



Undiagnosed Diseases Network Manual of Operations

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Table of Acronyms

Acronym	Definition
ACMG	American College of Medical Genetics and Genomics
API	Application programming interface
BCM	Baylor College of Medicine
bp	Base pairs
BWA	Burrows Wheeler Aligner

CC	Undiagnosed Diseases Network Coordinating Center
CDC	Centers for Disease Control and Prevention
CIRB	Central Institutional Review Board
CLIA	Clinical Laboratory Improvement Amendments
COI	Conflict of Interest
CPT	Cell Preparation Tube
CR	Continuing Review
CRC	Clinical Research Center
CS	Undiagnosed Diseases Network Clinical Site
CSF	Cerebrospinal fluid
CSL	Clinical Services Laboratory
dbGaP	Database of Genotypes and Phenotypes
DOB	Date of birth
EEG	Electroencephalogram
EMG	Electromyography
FFQ	Food Frequency Questionnaire
FIPS	Federal Information Processing Standards
FISMA	Federal Information Security Management Act
FWA	Federalwide Assurance
gDNA	Genomic DNA
GSL	Genomic Services Laboratory
HA	HudsonAlpha Institute for Biotechnology
HIPAA	Health Insurance Portability and Accountability Act
HPO	Human Phenotype Ontology
HRPP	Human Research Protections Program
ICD	International Classification of Diseases
ICF	Informed Consent and Assent Forms
IRB	Institutional Review Board
LCF	Lipidomics Core Facility
LIMS	Laboratory Information Management System
MC	Undiagnosed Diseases Network Metabolomics Core
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NCV	Nerve Conduction Velocity
NHANES	National Health and Nutrition Examination Survey
NHGRI	National Human Genome Research Institute
NHGRI-IRP	National Human Genome Research Institute-Intramural Research Program
NIH	National Institutes of Health
NIST	National Institute of Standards and Technology
NORD	National Organization for Rare Disorders
OMIM	Online Mendelian Inheritance in Man
ORDR	Office of Rare Diseases Research

OSC	Office of Strategic Coordination
PBMC	Peripheral Blood Mononuclear Cell
PCC	Participant Care Coordinator
PCR	Polymerase chain reaction
PE	Paired-end
PEER	Participant Engagement and Empowerment Resource
PhenX	Phenotypes and eXposures consensus measurement
PHI	Personal Health Information
PI	Principal Investigator
PII	Personally Identifiable Information
QC	Quality Control
QWES	Quick Whole Exome Sequencing
SC	Undiagnosed Diseases Network Sequencing Core
SNP	Single Nucleotide Polymorphism
SRC	Scientific Review Committee
SST	Serum separator tube
TAT	Turnaround time
UDN	Undiagnosed Diseases Network
UDNCB	Undiagnosed Diseases Network Central Biorepository
UDN NIH PO	Undiagnosed Diseases Network National Institutes of Health Program
UDP	Undiagnosed Diseases Program
UDPICS	Undiagnosed Diseases Program Integrated Collaboration System
UUID	Universally Unique Identifier
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing
WGL	Whole Genome Laboratory

I. Network Overview and Operating Procedures

A. Network Overview

The Undiagnosed Diseases Network (UDN) consists of 12 Clinical Sites (CSs), a Coordinating Center (CC), 2 DNA Sequencing Cores (SCs), a Model Organisms Screening Center, a Metabolomics Core (MC), and a Central Biorepository.

The CC is located at the following institution, with the following PIs:

- Harvard Medical School (with University of Alabama at Birmingham), Boston, MA - Isaac Kohane, MD, PhD; Matthew Might, PhD; and Alexa McCray, PhD

The CSs are located at the following institutions, with the following PIs:

- Baylor College of Medicine, Houston, TX - Brendan Lee, MD, PhD and Carlos Bacino, MD
- Children's Hospital of Philadelphia (with University of Pennsylvania), Philadelphia, PA - Reed Pyeritz, MD, PhD and Kathleen Sullivan, MD, PhD
- Duke University Medical Center (with Columbia University Medical Center), Durham, NC - David Goldstein, PhD and Vandana Shashi, MBBS, MD
- Harvard Teaching Hospitals (including Boston Children's Hospital, Brigham and Women's Hospital, and Massachusetts General Hospital), Boston, MA - Joseph Loscalzo, MD, PhD
- National Institutes of Health (NIH), Bethesda, MD - David Adams, MD, PhD; William Gahl, MD, PhD; Cynthia Tifft, MD, PhD; and Camilo Toro, MD
- Stanford Medicine, Palo Alto, CA - Euan Ashley, MD; Jonathan Bernstein, MD; Paul Fisher, MD; and Matthew Wheeler, MD, PhD
- University of California Los Angeles, Los Angeles, CA - Julian Martinez, MD, PhD; Stanley Nelson, MD; and Christina Palmer, PhD
- University of Miami, Miami, FL - Mustafa Tekin, MD and Stephan Züchner, MD, PhD
- University of Utah, Salt Lake City, UT - Lorenzo Botto, MD
- University of Washington (with Seattle Children's Hospital), Seattle, WA - Katrina Dipple, MD, PhD and Gail Jarvik, MD, PhD
- Vanderbilt University Medical Center, Nashville, TN - Joy Cogan, PhD; Rizwan Hamid, MD, PhD; John Newman, MD, PhD; and John Phillips III, MD
- Washington University in St. Louis, St. Louis, MO - F. Sessions Cole III, MD

The SCs are located at the following institutions, with the following PIs:

- Baylor College of Medicine, Houston, TX - Christine Eng, MD
- HudsonAlpha (with Illumina), Huntsville, AL - Liz Worthey, PhD

The Model Organisms Screening Center is located at the following institutions, with the following PI:

- Baylor College of Medicine (with University of Oregon), Houston, TX - Hugo Bellen, DVM, PhD
- Washington University in St. Louis, St. Louis, MO - Tim Schedl, PhD and Lilianna Solnica-Krezel, PhD

The Metabolomics Core is located at the following institutions, with the following PIs:

- Mayo Clinic, Rochester, MN - Devin Oglesbee, PhD and Ian Lanza, PhD

- Pacific Northwest National Laboratory (with Oregon Health & Science University), Richland, WA - Thomas Metz, PhD and David Koeller, MD

The Central Biorepository is located at the following institution, with the following PIs:

- Vanderbilt University Medical Center, Nashville, TN - Joy Cogan, PhD and John Phillips III, MD

The purpose of this cooperative research Network is to establish a national network added to and building upon the NIH Undiagnosed Diseases Program (NIH UDP). The objectives of this program are to: 1) improve the level of diagnosis and care for patients with undiagnosed diseases through the development of common protocols designed by a community of investigators; 2) facilitate research into the etiology of undiagnosed diseases, by collecting and sharing standardized, high-quality clinical and laboratory data including genotyping, phenotyping, and documentation of environmental exposures; and 3) create an integrated and collaborative research community across multiple CSs and among laboratory and clinical investigators prepared to investigate the pathophysiology of these new and rare diseases and share this understanding to identify improved options for optimal patient management.

The major funder of the UDN is the NIH Common Fund, which is managed by the Office of the Director/Office of Strategic Coordination (OSC).

B. Cooperative Agreement Responsibilities

The administrative and funding instrument used for the UDN is the cooperative agreement, an "assistance" mechanism (rather than an "acquisition" mechanism), in which substantial NIH programmatic involvement with the awardees is anticipated during the performance of the activities. Under the cooperative agreement, the NIH purpose is to support and stimulate the recipients' activities by involvement in and otherwise working jointly with the award recipients in a partnership role; it is not to assume direction, prime responsibility, or a dominant role in the activities. Consistent with this concept, the dominant role and prime responsibility resides with the awardees for the project as a whole, although specific tasks and activities may be shared among the awardees and the NIH as defined below.

NIH staff have substantial programmatic involvement that is above and beyond the normal stewardship role as described below:

The NIH Project Scientist(s) have substantial scientific and programmatic involvement during the conduct of this activity through technical assistance, advice, and coordination. However, the role of NIH staff is to facilitate and not to direct the activities. It is anticipated that decisions in all activities are reached by consensus of the UDN and that NIH staff are given the opportunity to offer input to this process. The Project Scientist(s) will participate as members of the Steering Committee and will have one vote. The Project Scientist(s) have the following substantial involvement:

1. Participating with the other Steering Committee members in addressing issues that arise with UDN planning, operation, and analysis. The Project Scientist(s) assist and facilitate the group process and do not direct it.
2. Serving as a liaison, helping to coordinate activities, including acting as a liaison to other NIH Institutes/Centers, and as an information resource for the awardees. The Project Scientist(s) also help coordinate the efforts of the UDN with other groups conducting similar efforts.

3. Attending all Steering Committee meetings as a voting member and all working group meetings, assisting in developing operating guidelines, quality control procedures, and consistent policies for dealing with situations that require coordinated action. The Project Scientist(s) are responsible for working with the grantee(s) as needed to manage the logistic aspects of the resource.
4. Reporting periodically on Network progress to the NIH UDN Working Group (a trans-NIH Common Fund working group made up of staff from multiple NIH Institutes and Centers) and through it to the NIH Common Fund and to the National Advisory Council of Human Genome Research Institute.
5. Serving on subcommittees of the Steering Committee, and Working Groups as appropriate.
6. Assisting awardees in the development, if needed, of policies for dealing with situations that require coordinated action.
7. Providing advice in the management and technical performance of the award.
8. Assisting in promoting the availability of the data and related resources developed in the course of this program to the scientific community at large.
9. Participating in data analyses, interpretations, and, where warranted, co-authorship of the publication of results of studies conducted through the program.
10. Other NIH UDN Working Group staff may assist the awardee as designated by the UDN NIH Program Official (The NIH official responsible for the programmatic, scientific, and/or technical aspects of the grant).

Collaborative Responsibilities:

Close interaction among the participating investigators is required, as well as significant involvement from the NIH, to develop and operate the UDN. Principal investigators participate in in-person Steering Committee meetings on a quarterly basis during the first year of funding cycles and subsequently three times per year; during months in which there are not in-person meetings, there are monthly conference calls as needed to share information on data resources, methodologies, analytical tools, as well as data and preliminary results. Key co-investigators and pre- and post-doctoral trainees, especially those who are members of under-represented minority groups or those from different but related disciplines, are also eligible to attend these meetings.

All Awardees agree to work collaboratively to:

1. Assist in refining a common approach to patients with undiagnosed diseases.
2. Work collaboratively with other UDN investigators to provide for secure, accurate and timely data submission.
3. Participate in presenting and publishing new processes and substantive findings.
4. Participate in the governance of the UDN as a member of the Steering Committee.
5. Interact with other relevant National Human Genome Research Institute (NHGRI) and NIH activities, as needed, to promote synergy and consistency among similar projects.

Additionally, the CS Awardees agree to work collaboratively to:

1. Participate in network-wide processes for patient selection and assignment to a specific CS for evaluation.
2. Identify 10 previously unidentified diseases network-wide per year in FY16 and FY17.
3. Fulfill all principal investigator (PI) primary responsibilities laid out in [RFA-RM-13-004](#).

Additionally, the CC Awardee agrees to work collaboratively to:

1. Share statistical experience and expertise across the UDN and provide advice on statistical methods design.
2. Participate with the current NIH UDP investigators to refine and adapt current single center activities to the requirements of the Network.
3. Fulfill all PI primary responsibilities laid out in [RFA-RM-12-020](#).

Additionally, the DNA SC Awardees agree to work collaboratively to:

1. Fulfill all PI primary responsibilities laid out in [RFA-RM-13-018](#).

Additionally, the Model Organisms Screening Center Awardee agrees to work collaboratively to:

1. Fulfill all PI primary responsibilities laid out in [RFA-RM-14-016](#).

Additionally, the Metabolomics Core Awardee agrees to work collaboratively to:

1. Fulfill all PI primary responsibilities laid out in [RFA-RM-15-001](#).

C. Steering Committee Policies

Guideline: A Steering Committee composed of PIs from all sites (including the CC, CSs (including the NIH UDP), and the other Core Laboratories (including the DNA SCs, Model Organisms Screening Center, MC, and Central Biorepository)) and the NIH Project Scientist(s) will be responsible for the scientific direction of the Network, as set forth in the FOAs RFA-RM-12-020, RFA-RM-13-004, RFA-RM-13-18, RFA-RM-14-016, and RFA-RM-15-001. The Steering Committee is responsible for the scientific direction of the Network.

Policies:

1. The Steering Committee is responsible for policy decisions regarding the Network, and for the discussion and resolution of procedural issues that affect the operation and status of the network as a whole.
2. The UDN Steering Committee will be the operational group through which the NIH UDN Working Group interacts with the UDN.
3. The Steering Committee will have at least monthly conference calls.
4. The Steering Committee will meet in person quarterly during the first year of funding cycles and three times per year or as needed subsequently.
5. The minutes for all Steering Committee discussion will be documented and posted on a CC website (viewable to Steering Committee members).
6. The voting members of the UDN Steering Committee include the Principal Investigator(s) of each CS, the PI(s) of the CC, the PI(s) of each Core Laboratory (including the DNA SCs, Model Organisms Screening Center, Metabolomics Core, and Central Biorepository), and the collective NIH IC Project Scientists. Each site has one vote (multiple PIs may all be members of the Steering Committee, but collectively have one vote for their site) and the NIH Project Scientists group collectively has one vote.
7. The Steering Committee may add additional members, and other NIH Program staff may attend the Steering Committee meetings as desired.

STEERING COMMITTEE

Co-Chairs: Cynthia Tifft (UDP) and Vandana Shashi (Duke)

Members:

CS PIs (1 vote for each CS):

1. Baylor College of Medicine - Brendan Lee and Carlos Bacino
2. Children's Hospital of Philadelphia (w/ University of Pennsylvania) - Reed Pyeritz and Kate Sullivan (contact)
3. Duke University Medical Center (w/ Columbia University Medical Center) - David Goldstein and Vandana Shashi (contact)
4. Harvard Teaching Hospitals - Joseph Loscalzo
5. NIH UDP - David Adams, William Gahl (contact), Cynthia Tifft, and Camilo Toro
6. Stanford Medicine - Euan Ashley (contact), Jon Bernstein, Paul Fisher, and Matthew Wheeler
7. University of California, Los Angeles - Julian Martinez, Stanley Nelson (contact), and Christina Palmer
8. University of Miami - Mustafa Tekin (contact) and Stephan Züchner
9. University of Utah - Lorenzo Botto
10. University of Washington (w/ Seattle Children's Hospital) - Katrina Dipple and Gail Jarvik (contact)
11. Vanderbilt University Medical Center - Joy Cogan, Rizwan Hamid, John Newman, and John Phillips III (contact)
12. Washington University in St. Louis - F. Sessions Cole III

CC Pls (1 vote):

1. Harvard Medical School (w/ University of Alabama at Birmingham) - Isaac Kohane (contact), Alexa McCray, and Matthew Might

Core Pls (1 vote for each core):

1. Baylor College of Medicine - Christine Eng
2. HudsonAlpha (w/ Illumina) - Liz Worthey
3. Baylor College of Medicine (w/ University of Oregon) - Hugo Bellen
4. Washington University in St. Louis - Tim Schedl and Lilianna Solnica-Krezel (contact)
5. Mayo Clinic - Ian Lanza (contact) and Devin Oglesbee
6. Pacific Northwest National Laboratory (w/ Oregon Health & Science University) - David Koeller and Thomas Metz (contact)
7. Vanderbilt University Medical Center - Joy Cogan (contact) and John Phillips III

NIH IC Project Scientists (1 collective vote):

1. Anastasia Wise

D. Election of Steering Committee Co-Chairs

Guideline: The position of Chairperson of the Steering Committee of the UDN is filled by 2-3 co-chairs who serve overlapping terms. Co-chairs are selected by a vote of the UDN Steering Committee.

Principles:

1. The term of the position of Chair is 1-2 years in duration unless an extension is agreed to by the Steering Committee.
2. The individual holding the position of Chair must be a current member of the Steering Committee.
3. The Chair must be either the Principal Investigator of one of the CS or a Core Laboratory or the CC.

E. Executive Committee

The Executive Committee consists of the 2-3 co-chairs of the Steering Committee, the PIs of the CC, and the NIH Project Scientists. The Executive Committee meets weekly to review and monitor UDN progress.

F. Other Network Committees

Guideline: The Steering Committee may establish working groups as needed to address particular issues, which will include representatives from the program, the NIH, and possibly other experts. The Steering Committee has the overall responsibility of assessing and prioritizing the progress of the various working groups and other needed subcommittees of the working groups.

Working Group Governance:

1. Any individual or group proposing a new working group will present their idea to the Steering Committee. A formal vote of the Steering Committee is needed to create a new working group.
2. Volunteers for chair or co-chairs of the new working group will be solicited when the new working group is proposed. A formal vote of the Steering Committee is needed to confirm the chair or co-chairs.
3. Co-chairs are not required for all working groups, but may be recommended by the Steering Committee.
4. Working group co-chairs may come from the same site.
5. If there are no volunteers, or only one, the Steering Committee may recommend a site or type of site that may be a good fit for the working group and one of the Steering Committee co-chairs will solicit the site(s) for a recommended chair.
6. Any working group proposing to close will present their idea to the Steering Committee. A formal vote of the Steering Committee is needed to close a working group.

Active Committees and Working Groups:

1. Billing, Utility, and Utilization (BU3) Working Group
2. Biosamples and Biorepository Working Group
3. Case Review Committee
4. Clinical Protocols Working Group
 - a. Subcommittee: Site Operations
5. Commercial Collaborations Working Group
6. Environmental Exposures Working Group
7. Genetic Counseling & Testing Working Group
8. Metabolomics Working Group
9. Model Organisms Working Group
10. Publications and Research Committee
 - a. Subcommittee: Survey Committee
11. Sequencing Working Group
12. Sustainability Working Group
13. Tool Building Coalition Working Group

G. Implementing and Revising the Manual of Operations

1. Working groups are responsible for development and maintenance of related chapters for the Manual of Operations.
2. Chapters of the Manual of Operations are ratified by the Steering Committee.
3. Working groups have the authority to make decisions regarding implementation of ratified chapters of the Manual of Operations that are assigned to the working group for implementation.
4. If a working group cannot resolve an implementation decision internally, the Steering Committee is consulted.
5. Working groups consult with other relevant working groups on implementation decisions that involve multiple areas of expertise. A cross-working group liaison may be assigned to facilitate these interactions.
6. All working groups make their agendas and minutes available to other working groups.
7. Working groups that would like to recommend: 1) a change to a ratified Manual of Operations chapter that affects network-wide operations, or 2) addition of a new chapter, should recommend the change to the Steering Committee for ratification.
8. Groups that would like to recommend a change to the network-wide Institutional Review Board (IRB) protocol or consents should recommend the change to the Steering Committee.

II. Milestones and Metrics

One of the core functions of the NIH Program and the CC is to monitor each component of the network (CC, CSs, SCs, other Cores) as well as the network as a whole. The rationale for this monitoring is two-fold: (1) to encourage understanding and continuous improvement of components of the network and (2) to codify the expectations of our funders in order to ensure that our efforts are aligned with expectations.

NIH milestones are listed in the table below. The NIH Program may update these measures over the course of the project. It is anticipated that these metrics will be compiled and reported quarterly to the Steering Committee during the first two years of the UDN, after which the frequency of evaluation should be revisited by the Steering Committee.

NIH Performance Milestones	
1	By Oct 1, 2014 – UDN to establish working groups to develop the network-wide protocols for the UDN, including design, maintenance, and dissemination of the UDN Manual of Operations and central IRB protocol.
2	By April 1, 2015 – Clinical Sites work together with the UDN Coordinating Center to establish a network-wide application process and data standards for above-the-line data.
3	By Oct 1, 2015 – Establish collaboration amongst laboratory and clinical researchers, and share resulting data and approaches throughout the scientific and clinical communities.
4	By Oct 1, 2015 – UDN Coordinating Center to work together with the Clinical Sites to establish Gateway infrastructure for the UDN application process and above-the-line data.

5	By Oct 1, 2015 - Define the mechanism of at least 1 candidate gene in the pathophysiology of a rare or yet-to-be-described disease.
6	By Oct 1, 2015 - the NIH UDP will identify at least 5 candidate genes annually by working jointly with the UDN through FY2017.
7	By Sept 1, 2016 – 6 UDN extramural Clinical Sites to accept 35 patients, per site, in order to initiate evaluations.
8	By Sept 1, 2016 – UDN Sequencing Cores produce exomes/genomes for analysis in collaboration with the UDN Clinical Sites to identify candidate genes by providing raw data (FASTQ and BAM files) to the UDN Clinical sites within 2 weeks of sample receipt.
9	By Sept 1, 2016 - UDN Model Organisms Screening Center to have established the capability to screen 200 variants per year in collaboration with the UDN Clinical Sites.
10	By Oct 1, 2016 – The NIH UDP to work with the UDN Sequencing Cores to analyze the sequencing results of 300 individuals annually (new and returning).
11	By Dec 1, 2016 – UDN Coordinating Center to promote collaboration and data sharing by posting 50 new cases in dbGaP and other relevant databases; 100 new cases by Oct 1, 2017; and 300 new cases by Oct 1, 2018.
12	By Dec 1, 2016 – create a process for sharing gene variants identified through the UDN with the basic science community and sharing results of these studies back with the UDN.
13	By Jan 1, 2017 – Identify 10 unidentified diseases (previously unknown diseases, novel gene associations with known diseases, or novel phenotype associations with known diseases); by Jan 1, 2018, identify a cumulative total of 20 unidentified diseases.
14	By Aug 1, 2017 - UDN Metabolomics Core to establish background controls for various UDN sample types; and by Aug 1, 2017 to have established the capacity to analyze 150 samples per year in collaboration with the UDN Clinical Sites.
15	By Sept 1, 2017 – All 6 extramural UDN Clinical Sites to accept patients at a rate of 50 patients per year, per site, through FY 2017.
16	By April 1, 2018 – All 6 extramural UDN Clinical Sites to see patients at a rate of 50 patients per year, per site, to continue evaluations.

The CC calculates a range of measures to assess the performance of the network. Select metrics are available on the public website at <https://undiagnosed.hms.harvard.edu/about-us/facts-and-figures/>.

CS metrics calculated by the CC are described in the table below. It is anticipated that the CC will update these metrics over the course of the project.

CS metrics	
1	Length of Application Review: percentage of applications with a decision within 6 months of submission (target: 100%)

2	External Acceptances: percentage of accepted applicants coming from external sites (target: at least 50% external)
3	Adult Acceptances: percentage of accepted applicants >=18 years old (target: 40%)
4	Time between Acceptance and In-Person Evaluation: percentage of participants with an evaluation start date within 6 months of acceptance (target: not defined)
5	In-Person Evaluations Complete: percentage of evaluations complete relative to relevant milestone
6	Participant records approved for data sharing: percentage of evaluated participants with data sharing approved (target: 100%)
7	Samples Submitted to Biorepository: percentage of evaluated participants with samples in the biorepository (target: not defined)
8	Wrap Up Documents: number of evaluated participants with wrap up documents in the Gateway (target: not defined)
9	Concept Sheets Submitted: number of concept sheets submitted (target: not defined)

III. Institutional Review Board Communications

I. Consent Form Development

1. The UDN PI, Central IRB (CIRB), and CC CIRB Liaison will develop model informed consent and assent form (ICF) templates for the UDN, noting sections of the template that must be customized by each CS.
2. The CC CIRB Liaison will make the template ICFs available to the CS Site Coordinators.
3. The CS Site Coordinators will customize only the areas of the ICFs specified in the template, including:
 - a. Placing the consent form on the institutional letterhead
 - b. Adding standardized language as required by the CS (due to local policy requirements)
 - c. Incorporating HIPAA authorization for use and disclosure of PII if HIPAA is not available as a separate document, as per with the CS institution's standard approach. If HIPAA is provided as a separate document, it does not need to be submitted.
4. The CS Site Coordinators will send the completed site specific ICFs to the CC CIRB Liaison.
5. The CC CIRB Liaison will review the ICFs and send them to the UDN PI to submit to the CIRB.
6. The CIRB will review the site specific ICFs with all of the other submitted site materials provided for site approval.
7. The CIRB will communicate the results of the review to the UDN PI, the CC CIRB Liaison, the local PIs, and the Institutional Designees.
8. The CC CIRB Liaison will communicate the results of the review to the CS Site Coordinators.
9. The CIRB will provide to the CC CIRB Liaison the approved ICFs for each CS. ICFs will have a version control reference in the header and on the last page of the form.
10. The CC CIRB Liaison will make the CIRB-approved ICFs available to the CS Site Coordinators and will store centrally for all CSs to access.

II. Site documentation

1. Site documentation includes:
 - a. The principal investigators who will be included on the protocol, a roster of associate investigators, the site name, a description of the site, its location and Federalwide Assurance (FWA) number, documentation that UDN Site Human Research Protections Program (HRPP) training requirements have been met, and the name and contact information of responsible institutional officials.
 - b. Documentation of the local conflict of interest (COI) review for all investigators on the protocol indicating whether there are any unmitigated or existing conflicts.
 - c. Information about the UDN site's local research context as relevant to the site's role in the protocol.
2. The CC CIRB Liaison will send requests for documentation to the CS and Core Site Coordinators.
3. The CS and Core Site Coordinators will send completed documentation to the CC CIRB Liaison.
4. The CC CIRB Liaison will review and send the documentation to the UDN PI to submit to the CIRB.

III. Reportable Events

1. Unanticipated problems involving risks to subjects or others (including adverse events and protocol violations) and/or serious or continuing noncompliance will be reported by the CS and Core PIs directly to the UDN PI, who will report them to the CIRB. CC will request problem reports from the CS and Core PIs on a regular basis.
2. The CC CIRB Liaison will make a form, generated by the CIRB, available to the CSs and Cores to use for reporting the unanticipated problems to the UDN PI.
3. The CS and Core PIs will report all unanticipated problems or serious and/or continuing noncompliance within 7 or 14 days (depending on the nature of the unanticipated problem) to the UDN PI and copy the CC CIRB Liaison on all correspondences.
4. The UDN PI will report the unanticipated problem or serious and/or continuing noncompliance to the CIRB within a timeframe that does not exceed the timing allowed for the PI.
5. The CIRB will communicate the results of the review to the UDN PI, the CC CIRB Liaison, the local PI, the Institutional Designee, and in some cases, the Institutional Official related to unanticipated problems or serious and/or continuing noncompliance.
6. The CC CIRB Liaison will be copied on all correspondences between the CC, UDN PI, and the CSs and Cores.

IV. Continuing Review

1. Three months prior to the continuing renewal deadline, the CIRB will notify the CC CIRB Liaison regarding information required for Continuing Review (CR) and provide the forms that all CSs and Cores, including the intramural site, must complete.
2. The CC CIRB Liaison will notify each CS and Core Site Coordinator regarding information required for CR, and any other forms required by the CIRB.
3. The CS and Core Site Coordinators will submit their responsive information for CR to the CC CIRB Liaison within 2 months of the CR deadline.
4. The CC CIRB Liaison will review the UDN site forms for accuracy and completeness.
5. The CC CIRB Liaison will provide the individual site-specific CR forms as well as submit a single CR Application to the UDN PI.
6. The UDN PI will review the applications and submit all documents to the CIRB.
7. The CIRB will conduct CR of all submitted materials.
8. The CIRB will communicate the results of the review to the UDN PI, the CC CIRB Liaison, the local PIs, and the Institutional Designees.
9. The CC CIRB Liaison will communicate the results of the review to the CS and Core Site Coordinators.
10. **NOTE: All NHGRI protocols undergo review by the Scientific Review Committee (SRC) every three years. The SRC provides the UDN PI with a written review and a summary of outstanding comments and concerns. The UDN PI will provide the required materials to the SRC in the form of an amendment and no less than two months prior to submission to the CIRB for CR in order to permit sufficient time for SRC review.**

V. Amendments

1. Study-wide amendments
 - a. Study-wide protocol amendments will be reviewed with the Steering Committee

- before submission to the CIRB.
 - b. A completed amendment form with all supporting documentation, including tracked and clean copies of any modified documents, will be submitted to the CC CIRB Liaison. Study-wide amendments will be submitted to the CC CIRB Liaison the first Monday of the month.
 - c. The CC CIRB Liaison will finalize study-wide amendments and submit to the UDN PI.
 - d. The UDN PI will submit study-wide amendments to the CIRB, including tracked and clean copies of all modified documents with updates to the version control of each document.
 - e. If the study-wide protocol requires changes to the ICF:
 - i. The CC CIRB Liaison will modify the ICF model template.
 - ii. The CC CIRB will send tracked and clean copies of the modified ICF model template to the UDN PI to submit for CIRB review.
 - iii. Once the model template ICFs are approved by the CIRB, the CC CIRB Liaison will work with the CS Site Coordinators to modify each site-specific document.
 - f. The CIRB will communicate the results of the review to the UDN PI, CC IRB Liaison, the local PIs, and the Institutional Designees.
 - g. The CC CIRB Liaison will communicate the results of the review to the CS and Core Site Coordinators.
 - h. The CC CIRB Liaison will make available to the CSs and Cores the approved amendment documents.
 - i. If there are updated CS ICFs, the CC CIRB Liaison will make the ICFs available to the CS Site Coordinators and will store centrally for all CSs to access.
2. Study staff changes
- a. The CC CIRB Liaison will provide an amendment template for the CSs and Cores to complete that fulfills the CIRB requirements.
 - b. The CS and Core Site Coordinators will submit completed site-specific amendment forms with all supporting documentation, with tracked and clean copies of any modified documents, to the CC CIRB Liaison. Site-specific amendments will be submitted to the CC CIRB Liaison on the first and third Mondays of the month.
 - iv. For study staff changes, the local PI must sign the completed amendment template indicating conflict of interest review and completed training requirements.
 - c. The CC CIRB Liaison will submit the amendments to the UDN PI.
 - d. The UDN PI will submit the amendments to the CIRB.
 - e. The CIRB will communicate the results of the review to the UDN PI, CC CIRB Liaison, the local PI, and Institutional Designee.
 - f. The CC CIRB Liaison will communicate the results of the review to the CS and Core Site Coordinators.
 - g. The CC IRB Liaison will make available to the CS or Core the approved amendment documents.
3. Affiliated studies
- a. CSs and Cores may propose studies involving UDN participants that are not network wide.
 - b. These studies will require permission from the Publications and Research Committee. The UDN site carrying out an affiliated study will provide information

about the study, which may include a brief summary of the study, sites involved, nature and characteristics of proband involvement and consent required, and lead UDN investigator.

- c. If the aims of the study are distinct from the aims of the broader UDN study, a separate IRB protocol through the site proposing the study will be submitted. If the aims of the study are consistent with the aims of the broader UDN study, an amendment to the CIRB detailing the site-specific research will be submitted.
- d. The CC CIRB Liaison will keep track of these studies.
- e. The CC CIRB Liaison will inform the UDN PI and the CIRB of affiliated studies.

DUE DATES

Site-specific amendments are due to CC CIRB Liaison on the 1st and 3rd Monday of the month.

Study-wide amendments are due to CC CIRB Liaison on the 1st Monday of the month.

IV. Publications and Research

One parameter of UDN success is the number and quality of its publications and presentations. The purpose of this document is to establish a framework that facilitates and streamlines collaborative manuscript submission, as well as antecedent work, including meeting abstracts and presentations. The Publications and Research Committee (PRC) oversees the activities set out herein on behalf of the UDN Steering Committee, and report to it. Changes to the policy described herein, which are expected from time to time, must be approved by the Steering Committee. The publication policy applies to a proposed publication if the results are the product of research that the NIH UDN prime or sub-award funded.

A. Scope

1. To facilitate manuscript submission.
2. To provide input on abstract submission and scientific presentation, when requested.
3. To help the CC with content for the website and, if required, social media.
4. To maintain an up-to-date list of all presentations, abstracts, publications and proposals. The CC assists in tracking and coordinating projects.
5. Notwithstanding anything to the contrary in this document, the scope of PRC does not include evaluation of the scientific merit of any publication produced as a result of UDN participation.

B. Membership

The PRC is made up of one representative from each UDN site, core, and the NIH program.

C. Concept Sheets

Concept sheets are required for research projects, data analysis projects, grant applications, and UDN-led manuscripts. This includes but is not limited to: single-site projects, case reports, analysis of Gateway data from participants evaluated at other sites, and biorepository specimen requests from external groups. Concept sheets are encouraged but not required for manuscripts led by external groups. Manuscripts led by external groups should follow all guidelines outlined in section IV.D. Manuscript Review.

Concept sheets may be submitted online at https://hms.az1.qualtrics.com/jfe/form/SV_3CyZOKOuiEvnCx7 or by email to the CC (see Appendix 8: Research Concept Sheet). Once a concept sheet is submitted, the CC forwards the concept sheet to the PRC for review. The PRC reviews all concept sheets within 2 weeks of submission. The CC notifies submitters of approval at the end of the review period, including any feedback received from the PRC.

Concept sheets must be reviewed and approved by the PRC before substantial work begins. Investigators are encouraged to review existing concept sheets and manuscripts in the Publications and Research Log (found at: <https://hms.harvard.box.com/v/prc>) to assess redundancy. In addition to PRC review, investigators should note the following requirements for research projects and grant applications:

1. Research projects involving surveys require review by the Survey Committee prior to the PRC.
2. Research projects involving human subjects research require IRB review and approval.
3. Grant applications and research projects led by external investigators require a Steering Committee presentation and vote.
4. Grant applications and research projects involving external investigators and network-wide data and/or samples require a Steering Committee presentation and vote.

Note that UDN investigators do not need to contact each site individually to obtain approval for analysis of Gateway data from participants evaluated at other sites. Only concept sheet submission is required.

Concept sheets are not required when UDN investigators request biorepository specimens from participants evaluated at their own site.

D. Manuscript Review

Review process

All manuscripts must be reviewed and approved by the PRC prior to publication. Final manuscripts should be submitted to the PRC and journal of interest simultaneously. The PRC reviews all manuscripts within 2 weeks of submission. The CC notifies submitters of approval at the end of the review period, including any feedback received by PRC members.

Corporate authorship

The UDN should be acknowledged as a corporate author in the author list as “Undiagnosed Diseases Network”.

Member list

The UDN member list should be submitted to the journal of interest in accordance with the journal’s requirements for consortia. The UDN member list is updated on a quarterly basis and includes members from the sites as determined by the site PIs. When preparing manuscripts, authors should go to <https://hms.harvard.box.com/udnmemberlist> to download the current version of the member list.

Author indexing

When possible, authors included in the UDN member list should be indexed in PubMed.

Funding acknowledgement

All UDN papers (network-wide and local) must include the following funding acknowledgement:

Research reported in this manuscript was supported by the NIH Common Fund, through the Office of Strategic Coordination/Office of the NIH Director under Award Number(s) [INSERT AWARD NUMBERS]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The funding acknowledgement statement and award numbers are available at <https://hmsharvard.box.com/udnfa>.

Conflict of interest

The CC maintains a list of Conflicts of Interest (COI) for all UDN members at <https://hmsharvard.box.com/udncoi>. COI updates should be sent to the CC.

Notification of acceptance

Investigators should notify the CC when manuscripts are accepted and provide the expected publication date.

Notification of publication

Investigators should notify the CC when manuscripts are published.

Authorship guidelines

Authors (first, middle, and senior) are determined by the type, scope and site of project. First author takes primary responsibility for the manuscript. Given the nature of the UDN's work, shared first or last authors should be remembered as an option. Generally, it is expected that authors would make contributions to any or all of the following including but not limited to the concept, design, acquisition and analyses of data, drafting of manuscript, editing, and revision of manuscript. The PRC resolves all authorship disagreements.

Scope of manuscript review

PRC approval shall be for purposes of satisfaction of the points in this section alone and, for purposes of clarity, this means that the PRC shall not withhold approval of a manuscript on the basis of its scientific merit.

E. Abstracts and Presentations

Abstracts and presentations do not require PRC review. Abstracts and presentations should reference UDN grant number(s) and include "Undiagnosed Diseases Network" in the author list. Accepted abstracts should be sent to the CC so that they may be posted to the UDN CC website and added to the Publications and Research Log.

If there is an NHGRI or Common Fund co-author, final versions of the abstract must be submitted to the PO for review and approval.

F. UDN Publications and Research Log

The PRC advises the CC in management and population of the log of concepts, presentations, abstracts, and publications submitted, finished, and in process. This log can be found in Box.com (<https://hmsharvard.app.box.com/v/prc>) and the link is sent to the PRC each time a concept sheet or manuscript is circulated. The publications update sent to the PIs quarterly includes all current accepted and published manuscripts and concept sheets.

G. Start of UDN Publication

The PRC developed a manuscript, which described, defined, and introduced the network to the medical and scientific community: Ramoni, Rachel B., et al. "The undiagnosed diseases network: accelerating discovery about health and disease." *The American Journal of Human Genetics* 100.2 (2017): 185-192.

H. End of Funding Cycle Publications

The PRC is responsible for stimulating the preparation of manuscripts that describe the UDN experience towards the end of each funding cycle.

V. Communications

The purpose of this section is to describe the public-facing UDN websites and outline how the UDN engages with the public. It addresses both official and unofficial UDN social media communications. It provides guidance for UDN investigators interested in sharing information about the UDN on personal social media accounts and profiles. Guidelines for responding to media inquiries are also provided.

A. Website

Information about the UDN is made available on public-facing websites maintained by the CC, NHGRI, NIH Common Fund, and CSs.

- CC: <https://undiagnosed.hms.harvard.edu/> (alias: udnconnect.org)
- NHGRI: <https://www.genome.gov/27550959/>
- NIH Common Fund: <https://commonfund.nih.gov/Diseases/index>
- CSs
 - Duke: <http://www.dumcudn.org/>
 - Harvard: <http://www.bostonudn.org/>
 - Stanford: <https://undiagnosed.stanford.edu/>
 - UCLA: <https://www.genetics.ucla.edu/udn/>
 - UDP: <https://www.genome.gov/27544402/>
 - Vanderbilt: <https://mc.vanderbilt.edu/undiagnosed/>

The CC website is considered the primary source of up-to-date information about the UDN, as it contains the link to the Gateway, content related to the application process, CSs and core laboratories, current research, and press coverage. The CC website also includes contact information for the Helpdesk located at the CC. The NHGRI, NIH Common Fund, and CS websites are primarily for informational purposes.

B. Social Media

Official UDN social media communications

Since the launch of the Network, the CC has worked to cultivate a social media presence to 1) engage with potential applicants, referring providers, and collaborators, and (2) share the work of the UDN with interested stakeholders (e.g., general public, advocacy organizations, scientific community). A secondary goal is to share the UDN participant pages to identify similar patients.

When representing the UDN on social media, the CC:

- Carefully reviews all content for accuracy and relevance prior to posting on any social media platform
- Deletes spam and comments that are unrelated to the topic in question
- Replies to questions and comments when appropriate
- Avoids making political statements
- Notifies the Executive Committee and CS, if applicable, of comments that rise above the level of general questions or concerns

Official social media accounts managed by the CC include Twitter, Facebook, Reddit, and Instagram, which can be accessed searching for UDNconnect or visiting the following links:

- Twitter: <https://www.twitter.com/udnconnect>
- Facebook: <https://www.facebook.com/udnconnect>
- Reddit: <https://www.reddit.com/user/udnconnect/>
- Instagram: <https://www.instagram.com/udnconnect>

Unofficial UDN social media communications

Unofficial UDN social media communications are defined as communications about the UDN that have not been reviewed by a UDN investigator or NIH program staff member. Individuals and groups engaging in unofficial UDN social media communications are advised to include a disclaimer such as “Views are my own and do not necessarily reflect the views of the UDN or the NIH” on their social media profiles. They are also asked to route inquiries that may warrant or benefit from an official response to the CC.

Institutional social media communications

The CC engages with institutional social media accounts belonging to the NIH and UDN sites as appropriate. A list of these institutional social media accounts is maintained by the CC and is available to UDN members (<https://hms.harvard.box.com/v/communications>).

Best practices for personal social media accounts and profiles

UDN investigators are encouraged to share the work of the UDN on their personal social media profiles and engage with the official UDN social media accounts with the following guidelines in mind:

- Do not share sensitive or confidential information about UDN research activities or participants
- Avoid expressions that could be misinterpreted and should not engage in discourse that may reflect poorly on the UDN or the NIH
 - Example: politically charged tweets related to UDN funding
- Avoid making statements about unpublished UDN metrics and outcomes without appropriate context
 - Example: public Facebook posts about the high number of diagnoses made at your site

If the CC observes public social media posts made by a UDN investigator violate any of the above guidelines, the CC contacts the individual to suggest that the post be removed or made private. The CC notifies the UDN Executive Committee of such posts when appropriate, as determined by internal review at the CC.

Social media content suggestions

All members of the UDN are invited to send content suggestions for the UDN websites and social media accounts to UDN@hms.harvard.edu.

C. Media Inquiries

General guidelines

In general, UDN sites are welcome to respond to media inquiries directed at their site. If a site determines that the inquiry may benefit from additional perspectives, the site may opt to contact other UDN sites or the NIH. Comment requests related to NIH funding or other administrative issues should be directed to the CC for routing to NIH, NHGRI, and NIH Common Fund staff, as appropriate. Note that any comments from NIH staff require approval by one or more NIH communications offices. In some circumstances, the CC may engage experts at the UDN sites (e.g., individual with experience in broadcast television) when responding to media inquiries.

These guidelines do not apply to press releases and content developed by the UDN sites. In addition, they do not override institutional policies at the individual UDN sites.

Requests for interviews with participants

The CSs coordinate requests for interviews with participants. Cores and other sites that do not interact with participants should direct interview requests to the CC and include the following in writing:

1. An outline of the proposed interview
2. Criteria for selecting interview participants
3. Expected publication date

Once this information is received, the CC works with the CSs to identify participants or families appropriate for the interview.

Unpublished metrics

Each UDN site is welcome to comment on unpublished metrics for their site. However, the only network-wide metrics that should be shared with the media are those displayed on the UDN website at <https://undiagnosed.hms.harvard.edu/about-us/facts-and-figures/>.

VI. Technology

The UDN Gateway (<https://undiagnosed.hms.harvard.edu/>) is the centralized web-based patient and investigator portal for the UDN. The Gateway was built by developers at the CC to meet the unique needs of the network. The Gateway and integrated applications are administered by the CC in a manner that is compliant with Health Insurance Portability and Accountability Act (HIPAA), National Institute of Standards Technology (NIST) guidelines, and the Federal Information Security Management Act (FISMA) Moderate regulations.

