12-1-2022

Whole-genome and long-read sequencing identify a novel mechanism in RFC1 resulting in CANVAS syndrome

Katherine Abell King
Washington University School of Medicine in St. Louis

Daniel J. Wegner
Washington University School of Medicine in St. Louis

Robert C. Bucelli
Washington University School of Medicine in St. Louis

Jessica Shapiro
Washington University School of Medicine in St. Louis

Alexander J. Paul
Washington University School of Medicine in St. Louis

See next page for additional authors

CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

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

Katherine Abell King, MD, Daniel J. Wegner, MS, Robert C. Bucelli, MD, PhD, Jessica Shapiro, BS, Alexander J. Paul, MS, Patricia I. Dickson, MD, and Jennifer A. Wambach, MD, MS, on behalf of the Undiagnosed Disease Network (UDN)

Correspondence
Dr. Wambach
wambachj@wustl.edu

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.

From the Edward Mallinckrodt Department of Pediatrics (K.A.K., D.J.W., J.S., P.I.D., J.A.W.); Department of Neurology (R.C.B.); and McDonnell Genome Institute (A.J.P.), Washington University School of Medicine, St. Louis, MO.

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.

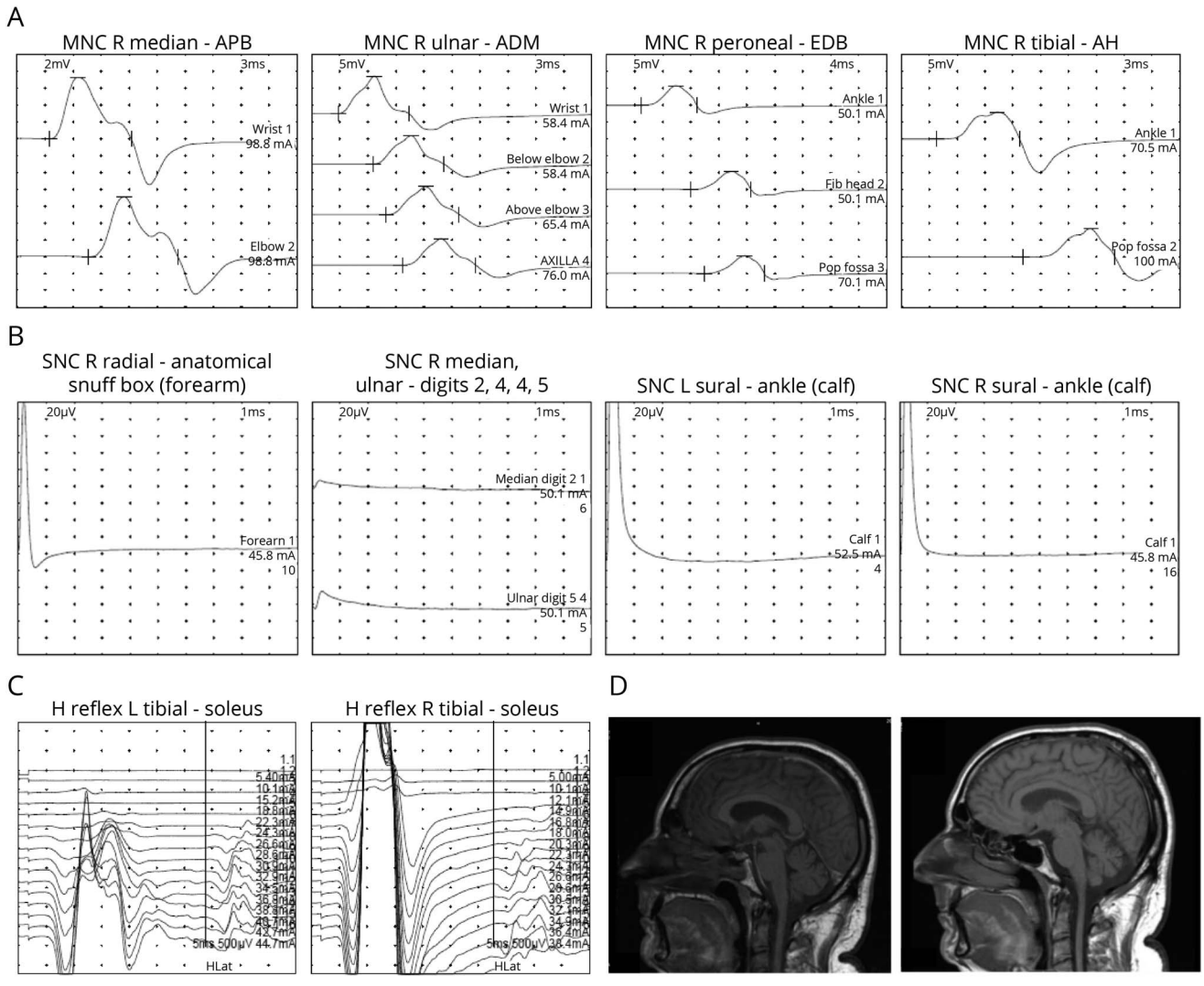
Cerebellar ataxia with neuropathy and bilateral vestibular areflexia syndrome (CANVAS) (MIM #614575) is a rare, adult-onset, neurodegenerative disorder attributed to biallelic intronic pentanucleotide expansions in *RFC1*, which encodes replication factor C, subunit 1,[1] the largest subunit of a DNA 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.

