CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

Full-length Isoform Sequencing for Resolving the Molecular Basis of Charcot-Marie-Tooth 2A

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Neurol Genet 2023;9:e200090. doi:10.1212/NXG.0000000000200090

Abstract

Objectives

Transcript sequencing of patient-derived samples has been shown to improve the diagnostic yield for solving cases of suspected Mendelian conditions, yet the added benefit of full-length long-read transcript sequencing is largely unexplored.

Methods

We applied short-read and full-length transcript sequencing and mitochondrial functional studies to a patient-derived fibroblast cell line from an individual with neuropathy that previously lacked a molecular diagnosis.

Results

We identified an intronic homozygous *MFN2* c.600-31T>G variant that disrupts the branch point critical for intron 6 splicing. Full-length long-read isoform complementary DNA (cDNA) sequencing after treatment with a nonsense-mediated mRNA decay (NMD) inhibitor revealed that this variant creates 5 distinct altered splicing transcripts. All 5 altered splicing transcripts have disrupted open reading frames and are subject to NMD. Furthermore, a patient-derived fibroblast line demonstrated abnormal lipid droplet formation, consistent with MFN2 dysfunction. Although correctly spliced full-length *MFN2* transcripts are still produced, this branch point variant results in deficient MFN2 levels and autosomal recessive Charcot-Marie-Tooth disease, axonal, type 2A (CMT2A).

Discussion

This case highlights the utility of full-length isoform sequencing for characterizing the molecular mechanism of undiagnosed rare diseases and expands our understanding of the genetic basis for CMT2A.

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Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

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The Article Processing charge was funded by the authors.

Glossary

CMT2A = Charcot-Marie-Tooth 2A; **LOF** = loss of function; **MFN2** = mitofusin 2; **NMD** = nonsense-mediated mRNA decay; **UDN** = Undiagnosed Diseases Network; **UPD[1]** = uniparental isodisomy of chromosome 1.

Introduction

Transcript sequencing is emerging as a powerful clinical tool and has been reported to increase diagnostic yield by 2%–24% vs DNA sequencing alone when evaluating cases of suspected Mendelian conditions.[1-5] These studies have used short-read sequencing to infer the identity of full-length transcripts.[6] However, exon skipping and alternative splice site usage within multi-intronic genes can limit the ability of short-read sequencing to accurately predict the coding impact of aberrantly spliced full-length transcripts. Distinguishing among potential full-length transcript outcomes is important for appropriately evaluating conditions whereby distinct phenotypes and inheritance patterns are associated with dominant-negative or loss-of-function (LOF) variants in the same gene.

Charcot-Marie-Tooth 2A (CMT2A) is an axonal peripheral nerve disorder characterized by motor, sensory, or autonomic neuropathy. Approximately 90% of individuals with CMT2A have monoallelic variants associated with a dominant-negative mode of action in mitofusin 2 (*MFN2*)[7] and a dominant mode of inheritance. By contrast, autosomal recessive inheritance is associated with biallelic LOF *MFN2* variants, which typically do not result in a clinical phenotype in the heterozygous state. Splicing variants in *MFN2* can cause both dominant and recessive forms of CMT2A,[8-10] indicating the need to accurately identify the effect of novel splicing variants. We report a patient found via short-read and full-length transcript sequencing to have a homozygous branch point variant in *MFN2* that causes MFN2 deficiency via the creation of 5 altered transcripts, all subject to nonsense-mediated mRNA decay (NMD).

Methods

Exome Sequencing and Analysis

Quad exome sequencing (proband, unaffected mother, unaffected brother, unaffected paternal half brother) was performed through the Undiagnosed Diseases Network (UDN) (Baylor College of Medicine).

Short-read Transcript Sequencing and Analysis

RNA extraction, library preparation, and short-read sequencing were performed on cultured skin fibroblasts from the proband, as previously described.[5] A control data set of short-read transcript/RNA sequencing from 236 skin fibroblast samples was used to identify RNA expression outliers and aberrant splicing products using OUTRIDER[11] and IRFinder,[12] respectively.

Full-length Transcript Sequencing and Analysis

Cultured fibroblasts were incubated with or without cycloheximide (100 μg/mL, Sigma-Aldrich) for 6 hours before RNA extraction (RNeasy Mini kit—Qiagen). cDNA synthesis was performed following the ISO-Seq protocol and sequenced using a Sequel II (PacBio, Menlo Park, CA). Iso-Seq data were processed using the Iso-Seq3 pipeline, mapped to GRCh38, and visualized using IGV.

Sanger Validation

cDNA was synthesized with random hexamers and SuperScript III reverse transcriptase (Invitrogen). The *MFN2* region of interest was PCR amplified for 35 rounds with an exon 5 sense primer (5′-GCCATGAGGCCTTTCTCCTT) and an exon 8 antisense primer (5′-AGACGCTCACTCACCTTGTG). PCR products were separated using 7% polyacrylamide, and then DNA bands were excised and incubated in water overnight to elute DNA, which was amplified using the primers mentioned earlier and subjected to Sanger sequencing.