Of note, each CS and Core uses local systems to track study data that falls outside the scope of the Gateway, such as medical records. The CC does not dictate which systems a given CS or Core chooses to use as long as that decision does not impair or threaten the overall security and compliance posture of the UDN data and technology network. Examples of local tracking systems include electronic medical record (EMR) systems, laboratory information management systems (LIMS), and REDCap. Data entered into these systems are stored securely and indefinitely under the oversight and policies of the individual CS and Cores.

A. UDN Gateway

Capabilities

The Gateway has two interfaces: patient-facing and investigator-facing. In the patient-facing interface, patients, parents/guardians, and referring providers are able to submit applications and study recommendation letters for consideration by the UDN. When an application is created, it is assigned a universally unique identifier (UUID) and a 6-digit UDN ID (e.g., UDN123456).

The investigator-facing interface is organized into CS- and Core-specific dashboards. This structure allows for sharing of personally identifiable information (PII) and personal health information (PHI) between UDN investigators.

Integrated applications

1. PhenoTips: The PhenoTips tool (<https://phenotips.org/>) is fully integrated into the Gateway to enable standardized phenotyping using the Human Phenotype Ontology (HPO). Features of PhenoTips include a dynamic pedigree editor, measurement and growth curves, and predictive ontology searches.
2. FileService: FileService is an application programming interface (API) that handles permissions, authentication, upload/download signing to cloud-based storage, metadata handling, searching and archiving of files. It is used as a service to the Gateway for managing secure authorized large file uploads and downloads.

Feature requests

The Gateway continues to evolve to meet the needs of the network. This evolution is primarily driven by requests for additional features. The feature request process described here applies to all Gateway feature requests, both large and small. Individuals or groups within the network are able to make feature requests. If a feature requester is able to provide funding for additional programming and support resources, the requested feature may be able to be addressed more quickly. To determine the resources required, the feature requester should speak with the CC

technology team. “Showstopper”/Critical bugs always jump the queue and take priority. These issues are sent to the CC technology team as they arise for immediate resolution.

Feature request process:

1. Feature requester completes the Feature Request Form (see Appendix 14: Feature Request Form) and sends the request to the CC at udngateway@hms-dbmi.atlassian.net.
2. The CC logs the feature request in the CC queue.
3. The CC technology team assesses the feasibility of the request from a technical and compliance standpoint. This may necessitate asking for additional information from the requester.
 - a. If the request is infeasible on a technical or compliance basis, the CC conveys this information to the feature requester and removes the feature request from the queue.
4. The CC technology team assigns an approximate time to complete the feature request.
5. The feature request is preliminarily prioritized by the CC, after which the Executive Committee vets the prioritization for presentation to and approval by the Steering Committee.
6. The CC technology team executes the feature requests in order of priority.

B. Data Security

Physical security

For physical records (paper, photographs, etc.), appropriate physical access controls (e.g., locked cabinet in a locked room) are used at all UDN sites.

Electronic security

For computer systems containing PII and PHI, security controls that are compliant with HIPAA, NIST, and FISMA are utilized and a proper Federal Information Processing Standard (FIPS) Publication 199 assessment will be performed prior to commissioning of these systems.

For computer systems that contain PII but not PHI, appropriate roles-based access and security controls are used and a third-party security assessment will be performed and documented.

For computer systems that contain neither PII nor PHI, roles-based controls are used and conform to the information security and compliance standards of affiliated institutions.

Auditing

The Gateway is assessed periodically via a risk-based approach to determine security, privacy, and compliance requirements. Local systems should have up-to-date virus scanners, disk encryption, workstation-level authentication, and lockouts on all machines accessing the Gateway.

VII. Data Sharing

A. UDN Data Sharing Principles

Data are expected to be shared so as to maximize the value of the significant public investment in the creation and operation of the network. In addition, given the special nature of the project, every effort should be made to share data in a timely and comprehensive manner. Individuals with undiagnosed conditions have often gone for extended periods of time without a diagnosis. Sharing results as rapidly and as broadly as possible holds the promise of accelerating diagnoses and identifying potential treatments for those with these often very serious conditions.

NIH policies

The UDN data sharing principles are consistent with the goals of the NIH Data Sharing Policy¹ which states that “Data should be made as widely and freely available as possible while safeguarding the privacy of participants, and protecting confidential and proprietary data.”² The UDN data sharing practices are aligned with the goals and requirements of the NIH Genomic Data Sharing Policy³. The Policy expects genomic data generated by NIH-funded research studies to be submitted to a relevant database no later than 3 months after data quality and control have been completed, and expects data to be released up to 6 months after data submission is initiated or at the time of acceptance of initial publication, whichever occurs first.

Expectations of research participants

Participants expect that their data will be shared as early and as broadly as possible in order to maximize the chances of reaching a diagnosis. Several participants have made their data available on the UDN website in the form of participant pages (<https://undiagnosed.hms.harvard.edu/participants/>).

Commitment of investigators

Investigators agree to further the mission of the UDN by creating new knowledge regarding the biochemistry, physiology, and mechanisms of undiagnosed diseases and improve diagnostic and management options for patients afflicted with them.

Investigators further agree to acknowledge that in pursuit of this mission, common UDN goals may supersede individual goals. Specifically, in the interest of rapid progress, investigators commit to:

1. Model a collaborative, open, interdisciplinary spirit, characterized by mutual trust and respect across disciplines, individuals, areas of expertise, institutions, and by demonstrating interest and engagement beyond their own specific domains.
2. Ensure that data generated at individual sites are comparable and additive by adhering to UDN data standards.
3. Make data available to the Gateway and external databases in a timely and effective manner.

The following outlines the minimum requirements for sharing the data that are collected in the course of participation in the UDN.

B. UDN Data Sharing Procedures

Data Sharing and Use Agreement

Policies and procedures for data sharing within and outside of the UDN are available (<https://hms.harvard.box.com/v/dsua>).

Informed consent and data security

At the time of application, individuals consent to have their data shared within the UDN. Each applicant is assigned a UUID and UDN ID by the Gateway. These identifiers are used for all data associated with that applicant. Role-based access and physical security controls that are fully aligned with the sensitivity of the data at each point of use and access are employed by the Gateway. De-identified data shared outside of the UDN does not reveal individual identifying information, consistent with the HIPAA Privacy Rule⁴. Individuals accepted into the Network give consent to have their data shared more broadly as part of a network-wide informed consent process.

Sharing identified data

With UDN investigators: All clinical, biospecimen, and sequencing data that are generated by the UDN effort are shared in a secure and compliant manner within the UDN. UDN data include data generated both in human subjects and laboratory research. All UDN investigators have access to the fully identified UDN data. UDN investigators who acquire UDN data must use the data responsibly and must monitor the use of the data by study personnel at their site.

With disease experts: In aid of a possible diagnosis, UDN data may be shared with disease experts who are not currently part of the UDN. In this case, the UDN CS that evaluated the participant must be notified that an outside expert has been consulted.

Sharing de-identified data

The UDN shares de-identified data in the following publicly accessible databases:

dbGaP: dbGaP⁵ (Database of Genotypes and Phenotypes) is an NIH-sponsored controlled access repository that was developed to archive and distribute the data and results from studies that have investigated the interaction of genotype and phenotype in humans. For each participant, phenotypic data entered into the Gateway and genotypic data, in the form of BAM and VCF files, are deposited in dbGaP.

PhenomeCentral: PhenomeCentral⁶ is a global repository and collaboration platform for secure data sharing in the rare and undiagnosed diseases community. PhenoTips⁷ is a web-based tool for collecting phenotypic data that is fully integrated with the Gateway. For each participant, phenotypic data entered through the Gateway and genotypic data, in the form of candidate genes, are deposited in PhenomeCentral.

ClinVar: ClinVar⁸ is a freely accessible, public archive of the relationships between specific human variations and phenotype. For each participant diagnosed with a genetic condition, variant level data will be shared in ClinVar. All data are submitted in accordance with ClinVar requirements. Currently, this includes: gene symbol, reference sequence, descriptions of DNA

and protein level sequence variants using Human Genome Variation Society (HGVS) nomenclature, variant type, transcript, cytogenetic and genomic positions, clinical significance and supporting evidence, demographic information, and phenotype information. This information is retrieved from the CS and the Gateway.

Additional databases: As data are generated by additional research cores, relevant databases for deposit of UDN data will be identified. These might include model organism databases, such as Flybase, Zebrafish Information Network, and the Mouse Genome Database, and metabolomics databases, such as the Human Metabolome Database and MetaboLights. In addition, the network will share data publicly on the UDN website. The UDN website⁹ is a resource that provides extensive information about the goals, activities, and outcomes of the network. Phenotypic data and candidate gene names are displayed. Sharing data on the UDN website could lead to the identification of additional patients with similar phenotypic and genotypic profiles, and could be useful for advancing scientific investigation and discoveries about these rare conditions.

Data sharing timelines

De-identified participant data must be ready for deposit in dbGaP and PhenomeCentral no later than 3 months after the in-person evaluation is complete and the initial sequencing report is uploaded to the Gateway. This means that phenotyping needs to be complete and, if relevant, candidate genes need to have been identified and entered into the Gateway.

De-identified participant data must be ready for deposit in ClinVar and for display on the UDN website no later than 6 months after the in-person evaluation is complete and the initial sequencing report is uploaded to the Gateway.

Although broad and timely data sharing are expected, UDN investigators have the option of delaying data sharing in certain unusual cases. A rationale for the delay and an estimated data release date must be submitted to the CC. The CC contacts the investigator shortly after the estimated data release date to assess progress and to determine if the data are ready to be shared.

Data release at publication

There is a separate publications section within this document that describes these policies. If broad data release is required as a condition of publication by the authors or the publisher, the CC should be contacted as soon as possible prior to making any commitments to ensure that the data release is feasible.

Role of the CC

The CC facilitates, monitors, and reports on the effective and timely sharing of data within the UDN and beyond.

C. References

1. NIH Data Sharing Policy. http://grants.nih.gov/grants/policy/data_sharing/
2. Final NIH Statement on Sharing Research Data, February 26, 2003. (<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html>)

3. NIH Genomic Data Sharing Policy. <https://www.federalregister.gov/documents/2014/08/28/2014-20385/final-nih-genomic-data-sharing-policy>
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9. The Undiagnosed Diseases Network. <https://undiagnosed.hms.harvard.edu/>

VIII. Clinical Protocols

A. Introduction

The clinical protocols component of the Manual of Operations “provides preliminary protocols and operating guidelines that will define an initial framework for common approaches to participant selection, data collection, laboratory investigation, and diagnosis, and serve as a base for further refinement by UDN investigators.” (From [RFA-RM-12-020](#)).

Background of the UDP (see Appendix 1: NIH UDP Protocol for additional information)

Delivery of medical care to patients with rare and yet-to-be described diseases can be fraught with repetitive, inconclusive efforts at diagnosis as patients and their families go from physician to physician in hopes of finding answers. The Office of Rare Diseases Research (ORDR) notes that 6% of individuals seeking their assistance have an undiagnosed disorder and as many as 15% remain in the undiagnosed category for at least 5 years as physicians labor to define cause and pathophysiology. To address these issues, the NIH UDP was established in May 2008, as a joint venture of the NIH ORDR, the National Human Genome Research Institute Intramural Research Program (NHGRI-IRP), and the NIH Clinical Research Center (CRC). The goals of the UDP were to:

1. Provide answers for patients with undiagnosed diseases;
2. Generate new knowledge about disease mechanisms;
3. Assess the application of new approaches to phenotyping and the use of genomic technologies;
4. Identify potential therapeutic targets, if possible.

UDN Clinical Protocols Working Group

The Clinical Protocols Working Group developed these clinical protocols as part of the Manual of Operations and, with input from the Steering Committee, continues to refine it. Should there be a need to vote on matters within the working group, each CS, the NIH Program, and the CC cast a single vote, for a total of 9 votes.

B. Detailed UDN Clinical Protocol

Study design

In this study, individuals with undiagnosed diseases, and their family members when applicable, are investigated. Applicants apply to the UDN through a secure website managed by the CC, called the Gateway, and are assigned to a CS based on an assignment algorithm. The CS collects and reviews the applicant’s medical records and accepts or does not accept the applicant. Accepted applicants typically are evaluated at the CS to which they were assigned; however, applicants may be reassigned to a different site based on presenting problems and the expertise of the site. Enrolled individuals undergo a comprehensive medical and family history, physical examination, laboratory testing, imaging studies, consultations, and biological specimen collection, typically over the course of up to a five-day evaluation. Follow-up visits may occur if indicated. See [ClinicalTrials.gov record](#) for a publicly available summary of the protocol.

Triaging and accepting applicants

Applicants (or their legal guardians) may learn about the UDN from a variety of sources, including the UDN website, publicity, or from another participant. Health care providers not associated with the UDN may learn of the UDN from sources including the UDN website, publicity, colleagues, or medical conferences or publications. Health care providers from CSs may refer their own patients for evaluation.

Individuals (or their legal guardians) register and apply to the UDN through the Gateway managed by the CC. In order to be considered for participation, individuals (or their legal guardians) are required to either electronically sign or verbally agree to a disclosure statement allowing the UDN to store the applicant data that is used to: a) assign the applicant to a CS for review, and b) collect characteristics of people who apply to the UDN. If an applicant (or legal guardian) does not speak English, a translator is used to facilitate the verbal consent process.

Once the applicant (or legal guardian) provides consent, either the applicant (or legal guardian), their referring provider, or a CC representative enters the following data into the Gateway:

1. Applicant's name, date of birth, gender, self-described race and ethnicity (for children <18 years the names of their parents are required).
2. Applicant's mailing address, contact information (email address, phone number). For children, the addresses and contact information for both parents are required. If parents are divorced or separated, they must provide information regarding who is legally permitted to sign a consent for medical research on behalf of the child. If parents are separated or divorced they must also both be willing to: (1) provide family history information, and (2) submit DNA samples for genomic analysis. If an adult applicant is unable to consent, the name and contact information of the individual with legal power of attorney who is able to consent on the applicant's behalf must be provided.
3. Evaluation history
4. UDN site preference
5. Travel limitations
6. Referring provider's name and contact information (mailing address, email address, phone number, fax number).
7. Applicant's chief complaint, identification of the system most involved (i.e., cardiac, gastrointestinal), and symptom onset.
8. Environmental exposures. Probands are sent an email asking them to complete an environmental exposures questionnaire. The survey is administered through Qualtrics and data is stored in Qualtrics and in the Gateway. If participants are unable to complete the survey online, paper surveys can be requested. Completed paper surveys are sent to the CSs for data entry. Survey responses are used by the CSs to better inform the evaluation.

Either the applicant (or legal guardian), their referring provider, or a CC representative also uploads to the Gateway a study recommendation letter (see Appendix 2: Example Study Recommendation Letters) summarizing the following: pertinent medical problems, prior diagnoses, history of evaluations and tests, medications, family history, review of systems, and provider's diagnostic impressions.

If an applicant (or legal guardian) does not speak English, a translator is used to facilitate the application process. If an applicant does not have access to the Internet, a paper application can be requested through the CC. Completed paper applications are mailed to the CC for data entry and CS assignment. Applicants (or their legal guardians) are instructed to refrain from

sending additional information, including any records, until assigned to a CS. If an applicant (or legal guardian) or their referring provider sends materials to the CC, the CC holds them until the applicant is assigned a CS, at which time the materials are sent to the CS.

The application is assigned to a CS for triage to determine if the applicant is appropriate for acceptance into the UDN at the assigned site. The assignment to a site is based on an assignment algorithm that takes into account the individual's location, pertinent medical problem(s), site workload and site preference. If a given site is the closest and/or best suited to see a given subject but has already reached enrollment quota, the case would likely be assigned to a different site.

If an application is deemed inaccurate or incomplete by a CS (e.g., study recommendation letter not written by a health care provider), the CS contacts the CC. The CC reaches out to applicants accordingly.

The CS makes initial contact with the applicant within 30 days of application submission to request that he/she send all information they have related to the reason for their application to the UDN. This may include: medical records, reports, laboratory studies, radiographic studies, photographs or videos, and pathology slides and reports. If the applicant (or legal guardian) has difficulty obtaining the medical records (including imaging and pathology materials), the site may also contact the referring provider for more information with appropriate release of information from the applicant. Records are reviewed by the CS for completeness. The CS requests any missing components (e.g., images, biopsy slides). The site director or his/her designee assigns the records for review to consultants based upon the specialty involved.

The CS reviews the applicant's records and study recommendation letter and makes a recommendation regarding acceptance into the UDN. (See Appendix 3: Applicant Review Form.) Since few individuals can be accepted into the UDN each year due to limited resources, preference is given to applicants for whom there is the greatest potential to provide a diagnosis or generate new knowledge about disease mechanisms. Inclusion and exclusion criteria are outlined below.

Inclusion criteria:

1. The applicant has a condition that remains undiagnosed despite thorough evaluation by a health care provide and has at least one objective finding.
2. The applicant (or legal guardian) agrees to the storage and sharing of information and biomaterials in an identified fashion amongst the UDN sites, and in a de-identified fashion to research sites beyond the network.

Exclusion criteria:

1. The applicant has a diagnosis that explains the objective findings.
2. Review of the records suggests a diagnosis and further evaluation by the UDN is deemed unnecessary.
3. The UDN is unlikely to improve on the comprehensive workup the applicant already received.
4. The applicant is too seriously ill to travel safely to the CS and telemedicine/distance consult is not possible or appropriate.

The Case Review Committee meets on a regular basis (weekly or biweekly) to review (at least initially) all cases at the CSs that have been recommended for acceptance, assign an applicant to a different CS if it feels that the applicant may be more appropriate for another CS based on expertise, and discuss challenging cases. Each CS and SC designate two Case Review Committee members and at least one alternate. Each CS has at least one clinician present at any Case Review Committee meeting. Ordinarily, a pediatrician and an adult internist from each CS are involved in each meeting. The co-chairs of the Committee rotate among the CSs every 6 months. One of them attends each meeting. If both are conflicted for a meeting, then a member of the Committee is asked to chair.

The Case Review Committee meeting is a forum for the CSs to concisely present participants to the UDN clinicians for review and input. The participants fall into two general categories, 1) those that the CS has accepted for evaluation and 2) those for whom the CS is uncertain, has questions about, or thinks may be better served at another CS. Sites are encouraged to present questionable cases for discussion rather than making acceptance decisions at the site level. Each CS presents a one-page summary of the case, and any imaging or pictorials that aid in the decision to accept for evaluation. Presentation and discussion of each case lasts no more than five minutes. If two CSs need prolonged discussion about an applicant, this is taken off line after the meeting. Cases are presented in the order they are submitted to the Case Review queue in the Gateway. The meetings terminate after an hour or 90 minutes. The Case Review Committee co-chairs move the meeting forward and take discussions off line if necessary. The decision to invite an applicant for evaluation is made primarily by individual CSs, except those for whom the CS is uncertain, has questions about, or thinks may be better served at another CS. The meeting exists to add value to the intended evaluation and to allow the UDN to be informed of the composition of the participant study population,

It is expected that about half of cases are pediatric, half adults. No more than half should be patients known to a CS, half completely new to any CS. "Patients known to a CS" are defined as any patient that is recommended by a health care provider from any of the institutions that are on that CS's award. A patient recommended by an outside provider is not considered "known to a CS" even if s/he has been seen previously at an institution on that CS's award.

A member of the Case Review Committee may also identify when s/he believes that an accepted or pending case falls outside of the inclusion and exclusion criteria specified in the protocol. In this case, a vote is called. Each eligible CS submits a vote to the Committee co-chairs. The CC, NIH program, and Cores do not have votes. If a majority vote of the Case Review Committee identifies a deviation, the CS may request the opportunity to do a more in-depth review of the case at the following meeting, which would be followed by a final majority vote. If a deviation is finally determined, a pending case would not be accepted, while an accepted case would be addressed in consultation with the IRB.

Application outcomes include:

1. Applicant and site appropriate for acceptance
2. Applicant appropriate for acceptance but reassigned to a different site
3. Applicant requires further testing or evaluation and may be reconsidered following receipt of the results
4. Applicant not appropriate for UDN
 - i. A diagnosis was identified based on the review.
 - ii. A potentially beneficial referral was identified based on review. An evaluation at the UDN may not be necessary to make a diagnosis.

- iii. The applicant is more appropriate for an expert site outside of UDN such as a research protocol at the NIH or elsewhere, the applicant is more appropriate for an expert at an academic medical center or elsewhere, or the applicant is not appropriate for the UDN and no alternative can be identified.

In all cases the referring provider and the applicant is informed of the decision, generally within 60 days after receipt of all the medical records. The CSs send out the disposition letters whether they accept or not accept. (See Appendix 4: Template Letters for Patients and Health Care Providers). When individuals are not accepted into the UDN, their application information is stored securely and indefinitely in the Gateway. More information related to applicants that are not accepted can be found in Appendix 11: UDN Standard Operating Procedures for Not Accepted Applicants.

Genetic testing

In some cases, it may be useful to have the results of genetic testing (whole-exome/whole-genome sequencing (WES/WGS)) from the enrolled proband and relevant family members prior to the clinical evaluation. Genetic testing is only performed on individuals accepted into the UDN. The sequencing is done at the Baylor College of Medicine. The decisions regarding timing of sequencing and WES versus WGS are made on a case-by-case basis as clinically indicated, and left to the discretion of the CS responsible for the evaluation.

If review of the proband's presenting medical problem(s) and medical records suggest that performing sequencing prior to the evaluation would be beneficial and aid in diagnosis, informed consent is obtained over the phone or by videoconferencing (or in person if reasonable) from the enrolled proband, parent, or guardian. The consent form is sent (by mail or email) to the proband (or legal guardian) prior to the remote or in-person consent. The PI, an associate investigator, a genetic counselor, or a project coordinator trained in consenting is available to answer questions and obtain consent. Consent is obtained at this time for the entire study, including for 1) obtaining blood for DNA extraction and sequencing, 2) any research studies performed as part of the evaluation, 3) obtaining other samples (blood, urine, etc.) during the evaluation for research, and 4) the collection of all of the clinical and research data by the UDN for research use. The signed consent forms are sent to the CS responsible for the evaluation and uploaded to the Gateway managed by the CC. The CS also records the consent version signed by the proband and proband's preferences in the Gateway managed by the CC. Genetic counseling is also provided by a physician or genetic counselor to all probands during the consent process. This genetic counseling includes a discussion of the types of results an individual may or may not receive (including primary and medically actionable findings), the likelihood of receiving these types of results, individual preferences for types of results returned, and limitations of the genetic testing including false negative and false positive results. Participants are given the choice of receiving medically actionable findings related to conditions with treatment or management options. Genetic counseling aids were developed to supplement this session.

Assent is obtained over the phone (or in person if reasonable) in the presence of a parent or guardian for all children ages 7-17 years old who are not decisionally impaired. Assent is given for the entire study, including assent for 1) obtaining blood for DNA extraction and sequencing, 2) any research studies performed as part of the evaluation, 3) obtaining other samples (blood, urine, etc.) during the evaluation for research, and 4) the collection of all of the clinical and research data by the UDN for research use. The signed assent form is sent to the CS

responsible for the evaluation and uploaded to the Gateway. The CS also records the assent version signed by the proband in the Gateway.

Once a signed consent form has been received from the proband (or legal guardian), a family history is obtained over the phone (or in person if reasonable) to identify other family members of interest related to the proband's phenotype. Family members are recruited through the proband, i.e., the researchers ask the proband (and/or legal guardian) for permission to contact the family members. Priority is given to those family members who would be most informative for sequencing analysis. Informed consent is obtained over the phone or by videoconferencing (or in person if reasonable) from interested family members. The consent form is sent (by mail or email) to the family member prior to the remote or in-person consent. The principal investigator, an associate investigator, a genetic counselor, or a project coordinator trained in consenting obtains consent and is available to answer questions. Consent is obtained for the collection of blood for DNA extraction and sequencing and for the collection of all of the clinical and research data and pertinent lab specimens for research use. The signed consent forms are sent to the CS responsible for the evaluation and uploaded to the Gateway. The CS also records the consent version signed by the family member and family member's preferences in the Gateway. Genetic counseling is also provided by a physician or genetic counselor to all family members. This genetic counseling includes a discussion of the types of results an individual may or may not receive, the likelihood of receiving these types of results, individual preferences for results returned, and limitations of the genetic testing including false negative and false positive results. During the consent, family members are given the choice of receiving medically actionable findings if they are identified. Genetic counseling aids were developed to supplement this session.

Once a signed consent or assent form has been received from the proband and family member(s), a kit is sent to the proband and family member(s) for obtaining DNA. The kit includes: tubes for blood collection, an order form, directions for payment by the CS (direct costs for the collection and shipping of samples are covered by the CS), and an addressed shipping container for the blood to be sent back to the CS. It is expected that the blood collection is completed with the assistance of the proband's local health care provider or local laboratory.

For probands selected for pre-evaluation sequencing, DNA is extracted at the CSs and sent to the SC, where the sequencing is performed and analyzed prior to the evaluation in order to have the analysis available by the time the plan for the proband's evaluation is finalized. Clinically significant variants are identified using standard programs for assessing pathogenicity, Mendelian segregation patterns, allele frequencies, and databases of benign variants. Through this process, medically actionable findings may be identified. Findings intended to be reported to probands or participating family members for use in clinical decision making are confirmed by Sanger sequencing in a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory.

If possible, results of genetic testing are disclosed to the proband (or legal guardian) or family member during an in-person genetic counseling session at the CS. However, since some participants may have to travel a significant distance to get to the CS, results of genetic testing may need to be disclosed to the participant (or legal guardian) in a genetic counseling session over the phone. This may occur when blood is drawn for sequencing as part of the evaluation or when the participation of a non-local family member is limited to a blood sample for DNA analysis and a medically actionable finding has been identified. CLIA-certified results of genetic testing are provided to probands (or legal guardians) during a genetic counseling session with a

qualified physician or genetic counselor. Genetic results related to the indication for testing are returned to all probands (or legal guardians) and their referring providers.

If a primary finding is identified, it is listed on the proband's genetic testing report. If other family members undergo genetic testing, the report may include information about inheritance and other family members that carry the primary finding. A parent of a proband may therefore learn information about his/her own genetic status or the status of his/her relatives when receiving his/her child's results. If this report is shared with relatives, they may also learn information relevant to their own genetic results. These results are discussed during a genetic counseling session with a qualified physician or genetic counselor.

The SC reports medically actionable findings in variants in the genes recommended for such reporting by the American College of Medical Genetics and Genomics. In addition, the SC reports other medically actionable findings beyond the currently recommended genes, provided these additional findings meet the threshold of having a defined medical treatment or specific management guidelines for disease surveillance. Stringent criteria for interpretation of variants in these medically actionable genes is applied; reported medically actionable variants have either been previously reported as pathogenic or expected to be pathogenic based on the usual molecular mechanism associated with the gene.

EXAMPLES OF MEDICALLY ACTIONABLE CONDITIONS NOT INCLUDED ON THE ACMG LIST	
Syndrome/Disorder	Gene
Progressive Familial Heart Block, Type 1B	<i>TRPM4</i>
Long QT type 4	<i>ANK2</i>
Familial Gastric Cancer	<i>CDH1</i>
Von Willebrand disease	<i>VWF</i>

Carrier status for autosomal recessive conditions may also be identified. Examples include cystic fibrosis and Tay-Sachs disease. Individuals who are carriers of a specific genetic disorder have one normal, working copy of the gene, and one changed copy of the gene. Because they have one working copy of the gene, carriers do not usually have symptoms of the disorder. Findings without medical treatment or specific management guidelines are not reported by the SC. Examples of these types of disorders include Alzheimer disease and late onset skeletal dysplasias (e.g., spondyloepiphyseal dysplasia - *TRAPPC2* gene).

During the pre-test genetic counseling/informed consent session, probands (or legal guardians) are given the option of receiving genetic results that are unrelated to the indication for testing, including results related to: (1) medical conditions with treatment or management options, known as medically actionable findings, and (2) carrier status findings (only applicable for adult probands). The proband's genetic testing results are shared with family members only if the proband (or legal guardian) provides permission. During the pre-test genetic counseling/informed consent session, family members are given the choice of receiving medically actionable findings if they are identified. A search for medically actionable findings will not routinely be performed in family members. However, medically actionable findings may be discovered by chance during the genetic testing process.

If a medically actionable finding is detected in a proband and is also present in a family member who consented to receive medically actionable findings, the finding will be returned on the family

member's report. If a medically actionable finding is present in a family member but not in the proband, it will not be detected or reported by the SC. If a medically actionable finding is detected in a proband and a family member who is not enrolled in the UDN is interested in learning if they also have this finding, the family member will be referred to a clinical genetics program for genetic counseling and testing. As this counseling and testing is not done as part of the UDN, cost associated with this clinical follow-up is billed to insurance. It is important to note that the lack of reportable medically actionable findings does not rule out any disease-causing genetic changes. If a medical concern is raised regarding a specific condition, further genetic testing may be warranted.

There are cases where the results of the sequencing performed prior to the evaluation point to a likely diagnosis and in these cases, the CSs are strongly encouraged to continue with the complete evaluation of the individual. The evaluation allows the site to collect phenotypic data about the condition, provide counseling, and make suggestions about management. Exceptions are cases where the diagnosed condition is common enough that established management standards exist and the presenting phenotype is a typical presentation of that disorder. If the clinical presentation varies from the typical clinical presentation of a well-recognized disorder, then phenotyping of the proband is still appropriate.

Planning the evaluation

Once a proband has been enrolled into the UDN and assigned to a CS, the site works with the proband (or legal guardian) and the local team to create a plan to maximize the efficiency of the evaluation. Information gathered by the CS prior to the evaluation includes:

1. Recent medical records (including consultation reports)
2. Previous tests and results
3. Pathology data/slides
4. Imaging/radiography results
5. Review of the medical history and review of systems
6. Family history (including name, age, and contact information of family members)
7. Medication list (including doses, schedule)
8. Contact information for the proband's relevant health care providers
9. Environmental assessment
10. Nutritional assessment
11. Proband needs for travel and admission (including ventilators, mobility issues, etc.).
12. Optional: additional surveys and interviews

The CS creates a plan for evaluation that includes:

1. Determining the lead physician.
2. Determining clinical tests, procedures, consults, or research studies to be performed, including a determination of whether additional IRB approvals are required.
3. Scheduling dates for the proband evaluation – typically evaluations occur over several days (expected to be five sequential days in most cases).
4. Determining the sequence and schedule of tests, procedures, and consults.
5. Arranging a “sedation day” if indicated (especially important for children to maximize the efficiency and minimize the number of times a child needs to be sedated for a procedure or test).
6. Arranging travel based on medical needs.

In-person evaluation schedule

On day 1 of the evaluation, if the proband (or legal guardian) has not already provided consent, consent is obtained for: 1) drawing blood for DNA extraction and sequencing, 2) any research studies performed as part of the evaluation, 3) obtaining other samples (blood, urine, etc.) during the evaluation for research, and 4) the collection of clinical and research data. If not already provided, assent is obtained for all non-decisionally impaired children 7-17 years of age. Following the consent, the CS records the consent version signed by the proband and proband's preferences in the Gateway. Probands may also be consented for other research projects at this time.

The initial visit with the primary care team, including the lead physician, also typically occurs on day 1. This visit often involves review of the medical history, review of the family history, performance of a physical examination, genetic counseling if results of genetic testing are available, surveys and interviews for research, and review of the goals of the visit and schedule. Changes in the schedule based on this initial visit are often made.

On subsequent evaluation days, all tests, procedures, and consultations take place. Clinical investigations during the evaluation may include: laboratory testing, imaging studies, and biological specimen collection. Genetic counseling may occur if results of genetic testing are available. Specialized research studies, such as proteomics, metabolomics, and functional studies, may also be performed to elucidate underlying mechanisms of disease. Surveys and interviews for research may also be administered and consultations/counseling sessions may be recorded (if proband and/or family members give permission). Surveys and interview guides are submitted to the IRB for review prior to their use. For individuals who did not undergo genetic testing prior to the evaluation, if it is determined during the visit that genetic testing is clinically indicated, blood is drawn and DNA is extracted and sent to a SC for sequencing.

On the last day of the evaluation, the team meets with the proband and family to summarize the evaluation and make plans for follow-up.

During the course of the evaluation, clinical diagnostic studies are performed as clinically indicated and within the standards of accepted medical practice. Specialized research studies are performed as deemed relevant. Clinical specimens are collected as medically indicated and at the discretion of the CS where the proband is being evaluated. Recommendations for research specimen collection are presented in the Biospecimens section of this document.

Remote evaluation

Remote evaluations are not generally expected but may occur if a proband is not able to travel to the CS due to medical instability or other reasons. CSs may collaborate with the referring health care provider(s) and/or local expert(s) to perform critical aspects of the evaluation, such as sample collection and physical examination. CSs may utilize videoconferencing to speak with the proband and/or family members regarding the evaluation; when doing so, CSs should select videoconferencing platforms that are consistent with their institutional policies. CSs may also send members of the study team to the participant's home to collect important samples, although this is expected to be a rare occurrence.

Unanticipated non-genetic medical information

During the course of this study, it is possible that unexpected medical information is discovered that is important to the proband's health care. This information is provided to the proband's health care provider. At the time, the proband (or legal guardian) is given the option of learning this information and referrals are provided as needed.

Change in clinical stability

If, during the course of the evaluation, the proband has a significant change in clinical stability requiring escalation of care or initiation of new treatments not covered by the research protocol, the proband may be offered completion of the UDN protocol at a later date. Probands and their caregivers, as well as referring providers, are apprised of this change of condition necessitating active treatment rather than research-based investigation. If the proband's condition does not allow discharge from the CS at the scheduled completion date, care is assumed either by the referring provider or appropriate clinical team members at the CS. Payment for further acute care is provided by the participant's insurance company.

Withdrawal procedures

During the UDN study, applicants and participants may withdraw or be withdrawn by UDN investigators. Withdrawal scenarios include, but are not limited to, the following:

1. Applicant or participant elects to withdraw
2. Applicant or participant is not compliant with study procedures or does not follow instructions given by UDN investigators
3. Applicant passes away before full study enrollment
4. Applicant is accepted, but is unable to be evaluated
5. Applicant is accepted, but site is unable to contact to schedule evaluation

If the applicant or participant needs to be withdrawn, the applicant or participant sends a written withdrawal to the PI at the CS (if possible). The site coordinator destroys the applicant or participant's research records and specimens at the CS. The site coordinator then contacts the CC to remove the applicant or participant's Gateway record. The CC changes the status to withdrawn on the Gateway and in written network records. If an individual was a participant, the CC marks the Gateway patient record as inactive. The site coordinator also contacts the biorepository to destroy the participant's research specimens and collaborators to destroy research records or specimens, if possible. If the individual was a participant, the site coordinator saves the signed consent form with the written withdrawal (if available).

Evaluation wrap-up

At the conclusion of the evaluation, and prior to discharge, the lead physician and other members of the care team as appropriate meet with the proband and family to: summarize the results of the clinical evaluation (clinical and research tests performed, procedures performed, consultations provided, results of testing received, and pending test results), provide genetic counseling as indicated, make recommendations for follow-up with the medical home team, provide clear instructions about how to contact UDN team members if additional questions or concerns arise, and answer any questions the proband or family may have. This wrap-up is facilitated using a structured wrap-up form (see Appendix 5: Wrap-up Template).

The wrap-up report and a short letter highlighting the key findings and follow-up recommendations accompanies the transfer of records to the referring health care provider and

other providers designated by the proband or family. The wrap-up report and a narrative summary of the evaluation are also uploaded to the Gateway.

All consultation and laboratory study reports pending at the time of discharge are included in a revised wrap-up report sent to the proband, referring provider, and any other care providers the proband has designated. If additional revisions occur, updated and revised wrap-up reports are issued.

Return visits to the UDN site

Follow-up visits to the CS are not generally expected but may occur under at least two circumstances: (1) the CS requests additional phenotyping of the proband or family members to clarify or inform “affected” status or further interrogate candidate genes, and (2) a diagnosis has been made and the family returns for delivery of results.

Billing procedures

The RFA stated that the CSs could bill participants’ health insurance for clinically indicated evaluations, procedures and tests, and use grant funds for underinsured or uninsured participants. The CSs were also required to provide participant transportation and lodging/meals during the one-week stay at the CS. These practices would ensure that participants did not incur out-of-pocket expenses and enable all subjects to have access to the UDN, irrespective of their health insurance status. This would also allow all participants the same experience as at the NIH UDP with no out-of-pocket expenses. However, while establishing billing procedures at the six CSs outside of the NIH UDP, it became evident that there were several challenges to implementing these practices. All the CSs were told by institutional representatives that insurance co-pays and deductibles could not be waived or reimbursed by the grant or institutional funds, due to a federal anti-inducement law that is framed for Medicare and Medicaid but is often applied to other insurance policies {42 U.S.C. § 1320a-7a(i)(6)}. Two sites were told by institutional representatives that they could not both bill insurance and pay for participant travel/lodging due to a federal anti-kickback law {42 U.S.C. § 1320a-7b}.

This led to the formation of a Billing Working Group to resolve the issues so as to not place the CSs and the participants at an undue disadvantage. After considering the legalities and the available choices, two billing options were created. The first option utilizes grant funds solely to cover all the participant evaluations, made feasible by institutional discounts (~80%) for participant care performed as part of NIH-funded studies. The second option bills the participant’s insurance companies for the clinical evaluations and cover underinsured/uninsured participants or tests not reimbursed by insurance with grant or institutional funds. Each CS can choose which option is best based on their institutional policies. In addition, each CS can re-evaluate and change to the other option based on their institutional policies.

To enable payment of co-pays and deductibles at sites that would bill insurance, the UDN is collaborating with the National Organization for Rare Disorders (NORD, <https://rarediseases.org/>). NORD has established a UDN patient assistance fund with contributions from the Running for Rare Diseases team. Each CS, including the NIH UDP, will receive \$5,000 of the NORD fund (total: \$35,000) to pay for tests needed for participants before being accepted into the UDN. This amount is utilized at the discretion of the CSs. Each CS will also receive \$5000 of the NORD fund (total: \$35,000) to pay for record retrieval through PicnicHealth. The remaining NORD fund is utilized by the three CSs that bill insurance to reimburse co-pays and deductibles for financially stressed participants (defined as those with an

income below 300% of federal poverty guidelines). This plan would allow for participants at each of these three CSs to be reimbursed \$6600 each, the maximum out-of-pocket expenses limit for an individual health insurance plan, as outlined by the Affordable Care Act (www.healthcare.gov).

Similarly, a collaboration with Mercy Medical Angels (<http://mercymedical.org/>) allows for provision of commercial air travel expenses for participants who are financially stressed. A memo of understanding has been signed between the UDN and Mercy Medical Angels. Each CS will decide if and when they want to use Mercy Medical Angels to arrange travel for the participant and one caretaker meeting the financial criteria (defined as having an income below 300% of the federal poverty guidelines). The CS will provide documentation of financial need and notify Mercy Medical Angels at least two weeks prior to the date of travel to allow sufficient time for them to make the travel arrangements. The CSs will pay Mercy Medical Angels a \$200 per ticket administrative fee from their grant funds and Mercy Medical Angels will arrange the travel for these participants and their family member.

The Billing Working Group merged with the Utility and Utilization in the UDN (U3) subcommittee to form a Billing/U3 (BU3) Working Group and continues to review issues that arise during the project, and significant changes to the billing structures and/or NORD fund distributions will be presented before the Steering Committee for consideration.

C. Post-evaluation Activities and Follow-up

Transitions of care

Transitions of care programs are designed to promote the safe and timely passage of patients between levels of health care and across care settings. In the context of patient management, suboptimal transitions of care may result in: readmissions, adverse drug events, use of higher-intensity setting of care, decreased functional status, reduced quality of life, unnecessary repetition of tests or procedures, avoidable costs, and/or additional stress on patients, families, and caregivers. Suboptimal transitions of care are a risk for participants undergoing diagnostic evaluations performed by the UDN due to the potential for poor communication between providers; inadequate participant, family, and/or caregiver understanding of findings and follow-up needs of the participant; incomplete diagnostic evaluations; and a lack of clear understanding of which results are returned and which are pending. The purpose of the transitions of care plan within the UDN is to avoid these outcomes.

Best practices at the CSs for transitions of care include providing a written summary of the diagnostic workup to the family upon departure (see section “Clinical evaluation wrap-up”), confirming that probands have made it home safely (via text message, email, or phone call), being available to families and caregivers in case any clinical issues arise, and maintaining open lines of communication.

In order to facilitate communication after the UDN site visit, the CC conducts a series of participant and family surveys.

Obtaining participant feedback

The CC and/or other UDN investigators remain in contact with participants and families after discharge from the CS, which may include contacting participants shortly after the visit to assess satisfaction with the UDN visit and understanding of recommendations, as well as contacting participants periodically to assess clinical and research status. Survey instruments and interview guides were created, vetted by the CC and Genetic Counseling and Testing Working Group, and approved by the CIRB.

Survey summary

Time point	Survey administered
Post-visit (ideally 7-14 days after the in-person evaluation ended)	Care outcomes (Interpersonal Processes of Care (IPC) Survey – modified)
6 months post-visit	Participant-specific clinical and research outcomes (6-month version)
Yearly post-visit	Participant-specific clinical and research outcomes (yearly version)

The immediate post-visit period is a delicate time for participants and families, but is also a unique opportunity to measure strengths and weaknesses of the UDN site visits. A participant and family satisfaction survey after the visit allows the CC to directly assess the participant experience at the CSs.

The CC measures aspects of the participant and family experience with the Interpersonal Processes of Care (IPC) Survey¹⁻³. The IPC Survey is a patient and family reported, multidimensional instrument appropriate for individuals from diverse racial and ethnic groups. The survey assesses several sub-domains of communication, patient-centered decision making, and interpersonal style. It is based on data from a sample of over 1,600 adult patients of diverse racial and ethnic background (African Americans, English- and Spanish-speaking Latinos, non-Latino Whites) and was developed to allow reliable, valid, and unbiased comparisons across these groups. The survey was also created to describe disparities in interpersonal care, predict patient outcomes, and examine outcomes of quality improvement efforts to reduce health care disparities. In addition, open-ended questions are asked to assess how the visit went, what went well and what did not, and areas for improvement.

