

Undiagnosed Diseases Network Manual of Operations

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Table of Acronyms

Acronym	Definition
ACMG	American College of Medical Genetics and Genomics
BCM	Baylor College of Medicine
bp	Base pairs
BWA	Burrows Wheeler Aligner
CAP	College of American Pathologists
CC	Undiagnosed Diseases Network Coordinating Center
CIRB	Central Institutional Review Board
CLIA	Clinical Laboratory Improvement Amendments
COI	Conflict of Interest
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
HGNC	HUGO Gene Nomenclature Committee
HIPAA	Health Insurance Portability and Accountability Act
HITECH	Health Information Technology for Economic and Clinical Health Act
HPO	Human Phenotype Ontology
HRPP	Human Research Protections Program
HUGO	Human Genome Organisation
ICD	International Classification of Diseases
ICF	Informed Consent and Assent Forms
IRB	Institutional Review Board
LIMS	Laboratory Information Management System
MC	Undiagnosed Diseases Network Metabolomics Core
MMA	Mercy Medical Angels
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy

NCBI	National Center for Biotechnology Information
NCV	Nerve Conduction Velocity
NGS	Next Generation Sequencing
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	Patient Care Coordinator
PCP	Primary Care Physician
PCR	Polymerase chain reaction
PE	Paired-end
PEG	UDN Participant Engagement Group
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
SNOMED	Systematized Nomenclature of Medicine
SNP	Single Nucleotide Polymorphism
SRC	Scientific Review Committee
TAT	Turnaround time
UDN	Undiagnosed Diseases Network
UDNCB	Undiagnosed Diseases Network Central Biorepository
UDN NIH PO	Undiagnosed Diseases Network National Institutes of Health Program Official
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 7 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, Boston, MA - Isaac Kohane, MD, PhD; Alexa McCray, PhD; and Rachel Ramoni, DMD, ScD

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

- Baylor College of Medicine, Houston, TX - Brendan Lee, MD, PhD
- Duke University (with Columbia University), 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 and Cynthia Tift, MD, PhD
- Stanford Medicine, Palo Alto, CA - Euan Ashley, MD; Jonathan Bernstein, MD; and Paul Fisher, MD
- University of California Los Angeles, Los Angeles, CA - Katrina Dipple MD, PhD; Stanley Nelson, MD; Christina Palmer, PhD; and Eric Vilain, MD, PhD
- Vanderbilt University Medical Center, Nashville, TN – Rizwan Hamid, MD, PhD, John Newman, MD, PhD, and John Phillips 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 - Howard Jacob, 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

The MC is located at the following institutions, with the following PIs:

- Battelle 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 clinical sites 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:

- 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.
- 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.
- 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.
- 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.

- Serving on subcommittees of the Steering Committee, and Working Groups as appropriate.
- Assisting awardees in the development, if needed, of policies for dealing with situations that require coordinated action.
- Providing advice in the management and technical performance of the award.
- Assisting in promoting the availability of the data and related resources developed in the course of this program to the scientific community at large.
- Participating in data analyses, interpretations, and, where warranted, co-authorship of the publication of results of studies conducted through the program.
- 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 Network operation 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:

- Assist in refining a common approach to patients with undiagnosed diseases.
- Work collaboratively with other UDN investigators to provide for secure, accurate and timely data submission.
- Participate in presenting and publishing new processes and substantive findings.
- Participate in the governance of the UDN as a member of the Steering Committee.
- 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 Clinical Site Awardees agree to work collaboratively to:

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

Additionally, the CC Awardee agrees to work collaboratively to:

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

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

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

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

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

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

- 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), 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:

- 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.
- The UDN Steering Committee will be the operational group through which the NIH UDN Working Group interacts with the UDN
- The Steering Committee will have at least monthly conference calls.
- The Steering Committee will meet in person quarterly during the first year and three times per year or as needed subsequently.
- The minutes for all Steering Committee discussion will be documented and posted on a CC website (viewable to Steering Committee members).
- 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.
- The Steering Committee may add additional members, and other government staff may attend the Steering Committee meetings as desired.