Lipid Droplet Analysis

Fibroblasts from the proband and a patient not harboring *MFN2* variants were separately plated and grown on glass bottom dishes and then incubated with 0.1 μg/mL Mitotracker Red CMX Ros (Molecular Probes), 5 mM BODIPY 493/503 (Invitrogen), and 3 drops of NucBlue (Invitrogen) for 15 minutes at 37°C with 5% CO_2. Cells were moved into complete media for ≥45 minutes and then imaged using a Z-series step size of 0.3 μm on a Nikon Ti-E widefield microscope with a 63X NA 1.4 oil objective (Nikon), solid-state light source (Spectra X, Lumencor), and an sCMOS camera (Zyla 5.5 megapixel). Each line was imaged on 3 separate occasions (n > 100 cells/experiment). Images were deconvolved using 7 iterations of 3D Landweber deconvolution. For each image, 13–34 cells were captured and analyzed together. The number and fluorescence intensity of lipid droplets was quantified using the automated Spot Detection Analysis after removing background signal (Nikon Elements). Detection was set to detect "bright spots" of different sizes (typical diameter 0.54 μm) with a contrast of 10.1. Function "detect all objects" was selected. Using the ROI statistics function, the number of spots detected and pixel intensity were quantified. Maximum intensity projections were generated using ImageJ (NIH). All quantification was performed by an experimenter blinded to sample identity.

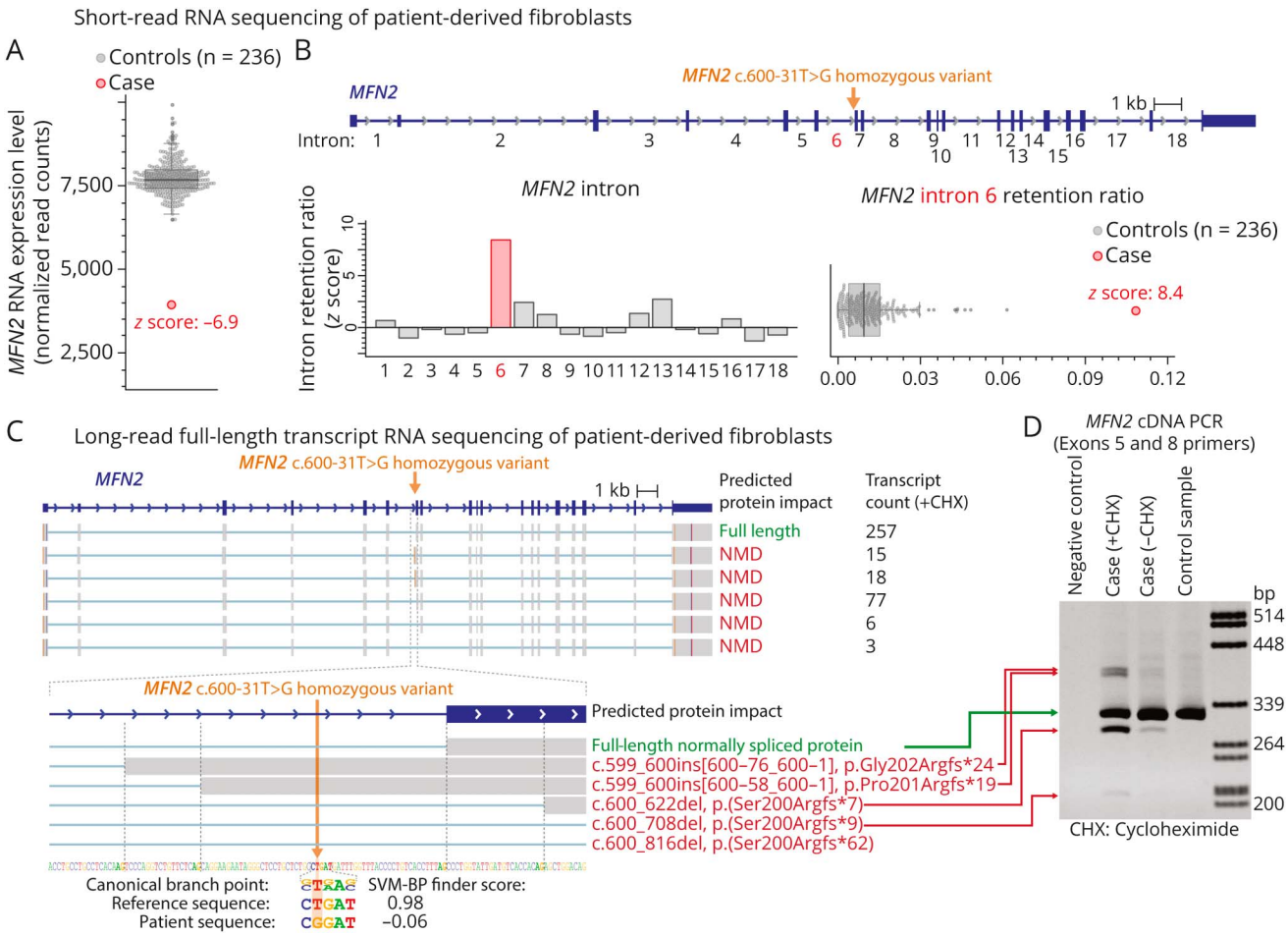
Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the National Institutes of Health Institutional Review Board (IRB) (IRB # 15HG0130), and written informed consent was obtained from all participants in the study.

