this scenario, novel tools that can help in the differential diagnosis between RFC1/CANVAS and other late-onset ataxias are clearly needed.

Magnetic resonance imaging (MRI) is a robust technique that enables detailed anatomic evaluation of the cerebellum and brainstem.[21,22] It has been extensively employed to characterize the structural signature of many ataxic disorders, including MSA-C, SCAs and more recently RFC1/CANVAS.[22–25] These efforts expanded our understanding of the mechanisms underlying each condition, but unfortunately they did not identify reliable diagnostic markers to separate RFC1/CANVAS, SCAs and MSA-C. There is indeed a huge overlap in the patterns of cerebellar as well as brainstem atrophy among these conditions.[10,23–27] Recently, thinning of pontine cranial nerves (V and VIII) was reported by Matos et al as a conspicuous and easy-to-recognize neuroradiological feature in RFC1/CANVAS.[28] This MRI sign may be a valid parameter to assist in the diagnosis of RFC1/CANVAS. However, its specificity remains to be established, because it was not previously assessed in SCA or MSA-C cohorts. In the current study, we took a step forward in the evaluation of cranial nerve thinning as a specific diagnostic marker for RFC1/CANVAS. To accomplish that, we assessed qualitatively as well as quantitatively cranial nerves V and VIII in high-resolution MRI scans of a representative cohort of subjects with RFC1/CANVAS, SCAs and MSA-C. Our ultimate goal was to determine the usefulness of this neuroradiological sign in the diagnosis of RFC1/CANVAS.

Methods

Subjects' Selection

Between October 2020 and January 2023, we enrolled 104 adult subjects who underwent MRI scans, including 89 with cerebellar ataxia and 15 age-and-sex matched healthy controls. In the ataxia group, two patients were excluded because they could not tolerate the MRI scans due to claustrophobia and two patients were excluded because of significant motion artifacts in the acquired images. In the final ataxia group, 17 individuals had molecular confirmation of RFC1/CANVAS, 57 had SCA (14 SCA2, 24 SCA3 and 19 SCA6 patients) and 11 MSA-C. The last subgroup was defined according to the latest MDS clinical criteria for MSA-C.[29] All patients with MSA-C had clinically established disease.

None of the subjects had comorbid neurologic or psychiatric disorders. Healthy controls also presented entirely normal neurological examination as well as lack of family history of neurologic or psychiatric conditions.

All ataxic patients were regularly followed either at the University of Campinas (UNICAMP) or at the Federal University of São Paulo (UNIFESP). The study protocol was approved by the local Ethics Committee of UNICAMP under the number CAAE 83241318.3.1001.5404 and written informed consent was obtained from all participants before inclusion.

Clinical Evaluation

All subjects underwent clinical and neurological evaluation on the same day MRI scans were obtained. Demographic and clinical data were collected using standardized questionnaires. The neurological examination of each individual was performed by the same board-certified neurologist (CCL). Ataxia severity was quantified using the Brazilian Portuguese validated version of the Scale for Assessment and Rating of Ataxia (SARA).[30,31] The progression rate of ataxia was estimated as the total SARA score divided by the duration of disease in years and was termed *SARA progression*. In the RFC1 group, the Sensory Ataxia Rating Scale (SEARS) and the Scales for Outcomes in Parkinson's Disease-Autonomic questionnaire (SCOPA-AUT) were also used to assess the severity of sensory neuronopathy and dysautonomia, respectively.[32,33]

MRI Acquisition

All subjects underwent MRI scans on the same 3 T Philips Achieva Scanner (Philips, Best, The Netherlands). Routine T2-weighted images were acquired to exclude unrelated abnormalities. All images were carefully reviewed by a board-certified neuroradiologist (GSOW) (Fig. 1). In all acquisitions, we used a standard 8-channel head coil. The Steady-State Free Procession (SSFP) sequence *Balanced Fast Field Echo* (bFFE) was used for qualitative and quantitative assessment of cranial nerves. The parameters for acquiring bFFE data were the following: acquiring voxel size $0.58 \times 0.58 \times 1.00\ mm^3$, interpolated to $0.28 \times 0.28 \times 0.5\ mm^3$, reconstructed matrix 640×640 mm, 75 slices, TR/TE 7.1/3.0 ms, and flip angle 45°.This sequence was used due to its short scanning time that enables the fast acquisition of high quality images, which is particularly interesting for ataxic patients who often find it hard standing still for long periods of time.[34] This dedicated sequence also provides high contrast and spatial resolution, which are useful for properly evaluating structures as small as cranial nerves.[34–36]

MRI Analyses

Semi-Quantitative Evaluation of Cranial Nerve Thinning

Semi-quantitative evaluation of cranial nerves V and VIII was accomplished by an experienced and board-certified neuroradiologist (GSOW), who was blind to the clinical status of each subject. He assessed separately each MRI scan and then classified