The 6-month and yearly post-visit surveys are participant-specific clinical and research outcomes surveys. The timing of these surveys was chosen for the following reasons: 1) accounts for possible delays in receiving final test results; 2) enough time has passed to assess new treatments; and 3) any problems with the transitions of care to the home team can be identified. At these time points, new topics are introduced, such as whether the investigators discussed research options. These surveys also address the “coordination between health settings” element of the participant experience in more detail.

The CC typically calls the participant or the participant’s representative, administers the IPC Survey, and asks the open-ended questions. Data is collected in real-time by the CC while on the call. At each time point, if the participant or participant’s representative is not reached via phone, an email is sent to collect the data via email. It is important for the person conducting the surveys to check the Gateway to confirm the participant is living. Surveys are not administered to representatives of participants that are deceased. If survey respondents have specific questions or areas that require follow-up, the CC reaches out to the participant’s CS.

Participant Engagement and Empowerment Resource (PEER)

Overview

The purpose of the UDN Participant Engagement and Empowerment Resource (PEER) is to provide the participant and family perspective on UDN research goals and participant experience. The PEER engages with UDN investigators in the development and assessment of

participant oriented materials and identifies best practices for receiving participant input in research. There are many potential benefits of the PEER, including fostering longstanding relationships among participants, families, and researchers; promoting participant empowerment; educating participants and families on the essential dual clinical/research mission of the UDN and similar initiatives; and encouraging future engagement in research studies.

PEER membership

The PEER includes up to 11 participants or legal guardians who are interested in partnering with UDN investigators to assess and contribute to the UDN research process and participant experience. Members of the PEER must be willing to engage in thoughtful conversation about the positive and negative aspects of the research process and respect the perspectives of others. Ideally there is adult, adolescent, and pediatric participant and family member representation.

All UDN participants or legal guardians are offered the opportunity to participate in the PEER following the Transitions of Care survey. The application form is sent to all individuals interested in participating. Applications received are sent to the coordinators of clinical site who know the participant or legal guardian for review. Based on the coordinator review, a small group of individuals are selected by the CC and the CC drafts a proposed member list. The proposed member list is then submitted to the UDN Executive Committee and ratified by the UDN Steering Committee.

Additionally, there are three *ex-officio* members: a CC representative, a Site Operations Working Group representative, and a Genetic Counseling and Testing Working Group representative.

Terms are one year in duration and renewable. Members can reapply for a second term.

Activities

The PEER is responsible for their structure and activities. Activities may include:

1. Providing input regarding various research questions, eligibility criteria, and recruitment and informed consent processes
2. Identifying unmet participant needs
3. Contributing perspectives on risk/benefits of research project
4. Connecting families with one another and to support groups
5. Collecting participant and family experiences with the UDN from participants
6. Providing support for families when they are visiting a site far from home
7. Being a resource for families who have questions or concerns
8. Developing educational materials
9. Organizing participant conferences
10. Leading awareness efforts

Schedule and travel reimbursement

Conference calls are organized by the CC at a frequency determined by the PEER. Annual in-person meetings are held. A PEER co-chair or other PEER members may be invited to attend one in-person Steering Committee meeting annually. Travel expenses are covered for the

annual in-person PEER meeting and for a PEER member to attend the Steering Committee meeting, as needed.

Evaluation

The CC may develop satisfaction surveys to be completed on a regular basis by PEER members. The PEER are asked to develop an annual report on activities to be shared with the Steering Committee. There is an annual Steering Committee review of the PEER.

Transitions to basic science

In most cases, the transition of participant data and/or samples to basic science occurs through the CS to which the participant was assigned, or through cross-site UDN collaborations. There are referrals to the basic science community based on candidate genes and other diagnostic information, and it is important to track these activities in the Gateway.

Participant web pages project

Sequencing has the potential of revealing variants of uncertain significance. The discovery of these types of variants leaves patients in a state of uncertainty, faced with the possibility of never knowing the cause of their symptoms. Finding another patient with the same, or similar, variant can reduce or eliminate this uncertainty and end the diagnostic odyssey. It can take years before enough patients with the same or similar variant are identified to confirm a diagnosis. By using web pages to identify second cases, the UDN hopes to shorten the diagnostic process for participants, generate new knowledge of rare conditions, and connect patients and families to one another for support.

All participants that are undiagnosed or diagnosed with rare conditions are asked if they would like to partake in this project. If a participant is interested in participating, the CS contacts the CC and the CC consents the participant. Consent is obtained over the phone or by videoconferencing (or in person if reasonable) from the enrolled participant, parent, or guardian. Assent is obtained over the phone (or in person if reasonable) in the presence of a parent or guardian for all children ages 7-17 years old who are not decisionally impaired. The consent and assent forms are sent (by mail or email) to the participant (or legal guardian) prior to the remote or in-person consent. The principal investigator, an associate investigator, a genetic counselor, or a project coordinator trained in consenting at the CC is available to answer questions and obtain consent. The signed consent and assent forms are sent to the CC. The CC provides pre-paid, self-addressed envelopes to mail these forms. The CC records the consent and assent versions signed by the participant in the Gateway.

A web page about the participant is created by the CC based on information in the Gateway. The web page is reviewed by the CS and participant or parent/guardian. The web pages may include the following:

1. Genetic variants (including gene name(s) and variant)
2. Symptoms/signs, both past and present, using both Human Phenotype Ontology (HPO) terms and simplified language
3. Past medical history (including suspected diagnoses with dates or on a timeline)
4. Past and current treatments, procedures, and medications and their outcome and effectiveness (treatments, procedures, and medications with dates or on a timeline)
5. Gender, race, and ethnicity

6. Pictures and/or videos, if participant consents (pictures at multiple time points would be ideal)
7. Contact information (phone number, email address) for CC

Google alerts for all gene names of interest, specific variants of interest, and rare symptoms are created. The CC utilizes search engine optimization techniques to ensure that the pages are found.

When searching for symptoms or genetic variants, clinicians and families may find a UDN participant web page. The CC triages and records contacts made regarding these web pages. For possible matches, additional information is collected. The CC facilitates conversations between the potential match or clinician, the volunteer CS, and the participant. Participants are asked if they would like to communicate with each potential match or clinician before they are connected. If true matches are made, the CC updates the web pages accordingly. The CC records the amount of time spent processing each inquiry, how many participants receive diagnoses as a result of the web pages, and the length of time to each diagnosis. The UDN collaborates with the UDN patient advisors and participants to further refine this process and foster the creation of rare disease patient communities.

D. References

1. Napoles AM, Gregorich SE, Santoyo-Olsson J, O'Brien H, Stewart AL. Interpersonal processes of care and patient satisfaction: do associations differ by race, ethnicity, and language? *Health Serv Res.* 2009;44(4):1326-1344. doi: 1310.1111/j.1475-6773.2009.00965.x. Epub 02009 Mar 00931.
2. Stewart AL, Napoles-Springer A, Perez-Stable EJ. Interpersonal processes of care in diverse populations. *Milbank Q.* 1999;1999;77(3):305-339.
3. Stewart AL, Napoles-Springer AM, Gregorich SE, Santoyo-Olsson J. Interpersonal processes of care survey: patient-reported measures for diverse groups. *Health Serv Res.* 2007;42(3 Pt 1):1235-1256.

IX. Surveys

A. Background

Surveys offer an opportunity to learn from the unique participant population with rare and undiagnosed diseases. The purpose of this section is to outline the survey implementation approach for the network.

B. Survey Committee

Purpose

The Survey Committee ensures that surveys are consistent with the UDN mission and that participants are not overburdened. The Survey Committee is under the umbrella of the Publications and Research Committee (PRC).

Members

The Survey Committee includes:

1. One voting member from each CS (7 members)
2. One voting member from the CC
3. At least 1 survey methodologist from a UDN site. The survey methodologist could also be the voting member from their site.
4. One member from the Clinical Protocols Working Group, who acts as a liaison
5. Optional: One voting member from each Core

Ad hoc members:

1. Members (1-2) from any working group impacted by a survey under review are asked to join the Committee as ad hoc members.
2. UDN members with expertise in an area covered by a survey under review may be asked to join the Committee as ad hoc members.

Tasks

The Survey Committee reviews all surveys proposed after the initiation of the Committee. For survey research using the CIRB, the Survey Committee makes a recommendation regarding whether or not the survey should be integrated into the UDN. For survey research not using the CIRB, the Survey Committee reviews the survey in order to have a complete picture of surveys being administered to UDN participants, but does not issue recommendations.

Each survey using the CIRB, including those approved prior to the initiation of the Survey Committee, are reviewed once a year, starting one year after IRB approval was obtained. A subgroup of the Survey Committee (2-3 members), and ad hoc members as appropriate, are tasked with reviewing the survey performance characteristics and make a recommendation to the Survey Committee regarding survey continuation. The Survey Committee decides who the reviewers are for each survey, with a goal of dividing up the surveys among the Committee members. Surveys not using the CIRB are not reviewed yearly, but are requested to inform the Survey Committee annually as to the status of the research (completed; ongoing; other).

C. Survey Development

Creation of survey plan

The group proposing a survey must formulate a plan, which should include:

1. Purpose of survey, hypotheses, and questions answered by the survey
2. Instrument that will be used and validation of that instrument
3. UDN sample population (what sites will be involved; what populations within the UDN sites will be surveyed, e.g., all members or a subset)
4. Plans for administering the survey to non-English speaking individuals
5. Administration timeline
6. Method of survey administration (online, phone, etc.)
7. Text for all correspondence with participants
8. Individuals administering survey
9. Metadata from the Gateway that should be associated with survey (e.g., dates of visit, demographics, etc.)
10. Where data will be kept (Gateway or separate from Gateway) and who should have access to the data
11. Analysis plan, including who will do the analysis
12. Requirement for and availability of additional funds to conduct and analyze the survey

Plan proposal and approval

The group proposing a survey must complete and submit a research concept sheet. Research concept sheets are submitted to the CC and sent directly to the Survey Committee for review.

The rest of this section is ONLY applicable to surveys using the CIRB

After receiving a research concept sheet, the Survey Committee chair informs working groups impacted by the survey, UDN members with expertise in an area covered by the survey, and the Steering Committee of the survey review date. Updates to the Steering Committee are provided during the working group updates segment of the meetings. Applicable working groups and experts are offered the opportunity to join the review as ad hoc members.

At least one week prior to a review, the Survey Committee chair distributes the research concept sheet to Committee members, including ad hoc members. The Survey Committee documents their deliberations and recommendations in meeting minutes.

Working group ad hoc members report back to their groups within 2 weeks regarding the Survey Committee recommendations. The Survey Committee provides the working groups with: 1) the research concept sheet and 2) meeting minutes. The ad hoc members bring questions and feedback from the working groups back to the Survey Committee for discussion. Based on this discussion, the Survey Committee finalizes their recommendation.

The Survey Committee may not come to a final recommendation and recommend that the research concept sheet be revised. In this case, the group proposing the survey provides the revised concept sheet to the Survey Committee. The Survey Committee votes on a final recommendation, with a majority vote needed to decide on an outcome.

The Survey Committee sends their final recommendation to the PRC for review, expected to be within 3 weeks of the review. The Survey Committee provides the PRC with: 1) the research concept sheet and 2) meeting minutes. Following PRC review, the CC sends the outcome of the review to the chair of the Survey Committee, chair(s) of the working groups impacted by the survey, members of the proposing group, and the CC. The CC adds the survey review to an upcoming Steering Committee meeting agenda, expected to be within 1 month of the completion of the PRC review. New surveys are presented to the Steering Committee by the proposing group for final consideration on the assigned date.

The Steering Committee may approve the survey research, raise concerns and send the research concept sheet back to the group for revision, or reject the survey research.

IRB amendment submission

If the survey research is approved by the Steering Committee, the group proposing the survey must complete an amendment form detailing the survey plan and submit the form to the CIRB Liaison (see Section III. Institutional Review Board (IRB) Communications). The amendment must include any required changes to the UDN protocol, the survey plan, a paper version of the survey, and all language for communications with participants.

If the survey is not integrated into the Gateway, the group can start administering the survey once IRB approval has been obtained.

Survey tracking

The Survey Committee keeps a record of all UDN surveys. For those using the CIRB, the table includes the date when the survey was approved by the CIRB and the date of the yearly review.

Gateway integration

If the survey needs to be integrated into the Gateway, the UDN group should contact the CC.

Ongoing review

Surveys are reviewed once a year by a subgroup of the Survey Committee to determine if the survey should continue to be administered. The Survey Committee contacts the group conducting the survey 3 months prior to the annual review date. The Annual Survey Review Worksheet (Appendix 9: Annual Survey Review Worksheet) should be submitted to the chair of the Survey Committee on or before the review date.

After receiving an annual survey review worksheet, the Survey Committee chair informs working groups impacted by the survey, UDN members with expertise in an area covered by the survey, and the Steering Committee of the survey review date. Updates to the Steering Committee are provided on at least a monthly basis. Applicable working groups and experts are offered the opportunity to join the review.

At least one week prior to a review, the Survey Committee chair distributes the Annual Survey Review Worksheet to subgroup members, including ad hoc members. The subgroup documents their deliberations and recommendations in meeting minutes.

Working group ad hoc members report back to their groups within 2 weeks regarding the subgroup recommendations. The subgroup provides the working groups with: 1) the Annual Survey Review Worksheet and 2) meeting minutes. The ad hoc members bring questions and feedback from the working groups back to the subgroup for discussion. Based on this discussion, the subgroup finalizes their recommendation. The subgroup may not come to a final recommendation and may recommend that the Annual Survey Review Worksheet be revised. In this case, the group conducting the survey provides the Annual Survey Review Worksheet to the Survey Committee before a final recommendation is made.

The Survey Committee votes on final recommendations, with a majority vote needed to decide on an outcome. Surveys recommended for continuation or discontinuation by the Survey Committee are sent to the PRC for review. The Survey Committee provides the PRC with: 1) the Annual Survey Review Worksheet and 2) meeting minutes. Following PRC review, the CC sends the outcome of the review to the chair of the Survey Committee, chair(s) of the working groups impacted by the survey, and members of the conducting group. Survey continuations and discontinuations are presented to the Steering Committee during the working group updates segment at the next meeting. The Steering Committee may approve the continuation of the survey research, raise concerns and send the Annual Survey Review Worksheet back to the group for revision, or reject continuation of the survey research.

Significant survey modifications

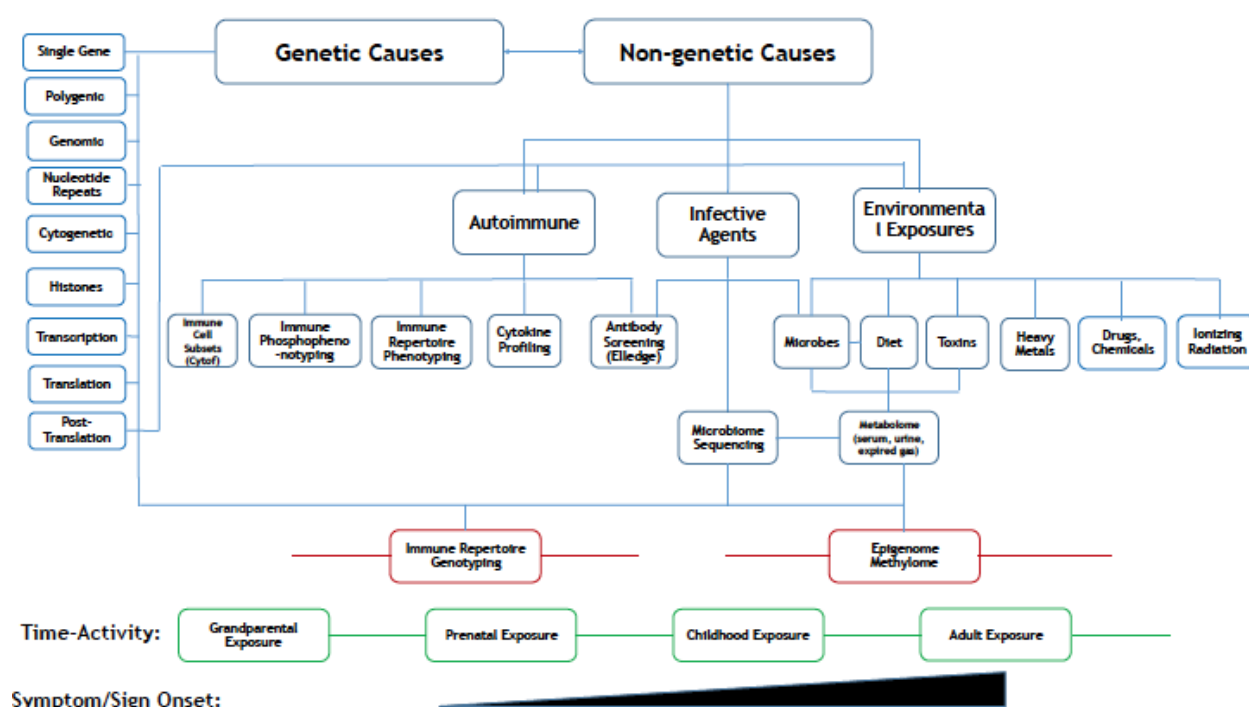
If significant changes are made to a survey (more than just minor edits), the group responsible for the survey submits the revised survey to the Survey Committee for review. The Survey Committee chair reviews the changes to determine if they require review by the Survey Committee. If the Survey Committee chair feels that the changes require Committee review, the chair informs the Committee, working groups impacted by the survey, UDN members with expertise in an area covered by the survey, and the Steering Committee, and schedules a survey review date. If there are no concerns, the review decision is expedited and the decision is reported to the Steering Committee during the working group updates.

X. Environmental Exposures

A. Background

The UDN fully recognizes that all diseases are likely determined by many factors, including “minor modifying” genes and environment, through molecular mechanisms such as by epigenetics, transcriptomics, post-translational modification of the proteome, the microbiome, infections, and immune responses. The UDN protocol for identifying possible environmental factors, outlined in the figure below, is intended to systematically screen all UDN applicants for such factors, expedite the efforts of clinical investigators, and perhaps improve the participants’ health by intervening against current environmental hazards.

Figure: A holistic view of the multifactorial, ecogenetic hypothesis of disease etiology.



In practice, this protocol begins with a screening of environmental exposures (exposome) via a self-administered survey completed by the UDN applicant (or their relatives). Environmental Exposures Working Group members with expertise in environmental and occupational medicine and teratology comment on responses that may deserve possible exploration by the UDN CS investigators. Recommendations may include further clinical evaluation by an Environmental and Occupational Medicine physician, a teratologist, or appropriate laboratory assays of drugs and environmental toxins, imprinting, RNA sequencing, immune response characterization, and possible infections.

B. The Environmental Exposures Working Group

Purpose

The Environmental Exposures Working Group facilitates: 1) the screening of UDN applicants for self-reported environmental exposures, 2) the interpretation of the screening questionnaire results, 3) recommendations for further evaluations of putative exposures by targeted clinical and laboratory investigations to corroborate the exposure(s), and 4) the preparation of presentations, abstracts, and manuscripts about the data related to environmental exposures. The Environmental Exposures Working Group follows the procedures of all Working Groups, as detailed elsewhere in this Manual of Operations.

Part of the consultation service of the Environmental Exposures Working Group is to facilitate the use of validated laboratory assays on the best specimen type to aid the UDN clinicians. It may invite laboratory consultations and/or assays, in coordination with the Metabolomics Core. Biospecimen handling follows procedures of this Manual, although special handling of rare sample types (teeth, hair, nails, etc.) and special specimen containers may supersede general recommendations.

The Environmental Exposures Working Group initiates aggregate analyses of the results of environmental exposure assessments as reported on the questionnaire, especially when corroborated by laboratory or other independent data. The procedures of the Publications and Research Committee are followed.

Members

The Environmental Exposures Working Group includes two co-chairs, to be approved by the UDN Steering Committee. Decisions are reached by consensus and are only advisory, so no distinction is made between voting and non-voting members. Other members include:

1. Members from any of the CSs
2. One member from the CC
3. Optional: One member from each Core
4. Optional ad hoc members: Up to three other interested parties, preferably with expertise in occupational and environmental medicine or teratology

C. Environmental Exposures Questionnaire

Current and previous versions of the Environmental Exposures Questionnaire are available (<https://hms.harvard.box.com/v/envsurvey>).

The initial application had two survey questions about any possible environmental factor related to the illness and, for affected children, any suspected preconceptual and prenatal exposures. Subsequently, a longer environmental exposures questionnaire was developed by an environmental exposures ad hoc group, which included experts in occupational and environmental medicine, teratology, toxicology, epigenetics, and genetic epidemiology. This questionnaire was designed for self-completion by participants or parents/guardians and was implemented with electronic data capture by the CC using Qualtrics. To create the questionnaire, existing questionnaires were leveraged including the American Academy of Clinical Toxicology Exposure History Form,¹ CDC Environmental Exposure History,² PhenX project,³ and NHANES,⁴ in addition to questions developed with the assistance of various experts in multiple environmental exposures.

The areas addressed by the questionnaire include:

- a) occupational exposures to dust and chemicals,
- b) residential exposures to chemicals and pollution,
- c) use of alcohol, tobacco, and other drugs (prescribed and recreational),
- d) exposures from hobbies and travel,
- e) exposures to infectious disease vectors and sexually transmitted diseases,
- f) unusual dietary histories, and
- g) parental environmental and occupational exposures before and during pregnancy that resulted in the birth of the participant.

This questionnaire is intended to be used as a clinical screening tool to identify applicants who would potentially benefit from a more intensive environmental exposure assessment at the UDN CSs. It was initially administered to accepted participants, but is to be administered to all applicants in the future. It is expected that revisions to this questionnaire will be made over time. After a year's experience, a shortened version was implemented.

As of November 2018, the Environmental Exposures Working Group suspended review of all questionnaires, pending design and implementation of improved procedures for efficiently identifying applicants who report highly likely significant environmental exposures. Clinical sites are urged to review applicants' Environmental Exposures Questionnaires and contact Working Group members for expert opinion in assessing alleged exposures of concern.

Dear Dr. X,

The UDN Environmental Exposures Working Group has reviewed additional Environmental Exposures Questionnaires that have been completed by UDN patients at the XX Clinical Site.

The following patient accepted by your site had potentially relevant environmental exposures identified from the Environmental Exposures Questionnaire:

UDNXXX: This patient reported exposures to xxx. Therefore, we recommend an Environmental/Occupational Medicine evaluation.

Please note that we did not evaluate the full medical record for this subject, and we are basing this recommendation on the limited information available to us. Please use your judgement to determine whether this recommendation is appropriate. If you have any questions, we would be happy to discuss them.

Thanks very much for your help.
UDN Environmental Exposures Working Group

The Environmental Exposures Working Group may, on infrequent occasions, present its rationale for recommending acceptance of an applicant mostly because of a very compelling environmental history of possible etiologic importance, one that is addressable by UDN clinicians and laboratory investigators. Guidance may be offered in preferring an alternate CS to be used rather than the initially assigned one, on the basis of expertise to address the alleged exposure and access to laboratory approaches to investigate the putative pathogenicity. Any time CS reviewers become aware of an environmental exposure of special concern, they can request urgent review by the Environmental Exposures Working Group, which strives to provide an interpretation within one week.

D. References

1. American Academy of Clinical Toxicology Exposure History Form
<http://www.clintox.org/sections/occupationalenvironmental>
2. Agency for Toxic Substances and Disease Registry. Case Studies in Environmental Medicine (CSEM): Taking an Exposure History.
https://www.atsdr.cdc.gov/csem/exphistory/docs/exposure_history.pdf
3. Hamilton CM, Strader LC, Pratt JG, Maiese D, Hendershot T, Kwok RK, Hammond JA, Huggins W, Jackman D, Pan H, Nettles DS, Beaty TH, Farrer LA, Kraft P, Marazita ML, Ordovas JM, Pato CN, Spitz MR, Wagener D, Williams M, Junkins HA, Harlan WR, Ramos EM, Haines J. The PhenX Toolkit: Get the most from your measures. *Am J Epidemiol*. 2011;174(3):253-60. PMID 21749974..
4. Centers for Disease Control and Prevention, National Center for Health Statistics, National Health and Nutrition Examination Survey.
http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm.

XI. Sequencing

A. Flow of Samples to Sequencing Core

I. Sample collection and DNA extraction

1. The clinical sites (CSs) arrange for blood sample collection before or during the clinical evaluation. If collected off-site, blood samples for DNA should be shipped to the clinical site (CS).
2. Additional sample collection considerations:
 - a. If the individual has had a bone marrow transplant or recent blood transfusion, DNA from fibroblasts is preferred.
 - b. Samples from deceased individuals should be discussed with the sequencing core (SC) on a case-by-case basis.
3. The CS arranges DNA extraction and QC. DNA samples submitted for sequencing should meet the following conditions:
 - a. WES: at least 6ug of 50-200ng/ul DNA
 - b. WGS: at least 10ug of 50-200ng/ul DNA
4. Additional DNA is stored at the CS with other biospecimens collected during the clinical evaluation.

II. Sample shipping guidelines

1. CS prepares DNA samples for shipment to the SC. DNA samples should be sent as complete families (including all family members that will be included in the analysis) excepting clinically urgent samples that warrant prioritized sequencing. Urgency is at the discretion of the CS.
2. CS completes a sequencing request form in the Gateway for each DNA sample being sent for sequencing.
3. CS enters and releases updated phenotype information (i.e., PhenoTips) in the Gateway for use by the SC in their analyses.
4. CS enters shipping information (date DNA sent and tracking number) in the Gateway and ships samples.
 - a. Please note that the Gateway provides alerts for shipping of UDN samples, but shipment tracking needs to occur at the CS/SC level.
5. Gateway sends an automated email to alert the appropriate SC of sample shipment and available phenotypic data.
6. SC acknowledges receipt of samples by entering date DNA received in the Gateway.
7. If a submitted DNA sample does not pass QC at the SC or is otherwise deemed unacceptable, the SC contacts the CS site directly via phone or email to request a replacement.
8. Sample labeling discrepancies are addressed on a case-by-case basis at the discretion of each SC.

B. Flow of Clinical Information to Sequencing Core

1. The CS are responsible for collection of blood specimens and DNA extraction. The CC and SC will not know about or track the DNA specimens until they are shipped to the SC. The CSs are encouraged to collect all specimens for a family before sending them, but additional family members may be added at a later date if necessary. Please note

that for sites without an option for DNA extraction from a CLIA certified laboratory, arrangements can be made with the SC for DNA extraction. Sequencing will commence when blood from all family members has been received. The 2 week TAT will commence after all samples have been extracted.

2. Typically, CSs send an aliquot of DNA extracted in a CLIA-certified lab and keep the remainder of the DNA for future procedures.
3. Samples may be sent either before or after the in-person evaluation of the proband. In either case clinical information and a pedigree, including the relationships of all submitted family members to the proband, should be added to the PhenoTips as soon as possible for samples submitted for sequencing. This information is used by the SC for their analysis.
4. DNA samples submitted for sequencing must be labeled with participant name, date of birth, and the initials "UDN".
 - a. Local identifiers may also be included at the discretion of the CS. Local identifiers will appear with participant name on sequencing reports (i.e., John Doe, UDP123).
5. Other required information for sample submission includes:
 - a. Gateway consent form
 - b. Gateway sequencing form
 - c. Affection status of family members

C. Exome Sequencing

This section covers sample intake, library preparation, whole exome capture, and sequencing at Baylor College of Medicine. It describes the sample flow from DNA sample receipt to production of WES data, including appropriate quality control and assurance procedures.

Sample intake

A visual inspection of the sample tubes is conducted when received by the SC. Sample tube label is compared with information entered in the Gateway to ensure consistency and completeness of the Gateway data, consent forms, proper sample labeling, and sample tube integrity. Samples are accepted if no discrepancies are found, sample labels match, and no tube damage is observed. If any of the above criteria is not met, Baylor notifies the referring CS.

Once accepted, samples are accessioned into the Laboratory Information Management System (LIMS) system. Sample information in the Gateway is entered into the WGL LIMS database. Each sample is assigned an internal six-digit lab number, as well as a six-digit family number in LIMS. 1D bar code labels with participant specific information (unique identifier) including participant name, date of birth (DOB), lab number and family number are attached to the stock DNA tube. Subsequently samples are aliquoted from the stock tubes into 2D barcode tubes. The samples in 2D bar code tubes are processed for exome sequencing. Before sample transfer, the record for a sample is first opened in LIMS, then the 1D barcode label on the stock tube of the sample is scanned and the LIMS automatically verifies if the sample ID in LIMS matches that on the label. Then, the 2D bar code on the aliquot tube is also scanned to link the two bar codes in LIMS before sample transferring occurs. These steps are to ensure the chain of custody remains intact during sample transfer.

DNA sample QC

DNA samples are screened to quantify DNA as well as determine DNA quality. To determine DNA concentration and purity, the samples are evaluated using the Quant-iT PicoGreen dsDNA assay on the BioTek Synergy 2 microplate reader. Passing criteria include:

1. The R-squared value for the standard curve must be $\geq 99.9\%$ DNA concentration
2. Sample contains 1ug of DNA

To verify DNA integrity and relative size, the same dilution of sample is loaded on a 0.8% E-Gel. Passing criteria include:

1. Gel image is clear and shows no DNA degradation

If a sample does not meet the criteria above, the CS is notified.

Pre-capture library preparation

In order to meet UDN sequencing objectives, we use our quick whole exome sequencing (QWES) protocol. QWES is an optimized version of the standard Illumina (ILM) library preparation workflow that reduces library construction time to 5-6 hours.

Library construction is a completely automated process on the Span-8 Biomek NXP with an incorporated LIMS tracking system. Before starting library preparation, all primers and adapters lots are validated and the appropriate dilutions are prepared. Negative (H_2O) and reagent blank controls are included. Robot operator closely monitors each transfer step. Pre-capture library preparation involves the following steps:

Normalization and shearing

DNA samples are normalized to 750 ng total. Samples are loaded into Covaris microtubes in 50 ul aliquots and sheared to approximately 250-500 bp using the Covaris E220 ultrasonicator. Shearing efficiency is assessed using a 2.2% flash gel. Fragments should range from 100-600bp with average of 250-500bp. If the majority of sheared fragments are larger than 800 bp, the sample is re-sheared.

End repair

Fragmented DNA samples are treated with NEBNext® END REPAIR Module (catalog#: E6050L) at 20°C for 20 minutes to make blunt ended DNA. Then 1.8X Beckman SPRI beads (Agencourt AMPure XP Solid Phase Reversible Immobilization magnetic beads) and 70% Ethanol are used for cleanup. Treated fragmented samples are eluted with 40 ul elution buffer while SPRI beads remaining in the solution.

3' Adenylation

The treated DNA samples are incubated with NEBNext® dA-Tailing Module (catalog#: E6053L) at 37°C for 20 minutes to incorporate a non-templated dAMP on the 3' end of a DNA fragment. The binding buffer (BB) made with Polyethylene Glycol (PEG) and 5 M Sodium Chloride (NaCl) (final concentration of PEG and NaCl: 20% and 2.5 M respectively) is applied to help dA-Tailing fragment DNA binding back to SPRI beads while the rest of solution is discard. 70% ethanol then is used for cleaning up the DNA bounded SPRI beads. DNA samples again are eluted with 40 ul elution buffer while SPRI beads remaining in the solution.

Ligation

Post dA-Tailing, DNA Samples are ligated with Illumina multiplexing paired-end (PE) adapters by using Invitrogen Expresslink ligase (catalog#: A13726101) and buffer at room temperature for 20 minutes. The same binding buffer is used to allow ligated DNA binding back to SPRI beads and 70% ethanol is used for cleaning up the SPRI beads. SPRI beads are removed post 40 ul elution buffer added.

Enrichment

Ligated DNA samples are enriched for total 6 cycles with 2X KAPA HiFi HotStart Ready Mix PCR kit (catalog#: KK2612) and Illumina PE PCR primers. AB GeneAmp PCR System 9700/Veriti are used for amplification Enrichment.

Post-enrichment QC

The enrichment PCR efficiency analysis is performed on 2.2% FlashGel by checking the product intensity. The FlashGel analysis is performed after 6 cycles and is re-run if the band is too weak. Additional PCR cycles can be added for samples with low yield.

1. No more than 9 cycles total can be run for samples
2. If the amount of the post-PCR product is insufficient after a total of 9 cycles, the whole process needs to be repeated.
3. The negative (H₂O) and reagents blank control should give no product

To check size distribution and quantify the final library, the sample is run on an Agilent Bioanalyzer 2100 DNA 7500 Chip.

1. The library sizes should range from 200 – 750 bp (Majority are 250-550bp) with the peak ranging from 250 - 350 bp.
2. The yield of library should be more than 1.5 ug. If pre-capture library yield is lower than 1ug, the library preparation is repeated. No adaptor dimer and free primers are visible.
3. See Figure 1 for an example of a passing pre-capture library.

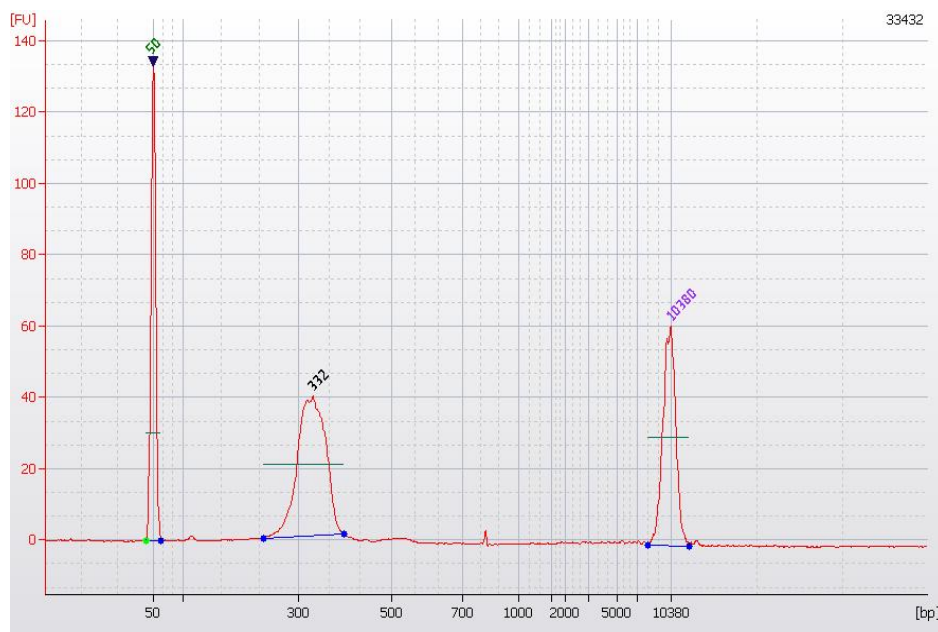


Figure 1: Illumina paired end DNA library on Agilent Bioanalyzer 2100

Whole exome capture

The whole exome capture utilizes the NimbleGen liquid capture on HGSC VCRome 2.1, that targets approximately 34Mbp of genomic DNA including all coding exons of currently known disease genes (OMIM, HGMD, and GeneTests). To enhance the coverage of clinically relevant disease genes, the currently developed spike-in probe set (Exome 3 – PKV2) is used in 1:1.25 equimolar ratio with the VCRome exome capture design in combination with the QWES protocol.

Solution capture is initiated by combining 1.5 ug of the pre-capture library, 40ul of 1mg/ml human Cot1 DNA, and adding 0.65 ul of each 1,000uM Hybridization enhancing (HE) oligos. Full-length hybridization enhancing oligos are used to augment capture efficiency.

This mixture is dried down in a DNA vacuum concentrator on high heat setting and re-suspended in Hybridization buffer and Formamide. The mixture is denatured for 10 minutes and VCRome probe with Panel Killer V2 combined in 1:1.25 ratio are added. The mixture is incubated at 56°C for 16 hours. The following day, captured DNA is washed and recovered. Post-Capture PCR amplification is performed using KAPA HiFi HotStart DNA polymerase with total 12 cycles.

Final library QC

FlashGel

Capture efficiency is checked using a 2.2% FlashGel. If the intensity is too low, the capture process needs to be repeated. Only primer bands should be seen for negative and reagent blank controls.

Bioanalyzer

To assess size distribution and quantify the final post-capture product, libraries are loaded onto DNA 7500 Chip for assessment on the Agilent 2100 Bioanalyzer. The majority size should be around 300-400 bp with the concentration above 20 nmol/L. See Figure 2 for an example of a passing final library.

qPCR

Capture efficiency evaluated by SYBR green-based qPCR with known four loci assays. Successfully enriched capture libraries have an average delta Ct of the four loci >6 with delta CT of the individual assay is >5.

Post-capture libraries must pass all three QC checks to proceed to cluster generation and sequencing. For each sequencing run, three individual barcoded libraries are normalized to 10nM and pooled into one pool.

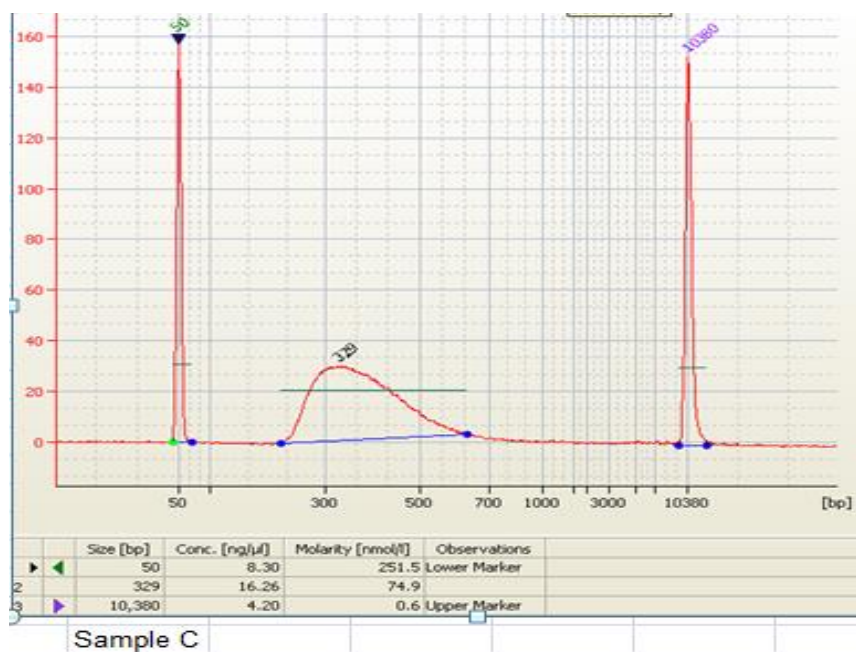


Figure 2: Final post-capture library on Agilent Bioanalyzer 2100

cBot cluster generation

Post-capture libraries are denatured prior to loading on the cBot. Denatured PhiX control is spiked-in into lanes 1 and 2 for HiSeq 2500 Rapid runs. The optimal library concentration for cluster generation is 10nM. Library and flow cell information are entered into WGL LIMS system prior to starting a cBot run.

After the cBot run is completed, each strip is checked to ensure that correct volumes were drawn for each lane. If volume of any reagent tube or DNA strip tube is not equal to the others (most frequently more volume is left), the reagent and/or DNA was not delivered properly for that lane.

Sequencing on HiSeq 2500

The HiSeq 2500 is employed for sequencing in rapid mode (27-hr cycle time) to generate 100bp paired-end reads in a format of 3 samples per lane to generate 10-12 GB per sample. Target coverage for proband and parental samples is >100x. The WGS LIMS system is utilized to track the run set up, status and quality metrics. Pertinent metrics and passing thresholds are provided in the tables that follow.

The run stops after imaging is complete for the first cycle and generates a first base report. The first report is confirmed by manual review and the run resumes if all the metrics correlate with the determined standards.

G intensity	>6000
Cluster density	>400k/mm2

The performance of the run is monitored, and the metrics below are recorded to assess quality at a particular step of the sequencing run, evaluate library quality and concentration, detect any potential sequencing reagents and/or optical issues.

Cluster density at cycle 5	900-1100 k/mm2
Phasing/pre-phasing at 25 cycles	<0.3/0.7%
Passing filter rate	>80%
PhiX error rate	<3%
Q30	>80%

After the run is complete, a comprehensive set of post-sequencing production metrics are continuously monitored and are reviewed at weekly meetings to facilitate timely troubleshooting to maintain overall pipeline performance. Overall run performance is evaluated by metrics from the off-instrument software (Casava) and from mapping results generated by the Mercury analysis pipelines using Burrows Wheeler Aligner (BWA) software to ensure that production standards are met.

Pass filter	>80%
Aligned reads	>80%
Error rate	<4%
Unique reads	>90%

The capture analysis is incorporated in the analysis pipeline and provides metrics to gauge the overall quality of the capture process. This pipeline reports:

1. Proportion of the aligned reads that map to the targeted region, which is relative to the effective enrichment of the capture
2. Distribution of coverage across the targeted bases; specifically, the fraction of targeted bases covered at 1x; 10x, 20x, 40x

The complexity of the capture library is assessed by calculating the number of alignment reads that occur from PCR duplicates. If needed, these reads can be removed from the analysis.

Key metrics that have been developed and are reviewed in weekly meetings are presented in the table below.

Reads Aligned to target	>50%
Target bases covered at >20x	>90%
Target bases covered at >40x	>80%
Mean coverage of target bases	>100x

As an additional quality control measure, samples are also analyzed by single nucleotide polymorphism (SNP) array. SNP data is compared with WES data to ensure correct sample identification and to assess sequencing quality. The data is analyzed using an automated pipeline that produces concordance and contamination scores.

Concordance	>90%
Contamination	<5%

D. Genome Sequencing

This section covers sample intake, library preparation, and whole genome sequencing at Baylor College of Medicine. This section describes the sample flow from DNA sample receipt to production of WGS data, including appropriate quality control (QC) and quality assurance procedures. The protocol described above for accessioning, DNA extraction, and DNA quality QC are to be followed for genome sequencing.

Sample intake

A visual inspection of the sample tubes is conducted when received by the SC. Sample tube label is compared with information entered in the Gateway to ensure consistency and completeness of the Gateway data, consent forms, proper sample labeling, and sample tube integrity. Samples are accepted if no discrepancies are found, sample labels match, and no tube damage is observed. If any of the above criteria is not met, Baylor notifies the referring CS.