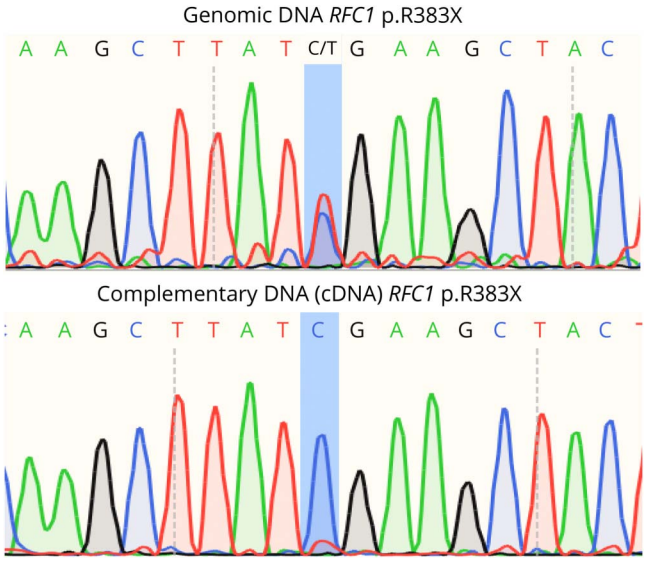
His neurologic examination demonstrated abnormal bilateral head impulse tests (large catch-up saccades bilaterally), downbeat nystagmus at the extremes of horizontal gaze,

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.

Publication History

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.

Appendix 1 Authors

Name	Location	Contribution
Katherine Abell King, MD	Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, MO	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Daniel J. Wegner, MS	Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, MO	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Robert C. Bucelli, MD, PhD	Department of Neurology, Washington University School of Medicine, St. Louis, MO	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Jessica Shapiro, BS	Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, MO	Major role in the acquisition of data and analysis or interpretation of data
Alexander J. Paul, MS	McDonnell Genome Institute, Washington University School of Medicine, St. Louis, MO	Major role in the acquisition of data and analysis or interpretation of data
Patricia I. Dickson, MD	Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, MO	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Jennifer A. Wambach, MD, MS	Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, MO	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

