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)

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.
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, 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. Here, we report an adult meeting clinical criteria for CANVAS 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.
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, 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 |

### 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. Craig | 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 |
| Name                  | Location                          | Role and Contribution                                |
|-----------------------|-----------------------------------|------------------------------------------------------|
| Richard A. Lewis      | Baylor College of Medicine Site   | Ophthalmology Project administration                  |
| Ronit Marom           | Baylor College of Medicine Site   | Sequence analysis team, pediatric genetics Project administration |
| Paolo M. Moretti      | Baylor College of Medicine Site   | Leadership team, adult neurology Project administration |
| Sarah K. Nicholas     | Baylor College of Medicine Site   | Allergy and immunology Project administration          |
| James P. Orenge       | Baylor College of Medicine Site   | Adult neurology Project administration                  |
| Jennifer E. Posey     | Baylor College of Medicine Site   | Adult genetics Project administration                  |
| Lorraine Potocki      | Baylor College of Medicine Site   | Pediatric genetics Project administration              |
| Jill A. Rosenfeld     | Baylor College of Medicine Site   | Site Manager Project administration                 |
| Daryl A. Scott        | Baylor College of Medicine Site   | Pediatric genetics Project administration              |
| Alyssa A. Tran        | Baylor College of Medicine Site   | Site coordinator Project administration                |
| Tiphanie P. Vogel     | Baylor College of Medicine Site   | Rheumatology Project administration                    |
| Monika Weisz Hubshman | Baylor College of Medicine Site   | Medical resident Project administration                |
| Kim Worley            | Baylor College of Medicine 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 Site   | Principal Investigator of Drosophila Core Project administration |
| Hugo J. Bellen         | Baylor College of Medicine Site   | Principal Investigator, SC Chair Project administration |
| Christine M. Eng      | Baylor College of Medicine Site   | Principal Investigator, Molecular Geneticist Project administration |
| Pengfei Liu           | Baylor College of Medicine Site   | Laboratory Director of Clinical Research Project administration |
| Patricia A. Ward      | Baylor College of Medicine Site   | 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 | Genetic counselor Project administration            |
| Gonench Kilich        | Children's Hospital of Philadelphia /University of Pennsylvania | 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                |
### 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             |
| Name           | Location                                      | Role                          | Contribution                               |
|---------------|-----------------------------------------------|-------------------------------|-------------------------------------------|
| Judy Schaecter| 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 |
| Name                     | Location                              | Role                          | Contribution                                  |
|--------------------------|---------------------------------------|-------------------------------|-----------------------------------------------|
| **Camilo Toro**          | National Institutes of Health,        | PI, Director Adult UDP Program| Project administration                        |
|                          | Undiagnosed Diseases Program          |                               |                                               |
| **Colleen E. Wahl**      | National Institutes of Health,        | Nurse practitioner            | Project administration                        |
|                          | Undiagnosed Diseases Program          |                               |                                               |
| **Eva Baker**            | National Institutes of Health,        | Radiologist                   | Project administration                        |
|                          | Undiagnosed Diseases Program, DRM     |                               |                                               |
| **Elizabeth A. Burke**   | National Institutes of Health,        | Postdoc investigator           | Project administration                        |
|                          | Undiagnosed Diseases Program, Program,|                               |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| **David R. Adams**      | National Institutes of Health,        | Principal Investigator, Deputy| Project administration                        |
|                          | Undiagnosed Diseases Program, Program,| Director for Clinical         |                                               |
|                          | National Human Genome Research Institute| Genomics, OCD/ NHGRI         |                                               |
| **William A. Gahl**     | National Institutes of Health,        | Principal Investigator, Clinical| Project administration                        |
|                          | Undiagnosed Diseases Program, Program,| Director NHGRI               |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| **May Christine V. Malicdan** | National Institutes of Health,      | Staff Scientist, Director of UDP Translational Research Laboratory | Project administration |
|                          | Undiagnosed Diseases Program, Program,|                               |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| **Cynthia J. Tifft**    | National Institutes of Health,        | Principal Investigator, Deputy| Project administration                        |
|                          | Undiagnosed Diseases Program, Program,| Clinical Director NHGRI      |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| **Lynne A. Wolfe**      | National Institutes of Health,        | Site coordinator and senior nurse practitioner | Project administration |
|                          | Undiagnosed Diseases Program, Program,|                               |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| **John Yang**           | National Institutes of Health,        | Research nurse coordinator    | Project administration                        |
|                          | Undiagnosed Diseases Program, Program,|                               |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| **Bradley Power**       | National Institutes of Health,        | Genetic counselor             | Project administration                        |
|                          | Undiagnosed Diseases Program, Program,|                               |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| **Bernadette Gochuico** | National Institutes of Health,        | Staff clinician               | Project administration                        |
|                          | Undiagnosed Diseases Program, Program,|                               |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| **Laryssa Huryn**       | National Institutes of Health,        | Staff clinician               | Project administration                        |
|                          | Undiagnosed Diseases Program, Program,|                               |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| **Lea Latham**          | National Institutes of Health,        | Nurse practitioner            | Project administration                        |
|                          | Undiagnosed Diseases Program, Program,|                               |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| **Joie Davis**          | National Institutes of Health,        | Nurse practitioner            | Project administration                        |
|                          | Undiagnosed Diseases Program, Program,|                               |                                               |
|                          | National Human Genome Research Institute|                               |                                               |
| Name                        | Location                               | Role           | Contribution                       |
|-----------------------------|----------------------------------------|----------------|------------------------------------|
| Deborah Mosbrook-Davis      | National Institutes of Health,         | Staff Project  | administration                     |
|                             | Undiagnosed Diseases Program,          |                |                                    |
|                             | National Human Genome Research Institute|                |                                    |
| Francis Rossignol           | National Institutes of Health,         | Staff clinician| Project administration             |
|                             | Undiagnosed Diseases Program,          |                |                                    |
|                             | National Human Genome Research Institute|                |                                    |
| Ben Solomon                 | National Institutes of Health,         | OCD Project    | administration                     |
|                             | Undiagnosed Diseases Program,          |                |                                    |
|                             | National Human Genome Research Institute|                |                                    |
| John MacDowall              | National Institutes of Health,         | Bioinformatician| Project administration             |
|                             | Undiagnosed Diseases Program,          |                |                                    |
|                             | National Human Genome Research Institute|                |                                    |
| Audrey Thurm                | National Institutes of Health,         | Psychologist    | Project administration             |
|                             | Undiagnosed Diseases Program,          |                |                                    |
|                             | National Human Genome Research Institute|                |                                    |
| Wadih Zein                  | National Institutes of Health,         | Staff clinician| Project administration             |
|                             | Undiagnosed Diseases Program,          |                |                                    |
|                             | National Human Genome Research Institute|                |                                    |
| Muhammad Yousef             | National Institutes of Health,         | Staff clinician| Project administration             |
|                             | Undiagnosed Diseases Program,          |                |                                    |
|                             | National Human Genome Research Institute|                |                                    |
| 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

| 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 Wengler          | 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              |
| 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              |
| 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
### References

1. Sullivan R, Kaiyryzhanov 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.

### Appendix 2 (continued)

| Name               | Location                  | Role                          | Contribution                      |
|--------------------|----------------------------|-------------------------------|-----------------------------------|
| F. Sessions Cole   | Washington University     | Coinvestigator                | Project administration            |
|                    | Clinical                   |                               |                                   |
| Nichole Hayes      | Washington University     | Site coordinator              | Project administration            |
|                    | Clinical                   |                               |                                   |
| Dana Kiley         | Washington University     | Research coordinator          | Project administration            |
|                    | Clinical                   |                               |                                   |
| Kathy Sisco        | Washington University     | Clinical site coordinator     | Project administration            |
|                    | Clinical                   |                               |                                   |
| Dustin Baldridge   | Washington University     | Coinvestigator                | Project administration            |
|                    | Clinical & Model Organization Screening Core | | |
| Stephen Pak        | Washington University     | Coinvestigator                | Project administration            |
|                    | Model Organization        |                               |                                   |
|                    | Screening Core            |                               |                                   |
| Timothy Schedl     | Washington University     | Principal investigator        | Project administration            |
|                    | Model Organization        |                               |                                   |
|                    | Screening Core            |                               |                                   |

**Appendix 2 (continued)**

| Name               | Location                  | Role                          | Contribution                      |
|--------------------|----------------------------|-------------------------------|-----------------------------------|
| Jimann Shin        | Washington University     | Senior scientist              | Project administration            |
|                    | Model Organization        |                               |                                   |
|                    | Screening Core            |                               |                                   |
| Lilianna Sолнica- Krezel | Washington University | Principal investigator | Project administration            |
|                    | Model Organization        |                               |                                   |
|                    | Screening Core            |                               |                                   |
Whole-Genome and Long-Read Sequencing Identify a Novel Mechanism in RFC1 Resulting in CANVAS Syndrome

Katherine Abell King, Daniel J. Wegner, Robert C. Bucelli, et al.

*Neurol Genet* 2022;8;
DOI 10.1212/NXG.0000000000200036

This information is current as of November 1, 2022

Updated Information & Services
including high resolution figures, can be found at:
http://ng.neurology.org/content/8/6/e200036.full.html

References
This article cites 9 articles, 1 of which you can access for free at:
http://ng.neurology.org/content/8/6/e200036.full.html##ref-list-1

Permissions & Licensing
Information about reproducing this article in parts (figures,tables) or in its entirety can be found online at:
http://ng.neurology.org/misc/about.xhtml#permissions

Reprints
Information about ordering reprints can be found online:
http://ng.neurology.org/misc/addir.xhtml#reprintsus