Once accepted, samples are accessioned into the Laboratory Information Management System (LIMS) system. Sample information in the Gateway is entered into the WGL LIMS database. Each sample is assigned an internal six-digit lab number, as well as a six-digit family number in LIMS. 1D bar code labels with participant specific information (unique identifier) including participant name, date of birth (DOB), lab number and family number are attached to the stock DNA tube. Subsequently samples are aliquoted from the stock tubes into 2D barcode tubes. The samples in 2D bar code tubes are processed for exome sequencing. Before sample transfer, the record for a sample is first opened in LIMS, then the 1D barcode label on the stock tube of the sample is scanned and the LIMS automatically verifies if the sample ID in LIMS matches that on the label. Then, the 2D bar code on the aliquot tube is also scanned to link the two bar codes in LIMS before sample transferring occurs. These steps are to ensure the chain of custody remains intact during sample transfer.

DNA sample QC

DNA samples are screened to quantify DNA as well as determine DNA quality. To determine DNA concentration and purity, the samples are evaluated using the Quant-iT PicoGreen dsDNA assay on the BioTek Synergy 2 microplate reader. Passing criteria include:

1. The R-squared value for the standard curve must be $\geq 99.9\%$ DNA concentration
2. Sample contains 1ug of DNA

To verify DNA integrity and relative size, the same dilution of sample is loaded on a 0.8% E-Gel. Passing criteria include:

1. Gel image is clear and shows no DNA degradation

If a sample does not meet the criteria above, the CS is notified.

Library preparation

The purpose of this SOP is to provide instructions on how to prepare a PCR free sample for whole genome sequencing by using KAPA hyper prep kit.

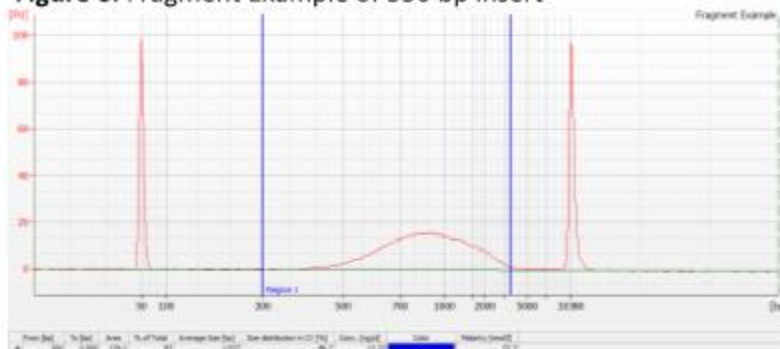
Reagents:

KAPA Hyper Prep Kit – KK8504

KAPA Library Quantification Kit

DNA samples are normalized to 30ng/ul in a final volume of 55ul. All gDNA samples require fragmentation in a random manner to create the fragments that become the inserts in the final library. The Covaris L-series and E-series instruments are used to shear the DNA to a final insert size of ~550bp. This longer insert size improves overall library performance and allows the longer sequencing read lengths on the Illumina NovaSeq platform (150bp) to be efficiently used without producing a significant number of over-lapping reads. QC is performed after sonication to ensure that yield and fragment size are within expected ranges. KAPA end repair and adaptor ligation and post ligation clean up-are performed. Insert size of the library is determined on Bioanalyzer.

Figure 6. Fragment Example of 550 bp insert



Note: Fragment average size should not fall below 900 bp. If so, repeat library.

After the library preparation is complete, final library yield, fragment size, and fragment distribution are measured. Finally, real-time PCR quantitation to determine the molar fraction of the sequenceable library is performed.

Sequencing on NovaSeq 6000

Kit: NovaSeq 5000/6000 S4 Rgt Kit (300 cycle) 20012866

Once a library passes QC, the production sequencing on the NovaSeq6000 is performed. PhiX must be loaded with the libraries to monitor the sequencing metrics. Record the quality metrics of the run at any time after the 26th cycle. The metrics are:

Cluster passing filter: >60%
Estimated yield (S4 2X151) 3TB
Q30 score >or equal to 80%

Clustering and sequencing are performed as per standard Illumina protocols for NovaSeq sequencing. Approximately 16 UDN samples will be sequenced on an S4 flowcell which will yield a minimum of 40X coverage for each sample.

D. Analysis

Overview

The steps in the analysis of WES or WGS data can generally be divided into 4 phases: primary analysis, secondary analysis, tertiary analysis, and interpretation. Secondary analysis can be further subdivided into read mapping and variant calling phases. Best-of-breed standards in analysis of WES or WGS sequence data are followed (as defined in this manual and agreed to across the SC, CSs, and the CC).

Annual review of current methodologies is performed by the SC with an aim of identifying and potentially incorporating advances of note in analytical approaches supporting interpretation of sequencing data. Any alterations that are considered for inclusion are shared with the Sequencing Working Group. Those prioritized are implemented leading to revision of the analysis steps outlined in this section. Each CS may conduct analyses on UDN cases sequence data as they see fit, but the SC undertakes primary, secondary, and tertiary analysis of the sequencing data. The purpose of this is to provide consistency in the format and quality of the data provided and to create maximal utility for the widest range of consumers of these data. For example, this method ensures that sites without existing clinically certified variant annotation and prioritization pipelines have access to richly annotated data.

Sequencing files in the Gateway

Output files from various stages in the analysis process derived from various applications are uploaded to the Gateway by the SC. Files may also be stored locally according to clinical data retention policies in place at the SC. As currently defined these are:

1. Standard compliant format FASTQ files
2. Standard compliant BAM files
3. Standard compliant VCF files
4. Annotated variant files (tab delimited text file format)
5. An interpreted clinical report is also be provided by the SC.
 - a. The format of this report follows the existing industry standards for clinical sequencing reports. Clinical reports include the following report sections:
 - i. Lab contact information and general test information
 - ii. Participant name and date of birth
 - iii. Indication for testing
 - iv. Primary findings (pathogenic, likely pathogenic, and variants of unknown clinical significance) in tabular format
 - v. Medically actionable findings in tabular format
 - vi. Interpretation of findings – textual discussion of the relevance of the findings given the clinical presentation of the proband

- vii. Specific recommendations
 - viii. A description of the methods used
 - ix. Limitations for both the sequencing technology and analytical processes
 - x. References
6. Each family member receives a report containing one of three possible results:
- a. Medically actionable finding identified
 - b. No medically actionable finding identified
 - c. Family member opted out of receiving medically actionable findings

Analysis, variant calling and annotation

For analysis, we will utilize the Dragen system (<http://www.edicogenome.com/dragen/dragen-genome-pipeline/>) to generate BAM and VCF analyses. Dragen processors enable NGS data analysis to be performed at extreme speed and with very high accuracy. Dragen uses field-programmable gate arrays (FPGA) - a reconfigurable processor to provide hardware-accelerated implementations of genome analysis algorithms. The main applications of the Dragen FPGA are Map/Align, sorting, duplicate marking haplotype variant calling and compression. Map/Align acceleration is about 2 orders of magnitude faster when compared to modern CPU analysis speed.

The output data from the Illumina HiSeq or NovaSeq are converted from BCL files to FastQ files according to each sample's specific adapter sequence using Illumina's recommended procedure. FastQ data are aligned to the human reference genome using the Edico Dragen BioIT Platform. The output of the alignment is a BAM file; QC metrics of the map-align process are recorded for quality review. QC statistics include coverage for exome genes and genes known to be implicated in human disease, mate-pair alignment information as well as number of total and duplicate reads. Variant calling on the BAM file is performed using the Edico Dragen haplotype-based variant calling system and the output is a VCF file. For indels and SNV, the variant calling step generates a "raw" VCF file containing a list of detected variants, which are then annotated using a locally installed annotation system. Edico Dragen output also includes gVCF genotype calls on a dense collection of approximately 500,000 exonic SNPs derived from the 1000 Genomes Project, and these SNPs are used for identity analysis as well as for analysis of copy number variation (CNV) and segments of homozygosity or uniparental inheritance. Our pipeline also includes a joint calling step to enable variant discovery leveraging data from multiple samples, allowing us to detect variants with greater sensitivity and genotype samples more accurately. BAM files are first generated per sample followed by creation of gVCF file. The last step, and the only step that involves all samples, is to process all previously generated gVCFs together, creating the joint calling of variants. All information is integrated to a single VCF that contains all family variants information.

Primary analysis – demultiplexing

Primary analysis (demultiplexing) is performed on the HiSeq or NovaSeq instrument workstation according to Illumina guidelines. The Dragen BCL conversion pipeline enables fast BCL to FASTQ conversion of NGS data including both HiSeq and NovaSeq chemistries. FASTQ files are written out to directory structure that identifies the run, flowcell, and each filename will contain the labnumber of the sample.

Secondary analysis – read mapping

SC performs clinically appropriate validation of all datasets and algorithms/software applications in use within its clinically validated analysis pipelines. Significant pipeline component changes undergo re-validation at the discretion of the SC.

Secondary analysis step 1 (mapping and realignment; FastQs serve as input to the Dragen software which performs the mapping/alignment, calculates coverage, performs variant calling, and joint analysis.

Alignment against Hg19build37 (b37) is performed on Dragen instrument according to the Dragen proprietary map-align algorithm. GRCh37/hg19 (b37d5) will continue to serve as the alignment template until it is superseded by GRCh38/hg38 and approved by the Steering Committee. This is expected during year 1 of UDN Phase II. Alignment of reads to GRCh37/hg19 (b37d5) is performed without truncation of the data. More specifically, duplicate rate is recorded, but not removed from the dataset.

Tertiary analysis – variant annotation

The variant annotation is performed by a locally installed program that leverages the GenomOncology Knowledge Management System API and provides annotations using open source data sets such as gnomAD, ExAC, EVS, and ClinVar and professional resources such as HGMD Pro. The API also provides HGVS nomenclature built using the Biocommons open-source suite of tools: HGVS python library, the UTA transcript repository, and SeqRepo sequence database. This annotation system reports zygosity as well as inference of mutation types including nonsense, missense, synonymous, splicing and frameshift, and others. Annotation is highly parallelized and operates on several thousand lines per second. The specific variant annotations and the tools used to produce them are outlined in Table

Table. A depiction of the set of variant annotations and tools used by SC.

Annotation	BCM
Alamut	yes
splice sites	yes
near splice site	yes
protein coding flag	yes
syn change flag	yes
non-syn change flag	yes
AA change	yes
Sift prediction	yes
Polyphen2 HVAR prediction	yes
Polyphen2 HDIV prediction	yes
Mutation Taster prediction	yes
MutationAssessor prediction	yes
stop gain flag	yes
stop loss flag	yes
frameshift flag insertion/deletion/indel	yes
non-frameshift flag insertion/deletion/indel	yes
location: intron/exon	yes
location: 5'UTR/3'UTR/Intergenic/Promotor	yes

HGNC appropriate Gene Symbol	yes
Transcript ID	yes
COSMIC	yes
HGMD ID	yes
HGMD variant level association	yes
HGMD gene level association	yes
OMIM ID	yes
OMIM variant level association	yes
OMIM gene level association	yes
ClinVar ID	yes
ClinVar metadata (various; to be clarified)	yes
dbSNP ID	yes
dbSNP AF	yes
1000 Genomes AF	yes
ESP EVS AF	yes
Mappability score	yes

Coverage analysis

Depth of coverage is calculated for the WES regions of interest by Dragen. For WES, the regions used in the coverage calculation are defined by the unpadded target file. The direct input for the coverage analysis is the BAM file produced in the preceding alignment step. The output is a genome coverage bed file, which is a tabular histogram of the coverage depths of the target regions. The resulting coverage bed is used as an input to the DART QC Scoring step for calculating various coverage statistics for each individual sample to be used during QC review.

Interpretation

The SC also provides an interpreted clinical report. A systematic process is followed in accordance with published American College of Medical Genetics and Genomics (ACMG) guidelines to determine the clinical significance of each variant considered for reporting. From the start of Phase II in September 2018, WGS clinical report will consist of reporting of the digital exome using the same exome BED file as the Baylor clinical exome. In addition, 5' and 3' UTR will be analyzed as well as deep intronic changes that were previously described. Further development of a clinical genome report including SNV, CNV, SV, mitochondrial genome, and trinucleotide repeat variants (validated by secondary methodology) is underway and will be introduced stepwise within year 1. The MOO will be updated when these additional elements are launched clinically.

Analysis output delivery turnaround times

Initial analysis (to end of tertiary analysis phase) is completed within a 2-week turnaround time (TAT). Preliminary clinical reports are typically available 8 weeks after raw data is uploaded to the Gateway. All variants included in clinical reports are confirmed by Sanger sequencing. CSs may request additional Sanger sequencing of variants identified during their analysis. Sanger sequencing is performed by the SC. The SC can Sanger confirm up to 8 variants per case. The turnaround time for final clinical reports depends on the timing of CS data analysis but will be 4-

6 weeks from receipt of the CS's additional variants. Please note that TAT may sometimes be delayed depending on the complexity of Sanger primer design for certain genes, particularly those not encountered before in the sequencing core.

Requesting release of sequencing data

The SC follows existing institutional policies for fulfilling raw data requests. Raw data is only released after a final report has been returned to the CS and test results have been communicated to the participant. Baylor College of Medicine can release WES data to physicians and qualified researchers at the participant's request. See <https://baylorgenetics.com/consent/>.

Variants that will appear on the clinical report include:

1. Pathogenic or likely pathogenic variants (SNVs, CNVs or SVs) related to clinical phenotype
2. Variants of unknown clinical significance (VUS) related to clinical phenotype
3. Medically actionable pathogenic variants unrelated to clinical phenotype, if opted for reporting
4. Pathogenic variant indicating carrier status for recessive Mendelian disorders, if opted for reporting

The final step in the interpretation process is a weighing of the evidence regarding each candidate variant. The clinical interpretation of variants and the preparation of the clinical report is conducted in three independent reviews, the first by a PhD-level scientist, the second by an ABMGG-certified clinical molecular geneticist, and the third by a clinical geneticist or genetic counselor. These three reviews are correlated to build a final report, with differing interpretations vetted by an advisory group of laboratory diagnosticians and clinicians who may have expertise in the gene of interest. The final weighing of evidence for pathogenicity of each candidate variant is a manual process that requires critical review of the primary literature, information from databases and in silico prediction programs, and correlation with the patient's phenotype and familial inheritance patterns. In addition to the categories of results listed above, during UDN Phase I we developed a collaborative interpretation process between the sequencing core lab and the clinical sites to confer on individual cases as needed. An extra table (referred to as "table 5") is communicated to the clinical sites which includes variants of interest that do not meet criteria for clinical reporting, such as novel genes. After the clinical site performs their independent analysis of the sequence data, they add additional variants of interest and indicate which of these they would like to have Sanger validation. Sanger is performed and a final report issued.

The clinical WES/WGS report will be issued for the proband to include variants belonging to sections 1-4 above and the allele transmissions of those variants in the parents. The clinical WES/WGS report will be issued for the unaffected parents for incidental findings only according to current UDN protocol. The clinical WES/WGS report includes patient phenotype, information at variant level, gene level, disease level, as well as result interpretation and recommendations for the patient and family. The timeline for providing a preliminary report will be reduced from 9 weeks in Phase I to 8 weeks following upload of raw data in Phase II. A final report which contains additional Sanger sequencing requested by the clinical site after their analysis will be issued 4-6 weeks after receiving the request.

E. Transcriptome Sequencing

This section covers sample intake, library preparation, and whole genome sequencing at Baylor College of Medicine. This section describes the sample flow from DNA sample receipt to production of transcriptome data, including appropriate quality control (QC) and quality assurance procedures. The protocol described above for accessioning, DNA extraction, and DNA quality QC are to be followed for exome and genome sequencing.

Intended use

RNA-Seq utilizes next generation sequencing to analyze the transcriptome. It has the capability to quantify gene expression and can also facilitate the discovery of novel transcripts, identification of alternatively spliced genes, and detection of allele-specific expression. In addition to poly A RNA(mRNA transcripts), other populations of RNA such as noncoding RNA and microRNA can be investigated. Following genomic sequencing, RNA-Seq can be used to provide evidence to further classify variants as disease causing or provide evidence for causality of new disease genes. This is primarily the role of RNA-Seq in the UDN. While WES or WGS analysis can detect SNVs, CNVs and SVs that are disease-causing, RNA-Seq may point to additional disease etiologies by identifying alternative splicing, differential expression of genes or alleles and gene fusions that cannot be readily seen at the DNA level. RNA-Seq provides a dynamic, in-depth view of the transcriptome by detecting and quantifying allele-specific expression arising from coding or non-coding variants and novel alternatively spliced RNA transcripts that may be the result of SNVs, CNVs and SVs as well as revealing the perturbative impact of pathogenic variation on gene expression networks and the larger transcriptome. Importantly a recent study describes a 35% increase in diagnoses using RNA-Seq in patients with a non-diagnostic WES or WGS. The purpose of this protocol is to generate RNA-Seq data for integration of functional omics data for variant prioritization and interpretation, which will be incorporated into the UDN workflow for selected cases. In addition, the analysis may help evaluate the utility of multi-omics data in diagnosing genetic disorders as well as establish procedures for integrating multi-omics into clinical practice.

In Phase 1, an RNA-Seq pilot study was undertaken with three labs providing the technical component. In addition, the groups agreed upon a harmonized analytical pipeline. Insofar as possible within the constraints of our laboratory, we will replicate these procedures that were developed during the pilot including sample types, type of RNA-Seq, cycles, number of reads per sample and analytics.

Test Order

We will follow the workflow for RNA-Seq requests that was established during the UDN RNA-Seq pilot study.

Clinical sites request RNA-Seq through the Gateway. Clinical sites should access the Sequencing Requests section of the Gateway and select Transcriptome from the drop down menu.

Sample intake

A visual inspection of the sample tubes is conducted when received by the SC. Sample tube label is compared with information entered in the Gateway to ensure consistency and completeness of the Gateway data, consent forms, proper sample labeling, and sample tube

integrity. Samples are accepted if no discrepancies are found, sample labels match, and no tube damage is observed. If any of the above criteria is not met, Baylor notifies the referring CS.

Once accepted, samples are accessioned into the Laboratory Information Management System (LIMS) system. Sample information in the Gateway is entered into the WGL LIMS database. Each sample is assigned an internal six-digit lab number, as well as a six-digit family number in LIMS. 1D bar code labels with participant specific information (unique identifier) including participant name, date of birth (DOB), lab number and family number are attached to the stock DNA tube. Subsequently samples are aliquoted from the stock tubes into 2D barcode tubes. The samples in 2D bar code tubes are processed for exome sequencing. Before sample transfer, the record for a sample is first opened in LIMS, then the 1D barcode label on the stock tube of the sample is scanned and the LIMS automatically verifies if the sample ID in LIMS matches that on the label. Then, the 2D bar code on the aliquot tube is also scanned to link the two bar codes in LIMS before sample transferring occurs. These steps are to ensure the chain of custody remains intact during sample transfer.

Assay description

RNA-Seq consists of isolating RNA, converting it to complementary DNA (cDNA), enriching for polyadenylated transcripts or ribo-depletion to remove ribosomal RNAs, preparing the sequencing library and sequencing on an NGS platform. Additional considerations include the use of biological and technical replicates, depth of sequencing, and desired coverage across the transcriptome.

RNA-Seq library prep and sequencing

The transcriptome data will be generated by RNA-Seq from RNA isolated from peripheral whole-blood samples or cultured fibroblasts available from the UDN clinical sites or the Biorepository. Extracted RNA shipped in dry ice will also be accepted. Sample collection and RNA purification will be performed according to PaxGene protocols. All RNA samples will be kept at -80C in 2-3 aliquots. Preparation of cDNA template will use New England Biolabs commercial kit. The quantity and size distribution of cDNA is determined by bioanalyzer with an average size of 250-300bp. Library construction will be carried out by using Illumina TruSeq stranded mRNA library construction kit. The quantity, library concentration and size distribution of the final library is assessed on bioanalyzer. The expected size distribution has an average size of 400bp with a range between 200-800bp. Sequencing is performed on HiSeq2500 or NovaSeq with 150 cycles of paired-end reads. Only the Q₃₀ score >85 data will be used. We aim at 50M reads per sample. We will include standards, from external RNA control consortium, containing 92 transcripts spanning 10⁶ fold concentration range.

RNA-Seq analysis

The RNA-Seq pilot task force devised a harmonized analytical pipeline that the SC will adopt. It is adapted from GTEx and TOPMed Analysis pipeline and is shared through github and Google doc:

https://github.com/furbelows/udn_rnaseq/blob/master/UCLA_UDN_DNAnexus.md

The SC will implement this pipeline in our laboratory and will update the MOO when implemented. Expected date for launch is within 2nd quarter 2019.

Output files generated from this pipeline will be uploaded to the Gateway. These files consist of:
Coordinate-sorted rmdupBAM
BAM index
Genecount table
Chimera table
Splice table
Transcriptome BAM
RSEM quantification – isoform quant, gene quant
RNASeQC summary – total number of reads, proportion of mapped reads, proportion of uniquely mapped reads, estimated library size, proportion of reads mapped to intron/exon, number of spliced alignments, proportion of ribosomal RNA

The turnaround time for RNA-Seq datafile deposition after receiving blood or fibroblasts is approximately 4 weeks.

F. Reanalysis and Reinterpretation of Exome and Genome Data

Reanalysis of previously sequenced exomes and genomes is a standard approach for clinical sequencing laboratories. The power from reanalysis comes from multiple activities within the field, changes in analytical pipelines and changes in clinical status of the patients/participants within the clinical sites. At the November 2016 in-person UDN Steering Committee meeting Baylor reported that reinterpretation for clinical exome sequencing demonstrates an additional diagnostic rate of 11% obtained three-to-four years after an initial report and 5% after a two year interval from the original report date. These results support the value of instituting reinterpretation and reanalysis for UDN cases that remain without a diagnosis. Reanalysis may also be helpful for those cases in which one diagnosis may not completely explain the observed phenotype (e.g., J. Posey, Resolution of disease phenotypes resulting from multilocus genomic variation, New Engl J Med 376:21-31, 2017).

Approach

There are two different analytic approaches proposed: reanalysis and reinterpretation.

Reanalysis

Definition: Start with raw data and rerun the entire analysis and interpretation. Recommend conducting with a significant pipeline change. Reanalysis is available for Phase 1 cases. Clinical sites request reanalysis for specific cases. Each clinical site will be allocated a certain number of reanalysis cases. Reanalysis will be requested through the Coordinating Center. Upon reanalysis, an amended report will be issued which communicates results, new findings, or no changes to original interpretation.

Reinterpretation

Definition: Review of variants in the context of new data in the literature, both new disease gene associations and changes in variant interpretation. Reinterpretation will be conducted annually in an automated fashion. Each clinical site's number of annual reanalyses will be equal to the number of undiagnosed cases at least one year old. The reinterpretation will be conducted on a

quarterly basis (all cases that are at least one year from original report issue). Amended reports will be issued indicating new interpretations, new diagnoses, or no new revised findings. Will commence in September 2019.

Detailed Baylor Reanalysis/Reinterpretation

Reanalysis/Reinterpretation Components	Task	Details
Re-review	Clinical records	Extract new clinical information and review by director (30 mins)
Re-review	Data files	Obtain and re-review genomic information for completeness and quality
Secondary reanalysis	FASTQ through VCF	Reprocessing through Baylor bioinformatics pipeline
*Tertiary analysis	VCF through primary review file	Reprocessing to obtain primary review file
Interpretation (analyst review)	4 hours for analysis	Initial analysis and subsequent discussions
Interpretation (director review)	3-4 hours for analysis	Initial analysis and subsequent discussions
Genetic counselor or medical director review	1 hour	
Report generation and sign-out	Accession (reviewing/compiling report loading the form)	
Return of findings	Review and preparation of packet and loading to Gateway	

*Reinterpretation starts with tertiary analysis and is automated process

XII. Biospecimens

A. Recommendations for Research Specimen Collection and Processing

Overview

The following specimens should be collected on all probands and affected family members unless they are refused by the participant or their collection would compromise participant safety. Clinical sample collection is understood to be the priority and blood draw volume limits are guided by institutional policies. Blood volume limits in pediatric probands are addressed below. Specimens may also be collected from unaffected family members at the discretion of the CS. When blood is drawn for research purposes, DNA collection and plasma for metabolomics should be given the highest priority. It is then up to the individual CS to prioritize the remaining available blood for serum, plasma, and peripheral blood mononuclear cells (PBMCs) based on planned studies.

Serum: 3 ml serum in 0.5 ml aliquots

Plasma: 3 ml plasma in 6 x 0.5 ml aliquots (for general use) and 3 x 0.1 ml (for untargeted metabolomics analyses)

DNA: at least 20 micrograms (with goal of 50 micrograms) at a target concentration of 100-200 ng/ul (with a minimum of 50 ng/ul)

PBMCs: stored in 4 aliquots of 2×10^6 cells followed by multiple aliquots of 5×10^6 cells each

Urine: 10 ml urine in 10 x 1.0 ml aliquots (for general use) and 3 x 0.2 ml (for untargeted metabolomics analyses)

In the instructions below, aliquots for the Metabolomics Core must be placed in 0.5ml Sarstedt Biosphere® SC Micro Tubes (Sarstedt Catalog # 72.730.217), snap frozen, and shipped to the lab within 3-6 months for analysis. Collection of samples for metabolomics analysis is at the discretion of the CS.

General sample collection guidelines

Blood samples for serum and plasma should be obtained in the fasting state, defined as an overnight fast for adults and at least 3 hours of fasting for children. If a subject is unable to fast, samples should still be obtained. The CS should record whether blood samples were collected as fasting (including duration) or non-fasting.

Blood for PBMCs should be collected in CPT Vacutainer® tubes with citrate (one 8 ml tube for pediatric subjects and as total blood draw volume allows, and two 8 ml tubes for adult subjects; if sample volumes are limited by proband body weight use the smaller 4 ml CPT tube). If the CS has expired 8 ml CPT tubes or wants to be cost-efficient and keep only 4 ml CPT tubes in stock, the CS uses as many 4 ml CPT tubes as needed.

Blood for DNA should be collected in one 10 ml purple top EDTA Vacutainer® tube and sent to a local CLIA laboratory for DNA extraction and quantification. If DNA from blood cannot be obtained, an alternative source of DNA such as skin fibroblasts should be considered.

Pediatric probands (and other participants with limited blood collections)

The volume of blood drawn should be consistent with the allowable blood collection based on participant body weight. In cases where the blood volume that can be obtained is the limiting factor, samples should be obtained in the following order:

1. 3 ml blood for DNA (EDTA)
2. 3 ml blood for plasma (EDTA)
3. 3 ml blood for serum (SST)
4. 4 ml blood for PBMCs (Citrate CPT tube)

Sample tracking and storage

Barcode labels of samples to be stored at a local or central biorepository should include the UDN ID, sample type, and date of sample collection.

Samples should be stored as follows:

- Serum: -80°C or colder (e.g., liquid nitrogen)
- Plasma: -80°C or colder (e.g., liquid nitrogen)
- Urine: -80°C or colder (e.g., liquid nitrogen)
- DNA: -20°C or colder
- PBMC: cryopreserved in liquid nitrogen

Note that aliquots for metabolomics analysis should not be subjected to freeze thawing.

Sample collection and processing protocols (I-VII)

I. Blood sample collection and processing

1. Overview
 - a. Processing of blood for plasma and serum, as well as urine, should be performed within two hours of sample collection. PBMCs should be processed within 24 hours of sample collection. All steps should be performed as quickly as possible and on wet ice or otherwise at 4°C, as appropriate, to minimize artifacts in metabolomics data.
 - b. The standard large serum, plasma, urine, DNA, and PBMC aliquots should be stored in screw-cap cryovials appropriate for ultra-cold storage (e.g., Nalgene NUNC 1.8 ml Cryovials, Fisher Scientific Catalog #: 12-565-170N).
 - c. Aliquots for untargeted metabolomics need to be stored in Sarstedt Biosphere® SC Micro 0.5ml Tubes, Sarstedt Catalog # 72.730.217.
2. Serum sample collection and processing
 - a. Blood for serum should be collected in one 10 ml red top Vacutainer® Serum Separator Tubes (SST) with clot activator
 - a. After obtaining the SST sample, allow sample to clot 30 minutes in a vertical position

- b. Centrifuge vacutainer tube at 2500 RPM ($1-1.3 \times 10^3$ g) for 10 minutes either at ambient temperature or at 4°C.
 - c. Carefully remove rubber stopper from vacutainer tube and remove caps from cryovial tubes
 - d. Aliquot 0.5 ml of serum into 6 cryovial storage tubes (3 ml total), and store samples in a -80°C or liquid nitrogen freezer with appropriate labels (UDN ID, sample type, and collection date). Pediatric samples generate fewer tubes.
3. Plasma sample collection and processing
 - a. Blood for plasma should be collected in one 10 ml purple top EDTA Vacutainer® tube
 - b. Centrifuge vacutainer tube at 2500 RPM ($1-1.3 \times 10^3$ g) for 10 minutes at 4°C
 - c. Aliquot 0.5 ml of plasma into 6 cryovial storage tubes (3 ml total), and store samples in a - 80°C or liquid nitrogen freezer with appropriate labels (UDN ID, sample type, and collection date). Pediatric samples generate fewer tubes.
Note: if metabolomic aliquots are needed (section d below) you may need to preserve fewer aliquots in this step.
 - d. Aliquot plasma to storage tubes for untargeted metabolomic studies (Sarstedt Biosphere® SC Micro Tube 0.5ml, Sarstedt Catalog # 72.730.217).
 - i. For untargeted metabolomics analyses: 3 aliquots of plasma (0.1 ml each). Otherwise, see the Metabolomics section for details regarding other analyses.
 - ii. For optional targeted analyses (available at the Metabolomics Core): 1 aliquot of plasma (1 ml). Please see the Metabolomics section for details regarding other analyses.
 - e. Flash freeze all samples in liquid nitrogen or quick freeze in dry ice/ethanol prior to storage in a -80°C freezer, or in liquid nitrogen, with appropriate labels (UDN ID, sample type, and collection date).
4. DNA extraction at CLIA laboratory
 - a. Multiple acceptable DNA extraction protocols for the EDTA Vacutainer tube blood samples can be used (Examples of suitable Extraction kits: Qiagen Gentra Puregene Blood Kit Catalog # 158445, or Qiagen DNeasy Blood and Tissue Kit Catalog# 69504). DNA should be stored in TE (Tris-EDTA) buffer).
 - b. DNA stored at the CS or UDN Central Biorepository (UDNCB) should be labeled with UDN ID, date of birth, sample type, and collection date.
 - c. DNA aliquots sent to the SC must be labeled with participant name, date of birth, and “UDN” (do NOT include UDN ID).
 - d. DNA quantification should be performed with PicoGreen (not NanoDrop), and DNA concentration should be between 100 to 200 ng/ul (with a minimum of 50 ng/ul).

II. PBMC isolation and cryopreservation

1. PBMC isolation
 - a. CPT tubes are the recommended cell separating device (refer to specific CPT tube manufacturer instructions for complete details for steps 1-4 and those below are offered as suggestions). Other cell separating devices may be utilized at individual sites for local biorepository.
 - b. Centrifuge blood collected in CPT tubes at room temperature at 1500 to 1800 x g in a swing bucket rotor for 20-30 minutes with no brake following specific instructions from the CPT tube documentation. Visually inspect the CPT gel plug in addition to other guidance in the manufacturer's instructions.

- c. Use an aspirating pipette to remove the PBMC layer located at the gel interface in the CPT tube.
 - d. Place the PBMCs in a new 50 ml conical tube.
 - e. Wash cells by gently resuspending the cell pellet in 10 ml sterile 4°C or ambient temperature PBS (or other physiologic buffer) followed by centrifugation at 250-400 x g. Repeat once.
 - f. Count the PBMCs on a hemocytometer, cellometer, or other standardized cell counting device.
 - g. Separate into 4 aliquots of 2×10^6 cells followed by multiple aliquots of 5×10^6 cells each in separate polypropylene tubes and centrifuge at 250-400 x g to create the final cell pellet. The maximum number of aliquots should be made to allow for as many separate PBMC samples as possible to be saved from any one donor.
2. Preparation of PBMCs for storage in a cryorepository
 - a. To prevent contamination, all processing shall be completed in a sterile biological safety cabinet by wiping all inside surfaces with 70% alcohol and performing UV light treatment for at least 5 minutes.
 - b. Remove as much of wash buffer as possible from each aliquoted PBMC pellet. You may gently flick pellet to loosen prior to next step (resuspension).
 - c. Resuspend each washed PBMC pellet containing 2 or 5 million cells in 1 ml of cold (2-8°C) CryoStor CS10 Freeze Media (BioLife Solutions).
 - d. Mix cells by gently tapping the tube; do not use a pipette.
 - e. Incubate resuspended cells at 2-8°C for 10 minutes.
 - f. Pipet gently to minimize shear force and transfer into a labeled cryopreservation vial.
 - g. For cryopreservation, transfer vials to a Controlled Rate Freezer to decrease the temperature in a controlled fashion (or if that is unavailable into an isopropanol containing cryopreservation system followed by transfer into a -80°C freezer for a minimum of 12 hours).
 - h. Once cryopreservation vials have been appropriately cooled and contents frozen, transfer to designated receptacle within the liquid nitrogen storage unit for long-term storage with appropriate labels (UDN ID, sample type, and collection date).

III. Urine sample collection and processing

1. Urine samples should be the first morning void urine collected in a polypropylene container. A 24-hour urine sample is not required, but may be elected by the CS.
2. Centrifuge urine at 1000 x g for 5 minutes at 4°C to remove any cells and particulates.
3. Transfer supernatant to storage tubes
 - a. Ten 1.0 ml aliquots in 1.8 ml screw-cap cryovials
 - b. Three 0.2 ml aliquots in Sarstedt Biosphere® SC Micro 0.5ml Tubes (for untargeted metabolomics analyses). For other metabolomics services, see the Metabolomics section for details.
4. Flash freeze all samples in liquid nitrogen or quick freeze in dry ice/ethanol prior to storage in a -80°C or liquid nitrogen freezer with appropriate labels (UDN ID, sample type, and collection date).

IV. Sample shipment to the Metabolomics Core

1. See the Metabolomics section for shipping details to the Metabolomics Core at Mayo Clinic.

B. Recommendations for Optional Research Biospecimen Collection and Processing

I. CSF collection and processing

CSF should be collected from neurological cases for clinical laboratory studies and for research use, including metabolomics, lipidomics, glycomics testing, and inflammome studies. All samples should be collected by lumbar puncture in the L3/L4 or the L4/L5 interspace. If neurotransmitters are to be analyzed, they should be collected first and will preempt the possibility of obtaining an opening pressure. Neurotransmitters are volatile and have a cephalo-caudal concentration.

1. Microtubes

- a. Microtubes 1-5 (4.5 ml total): for shipment to Medical Neurogenetics (Note: Medical Neurogenetics and Baylor both supply 5 pre-marked CSF microtubes (total volume if filled only to the lines is 4-4.5 ml) - the studies offered by each lab varies though the methods are the same.
- b. CSF must be collected from the 1st drop into the designated tubes in the order indicated in the following table. Do not collect the CSF in one large tube and aliquot into the tube set.
- c. Fill each tube to the marked line with the following volumes, indicated in the table below. The total CSF volume required is 4-4.5 ml.

Tube Number	Required Volume
1	0.5 ml
2	1.0 ml
3	1.0 ml
4	1.0 ml
5	1.0 ml

- d. If the samples are not blood contaminated, place the tubes on ice (or dry ice if available), and then transfer to a -80°C freezer. If the samples are blood contaminated, then centrifuge immediately (prior to freezing) and transfer the clear CSF to new similarly labeled tubes then freeze and store at -80°C.
- e. Microtubes are collected in 1 ml aliquots and placed in wet ice until they can be aliquoted: 5 x 1 ml (e.g., for energetics), 3 x 0.2 ml (for metabolomics, glycoproteomics, and lipidomics analyses), and the remainder in a final aliquot (e.g., Sarstedt Polypropylene 2ml tube #72.694.107). The Metabolomics Core requires that its 3 x 0.2 ml aliquots be in Sarstedt Biosphere® SC Micro Tube 0.5ml, Sarstedt Catalog # 72.730.217.
- f. Polystyrene tubes 1-3 (0.5 ml each minimum): for measurement of glucose, cell count, protein, sterile fluid culture (Note: a standard lumbar puncture tray contains 4 polystyrene tubes with caps).
- g. Polystyrene tube 4: for IgG index, Oligoclonal bands, etc. (Note: use 4th tube from lumbar puncture tray here).

2. CSF handling
 - a. CSF is collected by lumbar puncture, place the CSF on wet ice immediately and transport to the laboratory. If dry ice is available and the CSF is not bloody the microtubules for neurotransmitters can be placed in the dry ice immediately.
 - b. If CSF is bloody, excessive blood may interfere with neurotransmitter and metabolomics testing
 - c. Flash freeze all samples in liquid nitrogen or quick freeze in dry ice/ethanol prior to storage in the -80°C freezer with appropriate labels (UDN ID, sample type, and collection date)
 - d. Samples should not be subjected to freeze-thawing

II. Optional blood collection and processing

1. General guidelines
 - a. As noted above, blood is collected from all probands for DNA, plasma, serum, and PBMCs. Various other optional samples may be considered as well, including: PaxRNA, buffy coat, platelets, oxylipids, and blood spot cards.
 - b. Blood for RNA is collected in PAXgene blood RNA Vacutainer® tubes (VWR 77776-026).
 - c. Blood for additional PBMC or other buffy coat collection is collected in additional CPT (citrate) tubes or other site-specific collection tubes.
 - d. If platelets are to be collected, draw 31.5 ml blood in 7 tubes of light blue top sodium citrate tubes (BLU).
 - e. If oxylipids are to be analyzed, then blood should be collected in a chilled EDTA tube and placed on ice.
 - f. Blood spot cards may also be obtained and stored at room temperature.
2. RNA processing
 - a. Use PAXgene Blood RNA Kit (Qiagen 762164)
 - b. Aliquot in 80ul aliquots and store in 500ul sterile, RNase- DNase-free tubes at -20°C until needed
3. RBC isolation
 - a. After removal of plasma (see procedures above), discard the remaining supernatant above the porous barrier using a plastic Pasteur pipette (wide orifice)
 - b. Using a glass Pasteur pipette (narrow orifice), transfer the erythrocyte (RBC) pellet to a 50 ml conical tube
 - c. Fill 50 ml conical tube to 40 ml with Phosphate Buffered Saline (PBS), pH 7.4 and invert several times to mix
 - d. Centrifuge for 5min at 1811 x g at 4°C
 - e. Remove saline layer and discard
 - f. Repeat wash with PBS pH 7.4 until PBS is clear (minimum of 3 times)
 - g. Aliquot 1 ml of the erythrocyte (RBC) pellet to clean cryovials. Store in -80°C freezer with appropriate labels (UDN ID, sample type, and collection date). *Note: this fraction also contains granulocytes*
4. Platelet Isolation
 - a. Add 1 volume HEP buffer + PGE1
 - b. Mix very gently by inverting the tube slowly
 - c. Spin at 100 x g for 15-20 min at room temperature (with no brake applied) to pellet contaminating red and white blood cells
 - d. Transfer the supernatant into new plastic tube using a transfer pipette (wide orifice)

- e. Pellet platelets by centrifugation at 800 x g for 15-20 min at room temperature (with no brake applied). Discard the supernatant.
 - f. Rinse the platelet pellet two times with platelet wash buffer by gently adding wash buffer and removing it slowly with a pipette. (DO NOT RESUSPEND! to avoid platelet activation)
 - g. Store the dry platelet pellet at -80°C freezer with appropriate labels (UDN ID, sample type, and collection date). *Note: Freezing the pellet disrupts the platelet granules. This pellet is only to be used for determination of glycomics, lipidomics and proteomics that does not include the platelet granules.*
5. Lipidomics sample processing
- a. Centrifuge chilled blood for 10 min at 1200 x g at 4°C
 - b. Aliquot 1 mL plasma into a single storage vial
 - c. Keep on ice and flash freeze in liquid nitrogen or quick freeze in dry ice/ethanol the samples prior to storage at -80 °C within 4 hours (optimal is to freeze immediately and store at -80°C) and do not subject to freeze thaw cycles. If that is not possible then the time should be adjusted to be the same for all samples. Do not store at -20 °C. Redraw the sample if it is extensively hemolyzed.

III. Skin biopsy collection and processing

Skin biopsies are used for culturing fibroblasts. These cell lines are then be used for various research purposes as well as glycomics testing. For subjects who are unable to provide PBMCs, skin fibroblasts provide an alternate source of living cells for future research.