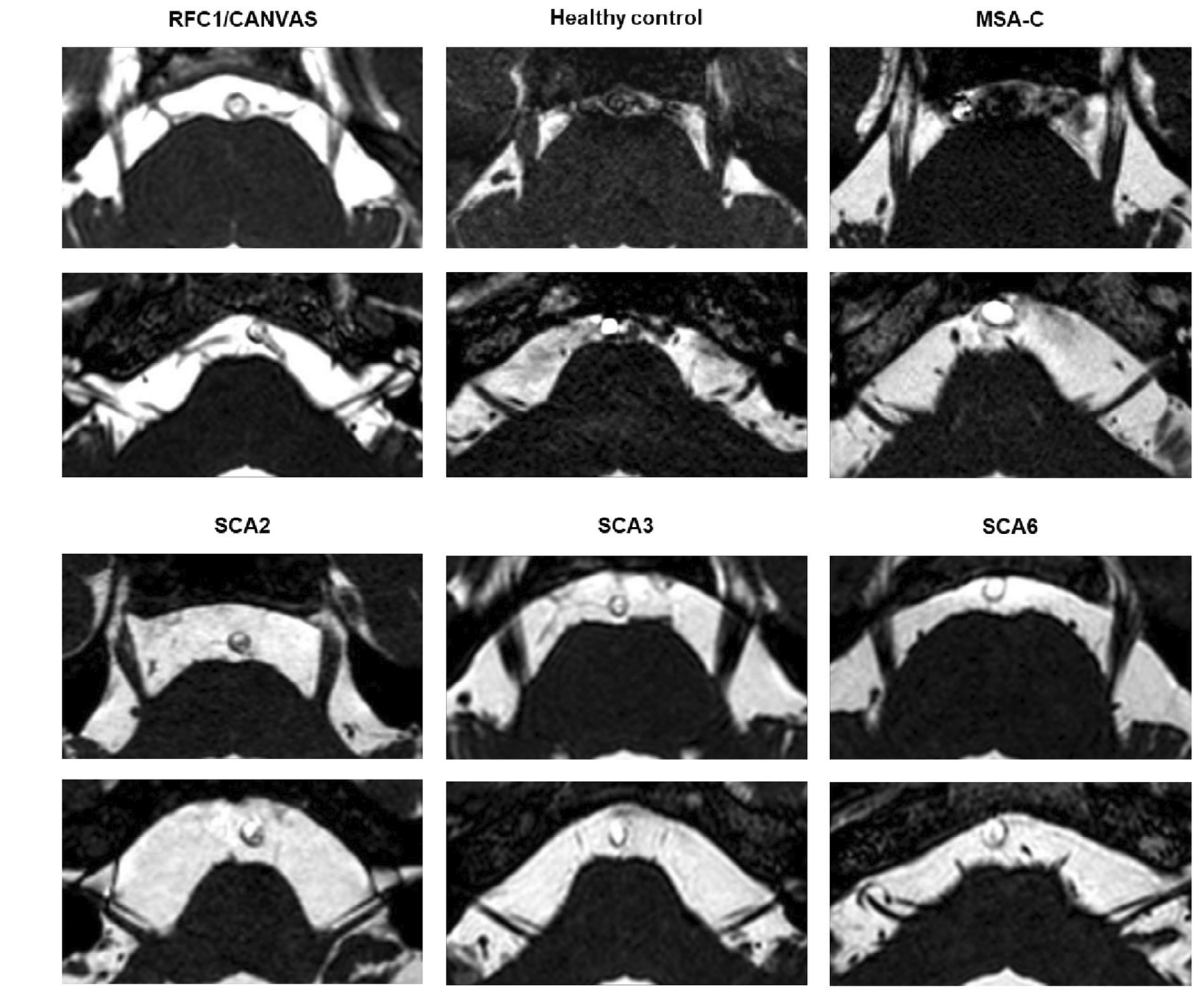

Figure 1. MRI scans showing cranial nerves V (top image) and VIII (bottom image) in late-onset ataxia patients and in healthy controls. Steady-state free procession (SSFP), axial plane.

cranial nerve V as well as VIII dichotomically as either normal or atrophic on both sides. All analyzes were based on the axial section of the bFFE sequence on the ARIA platform and the final classification of each nerve was reached after evaluating all slices in which they appeared.

Quantitative Evaluation of Cranial Nerve Thinning

Semi-quantitative analyses revealed that cranial nerve V thickness performed better than cranial nerve VIII to separate RFC1/CANVAS from the other ataxias. For this reason, we opted to perform manual segmentation of this nerve to obtain quantitative analyses. Cranial nerve V segmentation was performed by an experienced neurologist (CCL) using the same MRI sequence. She was also blind to the clinical status of the individuals and employed the same ARIA platform to manually segment the nerve. The diameter of the cranial nerve root (the most proximal limit of emergence in the brainstem) in the axial plane was measured in millimeters and used as a quantitative parameter (Fig. 2A). The slice used for measurement was the one with the largest diameter. There was no significant difference between the measurements of the right and left trigeminal nerves, therefore the mean diameter between both sides was used as a quantitative parameter for the analyses.