STEERING COMMITTEE

Co-Chairs: Euan Ashley, MD and William Gahl, MD, PhD

Members:

Clinical Site PIs (1 vote for each CS):

1. Baylor College of Medicine - Brendan Lee
2. Duke University (w/ Columbia) - David Goldstein and Vandana Shashi (contact)
3. Harvard Teaching Hospitals - Joseph Loscalzo
4. NIH - William Gahl (contact), Cynthia Tifft, and David Adams
5. Stanford Medicine - Euan Ashley (contact), Jon Bernstein, and Paul Fisher
6. UCLA - Katrina Dipple, Stanley Nelson, Christina Palmer, and Eric Vilain (contact)

7. Vanderbilt University Medical Center – Rizwan Hamid, John Newman, and John Phillips III (contact)

Coordinating Center PIs (1 vote):

8. Harvard Medical School - Isaac Kohane, Alexa McCray, and Rachel Ramoni (contact)

Core PIs (1 vote for each core):

9. Baylor College of Medicine - Christine Eng
10. HudsonAlpha (w/ Illumina) - Howard Jacob
11. Baylor College of Medicine (w/ University of Oregon) - Hugo Bellen
12. Battelle Pacific Northwest Laboratories (w/ Oregon Health & Science University) - David Koeller and Thomas Metz (contact)
13. Vanderbilt University Medical Center - Joy Cogan (contact) and John Phillips III

NIH IC Project Scientists (1 collective vote):

14. Anastasia Wise

D. Election of UDN Steering Committee Co-Chairs

Guideline: The position of Chairperson of the Steering Committee of the UDN will be filled by Co-chairs who serve overlapping terms. The first 2 Co-chairs will be selected by the NIH UDN Working Group. Subsequent Co-chairs will be selected by a vote of the UDN Steering Committee.

Principles:

1. The term of the position of Chair will be 1-2 years in duration.
2. The individual holding the position of Chair must be a current member of the UDN Steering Committee.
3. The Chair must be either the Principal Investigator of one of the CS or a Core Laboratory or the CC.

E. UDN Executive Committee

The UDN Executive Committee consists of the 2 Co-chairs of the UDN, the PIs of the Coordinating Center, 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 UDN Steering Committee will have 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:

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

Active Committees and Working Groups:

Billing Working Group

Chairs: Katrina Dipple (UCLA), Meredith Hanna (Harvard), and Vandana Shashi (Duke)

Biosamples and Biorepository Working Group

Chairs: Jordan Orange (BCM CS), Ed Silverman (Harvard), and Joy Cogan (Vanderbilt)

Case Review Committee

Chairs: Rotate every 3 months (see Appendix 4)

Clinical Protocols Working Group

Chairs: Cyndi Tifft (NIH UDP) and Katrina Dipple (UCLA)

Subcommittees: Site Operations, Utility & Utilization (U3)

Genetic Counseling & Testing Working Group

Chairs: Ingrid Holm (CC & Harvard CS), Allyn McConkie-Rosell (Duke), and Christina Palmer (UCLA)

Metabolomics Working Group

Chairs: Thomas Metz (PNNL), David Koeller (OHSU), and Gerard Berry (Harvard)

Model Organisms Working Group

Chairs: Hugo Bellen (BCM MOSC) and May Christine Malicdan (UDP)

Publications and Research Committee

Chairs: Rizwan Hamid (Vanderbilt) and Vandana Shashi (Duke)

Subcommittee: Survey Committee

Sequencing Working Group

Chairs: Christine Eng (BCM Seq) and Howard Jacob (HudsonAlpha)

G. Implementing and Revising the UDN Manual of Operations

- Working groups have been established to develop and maintain chapters for the UDN Manual of Operations.
- Chapters of the Manual of Operations are ratified by the UDN Steering Committee.
- 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.
- If a working group cannot resolve an implementation decision internally, the UDN Steering Committee will be consulted.
- Working groups will 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.
- All working groups will make their agendas and minutes available to other working groups.
- 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 UDN Steering Committee for ratification.
- Groups that would like to recommend a change to the UDN network-wide IRB protocol or consents should recommend the change to the UDN Executive Committee, who will determine the need for a Steering Committee vote.
- Ratified changes to the Manual of Operations will be submitted by the UDN working group recommending the change to the CC for the Manual of Operations to be updated.

II. UDN International Collaborative Clinical Sites

The UDN is open to International Collaborative Clinical Sites that agree to the criteria for participation described below.