1. Reagents
 - a. DMEM High Glucose (Invitrogen #11965-118)
 - b. Fetal Bovine Serum (FBS), Certified, Heat-Inactivated, US Origin (Invitrogen #10082-147)
 - c. 100X Antibiotic-Antimycotic (Invitrogen #15240-062)
 - d. 0.25% Trypsin-EDTA (Invitrogen #25200-056)
 - e. 1 x PBS pH 7.4 w/o Calcium or Magnesium (Invitrogen #10010-023)
 - f. Dimethyl sulfoxide (Sigma #D8418-100ML)
2. Collection protocol
 - a. 3-5mm punch full thickness skin biopsy obtained according to standard medical procedure
 - b. Place biopsy in sterile tissue culture medium (DMEM, 10% FBS, 1% antimycotic, antibiotic) contained in a 15 ml conical tube
 - c. Store and transport the biopsy at ambient temperature
 - d. Deliver the biopsy to the laboratory within 24 hours (Up to 96 hours is acceptable if shipped)
3. Initiation of skin fibroblast culture
 - a. Spray hood and scalpels with Ethanol and wipe with Kimwipe
 - b. Clean the biopsy tube by spraying well with Ethanol before placing in the hood
 - c. Label 6-well tissue culture (TC) plate and place in TC hood
 - d. Aspirate medium from biopsy sample using a 2 ml aspirating pipette
 - e. Remove biopsy sample and place in one well of the 6-well dish
 - f. Using the scalpels, cut the biopsy sample into 6 pieces (Try to attach the biopsy to the plate with the scratches made by the scalpel)

- g. Add 1 ml of pre-warmed culture medium (DMEM, 10% FBS, 1% antimycotic, antibiotic) to each well, being careful not to dislodge the biopsy
 - h. Gently swirl the 6-well dish to coat the wells with culture medium
 - i. Place in the 37°C, 5% CO₂ TC incubator for 4-5 days to allow the biopsy to attach to the well
 - j. Gently add 2 ml of fresh, pre-warmed culture medium to each well being careful to not dislodge biopsy sample
 - k. Allow the sample to remain in the 37°C incubator until a monolayer of cells is present in the wells, feeding cells with fresh culture medium every 3-4 days
 - l. Once adequate cells have grown out of the biopsy fragment, remove the cells from each of the 6-wells by washing with 2 ml PBS, and following removal of the PBS, by adding 800ul trypsin (0.25% Trypsin EDTA, Invitrogen). Incubate at 37°C until cells are released from substrate. Then add 2 ml of tissue culture medium and transfer cells to one T75 culture flask.
 - m. After 2 days, aspirate all but 1 ml of medium from flask. Scrape the bottom of the flask using a cell scraper, and wash cells with the 1 ml of medium remaining in the flask. Remove the 1 ml of culture, and place in a 1.5 ml eppendorf tube. Perform mycoplasma testing using ATCC Universal Mycoplasma Testing Kit (see section IX.B.III.7 below) on this 1 ml aliquot and record results.
 - n. Add 10 ml of fresh DMEM to flask and return to 37°C tissue culture incubator to allow the remaining cells to proliferate.
 - o. Allow samples to reach confluency
 - p. Remove medium and wash cells with 10 ml PBS
 - q. Detach cells as described below (Detaching and Passaging Cells). Add 8 ml of tissue culture medium to collect cell suspension, use 1 ml of the culture to start another T75 culture flask. As described below (Freezing Cells), count the cells remaining in suspension, centrifuge at 1000 RPM for 10 minutes, add 3 ml of freezing medium, and freeze the remaining culture into 3x 1 ml cryovials at -80°C in a cool cell for 3 days with appropriate labels (UDN ID, sample type, collection date, and passage number). Transfer to -150°C for permanent storage (Passage 1 cells).
 - r. Allow the second T75 flask to reach confluency, feeding with fresh DMEM every 2-3 days. Once confluent, trypsinize the flask, add 9 ml of DMEM count the cells, centrifuge at 1000 RPM for 10 minutes, aspirate supernatant, add 3 ml of freezing media, and freeze at -80°C in a cool cell for 3 days with appropriate labels (UDN ID, sample type, collection date, and passage number). Transfer to -150°C for permanent storage (Passage 2 cells).
4. Freezing fibroblast cells
- a. Prepare 3-4 ml of fresh freezing medium (10% DMSO, 90% FBS) per T75 flask and warm to 37°C.
 - b. Place the 9 ml fibroblast culture into a sterile 15 ml conical tube.
 - c. Count cells using the cell counter: Add 10ul of culture to BioRad cell counting slide and insert into the BioRad TC20 Automated Cell Counter. Multiply this number of cells by 9 (volume of total culture) and divide by the number of frozen culture aliquots you are making to determine the amount of cells frozen per tube.
 - d. Centrifuge culture at 1000 RPM for 5 minutes.
 - e. Aspirate off supernatant.
 - f. Add prepared freezing medium and mix by pipetting up and down.
 - g. Aliquot 1 ml into labeled cryovial.
 - h. Place cryovial into Cool-Cell that is labeled with your name and date.

- i. Place Cool-Cell into -80°C freezer for 3 days (36 hours) with appropriate labels (UDN ID, sample type, collection date, amount of cells, and passage number) and then transfer into -150°C storage.
5. Tissue culture for frozen skin fibroblasts
 - a. Once the tissue culture medium has warmed, remove cell vial from -150°C and immediately place at 37°C.
 - b. Prepare a T75 tissue culture flask or 10cm petri dish by adding 10 ml of pre-warmed tissue culture medium.
 - c. Remove cells from vial using a sterile 1 ml pipette tip or 1 ml pipette.
 - d. Add cells to flask and gently mix.
 - e. Place inoculated flask in a 37°C, 5% CO₂ tissue culture incubator for 24 hours to allow cells to attach to the dish.
 - f. After 24 hours, remove medium using a 2 ml aspirating pipette and replace with 10 ml of fresh tissue culture medium. Place at 37°C, 5% CO₂ in tissue culture incubator.
 - g. Feed cells every 2-3 days by aspirating off old tissue culture medium and replacing with fresh, pre-warmed, tissue culture medium.
 - h. Once cells have reached confluency, cells must be passaged and split into new T75 culture dishes.
6. Detaching and passaging cells
 - a. Warm 0.25% Trypsin EDTA to 37°C.
 - b. Warm DMEM, 10% FBS, and 1% Anti-Anti to 37°C.
 - c. Once reagents have warmed, aspirate medium from flask containing cells using a 2 ml aspirating pipette.
 - d. Rinse cells with 10 ml of 1X PBS pH 7.4 without Calcium or Magnesium.
 - e. Aspirate 1X PBS using 2 ml aspirating pipette.
 - f. Add 1 ml of pre-warmed 0.25% Trypsin EDTA to TC flask and spread across the attachment area by swirling the flask.
 - g. Incubate flask at 37°C for ~ 5 minutes (or until cells are rounding), then gently tap the flask to release the cells.
 - h. Add 8 ml of pre-warmed tissue culture medium and wash cells to the bottom of the flask.
 - i. Add 3 ml of culture to fresh T75 flask containing 7 ml of tissue culture medium. Place at 37°C, 5% CO₂ in tissue culture incubator.
7. Mycoplasma testing
 - a. After 2-3 days of cell growth, aspirate all but ~1 ml of medium from flask.
 - b. Using a cell scraper, scrape the cells from the bottom of the flask only.
 - c. Using a 1 ml serological pipette, wash cells using the 1 ml of media in the flask.
 - d. Remove the 1 ml aliquot and place in a sterile 1.5 ml eppendorf tube.
 - e. Add fresh pre-warmed DMEM to flask and place in 37°C, 5% CO₂ tissue culture incubator for future use.
 - f. Perform mycoplasma testing using the Universal ATCC Mycoplasma Testing Kit (ATCC #30-1012K) according to the protocol on the 1 ml aliquot.
 - g. Record mycoplasma results and upload gel image into LIMS. If mycoplasma free, you can continue to passage. If mycoplasma positive, cells must be treated with Plasmocin (InvivoGen #ant-mpt) according to the protocol for 2 weeks and retested.

8. Growth and isolation of fibroblasts for metabolomics analysis
 - a. Grow passaged fibroblasts (passage 1 or 2) to a density of ≥ 1 million cells, in replicate ($n = 3 - 5$)
 - b. Remove cell culture media, and place 1 mL of spent media into a storage tube. Also place 1 mL of fresh media into a storage tube (Sarstedt Biosphere® SC Micro Tube 0.5 ml, Sarstedt Catalog # 72.730.217)
 - c. Quench cell metabolism using a wash of ice-cold PBS
 - d. Scrape cells from culture plate into storage tube (Sarstedt Biosphere® SC Micro Tube 0.5 ml, Sarstedt Catalog # 72.730.217). If needed, then add a small amount of ice-cold PBS to facilitate scraping and collecting of cells, followed by centrifugation to pellet cells (remove PBS and discard).
 - e. Flash freeze all samples in liquid nitrogen or quick freeze in dry ice/ethanol prior to storage in a -80°C freezer, or in liquid nitrogen, with appropriate labels (UDN ID, sample type, and collection date)

XIII. Central Biorepository

The UDN Central Biorepository (UDNCB) will accept samples from approximately 1,200 participants total per year from all of the Clinical Sites. Probands and affected family members should be prioritized followed by unaffected family members that underwent sequencing. Samples from family members are not required. Clinical Sites evaluating 15 cases per year can expect to submit up to 55 individuals to the UDNCB annually while Clinical Sites evaluating 30 cases per year will submit up to 110 individuals to the UDNCB annually.

A. Flow of samples to UDNCB

Samples are submitted to the UDNCB through the Gateway. CS are able to submit samples, view samples available, request samples, and view contact information for the UDNCB in the Gateway.

Submitting samples

1. CS follows specimen collection, processing, and storage guidelines described in Biospecimen section.
2. CS selects half of the processed samples for shipping to the UDNCB. The following samples are prioritized for submission to the UDNCB when available: DNA, plasma, serum, urine, and PBMCs. Other samples such as fibroblasts, CSF, and RNA may be submitted at the discretion of the CS. Samples may be shipped in batches (recommended frequency: at least every 2 months).
3. CS enters the sample information in the Gateway by navigating to the Biorepository tab and clicking on “Submit Specimens>Create New Submission.”
4. Once the samples are submitted click the Submission ID link to download a CSV file containing the details of the submission. Keep this for your records.
5. CS prepares the samples for shipment on dry ice, FedEx priority overnight (Mon-Weds only), include printed copy of sample submission form (see step 3). No shipping during a holiday week or two weeks before Christmas through New Year’s due to issues with FedEx deliveries during these times.
6. Triple pack your samples, select an appropriate dry ice safe shipping box and enough dry ice to last 48 hours in case of shipping/delivery delays.
 - a. Example: Thermosafe EPS foam box w/corrugated carton #448UPS & 10 lbs dry ice
 - b. Triple Packing Components:
 - i. Primary container – to contain the sample
 - ii. Secondary container – to contain leaks, should include absorbent material
 - iii. Outer package – to give form to the package and protect inner contents (e.g., a fiberboard box surrounding the styrofoam box)
 - iv. Note: The styrofoam box doesn’t count as one of the 3 containers here (it is to keep samples frozen)
7. Ship samples to: Lynette Rives, Vanderbilt Medical Center, DD-2205 MCN, Nashville TN 37232-2578, Phone: 615-875-7198
8. UDNCB notifies CS when samples have safely arrived.
9. Questions about preparing samples to ship, sample submission form, etc.: email UDNCB@VUMC.org or phone 615-875-7198.

Viewing Samples Available

Samples available can be viewed two ways in Gateway:

1. Within the individual participant record, navigate to the Samples tab and click “View Inventory” to view all samples available for that participant.
2. From anywhere in the Gateway, navigate to the Biorepository tab and click “Specimen Inventory” to view all samples currently available from the UDNCB.

Requesting Samples

Samples stored by the UDNCB are available to UDN investigators and their collaborators. An investigator wishing to obtain samples from the UDNCB should check the availability of the samples of interest through the Gateway. If the investigator wishes to obtain samples from the UDNCB that were deposited by their own site, no approval by the Publications and Research Committee (PRC) is required.

Since quantities are limited, sample requests for samples from a site other than the investigator’s require prior approval from the UDN. If the investigator wishes to obtain samples from the UDNCB that were deposited by another site, they must first obtain approval from the UDN Publications and Research Committee (PRC). After confirming the availability of samples, the investigator must submit a research concept sheet through Qualtrics (https://hms.az1.qualtrics.com/jfe/form/SV_3CyZOKOuiEvnCx7). The concept sheet should describe the proposed studies and the samples needed from the UDNCB. The UDN Publications and Research Committee (PRC) reviews the application and the Director of the UDNCB, who is part of the PRC, reconfirms sample availability. If the request is approved, a UDNCB approval number is sent to the investigator.

The investigator then completes the Sample Submission Form in the Biorepository section of the Gateway. Since outside investigators do not have Gateway access, a member of the UDN with Gateway access may submit the request on the investigator’s behalf.

The UDNCB contacts the investigator to arrange a time to ship samples. The investigator is asked to notify the UDNCB when samples arrive. The UDNCB notifies the relevant CS whenever samples are released to a UDN investigator or third party.

B. UDNCB Procedures

Storing and archiving of biological specimens

Mailed samples are opened in a clean “no amplified DNA” laboratory. The frozen samples, pre-aliquoted in screw cap cryotubes and labeled by the sender, are placed on dry ice while the labels are checked against the Sample Submission Form accompanying the package for confirmation. This form is downloaded from the Gateway and must be included with all samples shipped to the biorepository. The Sample Submission Form contains the participant UDN ID (also printed on the sample tubes). All tubes have participant UDN ID, sample type, and date of collection typed on the label. Receipt of the samples is recorded in the laboratory sample intake book and their condition noted (if dry ice is gone, samples are partially thawed, any tubes are cracked, etc.). Samples are not required to have barcodes, but the UDNCB has the ability to read 1D and 2D barcodes. If barcodes are included on the labels by the CS, the information outlined in the Biospecimen section must still be typed legibly on the sample labels.

Storage of samples: The biological samples are placed into liquid nitrogen cryotanks (PBMCs and possibly fibroblasts) and -80°C freezers (DNA, serum, plasma, urine) for long-term storage in the locations assigned by the Progeny Laboratory Information Management System (LIMS) database. Samples with multiple tubes are divided into 2 separate freezers/cryotanks. All UDNCB equipment is on the Vanderbilt Delta alarm system with temperature and nitrogen fluctuation notification automatically going to the Director's and the senior Research Assistant's cell phone/pager.

Documentation: The sender is notified by email of shipment arrival and any problems that may have occurred with the shipment (late arrival, partially thawed tubes, broken tubes, etc.). Any problems with the shipment are also recorded into the Progeny LIMS database. Documentation into the Gateway Sample Submission Form is completed by the CS submitting the samples, and a printed copy of the CSV file containing the details of the submission is included with the samples when they are shipped. No participant names or identifying information is recorded in the Progeny LIMS, the sample intake notebook, or requested on the Sample Submission Forms.

Retrieval and shipping of biological specimens

The UDNCB retrieves biological samples from liquid nitrogen and/or -80°C freezers, packages samples in dry ice, and ships to UDN investigators and collaborators. Sample information in Progeny LIMS is used to track quantities and distribution of biological samples.

Locating biological specimens in storage: The Progeny LIMS database contains participant UDN ID, date of birth, sample type, date of collection, and sample location. In addition to sample and location information, the Progeny LIMS database keeps track of original and current quantities of the biological samples and record the distribution of samples to investigators.

Sample retrieval and transfer: The Biorepository Working Group and Steering Committee must approve sample requests by reviewing the Sample Submission Form prior to electronic application to the UDNCB. After UDN approval by the applicant's receipt of a UDN approval number and completion of a Sample Submission Form through the Gateway, the UDNCB contacts the CS or CS-designated investigator requesting the samples by email to pre-arrange a date for shipment. The samples are then located using the Progeny LIMS database, retrieved, and placed on dry ice to prepare for transfer to the investigator requesting the sample.

Documentation of retrieval: The type of sample, amount transferred, date of retrieval, and the CS-designated investigator receiving the sample are recorded in the Progeny LIMS database. The Biorepository sample inventory on the Gateway is updated so that all UDN investigators can see which samples and the amount(s) of each remain in the system.

Packaging and shipping: The biological samples, already labeled and in screw cap tubes, are packaged and shipped per International Air Transportation Association (IATA) requirements that apply to all dangerous goods (such as dry ice) by air. Samples must be triple packed which includes a leak proof bag with absorbent material. UDNCB ships frozen samples in EPS foam containers (1.5 inch minimum thickness) with corrugated cartons, 10 lbs dry ice by FedEx priority overnight (Monday–Wednesday). Average dry ice sublimation in a 1-1/2 inch thick wall EPS container with corrugated container is 5 pounds over 24 hours (< 10 lbs in 48 hours).

Delays can arise with FedEx and the extra dry ice is a safeguard to protect the samples in case of delays in delivery. A list of sample content is included with shipment and the CS and CS-

designated investigator(s) are notified by email that the sample has shipped, given the FedEx tracking number, and an electronic copy of the sample sheet. The email requests that the UDNCB be notified upon receipt of the shipment and of any problems with the samples (e.g., tubes thawed or damaged, etc.).

Packaging and shipping budget: Shipping samples to the UDNCB is at the CS expense and can be in batches to reduce costs. Shipping samples out from the UDNCB to investigators is at the expense of the UDNCB. The budget accommodates 50 shipments per year. If sample approvals by the UDN exceed the number of shipments allotted per year, then additional funds will need to be made available for the UDNCB or, alternatively, the CS-designated investigator(s) receiving samples will be required to pay the shipping costs.

Quality control: The UDNCB keeps records on the number of samples received, their condition, date shipped/date arrived, etc. The UDNCB also keeps records on the number of samples shipped out, date shipped/date received, condition upon arrival, any problems reported by the recipient, etc.

Updating UDNCB inventory in the Gateway

Samples collected by the CS and entered into the UDNCB Sample Submission Form prior to shipping are automatically entered into the sample inventory and can be viewed in the Gateway. When samples are requested and shipped out through the UDNCB, the UDNCB edits/updates the sample inventory on the Gateway.

XIV. Metabolomics

A. Introduction

UDN Metabolomics Core at Mayo Clinic provides untargeted and targeted/quantitative metabolomics approaches, bioinformatics, and clinical interpretation to the biological samples collected in the UDN. The Metabolomics Core will engage in the following activities to help accomplish the mission of the UDN:

- 1) The Core will perform global, untargeted metabolite profiling using analytical platforms including GC-MS, LC-MS/MS, and NMR.
- 2) The Core will perform targeted quantitative analysis of select metabolites including key priority targets (i.e., glycans, lipids, and mitochondrial metabolites) as well as a wide breadth of other targetable metabolites that will provide crucial metabolic insights on a case-by-case basis.
- 3) The Core will support assay development for new metabolite targets as necessary.
- 4) The Core will support analytical needs of Model Systems Cores.
The Core will provide clinical expertise, standardized reporting, and metabolite interpretation to the UDN.

When to consider metabolomics analyses

Metabolomic studies should be considered for all UDN patients. Known inborn errors of metabolism have been associated with a diverse range of phenotypes affecting all systems. Certain phenotypes should prompt strong consideration of an underlying metabolic etiology. We recommend reviewing Chapter 1 of *Inborn Metabolic Diseases* 5th Edition, Saudubray, van den Berge and Walter Eds. Springer-Verlag, Berlin, for further details regarding clinical manifestations and inborn errors of metabolism.

Table 1. A Brief List of Various Phenotypes for Strong Consideration of Metabolomics.

Neurologic phenotypes	Intellectual disability – GAMT deficiency, AGAT deficiency, serine biosynthetic defects Seizures – sulfite oxidase deficiency, non-ketotic hyperglycinemia Episodic encephalopathy – urea cycle defects, organic acidemias, maple syrup urine disease Movement disorders – glutaric aciduria type 1, aromatic amino acid decarboxylase deficiency Neurologic decline – various leukodystrophies, storage disorders, MELAS Macrocephaly – Alexander disease, Canavan disease Microcephaly – PKU, sulfite oxidase deficiency Stroke or stroke-like episodes – MELAS, homocystinuria, CDGs Gastroenterologic phenotypes Abdominal pain – porphyrias, MNGIE, Fabry Hepatic disease – CDGs, Citrin deficiency, Nieman-Pick diseases, Arginase deficiency, HFI Diarrhea – MNGIE, mevalonate kinase deficiency, CDGs, congenital diarrheas
Cardiac phenotypes	Cardiomyopathy – fatty acid oxidation defects, Barth syndrome, Fabry disease, Pompe disease Arrhythmias – Kearn-Sayre syndrome, Thiamine deficiency

Dermatologic phenotypes	Alopecia – biotinidase deficiency, multiple carboxylase deficiency, Menkes disease, porphyrias Angiokeratosis – Fabry, galactosialidosis Hair abnormalities – Argininosuccinic aciduria, menkes, trichothiodystrophy Ichthyosis – Refsum disease, steroid sulfatase deficiency Ulceration – prolidase deficiency Photosensitivity – porphyrias, xeroderma pigmentosa
Dysmorphic features	Coarse – storage diseases Structural malformations – Smith-Lemli-Opitz disease, CHILD syndrome, CDGs, Antley-Bixler disease
Renal phenotypes	Stones – cystinuria, xanthine oxidase deficiency, Lesh-Nyhan disease, hyperoxalurias Tubulopathies – respiratory chain disorders, galactosemia, tyrosinemia type I, cystinosis, Lowe syndrome Polycystic kidneys – CPT2 deficiency, CDGs, Zellweger syndrome Abnormal urine color – alkaptonuria, porphyrias
Bone disorders	Osteopenia – CDGs, glycogen storage disorders, organic acidurias Skeletal dysplasia – mucopolysaccharidoses, peroxisomal disorders
Rheumatologic/immunologic disorders	Immune deficiency - MVK deficiency, organic acidurias, GSD1b, CDGs, lysinuric protein intolerance,
Growth failure	Pearson syndrome, congenital chloride diarrhea, Schwachmann-diamond syndrome

B. General Workflow

Each CS is recommended to complete a Metabolomics Request in the Gateway and collect the appropriate specimen(s) for analysis. Requests for metabolomics analysis are reviewed by the Metabolomics Consultation Team at Mayo Clinic. In addition, Mayo Clinic's laboratory information system will be queried for potential patient laboratory data regarding past commercial testing results. If no specific hypotheses are generated, the MC will offer to perform untargeted metabolomics and/or lipidomics. If there are specific hypothesis regarding the participant's diagnosis that the CS wishes to explore, one or more targeted approaches to metabolomics will be available to pursue (see Table 2). The resultant data is initially analyzed and reviewed by the Metabolomics Reporting Team at Mayo Clinic, and if necessary, the data are reviewed by the Metabolomics Case Review Committee and requesting CS. If no diagnosis is identified, further rounds of analysis may be undertaken based on new hypotheses generated via discussions between the Metabolomics Case Review Committee and referring CS. Figure 1 outlines this workflow.

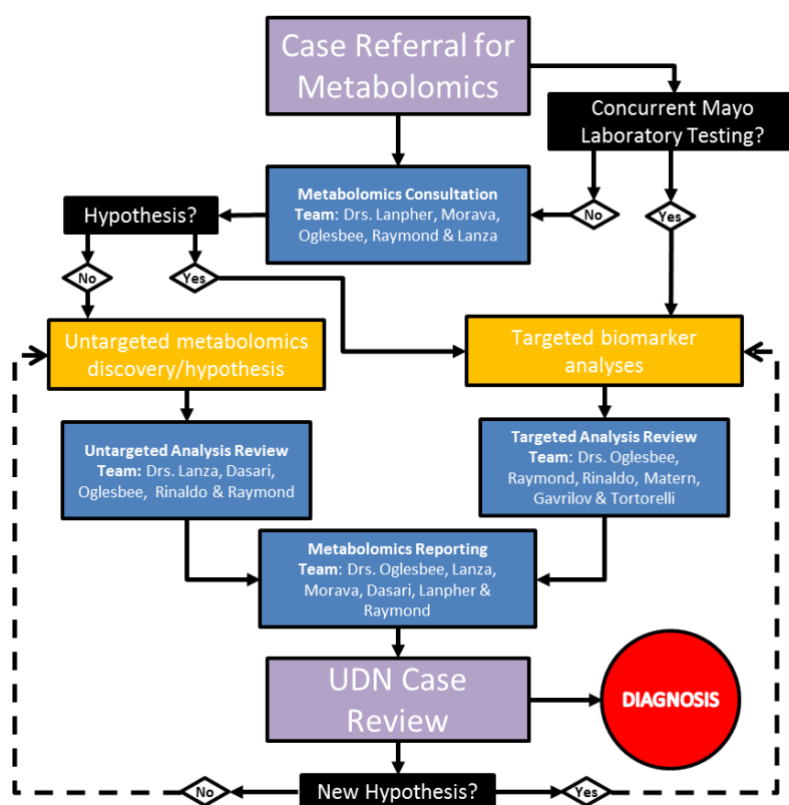


Figure 1: Metabolomics workflow diagram

C. Requesting Metabolomics Analyses

A Gateway Metabolomics Request is required to initiate MC services. The Metabolomics Request serves four purposes: 1) to alert the MC of the service request, 2) to provide a high-level summary of the proband and other family members, 3) to select the analytical method requested from the MC (Table 2), and 4) to establish a complete Metabolomics Request Form containing both the test method(s) and UDN ID for each shipped specimen.

Table 2. Targeted and Untargeted Metabolomic Analyses Offered by the MC.

Test Code	Targeted Analyses	Specimen Type	Volume
AACSF	Amino Acids, CSF	CSF	0.1 mL
AAPD	Amino Acids, Urine	Urine	1 mL
AAQP	Amino Acids, Plasma	Plasma	0.3 mL
ACRN	Acylcarnitines, Plasma	Plasma	0.1 mL
ACYLG	Acylglycines	Urine	4 mL
BAPS	Bile Acid Profile	Serum	0.3 mL
CDGN	Congenital Disorders of Glycosylation	Serum	0.1 mL
CRDPU	Creatine Disorder Panel	Urine	1 mL
FAO	Fatty Acid Oxidation Probe Assay	Cultured fibroblast	T-75
FAPCP	Fatty Acid Profile	Serum	0.2 mL
GDF15	Growth Differentiation Factor 15	Plasma	0.2 mL

HGEMP	Hydroxyglutaric Acids	Plasma	0.1 mL
HYOX	Hyperoxaluria Panel	Urine	1.1 mL
LYSDU	Lysosomal Storage Disorders Scrn	Urine	3 mL
OAU	Organic Acids Screen	Urine	4 mL
OXNP	Oxysterols, plasma	Plasma	0.3 mL
PIPU	Pipecolic Acid	Urine	2 mL
PLSD	Lysosomal and Peroxisomal Storage Disorders Screen	Blood spot, filter paper	2 circles
POXP	Peroxisomal Fatty Acid Profile, Plasma	Plasma	0.5 mL
PQNU	Porphyryns	Urine	varies
PUPYP	Purines and Pyrimidines Panel, Plasma	Plasma	0.2 mL
PUPYU	Purines and Pyrimidines Panel, Urine	Urine	2 mL
Q10	Coenzyme Q10	Plasma, sodium heparin	1 mL
STER	Sterols	Plasma	0.5 mL
TALDO	Polyols	Urine	1 mL
CTTMS	Androgens (Testosterone), ICL Method	Serum	0.5 mL
INFP1	Cytokine/Chemokine Panel, ICL Method	Serum	0.1 mL
Varies	Specific Peptide by RIA, ICL Method (contact for code)	Varies	1 mL
Test Code	Untargeted Metabolomics	Specimen Type	Volume
UTLCMS	Untargeted LCMS	Varies	0.1 mL
UTGCMS	Untargeted GCMS	Varies	0.1 mL
UTDMRM	Untargeted Central Carbon Metabolites	Varies	0.1 mL
UTLGT	Untargeted Lipidomics, Georgia Tech	Varies	0.1 mL
UTNMR	Untargeted NMR	Varies	0.25 mL

Metabolomics Service Request

1. CS completes a request for the desired metabolomics analysis (see Table 2) of the proband via the Gateway.
2. CS completes a request for the desired metabolomics analysis (see Table 2) for each additional family member to be evaluated via the Gateway.

Metabolomics Consultation Team Review

1. Requests for metabolomics analysis are reviewed by the MC Consultation Team.
2. Consultation with the referring CS is initiated by the MC for any questions or concerns regarding the participant's symptoms and/or analyses requested.

D. Flow of Samples to the Mayo Clinic Metabolomics Core

Recommendations for sample collection, processing, tracking, storage, and shipping

The Biospecimens section of this manual contains instructions for sample collection, processing, and storage. For shipping to the MC, the following instructions are provided:

Shipping samples

1. Consider specimen collection, labeling, processing, and storage guidelines described in Biospecimen section. All tubes must have the participant UDN ID, sample type, date of collection, and ordered service (MC Test Code, see Table 2) typed on the label.

2. CS enters the sample information in the Gateway by navigating to the Metabolomics tab and clicking on “Create New Submission.”
3. Once the samples are submitted, click the Submission ID link to download and print the Metabolomics Request Forms, and include them in the shipping box. Ensure that the tubes are correctly labeled and match the requested services listed on the submission form.
4. CS prepares the samples for shipment on dry ice, FedEx priority overnight (Mon-Weds only), include all printed copies of the Metabolomics Request Forms (see step 3). No shipping during a holiday week or two weeks before Christmas through New Year’s due to delays with FedEx deliveries during these times.
5. When packing samples, select an appropriate dry ice safe shipping box and enough dry ice for 48 hours in case of shipping/delivery delays. For example, use a Thermosafe EPS foam box w/corrugated carton #448UPS & 10 lbs dry ice.
Packing Components:
 - a. Primary container –a container that contains the sample
 - b. Secondary container – a Ziplock™ bag to contain leaks and should include absorbent material and labeled with “Biohazard specimen”
 - c. Tertiary container – Thermosafe styrofoam box with 10 lbs dry ice
6. Outer package – to give form to the package and protect inner contents (e.g., a fiberboard box surrounding the styrofoam box).
7. The Metabolomics Request Forms must be included with each shipment.

Ship samples to: Mayo Clinic Laboratories, 3050 Superior Drive NW, Rochester, MN 55901.
Tel. 507-284-1970.

The MC will notify CS via the Gateway when samples have safely arrived.

Questions about preparing samples to ship, sample submission form, etc., please email: biochemicalgenetics@mayo.edu, or Tel. 507-266-4996, and discuss these questions with one of the genetic counselors at the Biochemical Genetics Laboratory.

E. Flow of Clinical and Sequencing Information to the Metabolomics Core

Following submission of the Gateway Metabolomics Request, the MC reviews additional clinical and sequencing information available in the Gateway. The MC contacts the CS if more information is desired. The CS notifies the MC via email if new information becomes available after the metabolomics request form is submitted and this information is updated in the Gateway.

F. Targeted Analyses

This section covers sample intake, preparation, and analysis at Mayo Clinic. This section describes the sample flow from plasma, urine, CSF, or other tissue sample receipt to production of results for the UDN including appropriate quality control and assurance procedures.

Sample intake

Plasma, urine, CSF, serum, or other tissue samples are received from FedEx at Mayo Clinic Laboratories. The shipped material is forwarded to the Clinical Biochemical Genetics Laboratory (BGL), Hilton 314. At BGL, a visual inspection of the shipment of sample tubes is conducted to ensure that the correct number of samples have arrived as listed on the Metabolomics Request Form and that sample tube integrity has not been compromised. The sample tube labels are compared against information on the Metabolomics Request Form to ensure that the correct samples arrived for analysis. Samples will be accepted for analysis if no discrepancies are found, sample labels match, and no tube damage is observed. If any of the above criteria is not met, Mayo Clinic will notify the referring CS. Samples are distributed to each testing site (Targeted, Untargeted, or NMR) at the appropriate temperature. Long-term storage will occur between -70 to -80 °C. Once accepted, samples are accessioned into the BGL Laboratory Information System (LIMS, SoftBiochem) incorporating participant UDN ID, sample type, collection date, and ordered service, when available.

Sample QC

Prior to processing, samples are thawed and inspected for unusual characteristics such as hemolysis, precipitation, and discoloration. The sample tube label is compared with information on the Metabolomics Request Form and SoftBiochem order information. Samples are accepted for analysis if no discrepancies are found, sample labels match, and no tube damage is observed. If any of the above criteria is not met, the MC staff notify the referring CS. Any unusual findings are also recorded in the LIMS.

Sample processing and examples of available targeted analyses

Samples are batched and processed according the requested analyses and target analyte stabilities. Additional analytical information for quantitative methods is available at <https://www.mayocliniclabs.com/test-catalog/index.html> and searching by Test Code as listed in Table 2.

For every method, two levels of pooled QC samples, and blank specimens are included on all analytical batches. Internal standards are added to participant and QC samples, when needed by the analytical method.

Basic details of a few exemplary targeted metabolomics protocols are provided below. Additional method details can be provided upon request or found online at the link above to the Mayo Clinic test catalog.

CDGN, Congenital Disorders of Glycosylation Analysis

A technique of affinity chromatography coupled to ESI-MS is used to resolve the glycoforms of transferrin (Tf) and apolipoprotein CIII (Apo CIII). Hypoglycosylate Tf and/or Apo CIII are a biochemical marker for congenital disorders of glycosylation (CDG). The method is a qualitative assay which determines endogenous proteins and glycoforms using a combination of immunoaffinity capture and LC-MS analysis. This method is performed using an instrument with the Q1 quadrupole calibrated using unit mass resolution; the tandem mass spectrometer used is configured for LC-MS analysis for this application. If a sample provides an abnormal CDG result, a secondary MALDI-TOF/TOF 5800 Analyzer method of glycan analysis is performed for further characterization. An interpretive report is provided following analysis.

LYSDU, Lysosomal Disorder Screen

A sequential method to determine levels of ceramide trihexosides and sulfatides, mucopolysaccharides, and oligosaccharides by LC-MS/MS and MALDI-TOF. First, using a mobile phase to separate the ceramide trihexosides and sulfatides from specimen matrix, a MS/MS is operated in the multiple reaction monitoring (MRM) positive mode to measure ceramide trihexosides and multiple reaction monitoring (MRM) negative mode to measure sulfatides. For chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratan sulfate a LC-MS/MS analysis is used. Urine specimens are evaporated and the dry residue is subjected to methanolysis yielding the mucopolysaccharides for analysis as their unique repeating disaccharide units. LC-MS/MS is performed to separate the mucopolysaccharides from the bulk of the specimen matrix. Finally, for oligosaccharides, urine samples are extracted using Oasis HLB and carbograph columns and lyophilized overnight. Oligosaccharides are permethylated, the tubes centrifuged, and the supernatant removed. The supernatant is quenched with water, neutralized with acetic acid, extracted, eluted, and again lyophilized overnight. Specimens are resuspended, mixed 1:1 with a matrix solution, spotted onto a MALDI plate and allowed to air dry. The plate is then analyzed using a MALDI TOF/TOF 5800 Analyzer. An interpretative report is provided following analysis.

ACYLG, Quantitative Urine Acylglycine Profile

Urine volumes equivalent to 0.25 mg of creatinine are spiked with a mixture of labeled internal standards, allowed to equilibrate, acidified, and then extracted with ethyl acetate. After evaporation, the dry residue is derivatized to butyl esters. Specimens are analyzed by capillary GC/MS selected ion monitoring using ammonia chemical ionization and a stable isotope dilution method for quantification. A quantitative and interpretive report is provided.

AAQP, Quantitative Amino Acids

Quantitative analysis of amino acids (AA) is performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) by labeling amino acids present in plasma, CSF, and urine with aTRAQ® Reagent 121. Samples are combined with aTRAQ® Reagent 113-labeled Standard Mix and partially dried to remove readily volatile solvents. Amino Acids are separated and detected by LC-MS/MS (API 3200™). The concentrations of AA are established by comparison of their ion intensity (121-labeled amino acids) to that of their respective internal standards (113-labeled amino acids). Chromatography is performed using a C18 (150x4.6mm) column and total analysis time is 18 minutes. A quantitative and interpretive report is provided.

POXP, Peroxisomal Fatty Acids Plasma

Quantitative analysis of peroxisomal-related fatty acids is performed by acidic hydrolysis followed by basic hydrolysis and reacidification. Hexane extraction then proceeds to derivatization with pentafluorobenzyl bromide (PFB). Separation and detection of PFB-esters is accomplished by capillary GC/MS using electron capture ionization and selected negative ion monitoring. Quantitation is enhanced by the use of stable isotope-labeled internal standards. A quantitative report and interpretation is provided.

Instrument analyses

Depending on the requested method, samples are analyzed using either gas chromatography-mass spectrometry (GC/MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), matrix-assisted laser desorption/ionization mass spectrometry time-of-flight (MALDI-TOF/TOF). Each individual method has an standardized protocol and operating procedure that will be provided upon request.

Data processing

Metabolomics raw data are processed, depending on the method, using a combination of open-source, in-house developed, or commercial software tools to align data, identify, and quantify detected compounds. Raw data will be provided upon request.

G. Untargeted Analysis

Overview

This section covers sample preparation, analysis, and data reduction in support of discovery-based untargeted metabolite profiling that will take place in the Metabolomics Research Core at Mayo. This section describes the sample flow from plasma, urine, fibroblast, or CSF sample receipt through analysis, including appropriate quality control and assurance procedures.

Untargeted Metabolomics (Test UTLCMS) – Mayo Clinic Metabolomics Core Laboratory

Sample QC

Prior to processing, samples are thawed and inspected for unusual characteristics such as hemolysis, precipitation, and discoloration. The sample tube label is compared with information on the Metabolomics Request. Samples are accepted for analysis if no discrepancies are found, sample labels match, and no tube damage is observed. If any of the above criteria is not met, Mayo Clinic staff notify the referring CS. Any unusual findings are also recorded in the LIMS.

Sample processing

1. Thaw samples & QC (50ul plasma) on ice, spin 30 seconds @ 12000g & 4°C
2. Aliquot (35ul of Plasma/Serum, 50ul of CSF, 50ul 1xPBS with 2million cells, 50ul of tissue homogenate) of each sample and QC into a labeled 1.5 ml Eppendorf tube (on ice). Also, prepare a blank tube with 50ul of MeOH.
3. Add 2ul of 13C6 Phenylalanine Internal Standard to each sample tube and vortex
4. Add 3ul of 13C6 Phenylalanine Internal Standard to QC tube and vortex
5. Add 150ul of cold MeOH:ACN mix to each sample tube and vortex well
6. Add 300ul of cold MeOH:ACN mix to QC tube and vortex well
7. Keep on ice for 30 min with occasional vortexing (approx. every 10 min)
8. Centrifuge at 18000g for 20 min @ 4o C
9. Transfer 1/2 of supernatant (~75ul) to each of two 1 dram vials and dry under N2 on cold blocks (takes about 40 min)
10. Transfer 1/4 of supernatant (~75ul) to each of two 1 dram vials(QC) and discard remaining supernatant amount and dry under N2 on cold blocks
11. Store dry and capped @ -20°C until ready to reconstitute

Sample analysis

Samples are analyzed using a Quadruple Time-of-Flight Mass Spectrometer (Agilent Technologies 6550 Q-TOF) coupled with an Ultra High Pressure Liquid Chromatograph (1290 Infinity UHPLC Agilent Technologies). The dried samples are reconstituted in running buffer and analyzed within 24 hrs. Profiling data is acquired under both positive and negative electrospray ionization conditions over a mass range of 100 - 1200 m/z at a resolution of 10,000

(separate runs). Metabolite separation is achieved using two columns of differing polarity, a hydrophilic interaction column (HILIC, ethylene-bridged hybrid 2.1 x 150 mm, 1.7 mm; Waters) and a reversed-phase C18 column (high-strength silica 2.1 x 150 mm, 1.8 mm; Waters). For each column, the run time is 20 min using a flow rate of 400 μ L/min. A total of four runs per sample are performed to give maximum coverage of metabolites. Samples are injected in duplicate, and a quality control sample, made up of a pooled plasma sample, is injected several times during a run.

Chromatography

Metabolite separation is achieved using Infinity 1290 UPLC system (Agilent Technologies) with both hydrophilic interaction chromatography (HILIC) (ethylene-bridged hybrid 2.1x150 mm, 1.7 mm; Waters) and reversed-phase liquid chromatography C18 (RPLC) (high-strength silica 2.1x150 mm, 1.8 μ m; Waters). For C18 column, the run time is 28 min. Reverse-phase chromatography is performed using 99.9% solvent A (5 mmol/L NH₄ acetate, 0.1% formic acid, and 1% acetonitrile) to 100% solvent B (5 mmol/L NH₄ acetate, 0.1% formic acid, and 95% acetonitrile). The gradient is 0 min, 0.1% B (400 μ L/min); 1 min, 0.1% B (400 μ L/min); 7 min, 50% B (400 μ L/min); 11 min, 15% B (400 μ L/min); 13 min, 0.1% B (800 μ L/min); 25 min, 0.1% B (800 μ L/min); 25.5 min, 0.1% B (500 μ L/min); and 28 min, 0.1% B (400 μ L/min). For HILIC column, the run time is 28 min at a flow rate of 400 μ L/min. The hydrophilic interaction chromatography gradient is as follows: 0 min, 99.9% B; 1 min, 99.9% B; 9 min, 70% B; 11 min, 70% B; 12 min, 0.1% B; 13 min, 0.1% B; 14 min, 99.9% B; and 18 min, 99.9% B. The injection volume of each sample is 1 μ L and column is maintained at 45°C.

Mass spectrometry

A 6550 Q-ToF-MS (Agilent Technologies) is operated in both positive and negative electrospray ionization (ESI) modes using a scan range of 50–1,700 m/z. The instrument settings are as follows: nebulizer gas temperature 225°C, capillary voltage 3.5 kV, fragmentor voltage 150 V, octapole voltage 1000 V, cycle time 0.5 sec, and run time 15.0 min for HILIC and 18 min for C18 separation.

Data analysis

Figure 2 summarizes the design of the untargeted LC-MS-based metabolite profiling method. Triplicate samples will be collected from both affected and unaffected family members of each UDN patient. All samples will be included in a single batch to prevent batch effects confounding the study contrasts (like genotype, clinical phenotype, etc.). Since extracted metabolites from each sample are analyzed in four different modes, data from each mode is processed separately. For each mode, Mass Profiler (Agilent, Santa Clara, CA) software reads the chromatograms from Agilent instrument native files, filters the noise, and detects peaks.

Intensity of the peaks will be normalized either using the total ion current seen per sample or the QC reference sample (to reduce intra-batch variability). Detected peaks will be identified by matching their precursor masses, retention times, and MS/MS spectra against the NIST LC-MS (version 11) and METLIN metabolite databases for putative identification (IDs) with detection

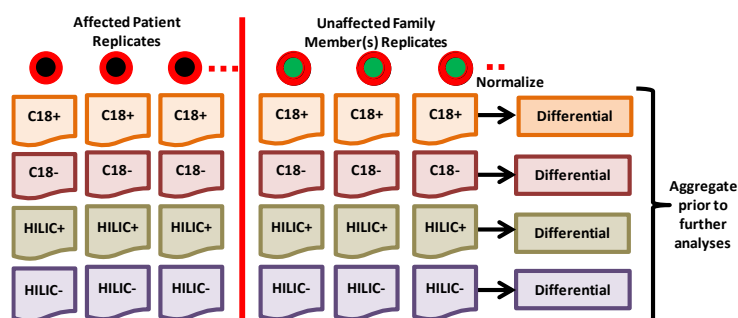


Figure 2. Untargeted LC-MS bioinformatics workflow for detecting metabolite changes.

window of <7 ppm. Mass accuracy of the Q-TOF method is <5 ppm with retention time precision better than 0.2%. Results from multiple modes will be combined for each batch and we will generate an “A-list” of identified compounds and their normalized intensities for differential expression analysis. A separate “B-list” of unidentified features and their intensities will also be reported (with full feature metadata like mass, RT, MS2, etc.) for future analysis. Metabolites detected in at least ≥50% of the samples are selected for differential expression analysis wherein normalized intensity of each metabolite is modeled using a generalized linear regression with all co-variables of interest (genotype, age, gender, etc.). Normalized data are analyzed by multivariate approach such as Principal Component Analysis (PCA) to reveal data homogeneity, groupings, outliers and trends. An unsupervised PCA and heat map of hierarchical clustering analysis (HCA) are performed to identify spiked, pooled and gender specific differences in the samples. ANOVA contrasts are utilized to detect metabolites that are significantly different between the patient and his/her unaffected family members. These results are utilized for pathway analysis using Ingenuity Pathway analysis and MetaCore to find dysregulated pathways. A 1.2x fold change can be detected with a precision of 4%.