Appendix 2 Coinvestigators

Name	Location	Role	Contribution
Mahshid S. Azamian	Baylor College of Medicine Clinical Site	Site coordinator, clinical assistant	Project administration
Carlos A.Bacino	Baylor College of Medicine Clinical Site	Co–principal investigator	Project administration
Ashok Balasubramanyam	Baylor College of Medicine Clinical Site	Lead clinician, adult	Project administration
Lindsay C. Burrage	Baylor College of Medicine Clinical Site	Sequence analysis team, pediatric genetics	Project administration
Hsiao-Tuan Chao	Baylor College of Medicine Clinical Site	Pediatric neurology	Project administration
Gary D. Clark	Baylor College of Medicine Clinical Site	Pediatric neurology	Project administration
William J. Craigen	Baylor College of Medicine Clinical Site	Leadership team, pediatric genetics, biochemical genetics	Project administration
Hongzheng Dai	Baylor College of Medicine Clinical Site	Sequence analysis team	Project administration
Lisa T. Emrick	Baylor College of Medicine Clinical Site	Leadership team, pediatric neurology	Project administration
Fariha Jamal	Baylor College of Medicine Clinical Site	Adult neurology	Project administration
Lefkothea Karaviti	Baylor College of Medicine Clinical Site	Pediatric endocrinology	Project administration
Shamika Ketkar	Baylor College of Medicine Clinical Site		Project administration
Seema R. Lalani	Baylor College of Medicine Clinical Site	Pediatric genetics, cytogenetics	Project administration
Brendan H. Lee	Baylor College of Medicine Clinical Site	Co–principal investigator	Project administration

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Richard A. Lewis	Baylor College of Medicine Clinical Site	Ophthalmology	Project administration
Ronit Marom	Baylor College of Medicine Clinical Site	Sequence analysis team, pediatric genetics	Project administration
Paolo M. Moretti	Baylor College of Medicine Clinical Site	Leadership team, adult neurology	Project administration
Sarah K. Nicholas	Baylor College of Medicine Clinical Site	Allergy and immunology	Project administration
James P. Orengo	Baylor College of Medicine Clinical Site	Adult neurology	Project administration
Jennifer E. Posey	Baylor College of Medicine Clinical Site	Adult genetics	Project administration
Lorraine Potocki	Baylor College of Medicine Clinical Site	Pediatric genetics	Project administration
Jill A. Rosenfeld	Baylor College of Medicine Clinical Site	Site Manager	Project administration
Daryl A. Scott	Baylor College of Medicine Clinical Site	Pediatric genetics	Project administration
Alyssa A. Tran	Baylor College of Medicine Clinical Site	Site coordinator	Project administration
Tiphanie P. Vogel	Baylor College of Medicine Clinical Site	Rheumatology	Project administration
Monika Weisz Hubshman	Baylor College of Medicine Clinical Site	Medical resident	Project administration
Kim Worley	Baylor College of Medicine Clinical Site	Sequence analysis	Project administration
Michael F. Wangler	Baylor College of Medicine MOSC	Principal Investigator of Drosophila Core	Project administration
Shinya Yamamoto	Baylor College of Medicine Model Organism Screening Core	Principal Investigator of Drosophila Core	Project administration
Hugo J. Bellen	Baylor College of Medicine Model Organism Screening Core	Principal Investigator, SC Chair	Project administration
Christine M. Eng	Baylor College of Medicine Sequencing	Principal Investigator, Molecular Geneticist	Project administration
Pengfei Liu	Baylor College of Medicine Sequencing	Laboratory Director of Clinical Research	Project administration

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Patricia A. Ward	Baylor College of Medicine Sequencing	Site coordinator and genetic counselor	Project administration
Edward Behrens	Children's Hospital of Philadelphia	Clinician, rheumatology	Project administration
Kosuke Izumi	Children's Hospital of Philadelphia	Clinician, genetics	Project administration
Marni Falk	Children's Hospital of Philadelphia	Clinician, mitochondrial disorders	Project administration
Kelly Hassey	Children's Hospital of Philadelphia	Site coordinator	Project administration
Kathleen Sullivan	Children's Hospital of Philadelphia	Principal investigator	Project administration
Anna Raper	Children's Hospital of Philadelphia /University of Pennsylvania	Genetic counselor	Project administration
Gonench Kilich	Children's Hospital of Philadelphia	Research assistant	Project administration
Zhe Zhang	Children's Hospital of Philadelphia	Bioinformatician	Project administration
Adeline Vanderver	Children's Hospital of Philadelphia	Clinician, neurology	Project administration
Vaidehi Jobanputra	Columbia	Principal investigator	Project administration
Heidi Cope	Duke	Genetic counselor	Project administration
Allyn McConkie-Rosell	Duke	Genetic counselor	Project administration
Kelly Schoch	Duke	Genetic counselor	Project administration
Vandana Shashi	Duke	PI	Project administration
Edward C. Smith	Duke	Clinician	Project administration
Rebecca C. Spillmann	Duke	Genetic counselor	Project administration
Jennifer A. Sullivan	Duke	Genetic counselor	Project administration
Queenie K.-G. Tan	Duke	Clinician	Project administration
Nicole M. Walley	Duke	Clinical research coordinator	Project administration
Pankaj B. Agrawal	Harvard	Coinvestigator	Project administration