Criteria for Participation in the UDN are:

1. Each organization will inform the UDN NIH Program Official and the UDN Steering Committee about their group's plans for a UDN International Collaborative Clinical Site.
2. Each organization will specify the sequencing, laboratory, and clinical evaluation plans for their proposed International Collaborative Clinical Site.
3. Each organization is expected to contribute significantly to the project, bringing their particular expertise to bear on accomplishing the goals of the UDN in a timely manner. Participation in the UDN should consist of more than submission of data to the UDN and should include substantial intellectual contributions to the Network.
4. Each organization will adhere to UDN publications policies, guidelines and agreements.
5. Each organization will have a data-sharing plan.
6. Each organization will take part in group activities, including attending some UDN Steering Committee meetings and working group calls and contributing to the products of these groups.
7. Each organization will agree that they will not disclose confidential information obtained from other members of the UDN.
8. Additional criteria may be added upon recommendations of the UDN Steering Committee, External Scientific Advisors, and the NIH UDN Working Group.

Affiliate Membership application process:

An organization that is interested in applying to be a UDN International Collaborative Clinical Site should complete the UDN International Collaborative Clinical Site Application form and return it to the CC for appropriate dissemination. Items that should be included in the application and that will be used to evaluate acceptance into the Network are:

1. A concise plan, including DNA sequencing, other laboratory, and clinical evaluation plans proposed and a rationale for how the proposed International Collaborative Clinical Site addresses the goals of the UDN. (Maximum length 3 pages, font 11, single spacing)
2. Evidence that the proposed International Collaborative Clinical Site's research has received appropriate Institutional Review Board (IRB) approvals and is consistent with participants' informed consent.
3. Evidence of funding to conduct the proposed research as an International Collaborative Clinical Site.
4. An agreement to abide by the UDN data sharing policies, along with all relevant UDN Policies and Procedures.
5. An agreement to participate in UDN activities, including attending some UDN Steering Committee meetings and working group calls and contributing to the products of these groups.

Applications will be reviewed by the UDN Steering Committee, UDN program staff, and the UDN External Scientific Advisors to determine whether the proposed International Collaborative Clinical Site will be accepted into the Network.

Evaluation for International Collaborative Clinical Site applications will include a determination that:

- The clinical evaluation plan is appropriate for the UDN;
- The applicant has sequence data available or funding available to sequence their patients; and
- The applicant has the requisite expertise to participate in the Network.

The participation of International Collaborative Clinical Sites will be reviewed yearly by the UDN Steering Committee, UDN program staff, and the External Scientific Advisors. A limited number of International Collaborative Clinical Sites may be approved and acceptance may be limited to one-year after which an assessment will be conducted for continuation. The UDN will begin accepting International Collaborative Clinical Site applications in July 2016.

III. Clinical Protocol

A. Introduction

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

I. Background of the UDP (see Appendices 1-3 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.

II. UDN Clinical Protocols Working Group

The UDN Clinical Protocols Working Group developed this Clinical Protocol as part of the Manual of Operations and with input from the UDN Steering Committee will continue to refine it. The Clinical Protocols Working Group currently consists of the members listed below. Should there be a need to vote on matters within the working group, each CS, the NIH Program, and the CC, will cast a single vote, for a total of 9 votes. Co-chairs of the Working Group are Cyndi Tiff (NIH UDP) and Katrina Dipple (UCLA).

- CC: Ingrid Holm, MD, MPH (primary representative); Rachel Ramoni, DMD, ScD; Kim Splinter, MS
- CSs:
 - Baylor College of Medicine: Carlos Bacino, MD (primary representative); Ashok Balasubramanyam, MD; Paolo Moretti, MD
 - Duke Medical Center: David Goldstein, PhD; Vandana Shashi, MD, MBBS (primary representative); Young-Hui Jiang, MD, PhD; Kelly Schoch, MS; Rebecca Spillman, MS
 - Harvard Medical School (Brigham and Women’s Hospital, Boston Children’s Hospital, Massachusetts General Hospital): David Sweetser, MD, PhD (primary representative); Ed Silverman, MD, PhD; Richard Maas, MD, PhD; Joan Stoler, MD; Calum MacRae, MD, PhD; Meredith Hanna; Wen-Hann Tan, MD;
 - NIH UDP: Cyndi Tiff, MD, PhD (primary representative); David Adams, MD, PhD; Bill Gahl, MD, PhD; Camillo Toro, MD