H1-NMR Small Metabolite Profiling (Test UTNMR)

Samples are thawed on ice at 4°C for 30–60 min. 50-200µl aliquots are transferred into 1.5ml Eppendorf tubes and 600µl of cold MeOH is added. The mixture is vortexed for 20s and then centrifuged at 13,300 RPM for 15 min. The supernatants (~760µl) are transferred into 1.5ml Eppendorf tubes and dried in a centrifugal vacuum evaporator for 12 hrs. In each dried sample, 500µl of 0.1M phosphate buffer (K₂HPO₄/KH₂PO₄, pH 7.4) and 50µl of 1mM TSP-d₄ in deuterium oxide (D₂O) are added. The mixture is vortexed for 20s and the solution is then transferred to a 5mm Sample Jet rack tube. All chemicals are purchased from Sigma-Aldrich (St. Louis, MO). The NMR spectra are acquired on a Bruker 600 MHz Avance IIIHD spectrometer equipped with a BBI room temperature probehead and SampleJet auto sampler (Bruker Biospin, Rheinstetten, Germany). ¹H NMR spectra are recorded using 1D noesy pulse sequence with presaturation (noesygppr1d), collecting 128 scans with 64k data points, 14 ppm spectral with calibrated 90 degree pulse (~11ms), 3.90s acquisition time, and 5s relaxation delay. Metabolites are identified and quantified using Chenomx NMR suite 8.2 software, by fitting the spectral lines of library compounds into the recorded NMR spectrum of samples. The quantification is based on peak area of TSP-d₄ signal. The metabolite concentrations are exported as µM in NMR sample and recalculated as µM.

Additional quantitative metabolomics methods

This section covers the analytical and preanalytical details of selected targeted metabolomics assays used to quantify levels of amines, tricarboxylic acid (TCA) cycle metabolites, acylcarnitines, and ceramides.

Amines

Samples and amino acid calibration standards are prepared with MassTrak Amino Acid Analysis Solution (AAA) kit from Waters according to instructions with slight modifications for detection on a mass spectrometer. A 10 point standard concentration curve is made from the calibration standard solution to calculate amino acid concentrations in samples. A solution containing U-¹³C₄-L-aspartic acid, U-¹³C₃-L-alanine, U-¹³C₄-L-threonine, U-¹³C₅-L-proline, U-¹³C₅-L-valine, U-¹³C₆-leucine, U-¹³C₆-phenylalanine all from Cambridge Isotope Laboratories, ¹³C₆-tyrosine from Isotec, L-arginine (15N₂, 2H₂) from MassTrace, norvaline from Sigma dissolved in 0.01N HCl is used as the internal standard solution. Frozen plasma samples are thawed, spiked with internal standard then deproteinized with cold MeOH followed by centrifugation at 10,000 g

for 5 minutes prior to derivatization according to MassTrak instructions. The amino acid derivatizing reagent used is 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. High resolution separation is done using an Acquity UPLC system, injecting 1 μ L of derivatized solution, with a UPLC BEH C18 1.7 micron 2.1 \times 150 mm column from Waters. Column flow is set to 400 μ L/min with a gradient from 99.9% A to 98% B where buffer A is 1% acetonitrile in 0.1% formic acid and buffer B is 100% acetonitrile. A column temp of 43 degrees Celsius and a sample tray temp of 6 $^{\circ}$ Celsius. Mass detection is completed on a TSQ Ultra Quantum from Thermo Finnigan running in ESI positive mode. A scan width of 0.002, scan time of 0.04 seconds per transition mass, collision energy of 25, collision gas pressure of 1.5 mTorr, tube lens value set to 90, monitoring a signature ion of the derivitized amines at m/z 171.04 by selected reaction monitoring.

TCA Cycle

Dried samples are derivatized with ethoxime and then with MtBSTFA + 1% tBDMCS (N-Methyl-N-(t-Butyldimethylsilyl)-Trifluoroacetamide + 1% t-Butyldimethylchlorosilane) before the analysis using Agilent 5977A gas chromatography/mass spectrometry (GC/MS) under electron impact and single ion monitoring conditions. Concentrations of lactic acid (m/z 261.2), fumaric acid (m/z 287.1), succinic acid (m/z 289.1), oxaloacetic acid (m/z 346.2), ketoglutaric acid (m/z 360.2), malic acid (m/z 419.3), cis aconitic acid (m/z 459.3), citric acid (m/z 591.4), isocitric acid (m/z 591.4), and glutamic acid (m/z 432.4) are measured against a 16-point calibration curve. Calibration curves are prepared fresh daily from standard and internal standard mixtures. A 16 point standard curve is prepared using an internal standard mixture containing lactate, succinate, fumarate, oxaloacetate, alpha-ketoglutarate, malate, aspartate, glutamate, 2-hydroxyglutamate, cis-aconitate, isocitrate, and citrate. For biological fluids such as plasma, urine, and CSF, 15 μ L of Internal Standard mix is added to 50 μ L sample. For cells, add 50 μ L in PBS, vortex and sonicate at 4 $^{\circ}$ C until the pellet is resuspended. For tissues, add 5 μ L per mg of tissue, vortex and sonicate at 4 $^{\circ}$ C until the pellet is resuspended. 260 μ L of cold 50:50 MeOH:AcCN is added, vortexed well and left on ice for 30 minutes with occasional vortexing. Samples are centrifuged for 15 minutes at 4 $^{\circ}$ C and 18000 g and supernatants transferred to pre-labeled 1 dram vials and dry under N₂ needles or by vacuum. A pooled plasma QC (50 μ L) is included. After drying, samples are derivatized and analyzed as follows:

1. Add 200 μ L of DCM to each vial, vortex, and re-dry.
2. 50 μ L of freshly made EOX in pyridine is added at 20 mg/mL.
3. Vortex and heat 1 hour at 35 $^{\circ}$ C
4. After drying, add 200 μ L of DCM to each vial, vortex, and re-dry.
5. Add 50 μ L of freshly made 20 mg/mL EOX in pyridine. Cap, vortex and heat 1 hour at 35 $^{\circ}$ C
6. Remove from heat block and spin 5 minutes at 4 $^{\circ}$ C and 3000 rpm.
7. Add 50 μ L of MtBSTFA; Cap, vortex and heat 1 hour @ 70 $^{\circ}$ C
8. Remove from heat block and spin 5 minutes at 4 $^{\circ}$ C and 3000 rpm
9. Let stand at room temp overnight
10. Analysis is performed on an GC-MS system (Agilent Technologies MSD 5977A) using a DB-5MS (30m \times 0.25mm \times 0.25 μ m) column (Chrom Tech #122-5532).

Acylcarnitines

Samples are reconstituted in 100 μ L buffer (99% MeOH, 1% H₂O, 1 mM ammonium formate, 0.1% formic acid) and analyzed on a Waters Acquity UPLC system (Milford, MA) coupled with a Thermo Quantiva tandem MS (West Palm Beach, FL) in positive (H)ESI mode. Concentrations of carnitine (162.11 to 85.02 m/z), acetylcarnitine (204.12 to 85.02 m/z), propionylcarnitine

(218.14 to 85.02 m/z), butyrylcarnitine (232.15 to 85.02 m/z), isovalerylcarnitine (246.17 to 85.02 m/z), octanoylcarnitine (288.22 to 85.02 m/z), lauroylcarnitine (344.28 to 85.02 m/z), myristoylcarnitine (372.36 to 85.02 m/z), palmitoylcarnitine (400.39 to 85.02 m/z), oleoylcarnitine (426.39 to 85.02 m/z), and stearoylcarnitine (438.39 to 85.02 m/z) are measured against an 11-point calibration curve (Cambridge Cat. # NSK-B-US, Sigma Cat. # L-3131, Avanti Polar Lipids Cat. # 870852, Chem-Impex Cat. # 01874) that underwent the same preparation (32).

1. Aliquot 25 uL matrix into Eppendorf tubes
2. Add 25 uL I.S. Mix Working Solution to each tube
3. Cap and vortex
4. Add 450 uL Extraction Solution (1:1 MeOH/DCM, cold)
5. Cap and vortex
6. Keep on ice for 30 min
7. Centrifuge 15 min @ 18,000 rpm and 4°C
8. Transfer supernatant into 1 dram vial
9. Dry under N₂ needles on room temperature blocks
10. Cap with Teflon lined caps and store at -20°C until analysis
11. Reconstitute in 50 uL Ceramide B Buffer
12. Inject 2 uL for analysis by LC/MS/MS using a Thermo Quantiva or Thermo Quantum Ultra
13. Note: Plasma QC is prepped with each set of samples

Ceramides

Sphingolipids are extracted using isopropanol-water-ethyl acetate (35:5:60, vol/vol/vol). Quantitative measurement of sphingolipids (Sph, dhSph, S1P, C14:0-Cer, C16:0-Cer, C18:1-Cer, C18:0-Cer, C20:0-Cer, C24:1-Cer, C24:0-Cer) is made using a Thermo TSQ Quantum Ultra mass spectrometer using positive ion ESI source with SRM. The chromatographic separation is performed using a Waters ACQUITY Ultra Performance Liquid Chromatography (UPLC). The analytic column is a reverse-phase Acquity C8 UPLC BEH column 2.1 × 150 mm, 1.7 μm (Waters, Milford, MA). Chromatographic separation is conducted in binary gradient using 2 mM ammonium formate, 0.15% formic acid in methanol as solvent A, and 1.5 mM ammonium formate, 0.1% formic acid in water as solvent B at the flow rate of 0.4 ml/min. C17Sph, C17S1P, and C17:0-ceramide are used as an internal standards. Standards utilizing 18C-sphingoid bases: sphingosine d18:1 (Sph), sphinganine d18:0 (dhSph), sphingosine-1-phosphate d18:1 (S1P), d18:1/14:0-Cer - ceramides containing myristic acid (C14:0-Cer), d18:1/16:0-Cer - ceramides containing palmitic acid (C16:0-Cer), d18:1/17:0-Cer - ceramides containing margaric acid (C17:0-Cer) - internal standard for ceramides, d18:1/18:0-Cer - ceramides containing stearic acid (C18:0-Cer), d18:1/18:1-Cer - ceramides containing oleic acid (C18:1-Cer), d18:1/20:0-Cer - ceramides containing arachidic acid (C20:0-Cer), d18:1/24:0-Cer - ceramides containing lignoceric acid (C24:0-Cer), d18:1/24:1-Cer - ceramides containing nervonic acid (C24:1-Cer), as well as internal standards utilizing 17C-sphingoid bases: sphingosine (d17:1-Sph) - internal standard for sphingosine and sphinganine, sphingosine 1-phosphate (d17:1-S1P) - internal standard for sphingosine 1-phosphate, are purchased from Avanti Polar Lipids.

An eight-point concentration standard curve is constructed by diluting the stock concentration solution with the 1% albumin solution. The sphingolipid extraction from samples is performed as follows: Ten μl of diluted stock internal standard solution (1:25 with 1% albumin solution) and 1.5 ml of extraction solution are added to samples and concentration standards. The mixture is vortexed, sonicated and then centrifuged for 10 min at 4000 rpm. The supernatant is transferred

to a new vial and pellet was extracted once more. The combined supernatants were evaporated under nitrogen on chilled blocks until dryness for UPLC/MS/MS analysis.

H. New Analytical Method Development

The Metabolomics Core has the expertise to co-develop, alongside a CS, novel targeted and untargeted methods for the UDN. New methods for analysis could include applications on gas chromatography-mass spectrometry (GC/MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and matrix-assisted laser desorption/ionization mass spectrometry time-of-flight (MALDI-TOF/TOF), but such proposals are not limited to these technologies as other platforms exist for development. The initiation of a joint effort towards co-development of new methods can be requested by emailing the PIs of the MC and discussing the potential application of new methods.

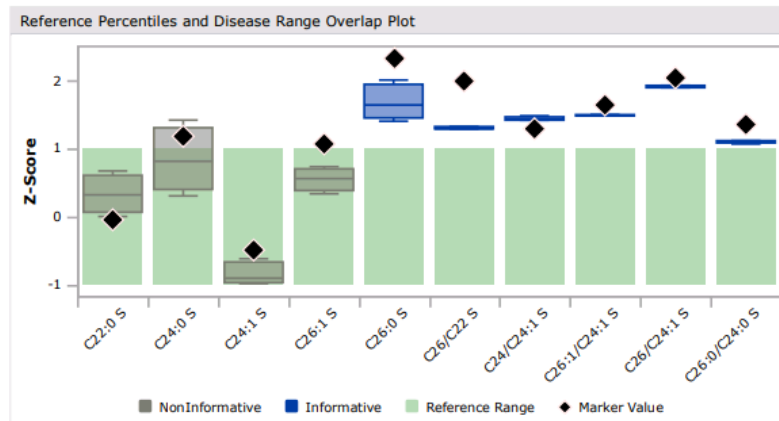
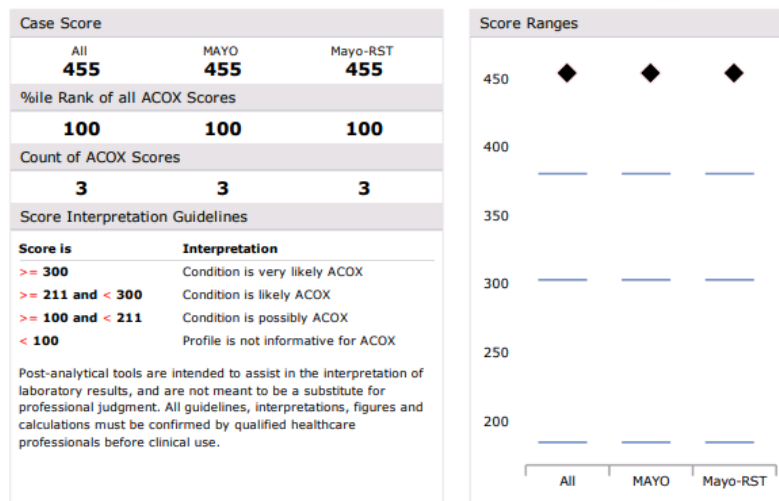
I. Return of Results of Metabolomics Analyses

The metabolomics data is initially processed and reviewed by the MC, and subsequently returned to the referring CS via the Gateway. If necessary, the data are also reviewed by the Metabolomics Case Review Committee in collaboration with the referring CS. If no diagnosis is identified, further rounds of metabolomics analysis, and/or other evaluations are undertaken based on new hypotheses generated via discussion between the Metabolomics Case Review Committee and referring CS (Figure 1).

Format of metabolomics results report

1. Summary of key findings as suggested by each analytical method
 - a. Untargeted metabolomics method
 - b. Targeted analysis
2. Primary diagnostic considerations
3. For targeted analyses, a Collaborative Laboratory Integrated Report (CLIR) (see Figure 3)
4. For untargeted NMR analyses, a Bruker-based report will be provided (see Figure 4)

Tool: ACOX 001



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Figure 3. An example Collaborative Laborative Integrated Report (CLIR) for the targeted analysis, Peroxisomal Fatty Acids, showing ACOX1 deficiency.

1 Creatinine

Compound	Conc. mmol/L	LOD mmol/L	95% Range mmol/L	Graphics ^(*)
Creatinine	0.02	0.01	0.06 - 0.14	

^(*) Gray horizontal boxes represent 95% concentration range, black vertical lines represent sample value.

2 Alcohols and derivatives

Compound	Conc. mmol/L	LOD mmol/L	95% Range mmol/L	Graphics ^(*)
Ethanol	< 0.10	0.10	≤ 0.80	













^(*) Gray horizontal boxes represent 95% concentration range, black vertical lines represent sample value.

3 Amines and derivatives

Compound	Conc. mmol/L	LOD mmol/L	95% Range mmol/L	Graphics ^(*)
Trimethylamine-N-oxide	< 0.05	0.05	≤ 0.05	

^(*) Gray horizontal boxes represent 95% concentration range, black vertical lines represent sample value.

4 Amino acids and derivatives

Compound	Conc. mmol/L	LOD mmol/L	95% Range mmol/L	Graphics ^(*)
Alanine	0.36	0.02	0.29 - 0.63	
Creatine	0.12	0.01	≤ 0.07	
Glutamic acid	0.13	0.05	≤ 0.25	
Glutamine	0.56	0.02	0.29 - 0.82	
Glycine	0.33	0.10	0.15 - 0.42	
Histidine	0.11	0.02	0.07 - 0.17	
Isoleucine	0.04	0.03	0.03 - 0.11	
Leucine	0.07	0.01	0.06 - 0.20	
Phenylalanine	< 0.05	0.05	≤ 0.06	
Threonine	0.13	0.06	≤ 0.51	
Tyrosine	0.71	0.03	≤ 0.08	
Valine	0.18	0.03	0.15 - 0.36	

^(*) Gray horizontal boxes represent 95% concentration range, black vertical lines represent sample value.

Figure 4. An example of an NMR report

XV. Model Organisms Screening

A. Goals

The goal of the Model Organisms Screening Center (MOSC) is to provide compelling data based on studies in worms (*Caenorhabditis elegans*), flies (*Drosophila melanogaster*), or zebrafish (*Danio rerio*) that refute or support the disease causality of specific genetic variants identified in participants by the Clinical Sites (CSs) and the Baylor Sequencing Core after genome or exome sequencing.

B. General Considerations

The MOSC began analyzing variants in Phase I in early 2016 with the *Drosophila* Core at Baylor College of Medicine and the Zebrafish Core at University of Oregon (BCM-UO). The MOSC had numerous discussions with the CSs about how to optimize the process of best diagnosing disease-associated variants in genes submitted by each site. MOSC investigators also had numerous interactions with human geneticists that helped shape approaches and logistics. The overall philosophy of the MOSC is that of a collaborative center for clinicians, human geneticists, and model organism researchers who work together to solve cases. This chapter of the Manual of Operations represents a summary of the general operations of the MOSC.

With the start of Phase II of the UDN in Fall 2018, there was an expansion of the MOSC to include a new *C. elegans* Core and a second Zebrafish Core at Washington University in St. Louis (WUSTL). The two groups (WUSTL and BCM-UO) function as a single multi-institutional core with the following components:

- I. Initial Variant Review Informatics Team - Dustin Baldrige (WUSTL) and Michael Wangler (BCM)
- II. *C. elegans* (Worm) Core - Tim Schedl and Stephen Pak (WUSTL)
- III. *Drosophila* (Fly) Core - Hugo Bellen, Shinya Yamamoto, and Michael Wangler (BCM)
- IV. Zebrafish (Fish) Core - Monte Westerfield and John Postlethwait (U Oregon) and Lilianna Solnica-Krezel (WUSTL)
- V. Administrative components - Angela Bowman (WUSTL), and Hugo Bellen, Michael Wangler (BCM)

C. Variant Submissions and Initial Variant Review

Gene variant submissions

Each CS submits cases to the MOSC for analysis, typically after sequencing is complete. Within the Gateway, CSs can enter clinical information about the participant, submit candidate variants for the MOSC, and explain the rationale for prioritizing candidate variants that may contribute to disease phenotypes. The Gateway tracks MOSC submissions and allows communication about prioritization and progress back to the site. The sites are encouraged to submit 1-5 variants per case.

Variants that will be considered higher priority have the following characteristics:

1. Come from an unsolved case.
2. Are in novel or candidate disease-causing genes.
3. Are novel variants (i.e. VUS) in known disease-causing genes, but with unique phenotypes (i.e. phenotype expansions).

Work that is outside the scope of the MOSC includes:

1. Studying complex multi-gene interactions.
2. Studying environmental triggers.
3. Solved cases: Pathogenic variants or variants of uncertain significance (VUS) in known genes when the phenotype matches the known disease phenotype.
4. Treatment/Drug screens (although the models generated in the MOSC will be made available for such studies).
5. Generating models for known disorders.

Each variant submitted by the CS through the Gateway will be analyzed in parallel by the Initial Variant Review Informatics Team at WUSTL and BCM. After parallel analysis, the two teams review the variants via online calls. The procedure is as follows:

- I. Variant submissions are submitted from Phase II UDN CSs using an updated version of the current Gateway MOSC request form developed in Phase I.
- II. Variant submissions are then independently assessed at WUSTL and BCM in a process called “Initial Review”, primarily from a medical genetics perspective (Figure 1). Initial review includes assessment of key human data, including confirmation of genomic coordinates, assessment of allele frequencies, search for additional cases with variants in the same gene, determination of whether there are existing disease-gene associations for each variant, and future filtering/prioritization based on non-human primate data sets. This process is a quality control step to ensure that the site has provided the correct information on good candidate variants to be further analyzed in the Cores through the secondary informatics analysis pipeline described below.

i. BCM review process

1. Using information submitted through the Gateway, the BCM site reviews clinical phenotypes of participants as documented by the CS during the visit, reviews the list of submitted variants and assesses which variants are suited for functional analyses in model organisms. This process is an important step because it shapes all downstream procedures. The MOSC carefully reviews each of the genes and variants that the CSs submit and explores the vast amounts of information that are available in public human and model organism databases. The MOSC performs expansive bioinformatic analyses to assess submitted genes and their variants. The MOSC developed a systematic Internet-based database search engine that integrates many of the steps that the MOSC first performed manually: MARRVEL (Model organism Aggregated Resources for Rare Variant ExpLoration; <http://marrvel.org/>). This search tool is publicly available and greatly reduces time spent to aggregate information used to prioritize variants (Luo et al., 2017).

2. Genes and variants that are being considered are available for review within the Gateway, providing all members of the UDN access to this information. Collaborative analyses are pursued as described (Wangler et al., 2017), involving the MOSC variants and those being analyzed by the Canadian Rare Disease Models and Mechanisms Network (RDMM), the Canadian equivalent of the UDN (<http://www.rare-diseases-catalyst-network.ca/>). In addition, the MOSC is currently considering expansion of this approach with the Japanese (Initiative on Rare and Undiagnosed Diseases) and Oceania (UDP Australia and New Zealand) consortia that attempt to diagnose rare or unknown diseases primarily to find matching cases. This communication avoids duplication of work and fosters collaborations to identify additional participants who have genotypes and symptoms similar to the UDN case. Similarly, the MOSC collaborates with the Baylor-Hopkins Center for Mendelian Genomics (CMG) and with Baylor Genetics (a clinical diagnostic sequencing facility) to attempt to identify additional patients who carry mutations in the same gene. A comparison of phenotypes of the additional patients may then lower or elevate the probability that the candidate variant is causative, and hence the priority with which the MOSC continues the characterization of gene and variant function *in vivo*. These collaborative searches and inquiries can exclude some variants or genes from functional analysis. In some cases, additional patients with similar phenotypes may be found that together lead to definitive diagnosis without the need of model organism data (Schoch et al., 2017). In other cases, the discovery of one or more additional patients with similar phenotypes in the collaborative search can significantly elevate the priority of the gene/variant to be studied in the MOSC.

ii. WUSTL review process

1. The WUSTL MOSC analyses submitted genes and variants in a parallel process that is similar to the above BCM review process. One reason for duplicate analysis is that the methods used are complementary to one another, such as RefSeq versus Ensembl for transcript annotation, allowing for a more comprehensive evaluation of submissions. Some tools are in common between both review processes, such as MARRVEL, as discussed above.
2. Variant nomenclature is assessed by use of the MutationTaster tool (<http://www.mutationtaster.org>), which is orthogonal to other methods used.
3. Variants are annotated by use of the ANNOVAR tool (<http://annovar.openbioinformatics.org/>): 1) genomic context and predicted effect on coding sequences (RefSeq), 2) population allele frequencies from multiple databases (gnomAD, ExAC, 1000 genomes), 3) multiple *in silico* nucleotide and protein functional predictions scores (e.g., SIFT, PolyPhen2, LRT, Mutation Taster, GERP++, PhyloP, CADD, REVEL, M-CAP), 4) splicing prediction (dbSNV), and 5) any previously observed clinical impact of variants (ClinVar, OMIM). Output from these analyses are placed onto a shared Google sheet to provide the “raw” data from which pathogenicity interpretations are made.

4. Literature searches are conducted to identify any human cases with disease-causing variants in the gene of interest, in order to compare to the phenotype of the UDN case.
5. Additional analysis modules are currently in development by WUSTL. One module involves the use of Population Sampling Probabilities (PSAP) (Wilfert et al., 2016), which leverages gene-specific models of pathogenicity derived from given pathogenicity scores (such as CADD) and the frequencies of variants observed in the unaffected population (such as ExAC). A second module uses recently available non-human primate (NHP) genomic sequence to interpret potentially pathogenic human variants

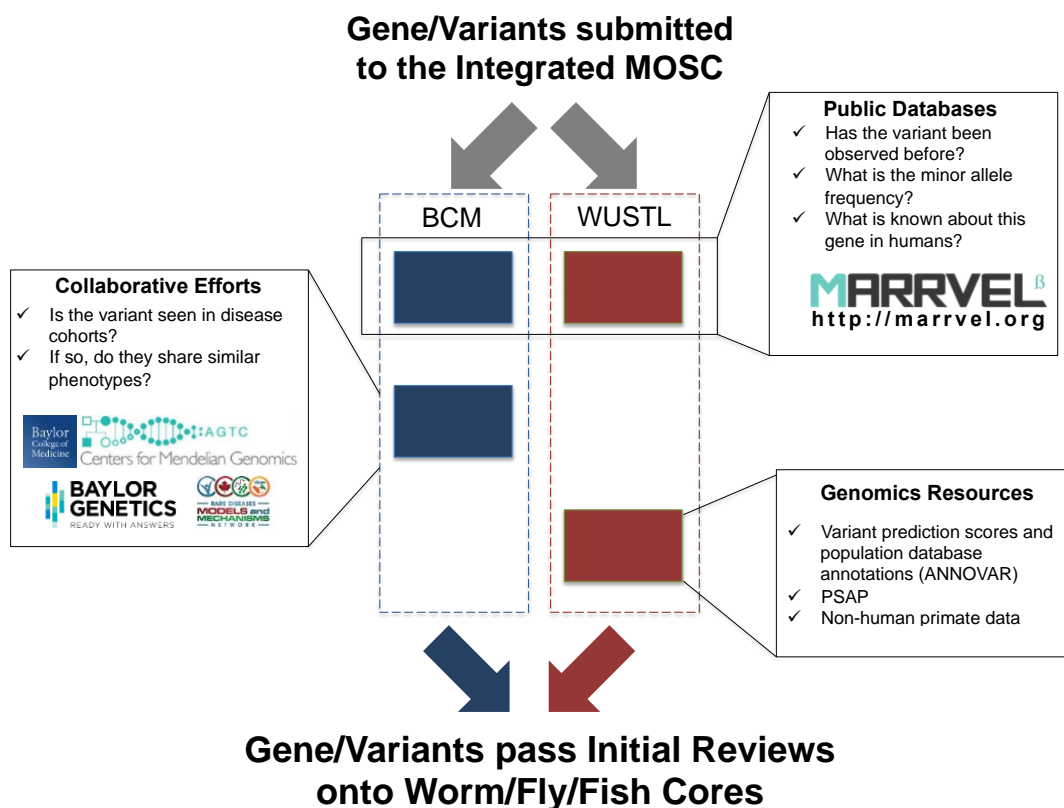


Figure 1. Workflow of the initial variant review plan

- III. At the conclusion of the initial review, BCM and WUSTL both assign priority values: “Pass” “Return to Site,” and “Pass with Concerns.” In principle, for cases where more than one gene is submitted, the BCM and WUSTL Initial Review will also prioritize amongst submitted genes for a single case and assign a “Top Candidate” status to one gene. This status will automatically be assigned to genes for cases with only one submitted gene/variant.
- IV. BCM and WUSTL communicate the results described in C.III into the Gateway. The two groups discuss any difference of opinion, and if there are any concerns for either group, the variant is likely assigned a “Pass with Concerns” designation.

- V. Notifications are provided via the Gateway with an “Initial Review” target turnaround time of 2 weeks from submission. Notification recipients depend on the variant designation as follows:
 - a. “Pass”- notification to Model Organism Core PIs (variants are processed as described in Part D below)
 - b. “Return to Site”- notification go to the CS. This notification states the reason for the submission return and that the site may contact the MOSC for clarification or additional information (including to make any necessary corrections to genomic coordinates) to resubmit the case. If no action is taken by the CS to resubmit the variant, these variants are not analyzed further.
 - c. “Pass with Concerns”- notification sent to Model Organism Core PIs (see Part D below).

D. Variant Assignment to Core

- I. The variants that have been designated as “Pass” (with or without concerns) from in the Initial Review (Section C-II) are analyzed at a “Quick Review” by each core (Figure 2). Examples of Quick Review determinations:
 - a. Worm Core might determine whether there is an ortholog and assess for paralogs, and whether the amino acid is conserved in worm so that the variant of interest could be modeled in this system.
 - b. Fly Core might determine whether there is an ortholog and assess for paralogs, determine whether there are published reagents available, whether there is a human cDNA available, and whether there is an alternative strategy to assess gene function other than the primary “humanization” approach.
 - c. Fish Core might determine whether there is an ortholog, assess for paralogs and known phenotypes, and might also assess whether the gene has been studied *in vivo* in mice and/or other vertebrate model organisms. Quick Review is carried out by UO and the results are discussed by the UO and WUSTL Fish Core teams to achieve a consensus regarding suitability of modeling this gene/variant in zebrafish. For genes/variants deemed suitable for modeling in zebrafish, further discussion leads to a decision about whether the variant would be better modeled at UO or WUSTL.
- II. Each core answers the question “Can the variant potentially be modeled in the core?” with “Yes”, “No” and “Maybe” based on the “Quick Review”, and may add comments.
- III. Gateway is configured to provide reminder notifications to ensure this step is completed no later than 4 weeks from submission.
- IV. Cores selecting “Yes” or “Maybe” will conduct a more thorough review if the variant was also classified as “Top Candidate.”
- V. Gateway notifications depend on outcomes, with examples shown below (Table 1).

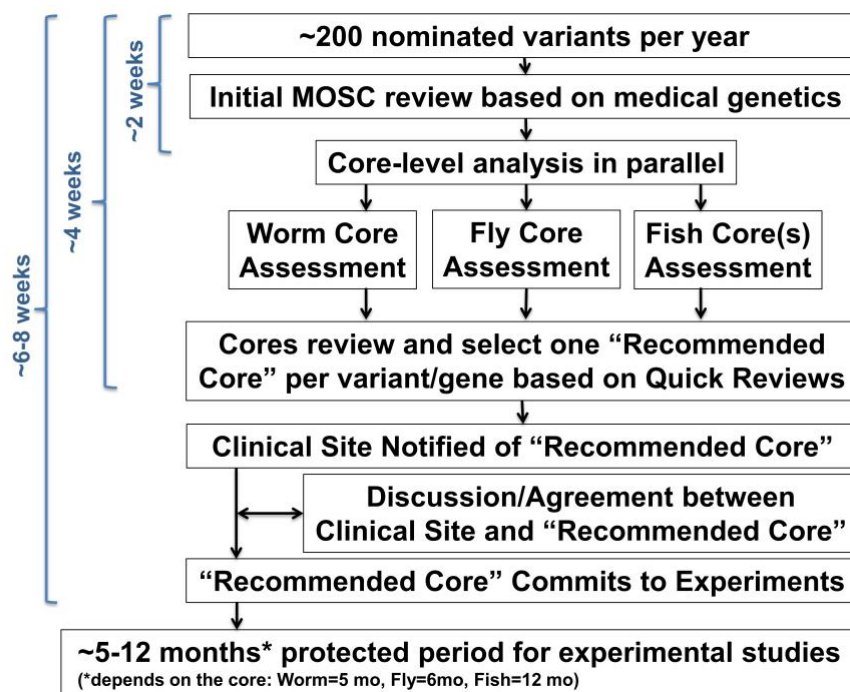


Figure 2. Schematic of the process of variant assignment and commitment

<u>Worm Core</u>	<u>Fly Core</u>	<u>Fish Core</u>	<u>Gateway Notification</u>
Yes	No	No	Worm Core
No	Yes	No	Fly Core
No	No	Yes	Fish Core
Yes	Yes	No	Worm and Fly Cores
Yes	No	Yes	Worm and Fish Cores
No	Yes	Yes	Fly and Fish Cores
Yes	Yes	Yes	All 3 cores
No	No	No	CS notified that the gene cannot be studied in the MOSC, asked to contact MOSC if they would like further information.

Table 1. Potential outcomes of the secondary informatics analysis

- VI. From this informatics analysis, a “Recommended Primary MO Core” is determined. For variants with only one “Yes”, the “Recommended Core” is automatic (**Table 1**). For variants with more than one “Yes”, the cores communicate within 2 weeks from the completion of the Quick Review, and prior to the next MOWG call, to decide which site is the “Recommended Primary MO Core”.

- VII. The CS is notified that a “Recommended Core” has been nominated. Once the core ‘commits’ to experimental studies, the variant is officially “assigned” to the specific core. This commitment and the transition from “Recommended Primary MO Core” to “Assigned Core” can either occur 1) immediately after the assignment of the “Recommended Primary MO Core” for some cases when a Core may unconditionally begin to work on the specific gene/variant, or 2) after a discussion between the Core and CS prior to the transition to “Assigned Core” is preferred and should be arranged. This discussion involves exchanging information regarding participant phenotypes, existing data regarding orthologous genes in MOs, the actual experimental strategies proposed by the Recommended Core and additional questions and answers the two parties may have regarding the case. A notification to the CS with instructions for establishing contact sent from the Gateway to prompt the initiation of the discussion. Upon the identification of the “Recommended Primary MO Core”, the CSs are responsible for establishing contact with the representatives of the specific MO Core, which is indicated in the Gateway. Ideally, the communication should take place within 2-4 weeks after the selection of the “Recommended Primary MO Core.”
- a. Phone calls or web meetings could be arranged by the CS to discuss the case with the Recommended MO Core.
 - b. The discussion could take place as part of the monthly Model Organism Working Group (MOWG) conference call. Other UDN Sites and Cores that are part of the MOWG may be involved in this discussion and may provide some additional input.
 - c. The case discussion can also take place through direct personal contact between the CS and Recommended Core representatives. Such communication can take place at an In-person Steering Committee meetings. E-mail communication may be sufficient for some cases (e.g., if Recommended Core is committed to the case unconditionally), but both the CS and core must be in agreement for the Recommended Cores to commit to the case experimentally. At minimum, the two parties should agree on the experimental strategies and the timeline (see section E).
 - d. Due to the need for time to perform model organism experiments, each core sets a recommended “protected period” (5 months for worm, 6 months for fly, 12 months for fish) during which the CSs are asked not to publish variants that are under study in the MOSC. After this period, the sites are asked to communicate directly with the MOSC core about the progress.

Furthermore, the MOSC collaborates with the Knockout Mouse Phenotyping Project (KOMP) at BCM to knockout the orthologous gene in the mouse if a null allele has not been published in the literature. These mouse knockout lines will undergo systematic phenotyping based on the standards of the International Mouse Phenotyping Consortium (IMPC; <http://www.mousephenotype.org/>). In addition, for specific genes in which experts in the broader *Drosophila*, *C. elegans* and zebrafish communities have established functional assays and are willing to collaborate with the UDN, the MOSC will facilitate communication between the CS and the model organism investigator on a case-by-case basis. The goal of these efforts is to provide new tools, accelerate future research on the gene for the model organism community, and ultimately to benefit the UDN by creating larger collections of models that were initiated from the UDN.

E. Model Organism Studies Timeline and Throughput

- I. The Worm core has a ramp up period of 5 variants in the first 6 months, 15 variants in the second six months, and potentially up to 60 variants per year in subsequent years, dependent on the number of accepted variants that can be tested in the worm. The Worm Core requires 5 months from the date of assignment, during which the sites cannot publish variants under study.
- II. The Fly Core will study 30 variants per year throughout. The Fly core will require 6 months from the date of assignment, during which the sites cannot publish variants under study.
- III. The Fly Core will also generate genetic and molecular resources (e.g. T2A-GAL4 lines, UAS-human cDNA transgenic flies and expression vectors) for genes that are not immediately actionable in the fly core to generate resources for the community to study the gene of interest as described in the original proposal.
- IV. The Fish core at Oregon will study 15 variants per year throughout. The Fish core at Oregon will require 12 months from the date of assignment, during which the sites cannot publish variants under study.
- V. The Fish core at WUSTL will aim to study 15 variants per year after 6 months and with the resources permitting ramp up. The Fish core at WUSTL will require 12 months from the date of assignment, during which the sites cannot publish variants under study.
- VI. When a variant is recommended to the Fish Core, a discussion between Oregon and WUSTL fish cores will determine in which of the two cores the variant will be functionally studied. The factors will include balancing the number of variants modeled in each core, and the expertise of each core: Oregon: neural, skeletal, muscular and reproductive phenotypes among others. WUSTL: developmental, cardiovascular, kidney, and liver phenotypes among others.

F. *C. elegans* Core Operations

The goal of the *C. elegans* Core is to determine if the candidate patient gene-variant, when examined in the worm, displays a phenotype(s). Observation of a phenotype provides experimental evidence that the gene-variant disrupts gene function in an animal. Additionally, the analysis can provide evidence supporting the participant's mode of inheritance (dominant, recessive or *de novo*) or allele status (homozygous, heterozygous, or compound heterozygous), and can potentially provide information on the nature of dominance (antimorph, hypermorph). In the analysis of phenotype, we employ the phenolog concept – distinct phenotypes that arise from genetic disruption of orthologous genes (McGary et al., 2010). Often patient presentation and *C. elegans* organismal phenotype are not obviously related; for example, a patient with a *TWIST*(E117V) *de novo* variant displays craniosynostosis, while the orthologous gene-variant in *C. elegans*, *hlh-8*(E29V), results in an egg laying defective phenotype. Nevertheless, both cases have the same underlying molecular defect, antimorphic poisoning of the *TWIST* transcription factor, reducing expression of downstream targets such as the FGF receptor and Notch ligand (Kim et al., 2017). Due to evolutionary distance, about 30% of patient gene-variants are conserved and can be modeled in *C. elegans*.

The assessment strategy involves knocking-in the patient gene-variant into the orthologous *C. elegans* gene, which is facile given the efficiency of CRISPR/Cas9 editing and the short generation time in the worm. The goal is to generate three variant edits and two control edits. Phenotypic analysis is guided by information on www.WormBase.org and in the literature. If no phenotypic information is available, then a multi-step high-throughput phenotyping pipeline is

employed. In most cases, variant edits are compared to null reference mutants, obtained from the *Caenorhabditis* Genetics Center or generated by the *C. elegans* CRISPR Knockout Project. Where appropriate, in some cases the *C. elegans* Core collaborates with *C. elegans* laboratories that have expertise in specific genes, pathways or phenotypes. Following completion of the project in conjunction with the relevant CS, genetic and phenotypic information is deposited into WormBase and the gene-variant and control variant strains are sent to the *Caenorhabditis* Genetics Center.

G. *Drosophila* Core Operations

The Baylor College of Medicine (BCM) *Drosophila* Core performs bioinformatic analysis of human and *Drosophila* databases. Upon further discussion with the CS that submitted the case, the core directors and scientists of the Fly Core design experimental strategies to pursue in *Drosophila melanogaster*.

The *Drosophila* Core tailors experiments to each gene and variant using the optimal genetic strategy for each individual case. One strategy depends on the versatile engineered transposable element MiMIC (*Minos*-mediated integration cassette) (Venken et al., 2011), which allows “humanization” of *Drosophila* genes (Bellen & Yamamoto, 2015). This pipeline allows for: (1) generation of strong loss of function (LOF) alleles in the fly gene of interest; (2) expression of the yeast GAL4 transcription factor driven by endogenous enhancers of the gene of interest; (3) functional replacement of the fly gene with the reference (wild-type) human gene to test for rescue of the fly mutant phenotype with the orthologous reference human cDNA expressed under control of GAL4 expressed in the proper spatial and temporal pattern; and (4) variant functional analysis through expression of the human disease allele variant in the same fly line. (1) and (2) are accomplished simultaneously by integrating a T2A-GAL4 cassette into a coding exon of a gene of interest (Chao et al., 2017; Wangler et al., 2017; Yoon et al., 2017) via recombinase-mediated cassette exchange of MiMIC, or via CRISPR-mediated homology directed repair (CRIMIC). Other strategies can also be used depending on the availability of pre-existing reagents (e.g., well characterized LOF alleles, experimentally verified RNAi lines), or when rescue with human cDNA has not been successful (Harel et al., 2016). For example, in cases in which the variant affects an amino acid conserved between the fly and human orthologs, a variant can be engineered in the context of a fly genomic construct to perform rescue experiments using previously characterized alleles with scorable phenotypes (Luo et al., 2017). Regardless of the exact methodology and phenotypes to be scored, the overarching goal of the *Drosophila* Core is to demonstrate whether the variant identified in the patient is functionally important, a knowledge that provides a molecular handle to further explore the molecular mechanism of disease.

H. Zebrafish Core Operations

The goal of the Zebrafish Core is to mimic in zebrafish the lesion found in the participant’s candidate gene and to provide a preliminary characterization of the phenotype of the mutation in zebrafish. The Zebrafish Core is composed of two research teams, one at the University of Oregon (UO) and the other at the Washington University in St. Louis (WUSTL). The two Zebrafish Core teams operate in a highly integrated manner to maximize communication and leverage the complementary expertise of the investigators. For the variants that have been designated as “Pass” (with or without concerns), during the “Quick Review”, the UO team initially performs extensive bioinformatic analyses that identify the zebrafish ortholog (or in a minority of

cases, co-orthologs) of the human candidate gene. Next, the UO team checks whether existing mutations or knockdowns have been characterized for the candidate gene in zebrafish and/or mouse, and if so, whether the described phenotypes support or fail to support the conclusion that the participant's clinical manifestation arises from a defect in the candidate gene. If no prior models exist, or if prior models have not been adequately investigated, the Zebrafish Core indicates that the variant potentially can be modeled in the Core. For genes/variants deemed suitable for modeling in zebrafish, initial discussion whether the variant could be modeled at UO or WUSTL also ensues.