Statistics

Data are shown using descriptive statistics. We compared the frequencies of combined and isolated thinning of cranial nerves V and VIII across groups using Fisher exact test. Sensitivity and specificity of each cranial nerve thinning to separate RFC1/CANVAS vs other ataxias was then computed. Comparison of CNV diameter between groups was performed using the

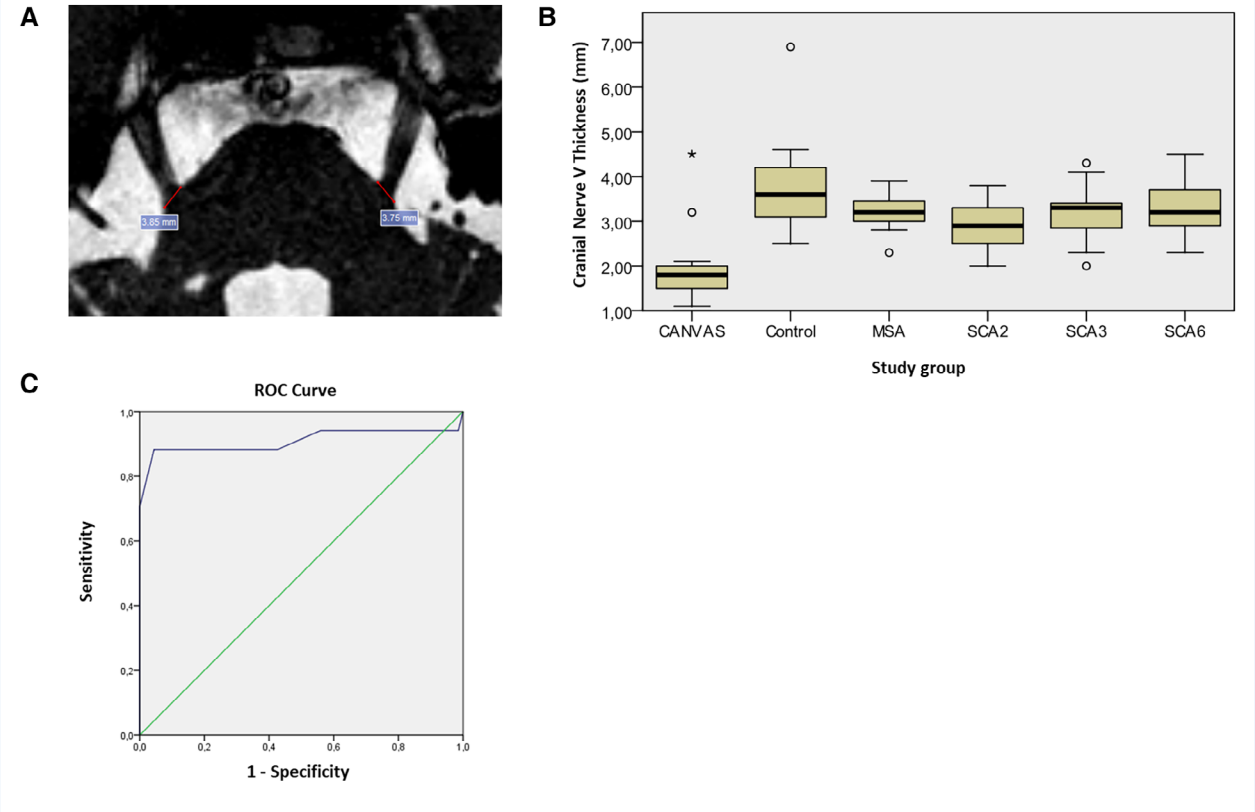

Figure 2. (**A**) Cranial nerve V segmentation exemplified in a healthy control. (**B**) Box-plot showing cranial nerve V diameter in all study groups. (**C**) ROC curve for the diagnostic performance of cranial nerve V thinning to differentiate RFC1/CANVAS from other ataxias (AUC = 0.91).

non-parametric Kruskal-Wallis test and Spearman's coefficient was used to assess potential correlations between CNV thinning and clinical data in the RFC1/CANVAS group. Receiver operating characteristic (ROC) analysis was performed and the area under the curve (AUC) was calculated to evaluate the overall diagnostic performance of CNV thickness in differentiating CANVAS/RFC1 patients from the other late-onset ataxia groups. All analyses were performed in IBM SPSS Statistics version 22 and the level of significance was set at 0.05 for all comparisons.