Continued

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Alan H. Beggs	Harvard	Site principal investigator	Project administration
Gerard T. Berry	Harvard	Coinvestigator	Project administration
Lauren C. Briere	Harvard	Site coordinator and genetic counselor	Project administration
Laurel A. Cobban	Harvard	Program Manager	Project administration
Matthew Coggins	Harvard	Clinician	Project administration
Cynthia M. Cooper	Harvard	Coinvestigator	Project administration
Elizabeth L. Fieg	Harvard	Site coordinator and genetic counselor	Project administration
Frances High	Harvard	Coinvestigator	Project administration
Ingrid A. Holm	Harvard	Coinvestigator	Project administration
Susan Korrick	Harvard	Coinvestigator	Project administration
Joel B. Krier	Harvard	Coinvestigator	Project administration
Sharyn A. Lincoln	Harvard	Site coordinator and genetic counselor	Project administration
Joseph Loscalzo	Harvard	Principal investigator	Project administration
Richard L. Maas	Harvard	Coinvestigator	Project administration
Calum A. MacRae	Harvard	Coinvestigator	Project administration
J. Carl Pallais	Harvard	Coinvestigator	Project administration
Deepak A. Rao	Harvard	Clinician	Project administration
Lance H. Rodan	Harvard	UDN fellow	Project administration
Edwin K. Silverman	Harvard	Coinvestigator	Project administration
Joan M. Stoler	Harvard	Coinvestigator	Project administration
David A. Sweetser	Harvard	Site principal investigator	Project administration
Melissa Walker	Harvard	Coinvestigator	Project administration
Chris A. Walsh	Harvard	Coinvestigator	Project administration
Cecilia Esteves	Harvard	Associate Director	Project administration

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Isaac S. Kohane	Harvard	Principal investigator	Project administration
Kimberly LeBlanc	Harvard	Director	Project administration
Alexa T. McCray	Harvard	Principal investigator	Project administration
Shilpa N. Kobren	Harvard	Research fellow	Project administration
Amelia L.M. Tan	Harvard	Research fellow	Project administration
Rachel Mahoney	Harvard	Project Manager	Project administration
Surendra Dasari	Mayo Clinic	Coinvestigator	Project administration
Brendan C. Lanpher	Mayo Clinic	Coinvestigator	Project administration
Ian R. Lanza	Mayo Clinic	Principal investigator	Project administration
Eva Morava	Mayo Clinic	Coinvestigator	Project administration
Devin Oglesbee	Mayo Clinic	Principal investigator	Project administration
Guney Bademci	Miami	Coinvestigator	Project administration
Deborah Barbouth	Miami	Coinvestigator	Project administration
Stephanie Bivona	Miami	Study coordinator	Project administration
Olveen Carrasquillo	Miami	Coinvestigator	Project administration
Ta Chen Peter Chang	Miami	Coinvestigator	Project administration
Irman Forghani	Miami	Coinvestigator	Project administration
Alana Grajewski	Miami	Coinvestigator	Project administration
Rosario Isasi	Miami	Coinvestigator	Project administration
Byron Lam	Miami	Coinvestigator	Project administration
Roy Levitt	Miami	Coinvestigator	Project administration
Xue Zhong Liu	Miami	Coinvestigator	Project administration
Jacob McCauley	Miami	Coinvestigator	Project administration
Ralph Sacco	Miami	Coinvestigator	Project administration
Mario Saporta	Miami	Coinvestigator	Project administration