When the Fish Core is the “Recommended Primary MO Core”, a discussion between UO and WUSTL fish cores determines in which of the two cores the variant will be studied functionally. Factors considered include balancing the number of variants modeled in each core, and the expertise of each core. The UO team will investigate variants implicated in neural, skeletal, muscular and reproductive phenotypes among others. The WUSTL team focuses on genes implicated in early developmental defects, developmental delay, cardiovascular, kidney, and liver phenotypes among others. Typically, individual variants are modelled by either Oregon or WUSTL teams; in some cases, a fish model can be generated at UP but aspect of its phenotype can be studied at WUSTL.

Either UP or WUSTL Fish Core develops knockout mutations for the candidate gene using CRISPR/Cas9 technology. The goal is to make at least two alleles for each gene tested, ideally one has an early stop codon to block the formation of any functional protein, and a second one removes the gene's promoter. The latter strategy aims at generation of a strong loss-of-function (LOF) allele that eliminates a transcript from this locus or a transcript with a nonsense mutation that can be associated with the compensation phenomenon, which can confound the phenotypic analyses (Rossi et al., 2015). In the third strategy, CRISPR/Cas9/oligonucleotide strategy is employed to introduce a candidate mutant variant into the endogenous zebrafish gene. Finally, the Zebrafish Core analyzes mutant phenotypes caused by the induced mutations and compares them to the participant's clinical findings. Results are discussed with the CSs upon completion of the phenotyping.

Future goals include experiments to test for variant function by learning whether the human wild-type cDNA or mRNA can rescue the zebrafish mutant phenotype but the human mutant cDNA cannot. In cases in which the participant's phenotype is thought to arise due to dominant gain-of-function mechanisms, experiments to determine whether over-expression of the variant in wild-type fish results in phenotypes that mimic the participant's symptoms can provide valuable mechanistic information. In addition, future studies may model the human mutation even better by making the precise homologous change in the zebrafish protein, a goal that requires methodological innovation for improving zebrafish knock-in technology.

I. Communications

The MOSC summarizes bioinformatic analyses in Gateway which sends the information back to the CSs for discussion. The MOSC also communicates the decision regarding which core will be performing the model generation and characterization.

Communication with Clinical Sites after data generation

After generation of mutant models and characterization of mutant phenotypes, the MOSC contacts CSs to discuss observations. The CSs often raise questions and issues that may be

addressed in subsequent experiments. Although the MOSC carries out characterization of the gene in mutant models, the CSs often pursue identification of additional patients to provide more robust evidence that the mutation observed in the participant is indeed associated with the observed phenotype. These data are integrated as part of the characterization to provide a final and compelling diagnosis.

Throughput and allocation

CSs submit approximately 200 variants per year. The *Drosophila* Core studies approximately 30 variants per year, the UO fish core studies approximately 15 per year, and the WUSTL Fish Core another 15 per year. The *C. elegans* core has a ramp up period of 5 variants in the first 6 months, 15 variants in the second 6 months, and potentially up to 60 variants per year in subsequent years, dependent on the number of accepted variants that can be tested in the worm.

Distribution of reagents

All *C. elegans* strains are sent to the *Caenorhabditis* Genetics Center. All the fly stocks are deposited in the Bloomington *Drosophila* Stock Center at Indiana University (BDSC, <http://flystocks.bio.indiana.edu/>). The fish resources are deposited at the Zebrafish International Resource Center (ZIRC, <https://zebrafish.org>). These facilities keep records of stocks they distribute, and these records document the usage of the MOSC materials. In addition, the vectors containing the UAS-human cDNAs are deposited in the *Drosophila* Genome Resource Center (DGRC) in Indiana and are publicly available. All these distribution centers are supported by the NIH. Finally, the reagents (worms, fish, flies and cDNAs) are or will be available for sharing with the CSs as soon as they are generated. Note that the MOSC acquired a human cDNA library containing about 40,000 unique cDNA clones assembled and generated in the past 6 years by the late Dr. Ken Scott at BCM.

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XVI. Appendices

APPENDIX 1: NIH UDP Protocols

Screening (30 inquiries each week)

A Patient Care Coordinator (PCC), selected for having pleasing but firm interpersonal skills, provides a central point for all inquiries that range in specificity from direct physician-to-physician referrals to cold calls to NIH Call Center (866-444-8806) from patients or family members seeking to learn more about the UDP. The NIH Call Center refers these calls to the PCC. Whatever the source of inquiries, the PCC mails the potential participant (or family, in the case of pediatric patients) an invitation package that includes a cover letter and an attached frequently asked questions document. A second letter is sent that the patient can share with his/her physician with an attached form for listing contact information of the current attending physician, a list of prior hospitalizations, and specialists that have been involved in the patient's care. This is often followed by phone exchanges with the PCC to clarify goals and structure of the program and the information required for further evaluation. Descriptions of the detailed flow of participants prior to CRC admission are below.

Substantial delays are often encountered at this phase of patient recruitment as families often request medical records from multiple institutions, reflective of the long diagnostic odyssey. The UDP believes it is essential to obtain a physician recommendation letter in order to provide a clear, current picture of the patient's illness and to ensure follow-up care after completion of the UDP evaluation.

As detailed later, initial UDP medical review requires complete records of previous care and evaluations. Patients and physicians may encounter problems with collection of results of prior blood work, imaging, and special tests as they negotiate retrieval of these materials from various health care facilities. A series of form letters are used to remind potential participants of documentation required, but not yet received, including prior phenotyping and a physician's recommendation letter.

Clarification of the goals of the UDP sometimes results in withdrawal of applicants who have been interested only in a 'second opinion' process. Potential participants who fail to provide the necessary phenotyping data, or for whom there is no physician recommendation letter, will not be further considered. Approximately two-thirds of patients who were initially interested in learning more about the UDP or in participating fail to complete the information gathering process trimming the 30 per week who express an interest in the program to 10 who remain interested and whose records can be gathered and reviewed. For pediatric patients only approximately one-third of families fail to complete the process or are found ineligible, usually because they already have a diagnosis (e.g., they have an unbalanced chromosome translocation with multiple malformation syndrome but the family does not think this is the answer).

Creation and careful review of a CRC medical record (10 applicants each week)

The next step in the recruitment process is to carry out a detailed review of each candidate's medical record, including the study recommendation letter from the current personal physician or physician- extender summarizing the salient features of the applicant's symptoms, with reports detailing already collected phenotyping. These reports might include personal and family

health history, physical examination, blood work and urine analysis/chemistries, imaging, and special studies such as CSF findings, electromyography (EMG), photos of skin lesions, and videos that display abnormalities of balance, gait, and strength. If biopsy or surgical procedures have been performed, biopsy slides may be reviewed by CRC pathologists if this appears to be essential for a decision. Prior imaging, especially CT and MR imaging, is extremely important in the review process, and every effort is made to obtain the images themselves, and not simply reports. The clinical records available vary across applicants, since some have had extensive prior evaluations by skilled physicians and others have had only a limited approach to finding a diagnosis.

The completed file is assigned to UDP team members and/or consultants to evaluate the likelihood that a rare or yet-to-be described disease is present and that the focused, systematic UDP approach might lead to a diagnosis. Useful indicators include other affected family members, objective physical findings, abnormalities found in blood work and/or imaging or other clues pointing to the presence of significant disease. A further consideration is whether, depending on family size and the availability of blood specimens on additional affected family members, the UDP's diagnostic armamentarium, especially SNP arrays or whole exome sequencing, could be useful in providing an answer. The review is physician intensive, and because records are often very extensive, the review process may be lengthy. Moreover, it may prove necessary to request additional information, or the advice of other UDP consultants. While the principal goal of this review process is to select participants for UDP evaluation, there are other potential results. Some applicants may be more suitable for referral to other open NIH research protocols. If, in the judgment of the review panel, there has been incomplete evaluation, the panel may choose to return the applicant to the referring physician with suggestions for further diagnostic approaches or recommend referral to an appropriate academic medical center.

The decision to invite applicants to travel, expense-free, to Bethesda, MD for a 5-day admission to the NIH CRC is made by Program Directors after detailed discussion with consultants and other members of the UDP team. The goal of this review process is to ensure, to the extent possible, that the accepted applicants are appropriate and match the resources of the UDP. A criterion for acceptance to the UDP is that the applicant is safe to travel. The pediatric applicants in particular are often medically fragile, medical clearance for commercial travel must be documented by the referring physician before applicants can be accepted and scheduled for evaluation. Pediatric applicants must have clearance from their physician one week prior to making the trip. In some cases, visits need to be rescheduled if the applicant is too sick to travel. UDP does not have the ability to pay for hospital-to-hospital transports, nor can they carry out these transports.

Preparation for the 5-day evaluation

Fitting the required diagnostic efforts into a 5-day evaluation requires careful planning to complete thorough phenotyping and place the findings in context for anxious participants and their families. This planning is complex and involves scheduling heavily used imaging resources and other diagnostic tests and insuring that initial evaluations by sub-specialists can be performed in a timely fashion. Participant-specific time constraints must also be considered.

Overall approach to phenotyping and specific data gathering in common subgroups

More than half of the participants accepted into the UDP have a neurological phenotype, and in children in particular this leads to a common phenotyping framework that includes intracranial

imaging (magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS)), neurologic consultation, electroencephalogram (EEG), EMG/nerve conduction velocity (NCV), lumbar puncture for CSF neurotransmitters and other special testing, skin biopsy both for fibroblast culture for functional verification of new candidate genes and for immunohistochemistry and electron microscopy, ophthalmologic exam under anesthesia, psychiatry consultation, and neurocognitive testing. In adult participants, CSF is also obtained for immunologic studies.

UDP metrics

The UDP has had much success, as can be seen from the following metrics:

- a) Metrics in the 4 years following the establishment of the UDP:
 - 6,300 inquiries evaluated
 - 2,300 physician referral letters with applicant medical records reviewed
 - 450 participants admitted to the NIH-CRC (Clinical Research Center)
- b) Weekly metrics:
 - 30 new inquiries
 - 10 applicants with completed referral letters and results from prior diagnostic efforts evaluated
 - 3 participants/families admitted for workup at the CRC
- c) Diagnostic metrics:
 - Approximately 100 participants (20-25%) were diagnosed with rare to extremely rare diseases
 - Two participants were found with diseases unknown to medicine
 - 15 genes not previously associated with human disease were discovered and tentatively related to disease phenotypes

Summary

The NIH UDP initial approach to identifying and evaluating participants with undiagnosed diseases has been refined and focused over nearly five years. It seeks to identify participants who are most likely to have a rare or unknown undiagnosed disease. The 5-day admission to the NIH is designed to define the underlying pathophysiology by careful phenotyping and to identify settings in which genomics may prove useful.

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Figure 1: NIH UDP participant flow

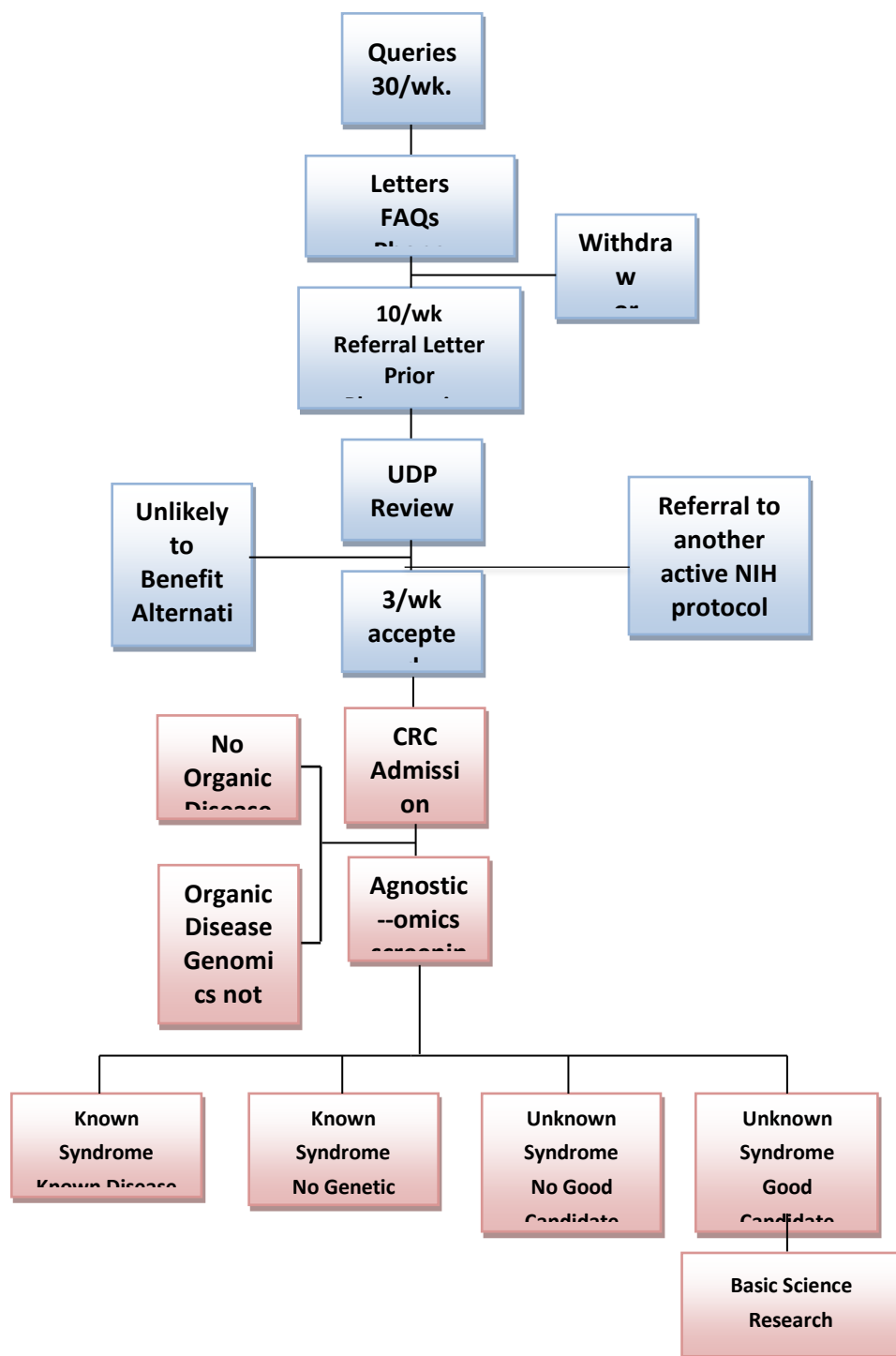
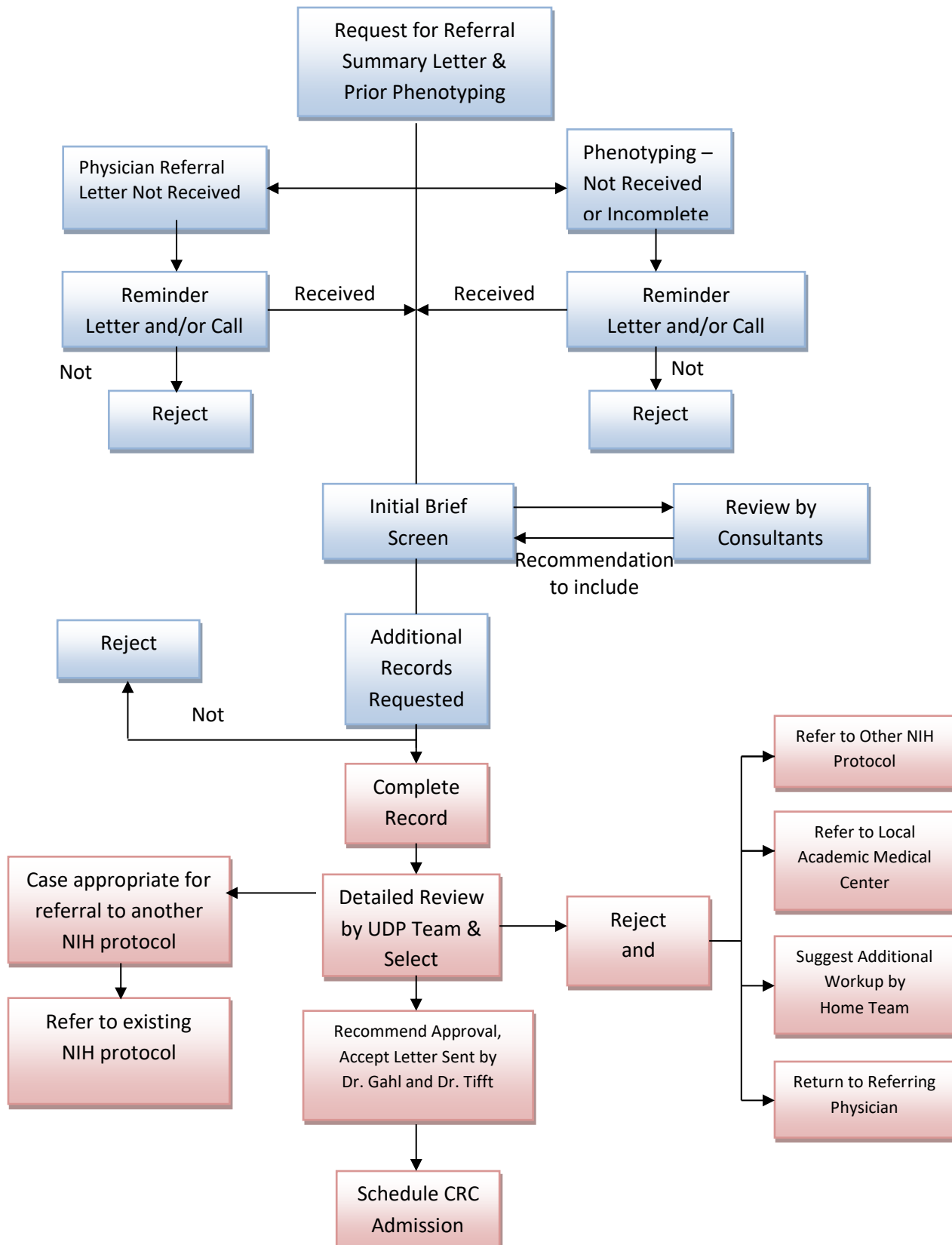


Figure 2: NIH UDP pre-CRC admission



APPENDIX 2: Example Study Recommendation Letters

Example Study Recommendation Letter #1 (pediatric)

Hospital/clinic logo here

To Whom it May Concern:

We are writing to you to request consideration of siblings, [patient names], for enrollment in the Undiagnosed Diseases Network (UDN). [Patient names] are followed by multiple specialists at [hospital name]. They are also followed by local pediatrician [physician name] for routine pediatric care.

[Patient name] is now a [age] year old [gender] with a history of dysmorphic features, failure to thrive, and hepatomegaly of unknown origin. Due to cryptogenic cirrhosis, liver transplant was performed at [age] months of age. Pathology results of [patient name]'s previous liver biopsy was suspicious for a [condition], specifically [specific condition], however, molecular testing for the [gene name] was negative. [Condition] enzyme screening and [condition] screen for the explanted liver sample came back in the low ranges, but not in the deficiency range usually seen. Additional extensive workup was unrevealing.

[Patient name] is now a [age] month old [gender] noted prenatally to have holoprosencephaly via fetal MRI at [time] weeks gestation. Brain MRI performed on DOL [time] was consistent with [description of MRI]. [He/she] was admitted at [age] months of life for evaluation of liver steatosis, microcephaly, and failure to thrive. At [age] months of age, [patient name] was identified to have new onset hepatomegaly in [month] with vomiting. A liver biopsy from [date] identified [results of liver biopsy]. Due to persistent FTT, G tube was placed in [month] with subsequent fungal peritonitis, now post-[time] day course of [medication]. [Patient name] continues to have daily emesis. [Patient name] is currently evaluated for liver transplantation (persistently elevated transaminases and synthetic dysfunction).

Of note, both siblings have a history of IUGR with failure to thrive, improved for [patient name] following liver transplantation. [Patient name] has a history of developmental delays, making significant progress with therapies, and now within normal limits. [Patient name] continues to have developmental delays and facial features similar to [his/her] [brother/sister] during infancy. Given the similarities in the presentation of these two siblings with an unremarkable family history (parents are not consanguineous), whole exome sequencing was obtained for [patient name] and identified a heterozygous mutation in the [gene name], which in the homozygous state is associated with [condition]. Subsequent deletion/duplication testing via the MitoMet oligonucleotide array returned as normal. Mitochondrial genome testing via

massively parallel sequencing was obtained for [patient name] and was unrevealing.

At this time, we are unable to identify a specific genetic etiology that would explain the findings seen in [patient name] and [patient name]. The presentation of two siblings with similar features, however, is suggestive of a possible autosomal recessive condition, which remains undiagnosed at this time. Parents are interested in identifying a diagnosis, and are also interested in having a third child. We would like to refer these siblings to the Undiagnosed Diseases Network for further evaluation to try and identify a diagnosis. Thank you for your review and consideration for acceptance into the program. Please do not hesitate to contact our office at [phone number] if you have any questions or require any additional materials.

Sincerely,

Healthcare provider signature

[Healthcare provider name]

Example Study Recommendation Letter #2 (pediatric)

Hospital/clinic logo here

To Whom it May Concern:

I have followed [patient name] since [age] months of age. [He/she] has a history of significant global developmental delay, [he/she] is nonverbal, has hyperoral behavior, macrocephaly, small stature, [further description]. [He/she] has a great disposition and visually interacts with [his/her] environment. [He/she] has continued to make very slow but steady motor development but has never developed speech. [He/she] has never had seizures or developmental regression. Significant genetic, metabolic, and neurodiagnostic evaluation (as listed below) has yet to yield an underlying diagnosis. I am referring [him/her] to the Undiagnosed Diseases Network in attempts to find a unifying diagnosis for [his/her] multitude of symptoms. I truly feel that there is an underlying metabolic or genetic cause for [his/her] symptoms that our testing thus far has not uncovered. [He/she] has been seen by numerous other specialists across the country.

[His/her] evaluation to date includes:

Normal or negative metabolic studies:

Urine organic acids

Serum amino acids

Creatinine guanidinoacetate

Etc.

Normal or negative genetic studies:

Routine chromosomes

Chromosomal microarray [year]

mtDNA point mutations and deletions

GeneDx 101 mitochondrial nuclear gene panel

Etc.

Neuroimaging/neurodiagnostics:

[year]--- MRI showed [results]

[year] CT showed [results]

Etc.

Normal or negative CSF studies:
Neurotransmitters
Biopterin

I truly appreciate your consideration for evaluation for [patient name]. This family has been on a very long quest to find a diagnosis and would be grateful for the opportunity to have [him/her] evaluated through the UDN.

Sincerely,

Healthcare provider signature

[Healthcare provider name]

Example Study Recommendation Letter #3 (pediatric)

Hospital/clinic logo here

Dear Undiagnosed Diseases Network,

I wholeheartedly recommend [patient name] to be evaluated by the Undiagnosed Diseases Network. [He/she] is a [age] year old with persistent myalgias, dyspnea, [description of condition] of unknown etiology. There are several other family members who are less severely affected with similar symptoms, suggesting a genetic etiology.

I recently met [patient name] to evaluate him for endocrinologic involvement of [his/her] presentation. While I did not find any endocrine pathology, I wanted to take the opportunity to refer him to the UDN. The notes from [his/her] neurologist Dr. [name] will have more details on his history, but I will describe the summary of what I learned.

[patient name] currently presents with [symptoms]. [Patient name]'s family reports that [patient name]'s symptoms initially began at [age] years of age when he began complaining of leg pain out of proportion to those expected for his age. He was evaluated at [age] years of age by a rheumatologist at [hospital], and then by Neurology where a deltoid biopsy was performed and reportedly normal. Additionally, other genetic testing for different forms of [condition] was negative. [He/she] was then referred to Dr. [name] at [hospital]. An EMG was normal, but a quadriceps biopsy showed a predominance of [finding] of unclear significance.

[Patient name]'s symptoms have all progressed over time. [He/she] complains of significant exercise intolerance and weakness in all muscles that have been slowly worsening over time. [His/her] weakness is particularly extreme after activity. Additionally, [patient name] has pain in [his/her] legs, around [his/her] neck, and lower back that is present all the time, although also worsened with activity. [He/she] has seen some improvement in the pain, especially in [area], after starting [medication]. [He/she] occasionally tries [medication] without much relief. The pain is particularly bad [time of day] whereas [his/her] other symptoms seem to be more extreme [time of day]. [His/her] [parent] notes that [he/she] also has some ptosis and [symptom] on several mornings when [he/she] wakes up that sometimes persists later in the day. Initially, this was one-sided, predominantly on the [side], but now appears to be bilateral. [Patient name] walks when [he/she] is at home but uses a wheelchair for transportation of further distances. [He/she] also appears to have [symptoms]. [His/her] motor strength and reflexes, however, are typically normal when [he/she] is evaluated in the neuromuscular clinic, suggesting that [patient name] has more trouble with fatigue than baseline muscle weakness. [He/she] also has a normal serum CK level. [He/she] has had evaluations for [syndromes] that were negative. [He/she] also had an empiric

trial of [medication] that did not improve [his/her] symptoms. Dr. [name] most recently requested a [test] given the [symptoms].

[Patient name] recently developed [symptom] on [his/her] back, which particularly precipitated the referral to my clinic. I did not feel any sign of excess [hormone]. [He/she] was also evaluated by dermatology who felt these to be [condition]. Additionally, [he/she] has a [birthmark].

[Patient name] has been seen by several other specialists. [He/she] follows with Dr. [name] at [hospital] for pulmonary and has been noted to have [symptom]. [He/she] also was briefly followed by Dr. [name] in [state] at [hospital] for some time, but no further diagnoses were noted. [He/she] has been evaluated by Cardiology with a normal echo and EKG. [He/she] has also been evaluated by Physical Therapy, who did not think that [he/she] would benefit from their intervention due to [his/her] exercise intolerance.

[Patient name]'s family history is of particular interest. [His/her] [Parent] is healthy other than migraines and is of [ethnicity] background. [His/her] [Parent] is healthy and of [ethnicity] descent. There is no consanguinity in the family. [Patient name] has [number] siblings. [His/her] oldest sibling is [age] years old with some slight degree of muscle weakness as well. [He/she] has [number] healthy child and is currently pregnant with no complications. [Patient name]'s oldest brother is [age] years old, and his next sibling is [age] years old. Both of them are healthy except for some asthma and allergies. [Patient name] has an [age]-year old sibling who has joint and muscle problems that are not as severe as [patient's].

Thank you for your consideration of [patient's] application.

Sincerely,

Healthcare provider signature

[Healthcare provider name]

Example Study Recommendation Letter #4 (adult)

Hospital/clinic logo here

Dear Undiagnosed Diseases Network Team,

I propose my patient [name] for your special protocol in the Undiagnosed Diseases Network. When I learned of your protocol, I immediately thought of [him/her]. [He/she] seems an ideal participant in your program.

Symptoms & History: [Name] suffers from an excruciating and bizarre illness that has devastated [his/her] life and gone undiagnosed for [number] years despite exhaustive workups at [institution] and here at [institution]. [He/she] has consulted over 100 medical specialists of whom many are at the pinnacle of their fields. [name] is a pleasant, intelligent [man/woman] and a motivated, cooperative patient.

- ☐ [Name] is a fair-skinned [age]-year old [man/woman] who has been disabled for the last [number] of years by burning facial pain and flushing of elusive etiology. [His/her] entire face and ears are involved; they are inflamed, red, and hot to the touch.
- ☐ Onset was rapid and for no apparent reason. Prior to the illness, [he/she] was in excellent health, a parent with a healthy child and successful professional who worked full-time.
- ☐ The facial pain requires [name] to remain nearly all the time in a cold room with a fan blowing directly on [his/her] face. [more explanation]
- ☐ While [name]'s face and ears are chronically hot, the rest of [his/her] body [description].
- ☐ [Name] has anhidrosis over 90% of [his/her] body. However, sweating that cannot be elicited by heat can sometimes be elicited with [system] stimulation.
- ☐ [He/she] developed [eye condition] in [his/her] [age], since remedied surgically.
- ☐ Other major symptoms include: [additional symptoms]

Diagnostics & Etiology: [Name]'s case is a medical mystery cutting across many organ systems/branches of medicine. One might describe it functionally as a putative sympathetic neurologic disorder of the thermoregulatory system that especially affects the vasculature and skin of the head. The origin of the proposed neuropathy could be genetic, autoimmune, infectious, toxicological, or some combination.

There are a number of tantalizing but unexplained clues including:

1. [He/she] is a carrier of one copy of the gene for the rare recessive genetic disease [condition], of which [his/her] relative died. But the [condition] experts have never seen

- symptoms manifested in a [condition] carrier.
2. [Protease] levels are chronically high, but not high enough for [condition]/
 3. [He/she] tests relatively normal on most blood and urine diagnostics, but with some curious exceptions: high on [tests]. Low on [tests].
 4. [Medication] has a minor positive effect on [his/her] symptoms and [he/she] takes it on an ongoing basis. This is the most helpful of the 100 or so medications that have been tried.
 5. [He/she] has idiosyncratic negative reactions to many medications, often responding to “subclinical” doses.
 6. [Name] was on a course of the medication [medication name] when [his/her] illness started, but there are no other documented cases of such a reaction to this medication.
 7. A number of surgical sympathetic blocks have been implemented on a temporary basis, sometimes with great beneficial effect and sometimes the opposite.
 8. Her illness bears some similarity to [condition], itself a rare and largely unexplained disease. However, [condition] affects the feet and sometimes the hands, and there is little or no reference in the literature to a similar disease affecting only the face.

Records: [Name] has carefully retained and organized the voluminous diagnostics and reports on [his/her] condition over [time] years seeking a diagnosis and treatment. This should be helpful to your efforts.

My role: While I am a [specialist] in private practice, I have served as [his/her] primary physician since very early in the illness. I would be pleased to support your efforts and provide follow-up. I understand that several other physicians that regularly see [name] are also in support of [his/her] application and would be available to communicate with you if requested.

Patient’s perspective: [Name] has been exhaustive and courageous in seeking an explanation for this illness. [He/she] read about your program in [magazine]. [He/she] fully understands that your program is primarily for research purposes and that the chances of significant benefits from participating are rather small. Please consider [him/her] for your program. My contact information and [his/hers] appears below.

Sincerely,

Healthcare provider signature

[Healthcare provider name]

APPENDIX 3: Applicant Review Form (Completed by Clinical Sites)

Applicant name: _____

UDN identifier: _____

Date of birth: _____

Date application submitted: _____

**Auto-populates from Gateway application*

UDN site: _____ **Auto-populates from site assignment*

Name of primary reviewer(s): _____

Category of primary condition (drop down list):

- Allergy/immunology
- Cardiology and vascular conditions
- Dentistry and craniofacial
- Dermatology
- Endocrinology
- Fibromyalgia/chronic fatigue syndrome
- Gastroenterology
- Gynecology and reproductive
- Hematology
- Infectious disease
- Musculoskeletal and orthopedics
- Nephrology
- Neurology
- Oncology
- Psychiatry
- Pulmonary
- Rheumatology
- Multiple pediatric (multiple congenital anomalies)
- Other
- None of the above

Please provide a narrative summary (150-200 words) of the applicant's condition. If applicable, please include: history of present symptoms, date symptoms first noted, past medical history, previous diagnoses/comorbidities (using ICD terms if possible), prior procedures and surgeries.

Height (%):

Weight (%):

Head circumference (%):

Prior genetic testing requested:

Exome sequencing ☐ Yes ☐ No ☐ Unknown
If yes: Relevant details: ☐ Trio ☐ Proband only ☐ Other

Genome sequencing ☐ Yes ☐ No ☐ Unknown
If yes: Relevant details: ☐ Trio ☐ Proband only ☐ Other

Single gene testing ☐ Yes ☐ No ☐ Unknown
If yes: Relevant details: ☐ Trio ☐ Proband only ☐ Other

Panel testing ☐ Yes ☐ No ☐ Unknown
If yes: Relevant details: ☐ Trio ☐ Proband only ☐ Other

Chromosomal microarray ☐ Yes ☐ No ☐ Unknown
If yes: Relevant details: ☐ Trio ☐ Proband only ☐ Other

Karyotype ☐ Yes ☐ No ☐ Unknown
If yes: Relevant details: ☐ Trio ☐ Proband only ☐ Other

Other ☐ Yes ☐ No ☐ Unknown
If yes: Relevant details: ☐ Trio ☐ Proband only ☐ Other

Was exome sequencing denied by insurance? ☐ Yes ☐ No ☐ Unknown

Please describe the applicant's pertinent prior evaluations.

Plan for UDN evaluation.

Other family members affected: Yes/No

- If yes:
 - How many affected? _____
 - How many available for analysis? Unknown/Some/All/None

Applicant images: *Attach files*

Other files: *Attach files*

Category 1: Inclusion/Exclusion Criteria

Inclusion Criteria

- ☐ Does Not Have Diagnosis Explaining Objective Findings
- ☐ Agrees to Storage and Sharing of Information & Biomaterials

Exclusion Criteria

- ☐ Has Diagnosis Explaining Objective Findings
- ☐ Diagnosis Suggested Based on Record Review; Further Evaluation Unnecessary
- ☐ Too Ill to Travel Safely to UDN site

Category 2: Strengths (≥3 Recommended)

- ☐ Objective Abnormal Finding(s)
- ☐ Unique Clinical Presentation
- ☐ Multiple Systems Affected
- ☐ Family History of Condition
- ☐ Relevant Family Members Available for Testing

- ☐ High Likelihood of Genetic Diagnosis
- ☐ Local Patient
- ☐ Relevant to Other UDN Participants
- ☐ Can Offer Sequencing
- ☐ Can Offer Additional Clinical Workup
- ☐ Other

Category 3: Limitations (<1 Recommended)

- ☐ No Relevant Family Members Available for Genetic Testing
- ☐ UDN Resources Not Appropriate for Case
- ☐ High Likelihood of Not Solving Case at Present
- ☐ Proband Likely to Refuse Certain Tests/Procedures
- ☐ No Objective Clinical Findings
- ☐ Other

Case Decision

- ☐ Recommended Acceptance at this Site
- ☐ Recommend Acceptance at Different Site
- ☐ Questionable Case
- ☐ Reconsider
- ☐ Not Accepted with Recommendations- Specific Testing
- ☐ Not Accepted with Recommendations- Seek Expert Care
- ☐ Not Accepted- Diagnosis Identified
- ☐ Not Accepted- UDN would likely not be able to find a diagnosis
- ☐ Not Accepted- Insufficient records made available to UDN site
- ☐ Withdrawn- Applicant request
- ☐ Withdrawn- UDN PI request
- ☐ Withdrawn- Lost to follow-up
- ☐ Withdrawn- Deceased

APPENDIX 4: Template Letters for Patients and Health Care Providers



Description: *Information for patients*

Date:

Address:

Dear [patient]:

Thank you for your interest in the Undiagnosed Diseases Network (UDN). Participants accepted into this program will be part of a clinical research study aimed at answering questions about medical conditions that have eluded diagnosis. We hope to advance medical knowledge in ways that can help improve health care for everyone.

Please discuss your participation in this program with your health care provider. Important considerations include:

- Many cases accepted will NOT result in a diagnosis.
- A recommendation by a health care provider is required.
- The provider who refers you will be asked to provide your medical information.
- The UDN will communicate the decision on accepting your case for evaluation in writing to you and your health care provider.
- If your case is accepted for UDN evaluation, the UDN will provide information from the evaluation to you and to your health care provider.
- Your health care provider will be responsible for your medical care after you have been evaluated in the UDN.

Details about the information needed from your health care provider are on the attached letter. Please insert information where requested and give the *Information for Referring Health Care Providers* document to your provider.

A UDN staff member will notify you when the information from your provider has been received. Once all materials are received, UDN review is expected to take about six to eight weeks.

Again, thank you for your interest in the UDN. Medical advances depend on individuals like you who volunteer as partners in medical discovery. More information about this program is available online at <https://undiagnosed.hms.harvard.edu>.

Best regards,

[name]



Description: *Information for health care providers*

Information for health care providers

Your patient has contacted the Undiagnosed Diseases Network (UDN) about participating in this research study. UDN participants are evaluated using a unique combination of scientific and medical expertise and resources. Participants must have a condition that has not been diagnosed following a thorough medical evaluation. More information about the UDN can be found here: <https://undiagnosed.hms.harvard.edu>.

If your patient decides to apply for this study, they will need to answer a series of brief questions through our application Gateway (<https://gateway.undiagnosed.hms.harvard.edu>) related to the following:

- Contact information
- Demographic information
- Health care provider information (name, address, phone, fax, email)
- Brief medical history questions
- Previous evaluations
- Travel limitations

A study recommendation letter from a licensed health care provider is also necessary in order to submit the online application to the UDN. Your patient will be asked to upload this letter to the online application. The study recommendation letter must include:

- A summary of the applicant's medical problems
- Date when symptoms were first noticed
- Previous diagnoses
- History of evaluations and testing
- History of treatments and medications
- Current medications
- Family history
- Health care provider's diagnostic impressions
- For pediatric patients: prenatal and birth history should also be provided.

If you would like to receive application updates, please ask your patient to list your contact information on the application.

After an application is submitted, it is then sent to one of the UDN clinical sites to review. The UDN clinical sites typically contact applicants within one month of application submission to request additional information, such as:

- Medical records
- Laboratory studies
- MRI, X-ray, or CT images (can be sent on CD)
- Photographs or videos
- Pathology slides and reports

- For pediatric patients: growth curves

In order to help with the application review process, the patient can begin to collect these records now. However, no medical records should be sent until specific instructions to do so are received from a UDN clinical site.

After all of the necessary information is received by a UDN clinical site, it typically takes 6-8 weeks for the network to decide whether to accept an applicant.

If your patient's case is accepted for UDN evaluation, the UDN will provide information from the evaluation to you and to your patient. You will be responsible for your patient's follow up medical care.

Thank you for considering this opportunity to consult with the UDN on your patient. We appreciate your commitment to providing the best possible care for your patients in ways that help advance medical knowledge and discovery.

Best regards,

[name]

Description: Medical record request- adult

Date:

Address:

Dear [patient]:

Thank you for your interest in the Undiagnosed Diseases Network (UDN). You have been assigned to the [site name] in [location] for consideration. In order to properly review your case, we will need the following medical records:

- _____ Admission and discharge summaries from hospitalizations related to your condition
- _____ Consultant notes (such as cardiologist, gastroenterologist, neurologist, geneticist)
- _____ Diagnostic laboratory studies
- _____ Biopsy reports and slides
- _____ Imaging studies (MRI, ultrasound, X-rays)
- _____ One or more photos of your face and anything that is relevant to your medical condition
- _____ Family history or drawn family tree
- _____ Other:

Please also complete and send to us contact information for each of your health care providers.

You can mail records to: [address].

Allow at least 8 weeks from the time we receive all of these materials for us to review your records and consult with relevant specialists. All records will be reviewed by our team, but not all cases will be accepted into our research study.

When the team has made a decision about your case you will be notified by mail.

Thank you again for your interest in the Undiagnosed Diseases Network research study.

Sincerely,

[name]
Principal Investigator at [Institution]

If you have any questions, please contact [Site Coordinator] at [phone number and email address].



Your institutional logo
here

Description: Medical record request- pediatric

Date:

Address:

Dear [patient's parent/guardian]:

Thank you for your interest in the Undiagnosed Diseases Network (UDN). Your child has been assigned to the [site name] in [location] for consideration. In order to properly review your child's case, we will need the following medical records:

- _____ Admission and discharge summaries from hospitalizations related to your child's condition
- _____ Consultant notes (such as cardiologist, gastroenterologist, neurologist, geneticist)
- _____ Diagnostic laboratory studies
- _____ Biopsy reports and slides
- _____ Imaging studies (MRI, ultrasound, X-rays).
- _____ One or more photos of your child's face and anything that is relevant to his/her medical condition
- _____ Family history or drawn family tree
- _____ Birth/Newborn records
- _____ Growth curves (height, weight, and head circumference)
- _____ Other:

Please also complete and send to us contact information for each of your child's health care providers.

You can mail records to: [address].

Allow at least 8 weeks from the time we receive all of these materials for us to review your child's records and consult with relevant specialists. All records will be reviewed by our team, but not all cases will be accepted into our research study.

When the team has made a decision about your child's case you will be notified by mail.

Thank you again for your interest in the Undiagnosed Diseases Network research study.

Sincerely,

[name]
Principal Investigator at [Institution]

If you have any questions, please contact [Site Coordinator] at [phone number and email address].



Your institutional logo
here

Description: *Records received*

Date:

Address:

Dear [patient]:

Thank you for your interest in the Undiagnosed Diseases Network (UDN). We are writing to inform you that your records have been received. Please allow 60 days from this time for us to review your records. Our team reviews all records, however, not all cases are accepted into the UDN.

When the team has made a decision about your case you will be notified.

Thank you again for your interest in our program.

Sincerely,

[name]
Principal Investigator at [Institution]

If you have any questions, please contact [Site Coordinator] at [phone number and email address].

Description: *Incomplete records*

Date:

Address:

Dear [patient]:

Thank you for your interest in the Undiagnosed Diseases Network (UDN). We are writing to inform you that some of your records have been received, however, they are not complete. When you send records: (1) please make sure there are not multiple copies of the same report, (2) organize the reports by subspecialty and date seen (i.e., genetics, gastroenterology, neurology etc.), and (3) Do NOT send double-sided copies. This will greatly speed processing and timely review of your case. Specifically, we are requesting:

- _____ Summary letter from health care provider
- _____ Medical records
- _____ Labs
- _____ Biopsy reports and slides
- _____ All imaging on CD, including brain
- _____ Anesthesiology records
- _____ Seizure medication levels within 30 days of the admission
- _____ Birth/Neonatal records **for pediatric patients**
- _____ Growth curves **for pediatric patients**
- _____ Photos **for pediatric patients**
- _____ Other:

Please allow 60 days from the time of our receipt of these materials for us to review your records. Our team reviews all records, however, not all cases are accepted into the UDN. When the team has made a decision about your case you will be notified.