Results

Demographic and Clinical Evaluation

Detailed demographic and clinical data of all subjects is provided in Table 1. Sex distribution was similar between the RFC1/CANVAS group and all other study groups (healthy controls: $P = 1.000$, MSA-C: $P = 1.000$, SCA2: $P = 0.275$, SCA3: $P = 0.1000$, SCA6: $P = 1.000$). Age distribution was similar between RFC1/CANVAS and controls ($P = 0.792$), MSA-C ($P = 1.000$), SCA3 ($P = 0.082$) and SCA6 ($P = 1.000$) groups. The median age at onset was also similar between RFC1/CANVAS and MSA-C ($P = 1.000$), SCA3 ($P = 0.183$) and SCA6 ($P = 1.000$) groups. However, the SCA2 group had a slightly younger age ($P = 0.015$) and earlier age at onset ($P = 0.014$) in comparison to the RFC1/CANVAS group. The RFC1/CANVAS group had a similar disease profile when compared with the spinocerebellar ataxias groups in relation to SARA scores (SCA2: $P = 1.000$, SCA3: $P = 0.405$, SCA6: $P = 1.000$), estimated rate of SARA progression (SCA2: $P = 1.000$, SCA3: $P = 0.661$, SCA6: $P = 0.441$) and median disease duration (SCA2: $P = 1.000$, SCA3: $P = 1.000$, SCA6: $P = 1.000$). Despite the similar SARA score ($P = 1.000$), the MSA-C group had an estimated rate of SARA progression significantly higher ($P = 0.027$) and shorter disease duration ($P = 0.010$).

Semi-Quantitative Evaluation of Cranial Nerve Thinning

Atrophy of CNV was significantly more frequent in the RFC1 group when compared to healthy controls (82% vs. 7%, $P < 0.001$), MSA (82% vs. 0%, $P < 0.001$), SCA2 (82% vs. 36%, $P = 0.012$), SCA3 (82% vs. 13%, $P < 0.001$) and SCA6 (82%

TABLE 1 *Demographic, clinical and imaging data of all subjects included in the study*

	RFC1 (n = 17)	MSA-C (n = 11)	SCA2 (n = 14)	SCA3 (n = 24)	SCA6 (n = 19)	Control (n = 15)	*P*-value
Age (y)	62 (12.5)	65 (10.0)	41 (22.3)	51 (18.8)	68 (9.8)	51 (16.0)	<0.001*
Male (%)	8 (47%)	5 (45%)	10 (71%)	11 (46%)	12 (63%)	8 (53%)	**
Age at onset (y)	49 (13.5)	60 (12.0)	26 (19.5)	40 (8.0)	54 (16.0)	-	<0.001*
Disease duration, (y)	10 (6.5)	4.0 (3.0)	8 (7.5)	10 (9.0)	14 (10.0)	-	0.001*
SARA scores	16 (15.75)	21.6 (14.5)	15.5 (12.25)	9 (12.0)	13 (11.5)	-	0.004*
SARA progression (per year)	1.72 (1.13)	5.38 (3.25)	1.49 (0.94)	1.04 (0.84)	1.10 (0.85)	-	<0.001*
CNV atrophy (%)	14 (82%)	0	5 (36%)	3 (13%)	1 (5%)	1 (7%)	**
CNVIII atrophy (%)	11 (65%)	1 (9%)	3 (21%)	5 (21%)	5 (26%)	0	**
Combined CNV and CNVIII atrophy (%)	11 (65%)	0	3 (21%)	3 (13%)	1 (5%)	0	**
NCV thickness (mm)	1.80 (0.5)	3.2 (0.6)	2.9 (0.9)	3.3 (0.58)	3.2 (1.0)	3.6 (1.3)	<0.001*

Note: Quantitative data are shown as Median (interquartile range).
Abbreviations: SARA, Scale for Assessment and Rating of Ataxia; CNV, cranial nerve V; CNVIII, cranial nerve VIII.
**P*-values referring to the distribution of medians among all groups.
***P*-values for comparisons between the RFC1/CANVAS group and the other groups are described in the results section, as a pairwise comparison was performed using Fisher's exact test.

vs. 5%, $P < 0.001$) groups (Fig. 1). Atrophy of CNVIII was also significantly more frequent in the RFC1 group when compared to healthy control (65% vs. 0%, $P < 0.001$), MSA (65% vs. 9%, $P = 0.006$), SCA2 (65% vs. 21%, $P = 0.029$), SCA3 (65% vs. 21%, $P = 0.009$) and SCA6 (65% vs. 26%, $P = 0.043$) groups. Combined atrophy of both nerves was more conspicuous in the RFC1 group relative to healthy control (65% vs. 0%, $P < 0.001$), MSA (65% vs. 0%, $P < 0.001$), SCA2 (65% vs. 21%, $P = 0.029$), SCA3 (65% vs. 13%, $P < 0.001$) and SCA6 (65% vs. 5%, $P < 0.001$) groups. The semi-quantitative analysis had a sensitivity and specificity for the diagnosis of RFC1-related disorder versus other late-onset ataxia groups of 82% and 88% for CNV atrophy, 65% and 83% for CNVIII atrophy, and 65% and 92% for combined CNV and CNVIII atrophy, respectively.