Data Availability

Sequencing data generated through the UDN are available at dbGaP Study Accession phs001232.v5.p2.

Figure 1 Identification of a Homozygous *MFN2* Branch Point Variant that Disrupts *MFN2* Splicing



(A) Short-read RNA sequencing identified *MFN2* as an expression outlier in this patient's sample, exhibiting 51% of the RNA expression level seen in control fibroblast samples. (B) Genetic testing of *MFN2* identified a deep intronic homozygous variant in intron 6 of *MFN2*. Short-read RNA sequencing identified that the intron retention ratio of intron 6 of *MFN2* was significantly abnormal compared with that in controls. (C) Long-read full-length transcript sequencing (ISO-Seq) of this patient's sample after treatment with the nonsense–mediated decay (NMD) inhibitor cycloheximide (CHX) identified 6 major *MFN2* transcripts that differ in their splicing patterns for intron 6. The predicted protein impact and transcript count of each are indicated to the right. Inset below shows the alternative splice acceptor sites used for each transcript, as well as the sequence context of the patient's variant relative to the canonical branch point sequence. (D) Polyacrylamide gel electrophoresis showing altered spliced products with and without CHX treatment affecting exon 7 of *MFN2* in cells from the patient and the normal splicing of *MFN2* in cells from a control sample.

Results

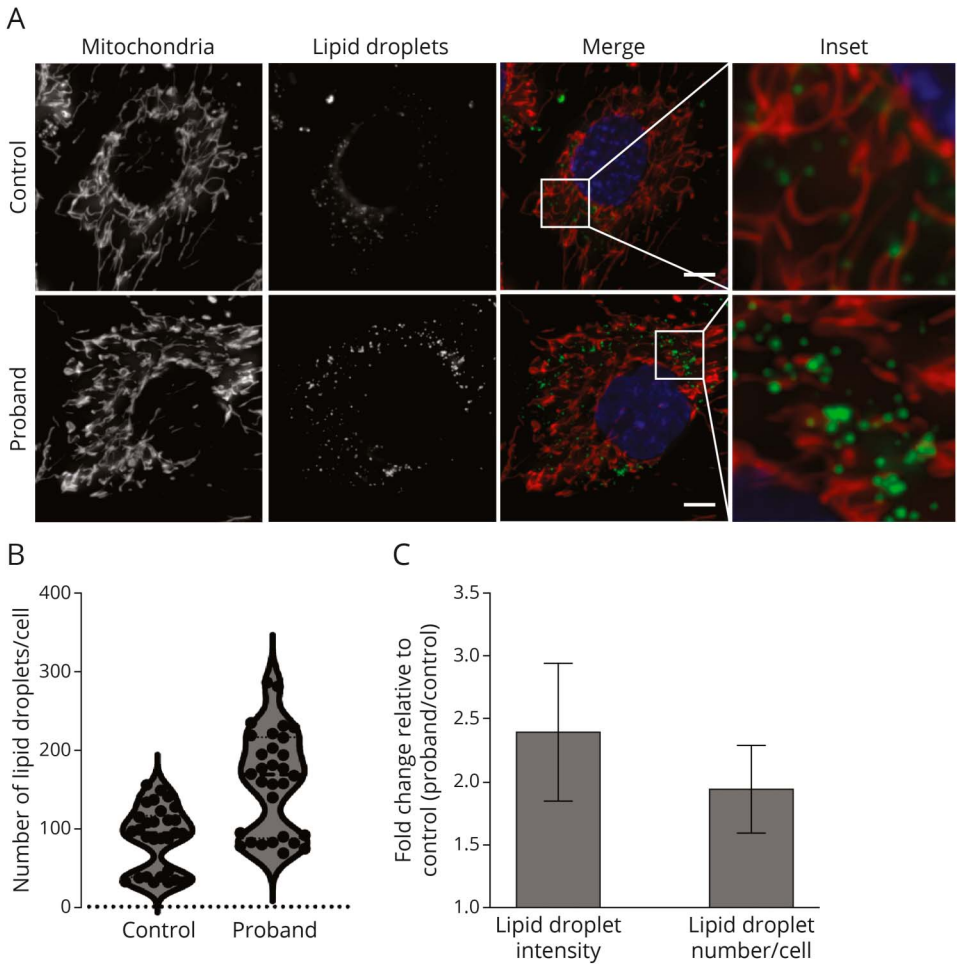
Clinical Phenotype

We evaluated a 42-year-old woman who initially presented with abnormal "foot-slapping" gait at 1 year of age that progressed into distal leg weakness requiring a wheelchair for mobility by age 8 years (eTable 1, links.lww.com/NXG/A621). She underwent spinal fusion and Harrington rod placement for scoliosis in her teens and developed respiratory involvement in her thirties. She had normal cognitive development and no family history of neuromuscular disease. EMG and nerve conduction velocity studies at age 2 years revealed distal motor and sensory polyneuropathy, with positive waves and fibrillation. Nerve and muscle biopsy revealed marked denervation atrophy. Neurologic examination at age 42 years showed normal facial strength, hypophonia, severe muscle wasting of arms and legs, and 1–2/5 proximal and 0/5 distal motor strength. Sensation was present but reduced to all modalities distally, and reflexes were absent throughout.