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Judy Schaechter	Miami	Coinvestigator	Project administration
Mustafa Tekin	Miami	Principal investigator	Project administration
Fred Telischi	Miami	Coinvestigator	Project administration
Willa Thorson	Miami	Coinvestigator	Project administration
Stephan Zuchner	Miami	Principal investigator	Project administration
Heather A. Colley	National Institutes of Health	Program Director	Project administration
Jyoti G. Dayal	National Institutes of Health	Program Director	Project administration
David J. Eckstein	National Institutes of Health	Senior Health Scientist Administrator	Project administration
Laurie C. Findley	National Institutes of Health	Scientific Program Analyst	Project administration
Donna M. Krasnewich	National Institutes of Health	Program Officer	Project administration
Laura A. Mamounas	National Institutes of Health	Program Director	Project administration
Teri A. Manolio	National Institutes of Health	Program Director	Project administration
John J. Mulvihill	National Institutes of Health	Senior consultant	Project administration
Grace L. LaMoure	National Institutes of Health	Scientific Program Analyst	Project administration
Madison P. Goldrich	National Institutes of Health	Scientific Program Analyst	Project administration
Tiina K. Urv	National Institutes of Health	Program Director	Project administration
Argenia L. Doss	National Institutes of Health	Program Lead/ Project Manager	Project administration
Maria T. Acosta	National Institutes of Health, Undiagnosed Diseases Program	Staff clinician	Project administration
Carsten Bonnenmann	National Institutes of Health, Undiagnosed Diseases Program	Staff clinician	Project administration
Precilla D'Souza	National Institutes of Health, Undiagnosed Diseases Program	Nurse practitioner	Project administration
David D. Draper	National Institutes of Health, Undiagnosed Diseases Program	Nurse specialist, research	Project administration
Carlos Ferreira	National Institutes of Health, Undiagnosed Diseases Program	Staff clinician	Project administration
Rena A. Godfrey	National Institutes of Health, Undiagnosed Diseases Program	Physician assistant	Project administration
Catherine A. Groden	National Institutes of Health, Undiagnosed Diseases Program	Nurse practitioner	Project administration
Ellen F. Macnamara	National Institutes of Health, Undiagnosed Diseases Program	Genetic counselor	Project administration
Valerie V. Maduro	National Institutes of Health, Undiagnosed Diseases Program	UDP Translational Laboratory Manager	Project administration
Thomas C. Markello	National Institutes of Health, Undiagnosed Diseases Program	Staff clinician	Project administration
Avi Nath	National Institutes of Health, Undiagnosed Diseases Program	Staff clinician	Project administration
Donna Novacic	National Institutes of Health, Undiagnosed Diseases Program	Staff clinician	Project administration
Barbara N. Pusey	National Institutes of Health, Undiagnosed Diseases Program	Informatician	Project administration

Continued

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Camilo Toro	National Institutes of Health, Undiagnosed Diseases Program	PI, Director Adult UDP Program	Project administration
Colleen E. Wahl	National Institutes of Health, Undiagnosed Diseases Program	Nurse practitioner	Project administration
Eva Baker	National Institutes of Health, Undiagnosed Diseases Program, DRM	Radiologist	Project administration
Elizabeth A. Burke	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Postdoc investigator	Project administration
David R. Adams	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Principal Investigator, Deputy Director for Clinical Genomics, OCD/ NHGRI	Project administration
William A. Gahl	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Principal Investigator , Clinical Director NHGRI	Project administration
May Christine V. Malicdan	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Staff Scientist, Director of UDP Translational Research Laboratory	Project administration
Cynthia J. Tifft	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Principal Investigator, Deputy Clinical Director NHGRI	Project administration

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Lynne A. Wolfe	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Site coordinator and senior nurse practitioner	Project administration
John Yang	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Research nurse coordinator	Project administration
Bradley Power	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Genetic counselor	Project administration
Bernadette Gochuico	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Staff clinician	Project administration
Laryssa Huryn	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Staff clinician	Project administration
Lea Latham	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Nurse practitioner	Project administration
Joie Davis	National Institutes of Health, Undiagnosed Diseases Program, NHGRI	Nurse practitioner	Project administration

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Deborah Mosbrook-Davis	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Staff	Project administration
Francis Rossignol	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Staff clinician	Project administration
Ben Solomon	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	OCD	Project administration
John MacDowall	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Bioinformatician	Project administration
Audrey Thurm	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Psychologist	Project administration
Wadih Zein	National Institutes of Health, Undiagnosed Diseases Program, NHGRI	Staff clinician	Project administration
Muhammad Yousef	National Institutes of Health, Undiagnosed Diseases Program, National Human Genome Research Institute	Staff clinician	Project administration
Margaret Adam	Pacific Northwest	Coinvestigator	Project administration

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Laura Amendola	Pacific Northwest	Genetic counselor	Project administration
Michael Bamshad	Pacific Northwest	Coinvestigator	Project administration
Anita Beck	Pacific Northwest	Coinvestigator	Project administration
Jimmy Bennett	Pacific Northwest	Coinvestigator	Project administration
Beverly Berg-Rood	Pacific Northwest	Division administrator	Project administration
Elizabeth Blue	Pacific Northwest	Coinvestigator	Project administration
Brenna Boyd	Pacific Northwest	Research coordinator	Project administration
Peter Byers	Pacific Northwest	Coinvestigator	Project administration
Sirisak Chanprasert	Pacific Northwest	Coinvestigator	Project administration
Michael Cunningham	Pacific Northwest	Coinvestigator	Project administration
Katrina Dipple	Pacific Northwest	Principal investigator	Project administration
Daniel Doherty	Pacific Northwest	Coinvestigator	Project administration
Dawn Earl	Pacific Northwest	Coinvestigator	Project administration
Ian Glass	Pacific Northwest	Clinical leader (pediatric)	Project administration
Katie Golden-Grant	Pacific Northwest	Genetic counselor	Project administration
Sihoun Hahn	Pacific Northwest	Coinvestigator	Project administration
Anne Hing	Pacific Northwest	Coinvestigator	Project administration
Fuki M. Hisama	Pacific Northwest	Clinical leader (adult)	Project administration
Martha Horike-Pyne	Pacific Northwest	Research coordinator	Project administration
Gail P. Jarvik	Pacific Northwest	Principal investigator	Project administration
Jeffrey Jarvik	Pacific Northwest	Coinvestigator	Project administration
Suman Jayadev	Pacific Northwest	Coinvestigator	Project administration
Christina Lam	Pacific Northwest	Coinvestigator	Project administration
Kenneth Maravilla	Pacific Northwest	Coinvestigator	Project administration
Heather Mefford	Pacific Northwest	Coinvestigator	Project administration

Continued

Appendix 2 *(continued)*

Name	Location	Role	Contribution
J. Lawrence Merritt	Pacific Northwest	Coinvestigator	Project administration
Ghayda Mirzaa	Pacific Northwest	Coinvestigator	Project administration
Deborah Nickerson	Pacific Northwest	Coinvestigator	Project administration
Wendy Raskind	Pacific Northwest	Coinvestigator	Project administration
Natalie Rosenwasser	Pacific Northwest	Pediatric rheumatologist	Project administration
C. Ron Scott	Pacific Northwest	Coinvestigator	Project administration
Angela Sun	Pacific Northwest	Coinvestigator	Project administration
Virginia Sybert	Pacific Northwest	Coinvestigator	Project administration
Stephanie Wallace	Pacific Northwest	Coinvestigator	Project administration
Mark Wener	Pacific Northwest	Coinvestigator	Project administration
Tara Wenger	Pacific Northwest	Pediatric geneticist	Project administration
Euan A. Ashley	Stanford	Principal investigator	Project administration
Gill Bejerano	Stanford	Coinvestigator	Project administration
Jonathan A. Bernstein	Stanford	Principal investigator	Project administration
Devon Bonner	Stanford	Genetic counselor	Project administration
Terra R. Coakley	Stanford	Project Manager	Project administration
Liliana Fernandez	Stanford	Study coordinator/ licensed Spanish interpreter/ curator	Project administration
Paul G. Fisher	Stanford	Principal investigator	Project administration
Jason Hom	Stanford	Clinician, adults	Project administration
Yong Huang	Stanford	Laboratory technician	Project administration
Jennefer N. Kohler	Stanford	Genetic counselor	Project administration
Elijah Kravets	Stanford	Study coordinator	Project administration
Beth A. Martin	Stanford	Clinician, adults	Project administration
Shruti Marwaha	Stanford	Bioinformatician	Project administration

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Archana N. Raja	Stanford	Bioinformatician	Project administration
Chloe M. Reuter	Stanford	Genetic counselor	Project administration
Maura Ruzhnikov	Stanford	Coinvestigator	Project administration
Jacinda B. Sampson	Stanford	Clinician, neurology	Project administration
Kevin S. Smith	Stanford	Laboratory technician	Project administration
Shirley Sutton	Stanford	Lab Manager	Project administration
Holly K. Tabor	Stanford	Coinvestigator	Project administration
Brianna M. Tucker	Stanford	Study coordinator	Project administration
Matthew T. Wheeler	Stanford	Principal investigator	Project administration
Diane B. Zastrow	Stanford	Curator	Project administration
Chunli Zhao	Stanford	Laboratory technician	Project administration
William E. Byrd	UAB CC	Bioinformatician	Project administration
Andrew B. Crouse	UAB CC	Study coordinator	Project administration
Matthew Might	UAB CC	Principal investigator	Project administration
Mariko Nakano-Okuno	UAB CC	Subinvestigator	Project administration
Jordan Whitlock	UAB CC	Subinvestigator	Project administration
Gabrielle Brown	UCLA	Research patient navigator/ coordinator	Project administration
Manish J. Butte	UCLA	Coinvestigator	Project administration
Esteban C. Dell'Angelica	UCLA	Coinvestigator	Project administration
Naghmeh Dorrani	UCLA	Site coordinator and genetic counselor	Project administration
Emilie D. Douine	UCLA	Research assistant	Project administration
Brent L. Fogel	UCLA	Coinvestigator	Project administration
Irma Gutierrez	UCLA	Study coordinator	Project administration
Alden Huang	UCLA	Sequencing interpretation	Project administration

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Deborah Krakow	UCLA	Coinvestigator	Project administration
Hane Lee	UCLA	Sequencing interpretation	Project administration
Sandra K. Loo	UCLA	Coinvestigator	Project administration
Bryan C. Mak	UCLA	Site coordinator and genetic counselor	Project administration
Martin G. Martin	UCLA	Coinvestigator	Project administration
Julian A. Martínez-Agosto	UCLA	Principal investigator	Project administration
Elisabeth McGee	UCLA	Research nurse	Project administration
Stanley F. Nelson	UCLA	Principal investigator	Project administration
Shirley Nieves-Rodriguez	UCLA	Graduate student researcher	Project administration
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

Continued

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

References

1. Sullivan R, Kaiyrzhanov R, Houlden H. Cerebellar ataxia, neuropathy, vestibular areflexia syndrome: genetic and clinical insights. *Curr Opin Neurol.* 2021;34(4):556-564.
2. 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.
3. Szmulewicz DJ, Roberts L, McLean CA, MacDougall HG, Halmagyi GM, Storey E. Proposed diagnostic criteria for cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS). *Neurol Clin Pract.* 2016;6(1):61-68.
4. Karczewski, K.J., Francioli, L.C., Tiao, G., et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581:434–443. https://doi.org/10.1038/s41586-020-2308-7. Accessed July 2022. gnomad.broadinstitute.org/.
5. Ahmad H, Requena T, Frejo L, et al. Clinical and functional characterization of a missense ELF2 variant in a CANVAS family. *Front Genet.* 2018;9:85.
6. Nanetti L, Di Bella D, Magri S, et al. Multifaceted and age-dependent phenotypes associated with biallelic PNPLA6 gene variants: eight novel cases and review of the literature. *Front Neurol.* 2021;12:793547.
7. Szmulewicz DJ, McLean CA, MacDougall HG, Roberts L, Storey E, Halmagyi GM. CANVAS an update: clinical presentation, investigation and management. *J Vestib Res.* 2014;24(5-6):465-474.
8. Traschutz A, Cortese A, Reich S, et al; RFC1 Study Group. Natural history, phenotypic spectrum, and discriminative features of multisystemic RFC1 disease. *Neurology.* 2021;96(9):e1369-e1382.
9. 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(1):151-165.