Quantitative Evaluation of Cranial Nerve V Thinning

Non-parametric analysis comparing CNV diameter among groups showed that CNV was significantly thinner in the RFC1 group than in the control ($P < 0.001$), MSA-C ($P < 0.001$), SCA2 ($P = 0.010$), SCA3 ($P < 0.001$) and SCA6 ($P < 0.001$) groups (Fig. 2B). The calculated area under the curve (AUC) after ROC curve analysis was 0.91 ($P < 0.001$; Fig. 2C). The cutoff CNV diameter value indicative of CANVAS/RFC1 was ≤2.2 mm. At this threshold, the sensitivity was 88.2% and specificity was 95.6%.

In the RFC1/CANVAS cohort, CNV thickness did not correlate with age, age at onset, disease duration, SEARS or SARA scores (Table S1). Interestingly, this imaging parameter presented significant correlation with the SCOPA-AUT total score ($\rho = -0.62$, $P = 0.011$), as well as with sub-scores in the urinary ($\rho = -0.57$, $P = 0.019$) and thermoregulatory ($\rho = -0.79$, $P < 0.001$) domains (Table S1).

Discussion

In the present study, we observed a high prevalence of CNV and CNVIII atrophy in patients with RFC1-related disorder, in consonance with previous neuroimaging focused studies.[28] However, for the first time, its specificity as a distinctive diagnostic feature between RFC1 and other late-onset ataxias was assessed. Indeed, we were able to uncover a novel neuroradiological sign that might be useful for practicing neurologists caring for ataxic subjects. This result expands the list of relevant imaging clues for specific ataxia subtypes, with comparable specificity and better sensitivity than classical signs, such as the "hot-cross-bun sign" for the diagnosis of MSA-C.[37] In particular, the description of an MRI biomarker that helps to distinguish between RFC1 and MSA-C patients is an important contribution to clinical practice. Both conditions may be remarkably similar on clinical grounds and in parallel, confirmatory testing may be challenging—patients with MSA-C may not fulfill all MDS criteria in the early stages, whereas genetic testing for RFC1 is not always straightforward.[38,39]

These neuroradiological signs in RFC1/CANVAS are in line with previous imaging and pathological reports.[40–43] Previous data with ultrasound imaging demonstrated smaller sensory nerves in patients with CANVAS compared to other sensory neuropathies, suggesting it as a possible marker of sensory ganglion involvement.[43] This finding is compatible with the

significant atrophy of the trigeminal nerve described in the present study. Szmulewicz et al. indeed described macro and microstructural findings in three autopsied cases. These authors noticed prominent atrophy not only of the vestibular component of CNVIII, but also of CNV.[41] Microscopic analyses of CNVIII revealed severe diminution in Scarpa ganglion cells.[40–42] Similarly, there was marked decrease in ganglion cells, loss of axonal fibers and formation of nodules of Nageotte at the trigeminal ganglion.[41] SCA2 and SCA3 may present similar pathological features, particularly regarding the vestibulo-cochlear nerve and nuclei.[44–46] However, neuronal depletion has not been depicted as severe as that seen in RFC1/CANVAS.[47] MSA-C is primarily an oligodendrogliopathy and the major pathological burden of the disease falls on the basal ganglia, brainstem and cerebellum.[48] Prominent cranial nerve involvement has not been reported in the available necropsy studies of patients with MSA-C.[48–50] Altogether, these data suggest that the neuroimaging abnormalities herein reported somehow recapitulate the pathological signature not only of RFC1/CANVAS, but also of the other late-onset degenerative ataxias.

As RFC1-related disorder is a late-onset and progressive disease, we would expect to find a correlation between trigeminal nerve thinning and disease duration. However, we failed to demonstrate such association, which may suggest that this sign is an early finding in the disease, or even that there is a hypoplastic component in the development of this structure in these patients. This theory is supported by the fact that in our dataset we have a patient with a disease duration as short as 4 years who already has significant thinning of the trigeminal nerve (<2.2 mm). Surprisingly, we found an association of trigeminal nerve thinning with the SCOPA-AUT score. This finding is in line with previous studies that demonstrated a similar pattern of trigeminal nerve thinning on MRI scans of patients with familial dysautonomia.[51] These authors also found no correlation between trigeminal thinning and age of the patients. As well as RFC1-related disorder, this entity impacts sensory and autonomic neurons.[52,53] Taken together, these results suggest that damage to autonomic and sensory fibers may be correlated in these monogenic conditions.