Identification of a Deep Intronic *MFN2* Variant

Initial genetic evaluation revealed paternal uniparental isodisomy of chromosome 1 (UPD[1]), while panel testing for neuromuscular disorder–associated genes was nondiagnostic. She was enrolled into the UDN, and initial exome analysis was nondiagnostic. Short-read transcript sequencing of patient-derived fibroblasts identified *MFN2*, located on chromosome 1, as an expression outlier with expression approximately half that of control fibroblasts (Z score −6.9) (Figure 1A) In addition, *MFN2* exhibited increased retention of intron 6 (Z score 8.6) (Figure 1B). Reanalysis of exome data identified a homozygous *MFN2* c.600-31T>G variant within intron 6 that is absent from population databases and is predicted to

Figure 2 *MFN2* Branch Point Variant Results in Abnormal Lipid Droplet Formation



(A) Representative images of control and proband fibroblast cells. Mitochondria were labeled with Mitotracker CMXRos, lipid droplets with Bodipy 493/503, and nuclei with NucBlue. Images represent maximum intensity projections. Scale bar = 5 μm. (B) Violin and swarm plots showing the number of lipid droplets per cell from 3 independent biological replicates where the number of lipid droplets per cell was quantified in 10 distinct fields each containing 13–34 cells. The median is indicated with a thick dashed line and quartiles with fine dashed lines. (C) Fold increase in lipid droplet fluorescence intensity and number in proband compared with that in control.

disrupt the U nucleotide in a yUnAy consensus branch point sequence[13] (Figure 1C).

Characterizing the Transcript Impact of an *MFN2* Branch Point Variant

Because branch point variants can induce complex splicing alterations,[14] we performed full-length isoform sequencing (ISO-Seq) to determine the identity of all full-length *MFN2* transcripts. ISO-Seq of patient-derived fibroblasts treated with the NMD inhibitor cycloheximide revealed 5 altered *MFN2* transcripts that each use a distinct splice acceptor site in lieu of the canonical exon 7 splice acceptor (Figure 1C). Notably, all 5 altered transcripts have disrupted open reading frames that make them subject to NMD, and none of them are present within control fibroblasts (Figure 1D).

MFN2 Branch Point Variant Causes *MFN2* Deficiency

To determine whether this branch point variant causes MFN2 deficiency, we analyzed patient-derived fibroblasts for hallmarks of *MFN2* dysfunction. Pathogenic *MFN2* variants are associated with diverse mitochondrial phenotypes, including increased lipid droplet formation.[15] We found that patient-derived fibroblast cells had both increased number and intensity of lipid droplets compared with control cells (Figure 2), consistent with this branch point variant causing MFN2 deficiency.

Discussion

We describe a pathogenic *MFN2* intronic branch point variant that causes autosomal recessive CMT2A—expanding our understanding of the molecular basis of CMT2A. Full-length transcript sequencing revealed that all altered transcripts induced by this variant are subject to NMD, consistent with an LOF mechanism for disease. Notably, the proband's asymptomatic father is presumed to be heterozygous for this variant, consistent with prior observations that LOF *MFN2* variants only cause disease in the recessive state.

Recent advances in long-read full-length transcript sequencing have the potential to transform clinical workflows for evaluating patients with suspected Mendelian conditions. This study provides a proof-of-concept for the utility of full-

length transcriptome data to identify disease-associated variants and to characterize the mechanism by which these variants cause disease. Further studies are needed to fully evaluate the utility of full-length transcript data in clinical practice.

Study Funding

A.B. Stergachis holds a Career Award for Medical Scientists from the Burroughs Wellcome Fund and is a Pew Biomedical Scholar. This study was supported by NIH grants 1U01HG010233, 1DP5OD029630, R01GM118509, and U01HG007703, in addition to funds from the Collagen Diagnostic Laboratory, University of Washington. Fibroblast data and analysis were partially supported by the California Center for Rare Diseases within the UCLA Institute of Precision Health. Sequence data analysis was supported by the University of Washington Center for Mendelian Genomics (UW-CMG), which was funded by NHGRI grant UM1 HG006493. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NG for full disclosures.

Publication History

Previously published on bioRxiv (doi.org/10.1101/2023.02.07.526487). Received by *Neurology: Genetics* February 3, 2023. Accepted in final form June 5, 2023. Submitted and externally peer reviewed. The handling editor was Margherita Milone, MD, PhD.