Thank you again for your interest in our program.

Sincerely,

[name]
Principal Investigator at [Institution]

If you have any questions or have difficulty requesting your medical records, please contact [Site Coordinator] at [phone number and email address].



Your institutional logo
here

Description: Partial application

Date:

Address:

Dear [patient]:

You have expressed interest in the Undiagnosed Diseases Network (UDN) and have submitted a partial application. We requested medical records on [date], however, these records have not been received. Since three months has passed since our initial request, we assume that you are no longer interested in being evaluated in the network and will be removing your name from our active rolls.

Sincerely,

[name]

Principal Investigator at [Institution]

If you have any questions or have difficulty requesting your medical records, please contact [Site Coordinator] at [phone number and email address].

Cc: [referring provider]

Description: Acceptance letter (pediatric) to health care provider

Date:

Address:

Dear [health care provider]:

Your patient _____ [DOB] has applied to the Undiagnosed Diseases Network (UDN). As part of a stringent 2 step review process, your patient's case has completed the first level of review and is being considered for evaluation at [your institutional name; city, state]. Consent to the UDN as a research study is the next step for enrollment in the UDN. If enrolled, participants in the Network will be examined using the unique combination of scientific and medical expertise and resources at [your institution]. This evaluation will require a 2-5 day visit for inpatient and/or outpatient care. There is no need to order additional tests or procedures for the purpose of preparing your patient for this evaluation.

Travel, meals, and lodging expenses will be covered for research participants according to our policies, to the extent allowed by law. A representative of the Network will contact the participant within the next few weeks. If the parents of your patient are separated or divorced we will need to receive the court paperwork specifying who is legally able to consent for the child to participate in medical research. We may also request blood specimens for DNA isolation from one or both parents prior to scheduling the child's visit to the [your institutional name].

Not all admissions will result in a diagnosis, but the evaluations should yield valuable information that medical researchers will use to: (1) help identify previously unrecognized rare diseases; (2) suggest new ways to treat and prevent common illnesses; and (3) determine promising options for continued medical research.

[Your institution] will provide information from the evaluation to you and to your patient as a part of this Network. You will be responsible for your patient's follow up medical care. Selected patients may be eligible for other ongoing research studies.

Thank you for consulting with the Undiagnosed Diseases Network on your patient. We appreciate your commitment to providing the best possible care for your patients in ways that help advance medical knowledge and discovery.

Sincerely,

[name]
Principal Investigator at [Institution]

If you have any questions, please contact [Site Coordinator] at [phone number and email address].

Cc: [participant]

Description: Acceptance letter (adult) to health care provider

Date:

Address:

Dear [health care provider]:

Your patient _____ [DOB] has applied to the Undiagnosed Diseases Network (UDN). As part of a stringent 2 step review process, your patient's case has completed the first level of review and is being considered for evaluation at [your institutional name; city, state]. Consent to the UDN as a research study is the next step for enrollment in the UDN. If enrolled, participants in the program will be examined using the unique combination of medical and scientific expertise and resources at [your institution]. This evaluation will require a 2-5 day visit for inpatient and/or outpatient care. There is no need to order additional tests or procedures for the purpose of preparing your patient for this evaluation.

Travel, meals, and lodging expenses will be covered for research participants according to our policies, to the extent allowed by law. A representative of the Network will contact the participant within the next few weeks.

Not all admissions will result in a diagnosis. In addition to contributing to the diagnosis of individual participants, UDN evaluations should yield valuable information that medical researchers will use to: (1) help identify previously unrecognized rare diseases; (2) suggest new ways to treat and prevent common illnesses; and (3) determine promising options for continued medical research.

[Your institution] will provide information from the evaluation to you and to your patient as a part of this Network. You will be responsible for your patient's follow-up medical care. Selected patients may be eligible for other ongoing research studies.

Thank you for consulting with the Undiagnosed Diseases Network on your patient. We appreciate your commitment to providing the best possible care for your patients in ways that help advance medical knowledge and discovery.

Sincerely,

[name]
Principal Investigator at [Institution]

If you have any questions, please contact [Site Coordinator] at [phone number and email address].

Cc: [participant]

Description: Acceptance letter to participant (optional)

Date:

Address:

Dear [participant/caregiver]:

As part of a stringent 2-step review process, [your/your child's] case has completed the first level of review and is being considered for evaluation at [your institutional name; city, state]. Consent to the UDN as a research study is the next step for enrollment in the UDN. If enrolled, participants in the program will be examined using the unique combination of medical and scientific expertise and resources at [your institution]. This evaluation will require a 2-5 day visit for inpatient and/or outpatient care.

We would like to schedule the evaluation for [approximate time] from now. Our team will be in contact with you regarding arranging travel to [Institution] and lodging. The research study will cover the cost of travel, meals, and lodging for [you/your child] and one caregiver. Any additional travelers will be at your expense. The research study will not cover additional expenses, like pay-per-view movies, room service, and mini-bar items.

Once scheduled, you may not reschedule the evaluation, except in the case of an emergency. The evaluation will be designed by the [Institution] research team and will be unique to [you/your child] & requires effort and care to plan. Visits may only be rescheduled in cases of adverse weather conditions, acute illness that would interfere with the planned evaluation, or otherwise at the discretion of the study investigators.

It is our hope that the care and evaluation [you/your child] will receive at [institution] will yield long awaited answers. Although [you/your child] may not initially receive a diagnosis, research into [your/your child's] condition will continue even after you return home.

Additionally, UDN evaluations should yield valuable information that medical researchers will use to: (1) help identify previously unrecognized rare diseases; (2) suggest new ways to treat and prevent common illnesses; and (3) determine promising options for continued medical research.

We look forward to meeting you [and your child] soon!

Sincerely,
The UDN team at [Institution]

If you have any questions, please contact [Travel Coordinator] at [phone number and email address].

Description: *Not Accept Letter to Healthcare provider*

Date:

Address:

Dear [health care provider]:

Your patient [name] [DOB] applied to participate in the Undiagnosed Diseases Network (UDN) research study. After a stringent review process, your patient was not accepted for participation in this study. As a research study with a defined set of resources, the UDN is not able to accept all who may benefit from this study.

[Insert one of these or another scenario below depending on whether you have additional suggestions for work up and whether or not you would be willing to reconsider the patient if the suggested work up comes back normal.]

[If no recommendations given and applicant has had extensive work-up]

Upon extensively reviewing [name of patient]'s records, we do not believe we can improve on the comprehensive work-up already done.

[If no recommendations given and site feels UDN would likely not be able to find a diagnosis]

Upon extensively reviewing [name of patient]'s records, we do not believe that our current research methods and technologies will identify a diagnosis.

[Required if recommendations given]

These comments are for consideration by [name of patient]'s medical team. The UDN does not coordinate medical care outside of the UDN evaluation, and all decisions about further workup are in the hands of the patient's local caregivers and their consultants.

[Required if patient would be reconsidered]

If a diagnosis remains elusive after further work up, we would be willing to reconsider [name of patient] for admission to the UDN. Once further work up is complete, [name of patient] can send an updated letter with the results of the work up to [contact information]. At that time, we will review the letter and previously submitted application.

Please note that decisions made by one site in the UDN are binding for the entire network. There is no process to request for another site to review an application after it is not accepted.

Thank you for consulting with the UDN on your patient. We appreciate your commitment to providing the best possible care for your patients in ways that help advance medical knowledge and discovery.

Sincerely,

[name]
Principal Investigator at [Institution]

If you have any questions, please contact [Site Coordinator] at [phone number and email address].

Cc: [participant]

Examples of recommendations that have been given for specific testing:

[If recommendations given]

Members of the Case Review Committee had a few thoughts for your consideration.

[Mitochondrial/Metabolic Recommendations] Pursuit of a mitochondrial and metabolic evaluation, including plasma and urinary amino acids, urinary organic acids, plasma lactate, pyruvate, carnitine (free and total), leukocyte CoQ, and acylcarnitine profile. For a complete metabolic and mitochondrial disease work up, you may consider contacting the Medical Genetics Laboratories at the Baylor College of Medicine: www.bcm.edu/geneticlabs/.

[Pitt-Hopkins syndrome] Testing for Pitt-Hopkins syndrome by looking at mutations or even a deletion of the TCF4 gene. For testing center sites, visit GeneTests at: <http://www.ncbi.nlm.nih.gov/sites/GeneTests/>.

[Congenital Disorders of Glycosylation] Congenital disorders of glycosylation (CDG) testing at the Mayo Medical Laboratories. Results are reported as the mono-oligosaccharide/di-oligosaccharide transferrin ratio, the a-oligosaccharide/di-oligosaccharide transferrin ratio, the tri-sialo/di-oligosaccharide transferrin ratio, the apolipoprotein CIII-1/apolipoprotein CIII-2 ratio and the apolipoprotein CIII-0/apolipoprotein CIII-2 ratio. For more information on how to send this test, visit: <http://www.mayomedicallaboratories.com/test-catalog/Overview/89891>

[PGM1 testing] PGM1 full gene sequencing, which is offered at multiple laboratories that can be identified at: <http://www.ncbi.nlm.nih.gov/sites/GeneTests/?db=GeneTests>.

[Lysosomal enzyme screening] Lysosomal enzyme screening. Information about this screening can also be found at the Emory Genetics Laboratory. Their website is: <http://genetics.emory.edu/egl/>.

Description: Welcome packet to patient

Welcome to the Undiagnosed Diseases Network

We want your visit to the UDN clinical site at [institution] to be as comfortable and productive as possible. We have created a package of information for you to review prior to your admission so you will know what to expect. The following are included in the package:

1. [institution] map and interior layout map of [institution]
2. Cafeterias [information]
3. Shuttle Service [information]
4. Airport transportation [information]
5. Security Procedures [information]
6. Patient Library [information]
7. Chapel [information]
8. Hospitality Services [information]
9. Gift Shops [information]

For further information, please visit the following websites:

<http://www>

Please be sure to bring the following with you:

- **List of medications and dosages in their original containers-** Please discuss any questions you may have regarding medications and/or equipment with your admitting Nurse Practitioner or Physician Assistant.
- **Any assistive devices that you use daily**, e.g., wheelchair, cane, walker, braces
- **Complete list of current physicians and their contact information**, i.e., addresses and phone numbers

A UDN staff member will contact you shortly to make travel arrangements and book local hotel accommodations. An additional member of the UDN team will contact you to review the admission process and ask additional questions about your medical history.

In the event you are unable to keep your scheduled visit, please contact your admitting Clinical Site Coordinator. You may be asked to re-schedule your visit.

Please feel free to contact our team if you have any questions or concerns. Generally, phone messages and e-mails are responded to promptly. We are looking forward to meeting you at [your institution].

[team contact information- names, emails, phone numbers]

Description: *Directions for remote blood draw*

DIRECTIONS: Remote Blood Draw

Date:

Address:

Dear [patient],

In order for your blood to be processed appropriately, please follow the directions below carefully. If possible, please arrange the blood draw through your health care provider.

Directions:

- 1) Draw [number] tubes of blood in the lavender topped tubes enclosed.
- 2) While talking to a UDN team member, review, sign, and date the consent form(s) enclosed and send the signed copy of the consent form(s) with your blood sample. **If we do not receive your signed consent form(s), we cannot process your blood.**
- 3) Ship the blood overnight (Monday-Thursday) to the UDN clinical site at [institution].
- 4) Call [number] or email [email] on the day that you ship the blood and provide the FedEx Tracking number.

Please contact us with any questions or concerns.

[name]

Principal Investigator at [Institution]

If you have any questions, please contact [Site Coordinator] at [phone number and email address].

Cc: [referring provider]



Your institutional logo
here

Description: *No diagnosis following evaluation*

Date:

Address:

Dear [patient]:

Thank you for your participation in the Undiagnosed Diseases Network (UDN) evaluation at [institution]. At this time, the clinical testing and evaluation phase is complete and has not yielded a definitive diagnosis. We will continue to pursue leads as they arise based upon ongoing research and new ideas that are generated among our expert consultants. We will keep the valuable information and biological samples collected during your visit in the hope that future research studies will be able to shed light on the medical problems that brought you to the UDN. If new prospects for investigation appear, we will contact you.

We very much appreciate your involvement in the UDN and your commitment to our joint goal of helping to advance medical knowledge, scientific discovery, and optimal care.

Sincerely,

[name]
Principal Investigator at [Institution]

If you have any questions, please contact [Site Coordinator] at [phone number and email address].

Cc: [referring provider]

APPENDIX 5: Wrap-up Template

Name:

DOB:

Dates of Visit:

Primary Clinician:

Attending Physician:

Presenting Symptoms/short summary of case:

Testing/Recommendations by System (as applicable):

GASTROENTEROLOGY

Consultant:

Testing/Results:

Recommendations:

NUTRITION

Consultant:

Testing/Results:

Recommendations:

NEUROLOGY

Consultant:

Testing/Results:

Recommendations:

PULMONOLOGY

Consultant:

Testing/Results:

Recommendations:

CARDIOLOGY

Consultant:

Testing/Results (EKG/ECHO):

Recommendations:

IMMUNOLOGY/INFECTIOUS DISEASE

Consultant:

Testing/Results:

Recommendations:

HEMATOLOGY

Consultant:

Testing/Results:

Recommendations:

ENDOCRINOLOGY

Consultant:

Testing/Results:

Recommendations:

DENTAL

Consultant:
Testing/Results:
Recommendations:

DERMATOLOGY

Consultant:
Testing/Results:
Recommendations:

OPHTHALMOLOGY

Consultant:
Testing/Results:
Recommendations:

AUDIOLOGY

Consultant:
Testing/Results:
Recommendations:

PHYSICAL MEDICINE & REHABILITATION

Consultant:
PT:
OT:
Speech:
Recommendations:

NEPHROLOGY

Consultant:
Testing/Results:
Recommendations:

RADIOLOGY

Test:
Results:
Test:
Results:

SPECIAL STUDIES

EMG/NCV:
Sleep Study:
Metabolic Cart:
Other:

OTHER

Consultant:
Testing/Results:
Recommendations:

SEND OUT AND PENDING TEST RESULTS

Test Name	Date Sent	Date Resulted Rev'd	Testing Lab	Result/Interp	Normal Range	Copies sent (date and person)
CSF studies						
CDT/N-glycan screen						
Urine Oligosaccharides						
WBC CoQ level						
Lysosomal Screen						
WBC Buffy Coat						
Urine Sulfocysteine						
MitoGEN						
POLG						
Muscle mtDNA Content						
Muscle ETC Enzymology & CoQ content						

PLEASE NOTE:

You will be contacted by the UDN for surveys to tell us about your experience. The first contact will be a week or so after you get home.

A list of patient resources, including support groups, can be found at:

<http://undiagnosed.hms.harvard.edu/resources/advocacy/>

APPENDIX 6: Participant Follow-up Surveys

7-14 days post visit survey: Interpersonal Processes of Care Survey: Short Form IPC-18 – UDN CC revised.

These questions are about your experience at _____/[CS] during your evaluation. When we ask “how often...?” we are referring to the many specialists you saw during your UDN visit. Please think about your entire UDN visit as you answer these questions.

	Never	Rarely	Sometimes	Usually	Always	N/A
1. How often did health care providers speak too fast or use words that were hard to understand?						
2. How often did health care providers listen carefully to what you had to say?						
3. How often did health care providers take your health concerns seriously?						
4. How often did health care providers explain your test results such as blood tests or X-rays?						
5. At the end of the visit, did the health care providers summarize the visit and results and answer your questions?						
6. How often were health care providers concerned about your feelings?						
7. How often did health care providers really respect you as a person?						
8. How often did health care providers treat you as an equal?						
9. How often did health care providers pay less attention to you because of your race or ethnicity?						

Open-ended questions:

10. How was your experience with the logistics of their UDN visit, including travel to and from the medical center, quality of their overnight accommodations, organization of the clinic visits/procedures?
11. What part/s of the UDN experience worked particularly well for you?
12. What part/s of the UDN experience did not work well?
13. What could we do to make the UDN experience better?
14. Did you feel the UDN visit was burdensome? If so, please explain.
15. Were changes to your medications suggested at the end of the UDN visit? If so, what were they and do you intend to follow the recommendations?

16. Were recommendations made for next steps (e.g., additional testing, referrals, etc.)? If so, what were they and do you intend to follow the recommendations?

6 months post-visit survey: Participant-specific clinical and research outcomes.

Please note:

1. There will be “yes”, “no”, “not applicable” and “refuse to answer” checkboxes for each question, in addition to text boxes.
2. “You” should be replaced by “your child”, “your spouse”, or “your family member” depending on who is answering the survey for the participant, i.e., the participant’s parent, spouse, or other family member, respectively.

Topic:	Question:	If yes:
<i>Diagnosis</i>	Did you receive a diagnosis at, or following, the UDN visit?	Please explain when you were diagnosed, and how this information was provided to you
<i>Follow-up</i>	Has follow-up with a health care provider occurred since the UDN visit?	Please describe the follow-up, including if the medical provider is at or in communication with the UDN site.
	Have you received results from tests that were pending at the time of the evaluation	Please describe who communicated to you and how those results were communicated.
<i>Treatment</i>	Was a new treatment started at the UDN visit?	Please describe the new treatment.
	Was a previous treatment discontinued at the UDN visit?	Please describe the discontinued treatment.
<i>Research</i>	Did the health care providers at the UDN site discuss research options at, or following, the visit?	Please describe the types of research discussed.
	Are you currently participating in research initiated by the UDN?	Please describe the research.
	Are you currently participating in research initiated outside of the UDN?	Please describe the research.
<i>Support</i>	Did the health care providers at the UDN site discuss support services (e.g., support groups, local and national resources, counseling) at, or following, the UDN visit?	Please describe the support services discussed.
	Are you participating in a support group (either in person or online)?	Please describe the support group.
<i>Continuing contact</i>	Have you been in contact with a member of the UDN since the time of the visit?	Please describe your communication with the UDN since the time of the visit.
	Do you have contact information for a team member, in case you would like to contact them later?	Please describe what type of contact information you have.
<i>Additional comments</i>	Is there anything that you would change about your UDN experience?	Please explain what you would change about your experience.

	Have you noticed any changes to yourself or your family members as a result of your UDN experience?	Please explain these changes.
	Are you having any issues with insurance billing for services from the UDN visit, such as uncovered expenses?	Please explain.

Yearly post-visit survey.

Please note that there will be “yes”, “no”, “not applicable” and “refuse to answer” checkboxes for each question, in addition to text boxes.

Topic:	Question:	If yes:
<i>Diagnosis</i>	If you did not have a diagnosis at the time of the last survey, have you received a diagnosis since the last survey?	Please explain how this information was provided to you.
<i>Follow-up</i>	Has follow-up with a health care provider occurred since the UDN visit?	Please describe the follow-up, including if the medical provider is at or in communication with the UDN site.
	Have you received the results from tests that were pending at the time of the last survey, including results from the genomics sequencing?	Please describe who communicated to you and how those results were communicated.
<i>Treatment</i>	Has a new treatment been started since the UDN visit?	Please describe the new treatment. What have been the effects of this treatment over the past year?
	Has a previous treatment been discontinued since the UDN visit?	Please describe the discontinued treatment.
<i>Research</i>	Have you discussed research options with the health care providers at the UDN site since the last survey?	Please describe the types of research discussed.
	Are you currently participating in research initiated by the UDN?	Please describe the research.
	Are you currently participating in research initiated outside of the UDN?	Please describe the research.
<i>Support</i>	Are you participating in a support group (either in person or online)?	Please describe the support group.
<i>Continuing contact</i>	Have you been in contact with a member of the UDN since the time of the visit?	Please describe your communication with the UDN since the time of the visit.
	Do you have contact information for a team member, in case you would like to contact them later?	Please describe what type of contact information you have.
<i>Additional comments</i>	Is there anything that you would change about your UDN experience?	Please explain what you would change about your experience.

	Have you noticed any changes to yourself or your family members since the last survey as a result of your UDN experience?	Please explain these changes.
	Are you having any issues with insurance billing for services from the UDN visit, such as uncovered expenses?	Please explain.

APPENDIX 7: Feature Request Form

Feature Requester Name(s):

Feature Requester Contact Information:

Name:

Institution:

Email:

Detailed Description of Feature:

Please provide a description of the feature and types of users that will interact with the feature and how they will access and use the feature. Describe the workflow from the perspective of each of these users. Screen shots of the Gateway where the new feature will exist are also preferable.

Importance to the UDN and Justification:

☐ Critical

☐ Major

☐ Minor

Justification:

APPENDIX 8: Research Concept Sheet

Thank you for your interest in collaborating with the UDN. Please complete the following application.

(To complete online, go to https://hms.az1.qualtrics.com/jfe/form/SV_3CyZOKOuiEvnCx7)

Type of submission:

- ☐ Research Project*
- ☐ Data Analysis*
- ☐ Manuscript
- ☐ Grant Application

*Distinction between research project and data analysis- research projects will involve the collection of new data while data analysis will involve new analyses of existing data.

Is this research project network wide?

- ☐ Yes
- ☐ No

Primary investigator contact information:

Name:

Institution:

Phone:

Email:

Are you a UDN Member?

- ☐ Yes
- ☐ No; Please list the name of your UDN contact:

Additional/Senior investigator(s) (specify institution):

Project title:

If the submission is a manuscript, please provide the following information:

Intended date (mm/dd/yyyy) and place of manuscript submission or meeting presentation:

Overall manuscript outline:

If the submission is a research project, data analysis, or grant application, please provide the following information:

Specific aims:

Funding plan:

Subject eligibility criteria:

Access to data and burden on subjects:

Consent required?

- ☐ Yes; Please describe:
☐ No

Methods:

Which (if any) UDN Working Groups are (or will be) involved? Include primary Working Group first.

Data management and statistical support:

- ☐ Required
☐ Not required

If required, please provide contact information for your local statistician:

Name:
Phone:
Email:

Biospecimens required:

Other needs:

Timeline:

Does this research project involve administering a survey to participants?

- ☐ Yes; Please attach a copy of the instrument to this application.
☐ No

If yes, is this research project under the UDN NIH General Medicine 1 Central IRB (falls under the UDN consent form) or will there be a separate IRB protocol and consent form through a local IRB?

- ☐ Will be under UDN Central IRB
☐ Will be under a separate local IRB

If the survey will be under the UDN Central IRB, the UDN Survey Committee will use the information requested below to make a recommendation as to whether or not this survey should be approved by the UDN. The goal is to ensure that approved surveys are consistent with the purpose of the UDN and that patients are not overburdened.

A. Survey Details

1. Survey Instrument
2. Construct measured
3. Description of validation of survey instrument
4. Rationale for using this particular survey instrument (as opposed to other instruments that measure the construct)

5. Translation of measure into other languages

B. Survey Population

1. UDN sites included in the study
2. Participant inclusion/exclusion criteria
3. Sample size

C. Study Logistics

1. Amount of time to administer
2. Method of administration (online [Qualtrics, other?], phone, mail, etc.)
3. Individuals administering survey (study staff, self, etc.)
4. # times survey is administered and when
5. Timeline for study: When will survey commence, for how long will you continue the survey, when will it end, when will data be analyzed

D. Please provide a plan for follow up if a problem or concern is identified on responses to the measure.

If the survey will be under a separate local IRB, the UDN Survey Committee will not be involved in determining whether or not this survey should be approved. However, the committee is requesting the following information in order obtain a complete picture of the surveys being administered to UDN patients at various sites.

A. Survey Details

1. Survey Instrument
2. Construct measured
3. Rationale for using this particular survey instrument (as opposed to a different instrument that measures the same construct)

B. Survey Population

1. UDN sites included in the study
2. Participant inclusion/exclusion criteria
3. Sample size

C. Study Logistics

1. Amount of time to administer
2. Method of administration (online, phone, mail, etc.)
3. # times survey is administered and when
4. Timeline for study: When will survey commence, for how long will you continue the survey, when will it end, when will data be analyzed

D. Please provide a plan for follow up if a problem or concern is identified on responses to the measure.

E. Data Sharing – who will have access to the data?

APPENDIX 9: Annual Survey Review Worksheet

Directions: Please provide the following information for the Survey Committee annual review. Please email the completed form to the chair of the Survey Committee: Ingrid A. Holm, MD, MPH- Ingrid.holm@childrens.harvard.edu

Due Date:

Date:

Date of initial IRB approval:

Name of study:

Name of survey instrument/s:

Names of UDN members/group responsible for conducting survey research:

Main contact person:

 Name:

 Site:

 Email address:

Summary of survey progress (*response should be no more than 2 pages*)

1. Survey implementation summary (include any changes to the survey or its implementation):
2. Number of UDN participants who qualified for the survey:
3. Number of UDN participants who participated/completed the survey:
4. Administration time:
5. The nature and amount of missing data:
6. Preliminary results (if interim analyses are explicitly allowed as part of the study design):

7. Impact on clinical care, if appropriate:
8. Describe any findings that impact the effectiveness of the survey or its implementation:

9. Any new knowledge gained based on the results to date:

APPENDIX 10: PEER Charter and Application

Participant Engagement and Empowerment Resource (PEER) Charter

PEER Mission Statement: The purpose of the UDN PEER is to support participants and family members in part by creating and sharing resources, and to provide the participant and family perspective on UDN research goals and participant experience. The PEER provides a “post-UDN visit voice”. Their goal is to advocate for participants, improve participant experience, “get the word out” about the UDN, and facilitate interactions between participants and the UDN.

Responsibilities of PEER Members: PEER members are expected to participate actively in the PEER and to respect each other’s views and opinions. Each team member is responsible for bringing and sharing experiences, and is expected to volunteer for tasks, be open, and be willing to talk.

PEER Membership: UDN Participant/Parent/Guardian

New member recruitment:

- Interest in joining the PEER is assessed on an ongoing basis by the Coordinating Center (CC) on the Transitions of Care calls.
- The CC sends interested members an application.
- By November 1 of each year, the PEER will review their membership status for openings, turnover of participants, need for new members, etc.
- After November 1 when the PEER has decided on how many new members are needed, applications undergo an initial review by the CC and the site coordinator at the UDN participant’s site.
- Applications passing the initial review are reviewed by the PEER, which votes on new member(s) and 1-2 back up choices if the first choice(s) decline. Each PEER member has one vote. The PEER will consider diversity (see below) and applications will be ranked in preference by the PEER based on the following attributes:
 - Initial application review feedback by CC and site coordinator
 - Application response content and length
 - Diversity of applicant in relation to current PEER members
- The CC contacts the invited members and back-up invited members if applicable.
- Once the new members are confirmed, the CC will contact the rest of the applicants to let them know they were not chosen but will be kept in the pool for the next year if desired.

PEER term: 1 year, may be renewed annually

Number of PEER participant/parent/guardian members: up to 11

PEER members who are inactive: Members who miss 2 consecutive meetings and/or we are not able to contact will be removed from the PEER.

Termination of membership: CC reserves the right to terminate membership of any member who does not act in accordance with the charter.

Diversity of members: The PEER prioritizes diversity in representation and will consider diversity in the acceptance process based on the following criteria:

1. Major criteria
 - a. UDN clinical sites (location of clinical evaluation)
 - b. Member type (Participant vs parent/guardian)
2. Minor criteria
 - a. Geographic location
 - b. Gender, race, ethnicity, urban/rural

PEER Membership: *Ex-officio* Members

Ex-officio members will include:

- 1 CC representative
- 2 site representatives from Site Operations Working Group and/or Genetic Counseling and Testing Working Group

Ex-officio members will be selected by the UDN Coordinating Center and UDN leadership and be rotated on a yearly basis.

Ex-officio member roles:

- *Ex-officio* members will participate on all calls and meetings.
- *Ex-officio* members will have one vote each on all votes except election of PEER leaders.

PEER Leadership:

- The PEER will nominate, agree, and vote on 1-2 co-chairs every year.
 - Co-chairs will be voted on during the annual in-person meeting.
 - Co-chair term: 1 year, January 1 – December 31; may be renewed annually.
 - Tasks of the co-chair(s): Engage the members, lead discussions, bring forward ideas, represent the PEER as needed.
- The PEER will nominate, agree, and vote on 1 secretary every six months.
 - Secretary will be voted on during conference calls.
 - Secretary term: 6 months; may be renewed every 6 months.
 - Tasks of the secretary: draft meeting agendas, take meeting minutes, circulate approved meeting minutes.
- The role of the UDN CC is to facilitate and provide the infrastructure to support the leader(s) and the PEER, and to serve as a liaison to the UDN Steering Committee

The Relationship Between the PEER and the UDN Steering Committee:

- The PEER will interface between the UDN Steering Committee and the UDN participants. The PEER will develop an annual report on activities to be shared with the Steering Committee. The UDN Steering Committee will send feedback on the annual report to the CC representative *ex-officio* member to be shared with the PEER. The

Coordinating Center will keep the UDN Steering Committee apprised of the PEER activities.

- Any concerns, comments, or questions between annual reports will be reported via the same mechanism.
- The PEER will respond to feedback from the UDN Coordinating Center within one month.
- Minutes from PEER meetings will be shared with the UDN Coordinating Center.

Conference Calls and Meeting:

1. Conference calls every 3 months (4 times a year) and ad hoc as needed.
2. Annual in-person meeting – cost is covered for up to 11 PEER members.
3. The agenda for meetings will be developed by the PEER with input from the UDN CC.
4. The PEER will set priorities with input from the UDN CC.
5. All meetings' minutes will be kept confidential within the UDN.
6. The PEER will create groups to work on specific projects as needed.

Application Form: UDN Participant Engagement and Empowerment Resource (PEER)

Overview of the UDN Participant Engagement and Empowerment Resource (PEER)

Purpose: The purpose of the UDN Participant Engagement and Empowerment Resource (PEER) is to provide the participant and family perspective on UDN research goals and participant experience. The PEER will engage with UDN investigators in the development and assessment of participant-oriented materials and identify best practices for receiving participant input in research.

Activities: The PEER will be responsible for its structure and activities. Activities may include, but are not limited to:

- Providing input regarding various research questions, eligibility criteria, and recruitment and informed consent processes
- Identifying unmet participant needs
- Contributing perspectives on risk/benefits of research project
- Connecting families with one another and to support groups
- Collecting participant and family experiences with the UDN from participants
- Providing support for families when they are visiting a site far from home
- Being a resource for families who have questions or concerns
- Developing educational materials
- Organizing participant conferences
- Leading awareness efforts

Membership: The PEER includes participants and family members. Members of the PEER must be willing to engage in thoughtful conversation about the positive and negative aspects of the research process and respect the perspectives of others. There will be adult, adolescent, and pediatric participant and family member representation.

Terms: Terms are one year in duration and are renewable on a yearly basis.

Meetings: Conference calls are expected to be held quarterly. In-person meetings are expected to be held annually. Travel expenses will be covered for the in-person meeting and the Steering Committee meeting as needed.

Acceptance into the PEER: Factors taken into consideration in the acceptance process include the answers on the application, diversity of sites represented on the PEER, and diversity of UDN participant age (PEER members who are an adult UDN participant or the parent of a child participant).

The UDN PEER is an equal opportunity organization and does not discriminate on based on any criteria including gender, race, ethnicity, or age.

Application Form:

Thank you for your interest in joining the UDN Participant Engagement and Empowerment Resource (PEER). If you have questions about this application, please contact the UDN Coordinating Center at 1-844-746-4836 or UDNPEER@hms.harvard.edu. Completed applications can be submitted to UDNPEER@hms.harvard.edu.

Application Deadline: November 1

Contact Information:

Last Name: First Name: UDN ID:
Street Address:
City: State: Zip Code:
Phone Number:
E-mail Address:

I am a:

- ☐ UDN participant
- ☐ Family member of UDN participant
- ☐ Other, please specify:

Areas of Interest:

Please describe in 50-100 words why you want to join the PEER:

Please indicate areas that you would be interested in (check all that apply):

- ☐ Providing input regarding various research questions, eligibility criteria, and recruitment and informed consent processes
- ☐ Identifying unmet participant needs;
- ☐ Contributing perspectives on risk/benefits of research project;
- ☐ Connecting families with one another and to support groups;
- ☐ Collecting participant and family experiences with the UDN from participants;
- ☐ Providing support for families when they are visiting a site far from home;

- ☐ Being a resource for families who have questions or concerns;
- ☐ Developing educational materials;
- ☐ Organizing participant conferences;
- ☐ Leading awareness efforts.

Summary of UDN Experience:

UDN Clinical Site:

Name of Primary UDN Clinical Site Contact:

Month of UDN Evaluation (MM/YYYY):

Please describe your overall experience with the UDN, including what went well and what can be improved:

Based on your experience with the UDN, please describe in 50-100 words what **went well**:

Based on your experience with the UDN, please describe in 50-100 words what you feel **could be improved**:

Is there anything that you feel is important for us to know about you? (limit to 50-100 words):

Availability:

Please list times when you are able to attend meetings:

- ☐ Daytime:
- ☐ Evening:
- ☐ Weekend:

APPENDIX 11: Standard Operating Procedures for Not Accepted Applicants

This Standard Operating Procedure (SOP) document was developed in November 2016 as a guideline for UDN investigators interacting with applicants not accepted or enrolled into the UDN study. Please note that this SOP is not intended to override institutional policy, but rather provide additional guidance for UDN investigators.

Stages of enrollment

Until an applicant has signed a consent form and enrolled in the UDN study, the term “accepted” should not be utilized in any communications with the applicant. Removing the use of the term “accepted” will help with confusion amongst applicants if any unforeseen reason prevents an individual from enrolling in the study between the time of application review by the CS and consent. This will lead to stages of enrollment before an applicant becomes a participant in the UDN study.

- Application Stage – from time of first inquiry or application start in the UDN Gateway through submission of an application to the UDN
- Review Stage – from submission of an application through medical records review and determination by the CS that an applicant should be “accepted” and referred to the consent stage
- Consent Stage – from decision of the CS to “accept” through consent of the applicant to participate in the UDN including second level review by the Case Review Committee and any other continuing review by the CS during the consent process
- Enrolled – participants in the UDN that are fully-consented to the study

Applicants that have completed the review stage may be informed that their application has completed the first level of review. Applicants should also be informed that they are not enrolled in the UDN study until consent is completed. These stages of enrollment will also be outlined on the UDN webpage FAQs.

Not accepted applicants

Applications that are not accepted during the review stage require the following information to be entered in the UDN Gateway:

- Applicant Review Form, Case Decision field – provides the reason for application to be not accepted
 - Not Accepted with Recommendations - Specific Testing
 - Not Accepted with Recommendations - Seek Expert Care
 - Not Accepted - Diagnosis Identified
 - Not Accepted - UDN would likely not be able to find a diagnosis
 - Not Accepted - Insufficient records made available to UDN site
- Not Accepted Letter – should be uploaded into the Gateway when sent to the applicant and referring provider

CC helpdesk communications

The applicant helpdesk email and phone number is staffed by personnel at the CC. When the helpdesk receives an applicant inquiry that rises above the level of general questions or

concerns, the CS involved is notified. Such inquiries will also be brought to the attention of the Executive Committee and CIRB. If an applicant expresses intent to contact Congress or any portion of the NIH directly, the Program Director will also be notified. A general log of applicant inquiries is kept at the CC.

Media inquiries

The CC will not comment on any applications to the media without the permission of the CS to which the participant has been assigned. All media inquiries related to specific applicants and participants will be referred to the CSs and logged by the CC. If a site receives a media inquiry directly, contact Kimberly LeBlanc who will log the inquiry and notify the Site Operations group. An example of language developed for media inquiries related to not accepted applicants is provided below:

“The Undiagnosed Diseases Network is not able to see all who apply. Many of our applicants have come to us as part of a long diagnostic odyssey in search of answers. With that in mind, we have to make difficult, often agonizing, decisions about whom to accept.”

Congressional inquiries

If a congressional communication related to an applicant or participant is received, the UDN Program Director should be notified as soon as possible so that a coordinated response can be initiated. Most congressional offices will reach out to the NIH directly as the federal agency involved; however, if a request for information is received by a CS or the CC, general guidelines on replies are detailed below.

Timeline for response: Technically, there is no timeline to respond to a letter from a personal office, but a response should be made as soon as possible. For an email inquiry, a response email should be sent immediately acknowledging receipt and noting that the requested information will be provided as soon as possible.

Example email language:

Dear XXX,

Thank you for your email. We are writing to acknowledge that we have received your letter and are working to pull together all of the information needed in order to address your questions. We will be in touch again as soon as we are able to provide an informed response.

*Sincerely,
XXX*

If the request was received via email, an identical postal mail can be sent if desired. If the request was received over the phone, a call to acknowledge receipt with similar information to the draft email above is appropriate.

Individuals to be contacted: The CS Principal Investigator, CC, and UDN Program Director should be contacted regarding any communications with a congressional office.

Official response: The NIH will notify the CS or CC when an official response can be distributed. The language below should only be sent with approval from the NIH, but is included as a reference.

Example reply language:

Dear XXX (the individual who contacted you from each congressional office),

Thank you for reaching out to us regarding XXX. The Undiagnosed Diseases Network (UDN) is a research study funded by the National Institutes of Health (NIH). It aims to improve the diagnosis and care of patients with undiagnosed diseases. Its goals are two-fold—to come up with a diagnosis for each participant and to learn more about rare and previously undiagnosed diseases. While one goal of the UDN is to successfully diagnose each participant in the study, it is important to note the current programmatic rate of diagnosis is under one-third of patients. Furthermore, the UDN does not provide treatment for participants; this responsibility rests with the participant and his or her health care provider.

As a research study with a defined set of resources, the UDN is not able to accommodate all applicants who want to be part of the study. Researchers at the XXX UDN site determined XXX was [insert reason for not acceptance here]. It is important to note that decisions made by one site in the UDN are binding for the entire network; and the UDN does not accept appeals related to application or enrollment decisions.

Please feel free to contact us if you have any further questions regarding this matter.

*Sincerely,
XXX*

Identification of a UDN participant

As general practice, no identifiable information should be shared with individuals not on the IRB protocol unless they have been directly contacted by an applicant or participant. If identifiable information needs to be shared with individuals not on the IRB protocol, sites should contact Kimberly LeBlanc at the CC.

Appeal process

The UDN will not accept appeals related to application or enrollment decisions. Language that there is no appeal process and that the UDN decision to not accept is binding should be communicated to applicants during the application process and in the FAQs online. Questionable cases should be sent to the CRC for review and will be prioritized for discussion.

APPENDIX 12: Diagnosis Coding Tool v1.0

Patient UDN ID
Diagnosis Name
Date Diagnosis Entered
Diagnosis Entered By
Phenotype MIM Number
Gene Name
Gene MIM Number
ICD Number
Additional Diagnosis Notes

Category 1: Certainty

The overall certainty of the diagnosis.

- ☐ Certain
- ☐ Highly Likely
- ☐ Tentative
- ☐ Low (still on differential diagnosis, but lacking clear evidence)

More information:

Category 2: Chief Method Used to Achieve Diagnosis

The single most important piece of evidence supporting the diagnosis. ☐

- ☐ Genome-scale sequencing including genome sequencing and exome sequencing (select all that apply)
 - ☐ Reanalysis of prior clinical exome data
 - ☐ Reanalysis of prior research exome data
 - ☐ Reanalysis of prior clinical genome data
 - ☐ Reanalysis of prior research genome data
 - ☐ Clinical site analysis of Baylor exome data
 - ☐ Clinical site analysis of HudsonAlpha genome data
 - ☐ Baylor – pathogenic
 - ☐ Baylor – likely pathogenic
 - ☐ Baylor – VUS
 - ☐ Baylor – research finding
 - ☐ HudsonAlpha – pathogenic
 - ☐ HudsonAlpha – likely pathogenic
 - ☐ HudsonAlpha – VUS
 - ☐ HudsonAlpha – research finding
 - ☐ Known prior to enrollment in UDN
 - ☐ Other
- ☐ Non-sequencing, genome-wide diagnostic assay (for example, SNP array, oligo array or karyotype)

- ☐ Microarray
- ☐ Karyotype
- ☐ Known prior to enrollment in UDN
- ☐ Other
- ☐ Directed clinical testing based on phenotype (may be molecular, including gene panels; biochemical; radiologic; or, other test type)
 - ☐ Panel testing
 - ☐ Single gene testing
 - ☐ Del/dup testing
 - ☐ Known prior to enrollment in UDN
 - ☐ Other
- ☐ Diagnosis made primarily on clinical grounds, including aggregate assessment of non-specific test results
- ☐ Other – please explain below

More information:

Category 3: Mechanistic Characterization

The degree to which the underlying mechanisms of disease pathology and causation are established.

- ☐ Biologically well-characterized disorder, e.g., high-specificity testing available; or, the gene, pathway, and/or cell biology is understood.
- ☐ Descriptive (no definitive testing available, specific mechanism unknown).
- ☐ Other – please explain below

More information:

Category 4: Timing and Source of Diagnosis

The source and timing of the diagnosis relative to evaluation by the UDN. ☐

- ☐ Diagnosis made before UDN application
- ☐ Diagnosis made by non-UDN entity after application but before UDN evaluation
- ☐ Diagnosis made by UDN after application but before evaluation
- ☐ Diagnosis made during UDN in-person evaluation
- ☐ Diagnosis made after UDN in-person evaluation by UDN
- ☐ Diagnosis made after UDN in-person evaluation by non-UDN entity
- ☐ Other, including misdiagnosis – please explain below

More information:

Category 5: Degree to Which This Diagnosis Explains Major Components of Phenotype

- ☐ Explains all major features of illness, including asserted phenotype expansion
- ☐ Explains some major features of illness, including asserted phenotype expansion
- ☐ Diagnosis does not explain any major features of illness
- ☐ Other – please explain below

More information:

Category 6: Consequence of Diagnosis

Was the diagnosis important in the future management of the study participant or family's overall health?

- ☐ The diagnosis had a major role in the subsequent management of the participant's and/or family's overall health
- ☐ The diagnosis had some role in the subsequent management of the participant's and/or family's overall health
- ☐ The diagnosis had no significant role in the subsequent management of the participant's and/or family's overall health
- ☐ Other – please explain below

More information:

Is there a readily available treatment?

- ☐ Yes
- ☐ No

If yes, please provide a brief description of the treatment:

More information:

Narrative: