CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

Whole-Genome and Long-Read Sequencing Identify a Novel Mechanism in *RFC1* Resulting in CANVAS Syndrome

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Neurol Genet 2022;8:e200036. doi:10.1212/NXG.0000000000200036

Abstract

Objectives

Cerebellar ataxia with neuropathy and bilateral vestibular areflexia syndrome (CANVAS) results from biallelic intronic pentanucleotide repeats in *RFC1*. We describe an adult male proband with progressive imbalance, cerebellar atrophy, somatosensory neuronopathy, and absence of peripheral vestibular function for whom clinical testing demonstrated a heterozygous *RFC1* expansion consistent with an unaffected carrier.

Methods

We performed whole-genome sequencing (WGS) on peripheral blood DNA samples from the proband and his unaffected mother. We performed DNA long-read sequencing and synthesized complementary DNA from RNA using peripheral blood from the proband.

Results

WGS confirmed the maternally inherited *RFC1* expansion and identified a rare, nonsense *RFC1* variant: c.C1147T; p.R383X in the proband but not the maternal DNA sample. *RFC1* variants were confirmed in *trans* with long-read sequencing. Functional studies demonstrated the absence of complementary DNA (cDNA) transcript from the c.C1147T; p.R383X variant supporting nonsense-mediated decay of this transcript.

Discussion

We report an adult with CANVAS due to compound heterozygous pathogenic *RFC1* variants: the pathogenic intronic pentanucleotide expansion confirmed in *trans* with a nonsense variant. This report represents a novel molecular mechanism for CANVAS. Sequencing for *RFC1* should be considered for adults meeting clinical criteria for the CANVAS phenotype if only a heterozygous pathogenic *RFC1* expansion is identified.

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Funding information and disclosures are provided at the end of the article. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

The Article Processing Charge was funded by the authors.

Undiagnosed Disease Network (UDN) coinvestigators are listed in Appendix 2 at the end of the article.

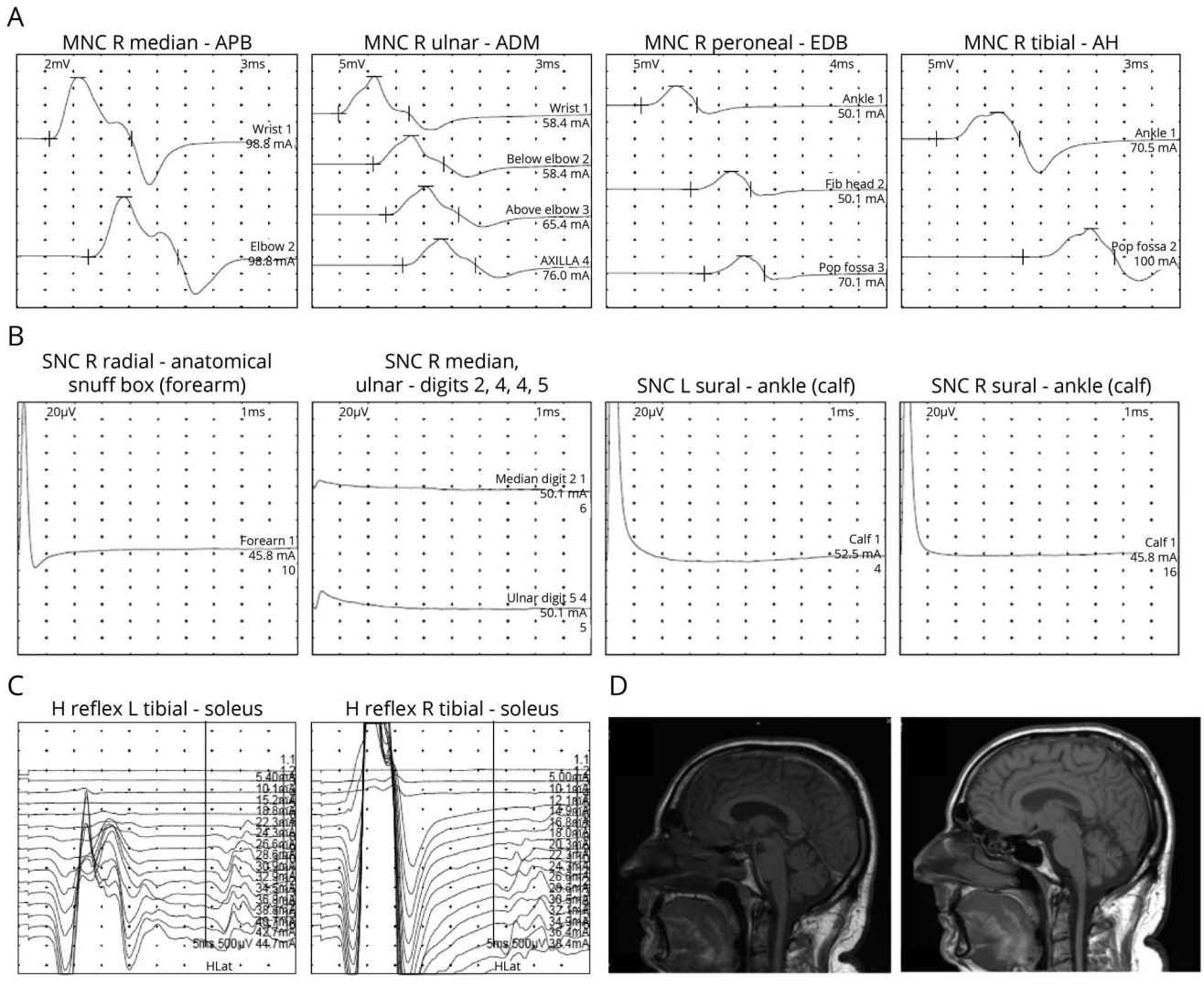
Cerebellar ataxia with neuropathy and bilateral vestibular areflexia syndrome (CANVAS) (MIM #614575) is a rare, adult-onset, neurodegenerative disorder attributed to biallelic intronic pentanucleotide expansions in *RFC1*, which encodes replication factor C, subunit 1,[1] the largest subunit of a DNA polymerase accessory protein important for DNA replication and repair. The mechanism by which biallelic intronic pentanucleotide repeats in *RFC1* result in neurologic disease is unknown but is unlikely to result from decreased expression of *RFC1*.[2] Here, we report an adult meeting clinical criteria for CANVAS[3] who was identified to be compound heterozygous for the *RFC1* expansion in *trans* with a nonsense variant, thereby identifying a new molecular mechanism for CANVAS.

Case Report

A 49-year-old man presented with a 5-year history of imbalance, most prominent in the absence of visual feedback and when walking on uneven ground. He reported a dry cough of 7-year duration. He endorsed symptoms consistent with oscillopsia but denied diplopia. He denied sensory loss, weakness, vertigo, change in hearing, or tinnitus. His mother reported vertigo and muscle cramps, and he has 3 healthy full siblings and 3 healthy children.

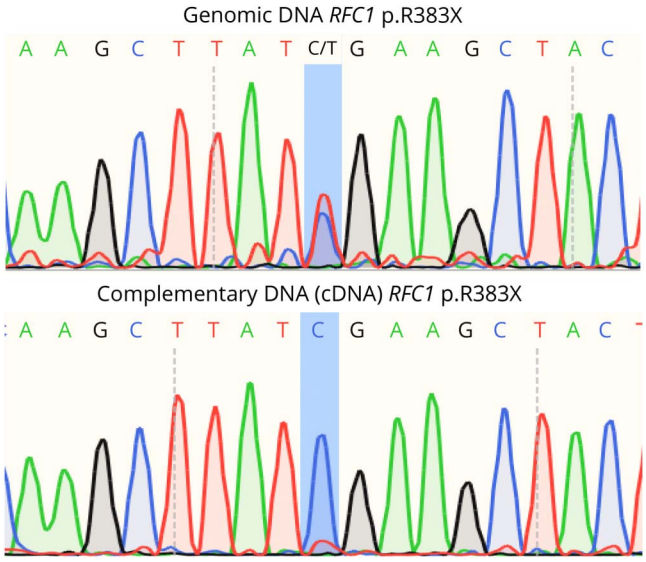
His neurologic examination demonstrated abnormal bilateral head impulse tests (large catch-up saccades bilaterally), downbeat nystagmus at the extremes of horizontal gaze,

Figure 1 Electrodiagnostic Studies and Brain MRI



(A) MNC, (B) antidromic SNC, and (C) tibial H-reflexes at age 49 years demonstrate diffuse loss of sensory responses with preserved motor responses and normal EMG, consistent with sensory neuronopathy. The preserved tibial H-reflexes, while atypical for most neuronopathies, have been identified as a unique attribute of neuronopathy associated with cerebellar ataxia with neuropathy and bilateral vestibular areflexia syndrome. (D) Sagittal T1-weighted MRI of the brain at age 44 years (left) and at age 49 years demonstrates interval development of mild cerebellar atrophy. ADM = abductor digiti minimi; AH = abductor hallucis; APB = abductor pollicis brevis; EDB = extensor digitorum brevis; MNC = motor nerve conduction; SNC = sensory nerve conduction.

Figure 2 Sanger Sequencing Files of the *RFC1* c.C1147T; p.R383X Variant Location (Blue Highlighted Box) in Genomic DNA and Complementary DNA



The presence of the heterozygous variant in genomic DNA is shown by 2 peaks (C/T), whereas only the wild-type peak (C) is seen in cDNA supporting nonsense-mediated decay of the transcript containing the *RFC1* c.C1147T; p.R383X nonsense variant. cDNA = complementary DNA.

panmodal sensory deficits in all limbs, and a positive Romberg sign. Reflexes were brisk throughout but symmetric. There was no appendicular ataxia. Stance in natural position was slightly wide, measuring 14 cm at the medial malleoli. He was unable to tandem but was otherwise steady on his feet.

Prior evaluations including brain MRI and extensive clinical laboratory testing were nondiagnostic. Electrodiagnostics showed diffusely absent sensory responses with normal motor studies and normal EMG, consistent with sensory neuronopathy (Figure 1, A–C). The cerebellum appeared smaller (mild atrophy) on subsequent brain MRI (Figure 1D). Vestibular testing demonstrated near absence of peripheral vestibular function bilaterally, with concurrent central features of abnormal optokinetic reflexes and side-to-side differences in quantitative measurements of smooth pursuits on videonystagmography. Because of clinical suspicion for CANVAS, clinical testing for the pathogenic *RFC1* intronic expansion was performed and demonstrated that the proband was heterozygous, consistent with an unaffected carrier.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the Washington University Human Research Protection Office. We obtained written informed consent from the proband and his mother as part of the Undiagnosed Disease Network (Supplement, links.lww.com/NXG/A552).

Sequencing

We performed whole-genome sequencing (WGS) on peripheral blood DNA from the proband and his mother. We used PacBio long-read sequencing (~20 kb reads, Pacific-Biosciences) to phase *RFC1* variants. We synthesized complementary DNA (cDNA) from RNA using proband peripheral blood.

Results

WGS confirmed the maternally inherited *RFC1* intronic pentanucleotide expansion and identified a *RFC1* nonsense variant c.C1147T; p.R383X in the proband that was not present in the maternal sample. Long-read sequencing confirmed the expansion (~600 AAGGG repeats), and phasing demonstrated that the *RFC1* variants are in *trans* (eFigure 1, links.lww.com/NXG/A552). The c.C1147T; p.R383X variant is extremely rare (gnomAD-v.3.1.2,[4] minor allele frequency 0.000007). Comparison of Sanger sequencing results for genomic and cDNA supported nonsense-mediated decay of the c.C1147T; p.R383X transcript (Figure 2). Alternative repeat disorders with ataxia and predicted damaging variants in *PNPLA6* and *ELF2* were excluded.[5,6]

Discussion

Adults with CANVAS typically display progressive ataxia from cerebellar dysfunction, vestibulopathy, and somatosensory deficits, deemed the CANVAS diagnostic triad.[1,7] Additional symptoms include chronic spasmodic cough, orthostatic hypotension, neuropathic pain, autonomic dysfunction, and sensory neuronopathy with preserved or even brisk reflexes.[1,7]

The molecular etiology of CANVAS was identified in affected unrelated adults using linkage analysis, exome/genome sequencing, and Sanger sequencing of long-range PCR products.[2] Affected adults were homozygous for a pentanucleotide intronic repeat, AAGGG(n) in *RFC1*, ranging in size from 400 to 2,000 repeats across families and stable within families.[2] Most affected individuals had ~1,000 repeats with no association between repeat size and age at onset.[2] This *RFC1* intronic expansion has been identified in diverse populations with an estimated carrier frequency of 0.7%–6.8%, with increased frequencies among certain ethnic groups.[1,8] The biallelic *RFC1* expansion is present in ~90% of individuals with the CANVAS triad and has been identified in individuals with related neurologic phenotypes including late-onset ataxia, peripheral neuropathy, and cerebellar dysfunction.[1,9] Our report demonstrates a novel mechanism for CANVAS with the identification of a nonsense variant in *trans* with the pathogenic *RFC1* expansion. *RFC1* sequencing should be considered if only a heterozygous pathogenic *RFC1* expansion is identified in an adult meeting clinical criteria for CANVAS.

Given the high carrier frequencies for the pathogenic *RFC1* expansion and the expanding number of neurologic disorders associated with *RFC1*,[8] the finding of this report may have implications for other neurodegenerative diseases.

Study Funding

This work was funded by National Human Genome Research Institute U01HG010215.

Disclosure

P. Dickson receives research support from Genzyme and M6P Therapeutics and is a consultant to Mandos, LLC. All other authors have no financial relationships to disclose. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

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Received by *Neurology: Genetics* May 9, 2022. Accepted in final form August 26, 2022. Submitted and externally peer reviewed. The handling editor was Stefan M. Pulst, MD, Dr med, FAAN.

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Christina G.S. Palmer	UCLA	Principal investigator	Project administration
Jeanette C. Papp	UCLA	Coinvestigator	Project administration
Neil H. Parker	UCLA	Coinvestigator	Project administration
Genecee Renteria	UCLA	Research assistant	Project administration
Rebecca H. Signer	UCLA	Site coordinator and genetic counselor	Project administration
Janet S. Sinsheimer	UCLA	Coinvestigator	Project administration
Jijun Wan	UCLA	Research assistant	Project administration
Lee-kai Wang	UCLA	Sequencing interpretation	Project administration
Katherine Wesseling Perry	UCLA	Clinician	Project administration
Jeremy D. Woods	UCLA	Medical fellow	Project administration
Justin Alvey	University of Utah	Coinvestigator	Project administration
Ashley Andrews	University of Utah	Clinical site coordinator	Project administration
Jim Bale	University of Utah	Coinvestigator	Project administration
John Bohnsack	University of Utah	Coinvestigator	Project administration
Lorenzo Botto	University of Utah	Principal investigator	Project administration

Appendix 2 *(continued)*

Name	Location	Role	Contribution
John Carey	University of Utah	Coinvestigator	Project administration
Laura Pace	University of Utah	Coinvestigator	Project administration
Nicola Longo	University of Utah	Coinvestigator	Project administration
Gabor Marth	University of Utah	Bioinformatician	Project administration
Paolo Moretti	University of Utah	Coinvestigator	Project administration
Aaron Quinlan	University of Utah	Bioinformatician	Project administration
Matt Velinder	University of Utah	Bioinformatician	Project administration
Dave Viskochil	University of Utah	Coinvestigator	Project administration
Pinar Bayrak-Toydemir	University of Utah/ARUP	Molecular geneticist	Project administration
Rong Mao	University of Utah/ARUP	Molecular geneticist	Project administration
Monte Westerfield	University of Oregon, Model Organism Screening Core	PI of Zebrafish Core	Project administration
Anna Bican	Vanderbilt	Study coordinator	Project administration
Elly Brokamp	Vanderbilt	Genetic counselor	Project administration
Laura Duncan	Vanderbilt	Genetic counselor	Project administration
Rizwan Hamid	Vanderbilt	Principal investigator	Project administration
Jennifer Kennedy	Vanderbilt	Genetic counselor	Project administration
Mary Kozuira	Vanderbilt	Nurse practitioner	Project administration
John H. Newman	Vanderbilt	Principal investigator	Project administration
John A. Phillips III	Vanderbilt	Principal investigator	Project administration
Lynette Rives	Vanderbilt	Lab Manager	Project administration
Amy K. Robertson	Vanderbilt	Nurse practitioner	Project administration
Emily Solem	Vanderbilt	Genetic counselor	Project administration
Joy D. Cogan	Vanderbilt	Principal Investigator, Director, Central Biorepository	Project administration

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Appendix 2 *(continued)*

Name	Location	Role	Contribution
F. Sessions Cole	Washington University Clinical	Coinvestigator	Project administration
Nichole Hayes	Washington University Clinical	Site coordinator	Project administration
Dana Kiley	Washington University Clinical	Research coordinator	Project administration
Kathy Sisco	Washington University Clinical	Clinical site coordinator	Project administration
Dustin Baldridge	Washington University Clinical & Model Organism Screening Core	Coinvestigator	Project administration
Stephen Pak	Washington University Model Organism Screening Core	Coinvestigator	Project administration
Timothy Schedl	Washington University Model Organism Screening Core	Principal investigator	Project administration
Jimann Shin	Washington University Model Organism Screening Core	Senior scientist	Project administration
Lilianna Solnica-Krezel	Washington University Model Organism Screening Core	Principal investigator	Project administration

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