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Continued

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Page C. Goddard, MS	Stanford University, Stanford	UDN Investigator	UDN Research Assistant
Rena A. Godfrey, PA	NIH UDP, Bethesda	UDN Investigator	UDN PA
Katie Golden-Grant, MS, CGC	Seattle, Washington	UDN Investigator	Genetic Counselor
Katherine Gomeztagle-Burgess	University of Washington	UW-CMG investigator	UW-CMG scientist
William W. Gordon	University of Washington	UW-CMG investigator	UW-CMG scientist
Alana Grajewski, MD	University of Miami, Miami	UDN Investigator	UDN MD
Don Hadley, MS, CGC	NHGRI, Bethesda	UDN Investigator	UDN Genetic Counselor
Sihoun Hahn, MD, PhD	Seattle Children's Hospital, Seattle	UDN Investigator	UDN MD

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Name	Location	Role	Contribution
Meghan C. Halley, PhD, MPH	Stanford University, Stanford	UDN Investigator	UDN Bioethics Advisor
Rizwan Hamid, MD, PhD, FAAP	Vanderbilt University, Nashville	UDN Investigator	UDN MD
Kelly Hassey, MSN, CRNP	Children's Hospital of Philadelphia, Philadelphia	UDN Investigator	UDN Research Coordinator
Nichole Hayes, BS	Washington University of St. Louis, St. Louis	UDN Investigator	UDN Site Coordinator
Frances High, MD, PhD	Harvard Medical School, Boston	UDN Investigator	UDN MD
Anne Hing, MD	Seattle Children's Hospital, Seattle	UDN Investigator	UDN MD
Ingrid A. Holm, MD, MPH	Harvard Medical School, Boston	UDN Investigator	UDN MD
Jason Hom, MD	Stanford University, Stanford	UDN Investigator	UDN MD
Alden Huang, PhD	University of California, Los Angeles	UDN Investigator	UDN Sequencing
Jameson R. Hurless	University of Washington	UW-CMG investigator	UW-CMG scientist
Sarah Hutchison, BS	NHGRI, Bethesda	UDN Investigator	UDN Program Analyst
Wendy Introne, MD	NIH, Bethesda	UDN Investigator	UDN MD
Rosario Isasi, JD, MPH	University of Miami, Miami	UDN Investigator	UDN Bioethics Advisor
Kosuke Izumi, MD, PhD	Children's Hospital of Philadelphia, Philadelphia	UDN Investigator	UDN MD
Fariha Jamal, MD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
Jeffrey Jarvik, MD, MPH	University of Washington, Seattle	UDN Investigator	UDN MD
Suman Jayadev, MD	University of Washington, Seattle	UDN Investigator	UDN MD
Orpa Jean-Marie, MSN	NIH, Bethesda	UDN Investigator	UDN Research Coordinator
Vaidehi Jobanputra, PhD, FACMG	Columbia University Irving Medical Center, New York	UDN Investigator	UDN MD

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Name	Location	Role	Contribution
Eric Johanson	University of Washington	UW-CMG investigator	UW-CMG scientist
Lefkothea Karaviti, MD, PhD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
Shamika Ketkar, PhD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
Dana Kiley, BS	Washington University, St. Louis	UDN Investigator	UDN Research Coordinator
Gonench Kilich, MD	Children's Hospital of Philadelphia, Philadelphia	UDN Investigator	UDN MD
Shilpa N. Kobren, PhD	Harvard Medical School, Boston	UDN Investigator	UDN Research Fellow
Isaac S. Kohane, MD, PhD	Harvard Medical School, Boston	UDN Investigator	UDN PI
J. Thomas Kolar	University of Washington	UW-CMG investigator	UW-CMG scientist
Jennefer N. Kohler, MS, CGC	Stanford University, Stanford	UDN Investigator	UDN Genetic Counselor
Susan Korrick, MD, MPH	Harvard Medical School, Boston	UDN Investigator	UDN MD
Mary Kozuira, DNP	Vanderbilt University, Nashville	UDN Investigator	UDN Nurse Practitioner
Deborah Krakow, MD	University of California Los Angeles, Los Angeles	UDN Investigator	UDN MD
Donna M. Krasnewich, MD, PhD	NIH, Bethesda	UDN Investigator	UDN Program Officer
Elijah Kravets, BS	Stanford University, Stanford	UDN Investigator	UDN Study Coordinator
Seema R. Lalani, MD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
Byron Lam, MD	University of Miami, Miami	UDN Investigator	UDN MD
Christina Lam, MD	Seattle Children's Hospital, Seattle	UDN Investigator	UDN MD
Brendan C. Lanpher, MD	Mayo Clinic, Rochester	UDN Investigator	UDN MD
Ian R. Lanza, PhD	Mayo Clinic, Rochester	UDN Investigator	UDN PI

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Name	Location	Role	Contribution
Suzanne M. Leal	Columbia University	UW-CMG investigator	UW-CMG scientist
Kimberly LeBlanc, MS	Harvard Medical School, Boston	UDN Investigator	UDN Associate Director of Research Operations
Brendan H. Lee, MD, PhD	Baylor College of Medicine, Houston	UDN Investigator	UDN Co-PI
Roy Levitt, MD	University of Miami, Miami	UDN Investigator	UDN MD
Richard A. Lewis, MD, MS	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
Pengfei Liu, PhD	Baylor College of Medicine, Houston	UDN Investigator	UDN Laboratory Director
Xue Zhong Liu, MD, PhD, FACS	University of Miami, Miami	UDN Investigator	UDN MD
Nicola Longo, MD, PhD	University of Utah, Salt Lake City	UDN Investigator	UDN MD
Sandra K. Loo, PhD	University of California Los Angeles, Los Angeles	UDN Investigator	UDN MD
Joseph Loscalzo, MD, PhD	Harvard Medical School, Boston	UDN Investigator	UDN PI
Richard L. Maas, MD, PhD	Harvard Medical School, Boston	UDN Investigator	UDN MD
Ellen F. Macnamara, MS, CGC	NIH, UDP, Bethesda	UDN Investigator	UDN Genetic Counselor
Calum A. MacRae, MD, PhD	Harvard Medical School, Boston	UDN Investigator	UDN MD
Valerie V. Maduro, BS	NIH, UDP, Bethesda	UDN Investigator	UDP Translational Laboratory Manager
AudreyStephannie Maghiro, BS	Harvard Medical School, Boston	UDN Investigator	UDN Coordinating Center Lead
Rachel Mahoney, MS, CGC	Harvard Medical School, Boston	UDN Investigator	UDN Genetic Counselor, Project Manager
May Christine V. Malicdan, MD, PhD	NIH UDP, NHGRI	UDN Investigator	UDN Staff Scientist, Director of UDP Translational Research Laboratory
Laura A. Mamounas, PhD	NIH, Bethesda	UDN Investigator	UDN Program Director
Teri A. Manolio, MD, PhD	NIH, Bethesda	UDN Investigator	UDN Program Director
Rong Mao, MD, FACMG	University of Utah, Salt Lake City and ARUP	UDN Investigator	UDN Molecular Geneticist

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Name	Location	Role	Contribution
Kenneth Maravilla, MD	University of Washington, Seattle	UDN Investigator	UDN MD
Ronit Marom, MD, PhD	Baylor College of Medicine, Houston	UDN Investigator	UDN Sequence Analyst
Gabor Marth, DSc	University of Utah, Salt Lake City	UDN Investigator	UDN Bioinformatician
Beth A. Martin, MD	Stanford University, Stanford	UDN Investigator	UDN MD
Martin G. Martin, MD	University of Utah, Salt Lake City	UDN Investigator	UDN Bioinformatician
Julian A. Martínez-Agosto, MD, PhD	University of California, Los Angeles	UDN Investigator	UDN PI
Colby T. Marvin	University of Washington	UW-CMG investigator	UW-CMG scientist
Shruti Marwaha, PhD	Stanford University, Stanford	UDN Investigator	UDN Bioinformatician
Jacob McCauley, PhD	University of Miami, Miami	UDN Investigator	UDN MD
Allyn McConkie-Rosell, PhD	Duke University, Durham	UDN Investigator	UDN MD
Alexa T. McCray, PhD	Harvard Medical School, Boston	UDN Investigator	UDN PI
Elisabeth McGee, CNS, APRN, CPN	University of California, Los Angeles	UDN Investigator	UDN Nurse Practitioner
Sean McGee	University of Washington	UW-CMG investigator	UW-CMG scientist
Daniel J. McGoldrick	University of Washington	UW-CMG investigator	UW-CMG scientist
Betselote Mekonnen	University of Washington	UW-CMG investigator	UW-CMG scientist
Heather Mefford, MD, PhD	Seattle Children's Hospital, Seattle	UDN Investigator	UDN MD
J. Lawrence Merritt, MD	Seattle Children's Hospital, Seattle	UDN Investigator	UDN MD
Matthew Might, PhD	University of Alabama, Birmingham	UDN Investigator	UDN PI
Eva Morava, MD, PhD	Mayo Clinic, Rochester	UDN Investigator	UDN MD
Paolo Moretti, MD	University of Utah, Salt Lake City	UDN Investigator	UDN MD

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Name	Location	Role	Contribution
John Mulvihill, MD	NIH, Bethesda	UDN Investigator	UDN Senior Consultant
Mariko Nakano-Okuno, PhD	University of Alabama, Birmingham	UDN Investigator	UDN Bioethics Advisor
John H. Newman, MD	Vanderbilt University, Nashville	UDN Investigator	UDN PI
Sarah K. Nicholas, MD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
Deborah Nickerson, PhD	University of Washington, Seattle	UDN-CMG Investigator	CMG PI
Patrick M. Nielsen	University of Washington	UW-CMG investigator	UW-CMG scientist
Shirley Nieves-Rodriguez, PhD Candidate	University of California, Los Angeles	UDN Investigator	UDN Researcher
Donna Novacic, MD	NIH UDP, Bethesda, MD	UDN Investigator	UDN MD
Devin Oglesbee, PhD	Mayo Clinic, Rochester	UDN Investigator	UDN PI, Metabolomics Core
James P. Orengo, MD, PhD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
Laura Pace, MD, PhD	University of Utah, Salt Lake City	UDN Investigator	UDN MD
Stephen Pak, PhD	Washington University in St. Louis, St. Louis	UDN Investigator	UDN MD – Model Organisms
J. Carl Pallais, MD	Harvard Medical School, Boston	UDN Investigator	UDN MD
Christina G.S. Palmer, PhD, CGC	University of California, Los Angeles	UDN Investigator	UDN PI
Jeanette C. Papp, MD	University of California, Los Angeles	UDN Investigator	UDN MD
Neil H. Parker, MD	University of California, Los Angeles	UDN Investigator	UDN MD
Karynne Patterson	University of Washington	UW-CMG investigator	UW-CMG scientist
John A. Phillips III, MD	Vanderbilt University, Nashville	UDN Investigator	UDN MD
Jennifer E. Posey, MD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
Lorraine Potocki, MD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD

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Name	Location	Role	Contribution
Barbara N. Pusey Swerdzewski, MS	NIH, UDP, Bethesda, MD	UDN Investigator	UDN Bioinformatics
Aaron Quinlan, PhD	University of Utah, Salt Lake City	UDN Investigator	UDN Bioinformatics
Aparna Radhakrishnan	University of Washington	UW-CMG investigator	UW-CMG scientist
Deepak A. Rao, MD, PhD	Harvard Medical School, Boston	UDN Investigator	UDN MD
Anna Raper, MS, CGC	University of Pennsylvania, Philadelphia	UDN Investigator	UDN Genetic Counselor
Genecee Renteria, BS	University of California, Los Angeles	UDN Investigator	UDN Research Assistant
Chloe M. Reuter, MS, CGC	Stanford University, Stanford	UDN Investigator	UDN Genetic Counselor
Matthew A. Richardson	University of Washington	UW-CMG investigator	UW-CMG scientist
Lynette Rives, BS	Vanderbilt University, Nashville	UDN Investigator	UDN Lab Manager
Amy K. Robertson, MSN, BSN, FNP	Vanderbilt University, Nashville	UDN Investigator	UDN Nurse Practitioner
Lance H. Rodan, MD	Harvard Medical School, Boston	UDN Investigator	UDN MD
Gwendolin T. Roote	University of Washington	UW-CMG investigator	UW-CMG scientist
Jill A. Rosenfeld, MS, CGC	Baylor College of Medicine, Houston	UDN Investigator	UDN Site Manager
Natalie Rosenwasser, MD	Seattle Children's Hospital, Seattle	UDN Investigator	UDN MD
Francis Rossignol, MD	NIH, UDP, NHGRI Bethesda, MD	UDN Investigator	UDN MD
Maura Ruzhnikov, MD	University of Miami, Miami	UDN Investigator	UDN MD
Erica L. Ryke	University of Washington	UW-CMG investigator	UW-CMG scientist
Ralph Sacco, MD	University of Miami, Miami	UDN Investigator	UDN MD
Jacinda B. Sampson, MD	Stanford University, Stanford	UDN Investigator	UDN MD
Mario Saporta, MD, PhD	University of Miami, Miami	UDN Investigator	UDN MD

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Name	Location	Role	Contribution
Judy Schaechter, MD, MBA	University of Miami, Miami	UDN Investigator	UDN MD
Timothy Schedl, PhD	Washington University in St. Louis, St. Louis MOSC	UDN Investigator	UDN PI – Model Organisms
Kelly Schoch, MS, CGC	Duke University, Durham	UDN Investigator	UDN Genetic Counselor
Isabelle Schrauwen	Columbia	UW-CMG investigator	UW-CMG scientist
Daryl A. Scott, MD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
C. Ron Scott, MD	University of Washington, Seattle	UDN Investigator	UDN MD
Vandana Shashi, MD	Duke University, Durham	UDN Investigator	UDN PI
Jimann Shin, PhD	Washington University in St. Louis, St. Louis MOSC	UDN Investigator	UDN Scientist – Model Organisms
Kathryn M. Shively	University of Washington	UW-CMG investigator	UW-CMG scientist
Edwin K. Silverman, MD, PhD	Harvard Medical School, Boston	UDN Investigator	UDN MD
Janet S. Sinsheimer, PhD	University of California, Los Angeles	UDN Investigator	UDN MD
Kathy Sisco, RN, CPNP	Washington University in St. Louis, St. Louis	UDN Investigator	UDN Clinical Site Coordinator
Edward C. Smith, MD	Duke University, Durham	UDN Investigator	UDN MD
Joshua D. Smith	University of Washington	UW-CMG investigator	UW-CMG scientist
Kevin S. Smith, PhD	Stanford University, Stanford	UDN Investigator	UDN Research Scientist
Lilianna Solnica-Krezel, PhD	Washington University in St. Louis, St. Louis MOSC	UDN Investigator	UDN PI
Ben Solomon, MD	NIH, UDP, NHGRI Bethesda, MD	UDN Investigator	UDN MD
Rebecca C. Spillmann, MS, CGC	Duke University, Durham	UDN Investigator	UDN Genetic Counselor

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Name	Location	Role	Contribution
Joan M. Stoler, MD	Harvard Medical School, Boston	UDN Investigator	UDN MD
Kathleen Sullivan, MD, PhD	Children's Hospital of Philadelphia, Philadelphia	UDN Investigator	UDN PI
Jennifer A. Sullivan, MS, CGC	Duke University, Durham	UDN Investigator	UDN Genetic Counselor
Angela Sun, MD	Seattle Children's Hospital, Seattle	UDN Investigator	UDN MD
Shirley Sutton, BS	Stanford University, Stanford	UDN Investigator	UDN Lab Manager
David A. Sweetser, MD, PhD	Harvard Medical School, Boston	UDN Investigator	UDN PI
Holly K. Tabor, PhD	Stanford University, Stanford	UDN Investigator	UDN MD
Monica Tackett	University of Washington	UW-CMG investigator	UW-CMG scientist
Queenie K.-G. Tan, MD	Duke University, Durham	UDN Investigator	UDN MD
Amelia L. M. Tan, PhD	Harvard Medical School, Boston	UDN Investigator	UDN Bioinformatics
Mustafa Tekin, MD	University of Miami, Miami	UDN Investigator	UDN PI
Fred Telischi, MD, FACS, MEE	University of Miami, Miami	UDN Investigator	UDN MD
Willa Thorson, MD	University of Miami, Miami	UDN Investigator	UDN MD
Cynthia J. Tifft, MD, PhD	NIH, UDP, NHGRI Bethesda, MD	UDN Investigator	UDN PI, Deputy Clinical Director NHGRI
Camilo Toro, MD	NIH, UDP, Bethesda, MD	UDN Investigator	UDN PI, UDP Program
Alyssa A. Tran	Baylor College of Medicine, Houston	UDN Investigator	UDN Site Coordinator
Rachel A. Ungar, PhD Candidate	Stanford University, Stanford	UDN Investigator	UDN Researcher
Tiina K. Urv, PhD	NIH, Bethesda	UDN Investigator	UDN Program Director
Adeline Vanderver, MD	Children's Hospital of Philadelphia, Philadelphia	UDN Investigator	UDN MD

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Name	Location	Role	Contribution
Matt Velinder, PhD	University of Utah, Salt Lake City	UDN Investigator	UDN Bioinformatics
David Viskochil, MD, PhD	University of Utah, Salt Lake City	UDN Investigator	UDN MD
Tiphanie P. Vogel, MD, PhD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
Colleen E. Wahl, ARNP	NIH, Bethesda	UDN Investigator	UDN Nurse Practitioner
Melissa Walker, MD, PhD	Harvard Medical School, Boston	UDN Investigator	UDN MD
Stephanie Wallace, MD	Seattle Children's Hospital, Seattle	UDN Investigator	UDN MD
Nicole M. Walley, MS	Duke University, Durham	UDN Investigator	UDN Research Coordinator
Jennifer Wambach, MD, MS	Washington University in St. Louis, St. Louis	UDN Investigator	UDN MD
Jijun Wan, BS	University of California, Los Angeles	UDN Investigator	UDN Research Assistant
Gao Wang	Columbia	UW-CMG investigator	UW-CMG scientist
Lee-kai Wang, BS	University of California, Los Angeles	UDN Investigator	UDN Bioinformatics
Michael F. Wangler, MD	Baylor College of Medicine, Houston	UDN Investigator	UDN PI – Model Organisms
Patricia A. Ward, MS, CGC	Baylor College of Medicine, Houston	UDN Investigator	UDN Genetic Counselor and Site Coordinator
Daniel Wegner, MS	Washington University in St. Louis, St. Louis	UDN Investigator	UDN Lab Manager – Model Organisms
Jeffrey M. Weiss	University of Washington	UW-CMG investigator	UW-CMG scientist
Monika Weisz Hubshman, MD, PhD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD
Tara Wenger, MD	Seattle Children's Hospital, Seattle	UDN Investigator	UDN MD
Monte Westerfield, PhD	University of Oregon MOSC, Eugene	UDN Investigator	UDN PI – Model Organisms
Marsha M. Wheeler	University of Washington	UW-CMG investigator	UW-CMG scientist
Matthew T. Wheeler, MD, PhD	Stanford University, Stanford	UDN Investigator	UDN PI
Jordan Whitlock, PhD Candidate	University of Alabama, Birmingham	UDN Investigator	UDN Researcher
Lynne A. Wolfe, MS, CRNP, BC	NIH UDP, NHGRI, Bethesda, MD	UDN Investigator	UDN Site Coordinator and Senior Nurse Practitioner
Kim Worley, PhD	Baylor College of Medicine, Houston	UDN Investigator	UDN MD – Sequencing
Changrui Xiao, MD	CHOC, Orange	UDN Investigator	UDN MD
Shinya Yamamoto	Baylor College of Medicine, Houston	UDN Investigator	UDN PI – Model Organisms
John Yang	NIH UDP, NHGRI	UDN Investigator	UDP Research Nurse Coordinator
Qian Yi	University of Washington	UW-CMG investigator	UW-CMG scientist
Xiaohong Zhang	University of Washington	UW-CMG investigator	UW-CMG scientist
Zhe Zhang, PhD	Children's Hospital of Philadelphia, Philadelphia	UDN Investigator	UDN Bioinformatics
Stephan Zuchner, MD, PhD	University of Miami School of Medicine, Miami	UDN Investigator	UDN PI

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