Despite the original results, the present study has some noteworthy limitations. There are other relevant differential diagnoses of RFC1/CANVAS that could not be assessed, such as late-onset Friedreich's ataxia and paraneoplastic cerebellar degeneration. Furthermore, one should remember this is a long-standing RFC1/CANVAS cohort, with a median duration of 10 years (IQR 6.5). Hence, we would still need to confirm that the same pattern of cranial nerve atrophy occurs in patients with shorter disease duration, such as those presenting with isolated sensory neuronopathy. Further studies with larger sample sizes and including additional causes of late-onset ataxia will be helpful to address these points.

In conclusion, atrophy of cranial nerves V and VIII is a frequent and specific finding in RFC1-related disorder MRI scans. The evaluation of these structures using a dedicated sequence is an easy-to-use tool that helps to distinguish these patients from MSA-C and SCA. Therefore, we propose that this new neuroradiological sign should be sought in the routine evaluation of late-onset degenerative ataxias.

Acknowledgment

We would like to thank the participants, staff and FAPESP that made this study possible.

Author Roles

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical analysis: A. Design, B. Execution, C. Review and critique; (3) Manuscript: A. Writing of the first draft, B. Review and critique.

C.C.L.: 1, 2A, 2B, 3A.
G.S.O.W.: 1C, 2B, 3B.
G.S.S.: 1C, 2C, 3B.
P.C.A.A.P.M.: 1C, 2C, 3B.
T.J.R.R.: 1B, 2C, 3B.
J.M.S.: 1C, 2C, 3B.
F.C.B.: 1C, 2C, 3B.
F.D.dL.: 1C, 2C, 3B.
A.R.M.M.: 1B, 2C, 3B.
O.G.P.B.: 1A, 1B, 2C, 3B.
J.L.P.: 1A, 1B, 2C, 3B.
W.M.Jr.: 1A, 1B, 2C, 3B.
M.C.F.Jr.: 1, 2A, 2C, 3B.

Disclosures

Ethical Compliance Statement: The study protocol was approved by the local Ethics Committee (Comitê de Ética e Pesquisa–UNICAMP) under the number CAAE 83241318.3.1001.5404 and written informed consent was obtained from all participants before inclusion. 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.

Funding Sources and Conflict of Interest: This study was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo–FAPESP, São Paulo, Brazil (grant numbers 2013/07559-3 and 2021/06739-4). Drs J.L.P, O.G.P.B, W.M.Jr and M.C.F.Jr are supported by FAPESP and CNPq (Conselho Nacional de Pesquisa—BRAZIL). Drs TJRR and MCFJ received funding from FARA (Friedreich's ataxia research alliance). The funding agencies did not interfere with the design of the study, collection of data or drafting of the manuscript. The authors report no conflicts of interest regarding this research.

Financial Disclosures for the Previous 12 Months: C.C.L.: none. G.S.O.W.: none. G.S.S.: none. P.C.A.A.P.M.: none. T.J.R.R.: none. J.M.S.: none. F.C.B.: none. F.D.L.: none. A.R.M.M.: none. J.L.P.: none. O.G.P.B.: none. W.M.Jr: none. M.C.F.: none. The authors declare that there are no additional disclosures to report. ■

References

1. Cortese A, Simone R, Sullivan R, et al. Biallelic expansion of an intronic repeat in RFC1 is a common cause of late-onset ataxia. *Nat Genet* 2019; 51(4):649–658.
2. Akçimen F, Ross JP, Bourassa CV, et al. Investigation of the RFC1 repeat expansion in a Canadian and a Brazilian ataxia cohort: identification of novel conformations. *Front Genet* 2019;10:1219.
3. Montaut S, Diedhiou N, Fahrer P, et al. Biallelic RFC1-expansion in a French multicentric sporadic ataxia cohort. *J Neurol* 2021;268:3337–3343.
4. Kontogeorgiou Z, Kartanou C, Tsirligkani C, et al. Biallelic RFC1 pentanucleotide repeat expansions in Greek patients with late-onset ataxia. *Clin Genet* 2021;100:90–94.
5. Lipponen J, Helisalmi S, Raivo J, et al. Molecular epidemiology of hereditary ataxia in Finland. *BMC Neurol* 2021;21:382.
6. Syriani D, Wong D, Andani S, et al. Prevalence of RFC1-mediated spinocerebellar ataxia in a north American ataxia cohort. *Neurol Genet* 2020; 6:e440.
7. Rafehi H, Szmulewicz DJ, Bennett MF, et al. Bioinformatics-based identification of expanded repeats: a non-reference intronic pentamer expansion in RFC1 causes CANVAS. *Am J Hum Genet* 2019;105:151–165.
8. Dominik N, Deforie V, Cortese A, Houlden H. CANVAS: a late onset ataxia due to biallelic intronic AAGGG expansions. *J Neurol* 2021;268: 1119–1126.
9. Infante J, Garcia A, Serrano-Cardenas KM, et al. Cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS) with chronic cough and preserved muscle stretch reflexes: evidence for selective sparing of afferent Ia fibres. *J Neurol* 2018;265:1454–1462.
10. Traschutz A, Cortese A, Reich S, et al. Natural history, phenotypic spectrum, and discriminative features of multisystemic rfc1-disease. *Neurology* 2021;96:e1369–e1382.
11. Krismer F, Wenning GK. Autonomic failure in CANVAS syndrome. *Brain* 2014;137:2625–2626.
12. Wu TY, Taylor JM, Kilfoyle DH, et al. Autonomic dysfunction is a major feature of cerebellar ataxia, neuropathy, vestibular areflexia 'CANVAS' syndrome. *Brain* 2014;137:2649–2656.
13. Schmitt GS, Lima FD, Matos PCAAP, et al. Dysautonomia in RFC1-related disorder: clinical and neurophysiological evaluation. *Clin Neurophysiol* 2022;142:68–74.
14. Schmitt GS, Martinez ARM, Graça FF, et al. DOPA-responsive parkinsonism in a patient with homozygous RFC1 expansions. *Mov Disord* 2020;10:1889–1890.
15. Rossi M, Perez-Lloret S, Doldan L, et al. Autosomal dominant cerebellar ataxias: a systematic review of clinical features. *Eur J Neurol* 2014;21(4): 607–615.
16. Sullivan R, Yau WY, O'Connor E, Houlden H. Spinocerebellar ataxia: an update. *J Neurol* 2019;266(2):533–544.
17. van de Warrenburg BPC, Notermans NC, Schelhaas HJ, et al. Peripheral nerve involvement in spinocerebellar ataxias. *Arch Neurol* 2004;61(2): 257–261.
18. Burk K, Fetter M, Abele M, et al. Autosomal dominant cerebellar ataxia type I: oculomotor abnormalities in families with SCA1, SCA2, and SCA3. *J Neurol* 1999;246:789–797.
19. Gordon CR, Joffe V, Vainstein G, Gadoth N. Vestibulo-ocular arreflexia in families with spinocerebellar ataxia type 3 (Machado-Joseph disease). *J Neurol Neurosurg Psychiatry* 2003;74:1403–1406.
20. Klockgether T. Sporadic ataxia with adult onset: classification and diagnostic criteria. *Lancet Neurol* 2010;9:94–104.
21. Deistung A, Stefanescu MR, Ernst TM, Schlamann M, Ladd ME, Reichenbach JR, Timmann D. Structural and functional magnetic resonance imaging of the cerebellum: considerations for assessing cerebellar ataxias. *Cerebellum* 2016;15(1):21–25.
22. Mascalchi M, Vella A. Neuroimaging applications in chronic ataxias. *Int Rev Neurobiol* 2018;143:109–162.
23. Watanabe H, Riku Y, Hara K, et al. Clinical and imaging features of multiple system atrophy: challenges for an early and clinically definitive diagnosis. *J Mov Disord* 2018;11(3):107–120.
24. Adanyeguh IM, Perlbarg V, Henry PG, et al. Autosomal dominant cerebellar ataxias: imaging biomarkers with high effect sizes. *Neuroimage Clin* 2018;19:858–867.
25. Klaes A, Reckziegel E, Franca MC Jr, et al. MR imaging in spinocerebellar ataxias: a systematic review. *AJNR Am J Neuroradiol* 2016; 37(8):1405–1412.
26. Cortese A, Tozza S, Yau WY, et al. Cerebellar ataxia, neuropathy, vestibular areflexia syndrome due to RFC1 repeat expansion. *Brain* 2020; 143(2):480–490.
27. Reetz K, Rodríguez-Labrada R, Dogan I, et al. Brain atrophy measures in preclinical and manifest spinocerebellar ataxia type 2. *Ann Clin Transl Neurol* 2018;5(2):128–137.
28. Matos PCAAP, Rezende TJR, Schmitt GS, et al. Brain structural signature of RFC1-related disorder. *Mov Disord* 2021;36(11):2634–2641.
29. Wenning GK, Stankovic I, Vignatelli L, et al. The Movement Disorder Society criteria for the diagnosis of multiple system atrophy. *Mov Disord* 2022;37(6):1131–1148.
30. Schmitz-Hübsch T, du Montcel ST, Baliko L, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. *Neurology* 2006;66(11):1717–1720. Erratum in: Neurology 2006;67(2):299.
31. Braga-Neto P, Godeiro-Junior C, Dutra LA, Pedroso JL, Barsottini OG. Translation and validation into Brazilian version of the scale of the assessment and rating of ataxia (SARA). *Arq Neuropsiquiatr* 2010;68(2): 228–230.
32. Martinez ARM, Martins MP, Martins CR Jr, Faber I, de Rezende TJR, Nucci A, França MC Jr. Sensory ataxia rating scale: development and validation of a functional scale for patients with sensory neuronopathies. *J Peripher Nerv Syst* 2019;24(3):242–246.
33. Carod-Artal FJ, Ribeiro Lda S, Kummer W, Martinez-Martin P. Psychometric properties of the SCOPA-AUT Brazilian Portuguese version. *Mov Disord* 2010;25(2):205–212.
34. Chavhan GB, Babyn PS, Jankharia BG, Cheng HL, Shroff MM. Steady-state MR imaging sequences: physics, classification, and clinical applications. *Radiographics* 2008;28(4):1147–1160.
35. Sheth S, Branstetter BF 4th, Escott EJ. Appearance of normal cranial nerves on steady-state free precession MR images. *Radiographics* 2009; 29(4):1045–1055. Erratum in: Radiographics. 2009;29(5):1544.
36. Romano N, Federici M, Castaldi A. Imaging of cranial nerves: a pictorial overview. *Insights Imaging* 2019;10(1):33.
37. Carré G, Dietemann JL, Gebus O, et al. Brain MRI of multiple system atrophy of cerebellar type: a prospective study with implications for diagnosis criteria. *J Neurol* 2020;267(5):1269–1277.
38. Sullivan R, Yau WY, Chelban V, et al. *RFC1*-related ataxia is a mimic of early multiple system atrophy. *J Neurol Neurosurg Psychiatry* 2021;92(4): 444–446.
39. Wan L, Chen Z, Wan N, et al. Biallelic Intronic AAGGG expansion of RFC1 is related to multiple system atrophy. *Ann Neurol* 2020;88(6): 1132–1143.
40. Ishai R, Seyyedi M, Chancellor AM, et al. The pathology of the vestibular system in CANVAS. *Otol Neurotol* 2021;42(3):e332–e340.
41. Szmulewicz DJ, McLean CA, Rodriguez ML, et al. Dorsal root ganglionopathy is responsible for the sensory impairment in CANVAS. *Neurology* 2014;82(16):1410–1415.
42. Szmulewicz DJ, Merchant SN, Halmagyi GM. Cerebellar ataxia with neuropathy and bilateral vestibular areflexia syndrome: a histopathologic case report. *Otol Neurotol* 2011;32(8):e63–e65.
43. Pelosi L, Mulroy E, Leadbetter R, et al. Peripheral nerves are pathologically small in cerebellar ataxia neuropathy vestibular areflexia syndrome: a controlled ultrasound study. *Eur J Neurol* 2018;25(4):659–665.
44. Gierga K, Bürk K, Bauer M, et al. Involvement of the cranial nerves and their nuclei in spinocerebellar ataxia type 2 (SCA2). *Acta Neuropathol* 2005;109(6):617–631.
45. Hoche F, Seidel K, Brunt ER, et al. Involvement of the auditory brainstem system in spinocerebellar ataxia type 2 (SCA2), type 3 (SCA3) and type 7 (SCA7). *Neuropathol Appl Neurobiol* 2008;34(5):479–491.
46. Rüb U, Brunt ER, de Vos RA, et al. Degeneration of the central vestibular system in spinocerebellar ataxia type 3 (SCA3) patients and its possible clinical significance. *Neuropathol Appl Neurobiol* 2004;30(4): 402–414.
47. Davies K, Szmulewicz DJ, Corben LA, Delatycki M, Lockhart PJ. *RFC1*-related disease: molecular and clinical insights. *Neurol Genet* 2022; 8(5):e200016.
48. Jellinger KA. Neuropathology of multiple system atrophy: new thoughts about pathogenesis. *Mov Disord* 2014;29(14):1720–1741.

49. Ahmed Z, Asi YT, Sailer A, Lees AJ, Houlden H, Revesz T, Holton JL. The neuropathology, pathophysiology and genetics of multiple system atrophy. *Neuropathol Appl Neurobiol* 2012;38(1):4–24.

50. Ozawa T, Paviour D, Quinn NP, et al. The spectrum of pathological involvement of the striatonigral and olivopontocerebellar systems in multiple system atrophy: clinicopathological correlations. *Brain* 2004;127(Pt 12):2657–2671.

51. Won E, Palma JA, Kaufmann H, et al. Quantitative magnetic resonance evaluation of the trigeminal nerve in familial dysautonomia. *Clin Auton Res* 2019;29(4):469–473.

52. Axelrod FB. Familial dysautonomia. *Muscle Nerve* 2004;29(3):352–363.

53. González-Duarte A, Cotrina-Vidal M, Kaufmann H, Norcliffe-Kaufmann L. Familial dysautonomia. *Clin Auton Res* 2023;33:269–280.

Supporting Information

Supporting information may be found in the online version of this article.

Table S1. Correlation between clinical parameters and trigeminal nerve thickness in RFC1/CANVAS patients.