- Stanford Medical Center: Jon Bernstein, MD, PhD (primary representative); Matthew Wheeler, MD, PhD
- University of California, Los Angeles Medical Center (UCLA): Katrina Dipple, MD, PhD (primary representative); Stan Nelson, MD; Christina Palmer, PhD; Eric Vilain, MD, PhD
- Vanderbilt Medical Center: John Newman, MD (primary representative); John Phillips III, MD; Rizwan Hamid, MD, PhD; Amy Robertson, NP

B. Detailed UDN Clinical Protocol

Study Design

In this study, individuals with undiagnosed diseases, and their family members when applicable, will be investigated. Applicants will apply to the UDN through a secure website managed by the CC, called the Gateway, and will be assigned to a CS based on an assignment algorithm. The CS will collect and review the applicant's medical records and will make a recommendation to accept or reject the applicant. Final approval to accept will be given by the UDN Case Review Committee (see Appendix 4: Case Review Committee of the UDN). Accepted applicants will typically be 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 will 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 Appendix 5: ClinicalTrials.gov Record for a publicly available summary of the protocol.)

I. Triaging and accepting applicants into the UDN

1. Types of referrals:
 - a. Applicant initiated: applicants (or their legal guardians) may learn about the UDN from a variety of sources, including the UDN website, publicity, or from another patient.
 - b. Healthcare provider initiated:
 - i. Healthcare providers not associated with the UDN may learn of the UDN from sources including the UDN website, publicity, colleagues, or medical conferences or publications.
 - ii. Healthcare providers from CSs may refer their own patients for evaluation.
2. UDN application:
 - a. Individuals (or their legal guardians) will register and apply to the UDN through the Gateway managed by the CC.
 - i. The website will include:
 1. Information about the UDN and the application process
 2. A link to the Gateway
 - ii. The Gateway will include:
 1. A disclosure statement – in order to be considered for participation in the UDN, individuals (or their legal guardians) will be required to either electronically sign or verbally agree to a disclosure statement allowing the UDN to store the applicant data that will be 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 will be used to facilitate the verbal consent process.
- iii. Once the applicant (or legal guardian) provides consent, either the applicant (or legal guardian), their referring provider, or a CC representative will enter the following data into the Gateway, which will be stored in the UDN database:
 1. Applicant's name, date of birth, gender, self-described race and ethnicity (for children <18 years the names of their parents will be required).
 2. Applicant's mailing address, contact information (email address, phone number). For children, the addresses and contact information for both parents will be 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 (this information does not refer to the Environmental Exposures Questionnaire, which is administered after acceptance)
 - iv. Either the applicant (or legal guardian), their referring provider, or a CC representative will also upload to the Gateway a referral letter (see Appendix 6: Example Referral Letters) summarizing the following:
 1. Pertinent medical problems
 2. Prior diagnoses
 3. History of evaluations and tests
 4. Medications
 5. Family history
 6. Review of systems
 7. Physician's diagnostic impressions
 - v. If an applicant (or legal guardian) does not speak English, a translator will be used to facilitate the application process.
 - vi. If an applicant does not have access to the Internet, a paper application can be requested through the CC. Completed paper applications will be mailed to the CC for data entry and CS assignment.
 - vii. Applicants (or their legal guardians) will be 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, it will hold them until the applicant is assigned a CS, at which time the materials will be sent to the CS.
3. GenomeConnect self-phenotyping survey:

- a. Applicants will be sent an email asking them to complete the GenomeConnect self-phenotyping survey after submitting an application to the UDN. The survey is estimated to take 30 minutes to complete. The survey will be administered through Qualtrics and data will be stored in Qualtrics and in the UDN database.
 - b. Survey responses will be used by the CSs and SCs to better understand applicant phenotypes.
4. Applicant triaging:
- a. The application will be 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 will be 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.
 - b. If an application is deemed inaccurate or incomplete by a CS (example: referral letter not written by a healthcare provider), the CS will contact the CC. The CC will reach out to applicants accordingly.
 - c. The CS will make initial contact with the applicant within 30 days of application submission.
 - d. The CS will gather the information needed to make a decision regarding acceptance (see Appendix 7: Suggested Triage Methods). Typically, this will involve collection of medical records from the applicant. 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.
 - e. The CS will review the applicant's records and referral letter and make a recommendation regarding acceptance into the UDN. (See Appendix 8: Applicant Review Form.)
 - f. The UDN Case Review Committee (see Appendix 4: Case Review Committee of the UDN) will meet on a regular basis (weekly or biweekly) to:
 - i. Finalize decisions (at least initially) for all cases at the CSs that have been recommended for acceptance.
 - ii. Assign an applicant to a different CS if it feels that the applicant may be more appropriate for another CS based on expertise.
 - iii. Review challenging cases
5. Guidelines for applicant selection:
- Since few individuals can be accepted into the UDN each year due to limited resources, preference will be given to applicants for whom there is the greatest potential to provide a diagnosis or generate new knowledge about disease mechanisms.
- a. More likely to be accepted:
 - i. The applicant does not have a diagnosis that explains the objective findings.
 - ii. 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.
 - b. Less likely to be accepted:
 - i. The applicant has a diagnosis that explains the objective findings.
 - ii. Review of the records suggests a diagnosis and further evaluation is deemed unnecessary.
 - iii. The applicant is too seriously ill to travel safely to the CS.
 - c. Preference may be given to individuals with one or more of the following characteristics:
 - i. Novel clinical findings
 - ii. Previous evaluations that have been non-diagnostic

- iii. A genetic diagnosis that has a poorly defined phenotype and no molecular mechanism
 - iv. Multiple family members affected
 - v. An objective laboratory or imaging clue to pursue
 - vi. If a genetic origin is considered, biological parents are available to obtain blood for DNA sequencing (these families will be the most informative for gene discovery)
 - vii. The individual is a member of an under-represented minority group
- 6. Application outcomes:
 - I. Applicant and site appropriate for acceptance
 - II. Applicant appropriate for acceptance but reassigned to a different site
 - III. Applicant requires further testing or evaluation and may be reconsidered following receipt of the results
 - IV. 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.
 - An expert at an academic medical center or elsewhere.
 - iv. The applicant is not appropriate for the UDN and no alternative can be identified.

In all cases the referring provider and the applicant will be informed of the decision, generally within 60 days after receipt of all the medical records. The CSs will send out the disposition letters whether they accept or not accept. (See Appendix 9: UDN Generic Letters.) When individuals are not accepted into the UDN, their application information will be stored securely and indefinitely in the database managed by the CC.

II. Sequencing prior to the clinical visit (optional)

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 will only be performed on individuals accepted into the UDN. The sequencing will be done at the Baylor College of Medicine and/or HudsonAlpha. The decisions regarding timing of sequencing and WES versus WGS will be 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 clinical evaluation would be beneficial and aid in diagnosis, the CS will follow the following protocol:

1. Informed consent: Informed consent will be obtained over the phone or by videoconferencing (or in person if reasonable) from the enrolled proband, parent, or guardian. The consent form will be 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 will be available to answer questions and obtain consent. Consent will be 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 will be sent to the CS responsible for the clinical evaluation and uploaded to the Gateway managed by the CC. The CS will also record the consent version signed by the proband and proband's preferences in the Gateway managed by the CC. Genetic counseling will also be provided by a physician or genetic counselor to all probands during the consent process. This genetic counseling will include a discussion of the types of results an individual may or may not receive (including primary, secondary, and incidental 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. Probands will be given the choice of learning secondary and incidental findings related to conditions with treatment or management options. "Secondary findings" are findings that the laboratory will look specifically for. "Incidental findings" are findings discovered by chance during the genetic testing process. Genetic counseling aids were developed to supplement this session.

2. Assent: Assent will be 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 will be 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 will be sent to the CS responsible for the clinical evaluation and uploaded to the Gateway managed by the CC. The CS will also record the assent version signed by the proband and in the Gateway managed by the CC.
3. Family history: Once a signed consent form has been received from the proband (or legal guardian), a family history will be obtained over the phone (or in person if reasonable) to identify other family members of interest related to the proband's phenotype.
4. Enrollment of family members: Family members will be recruited through the proband, i.e., the researchers will ask the proband (and/or legal guardian) for permission to contact the family members. Priority will be given to those family members who would be most informative for sequencing analysis. Informed consent will be obtained over the phone or by videoconferencing (or in person if reasonable) from interested family members. The consent form will be 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 will obtain consent and be available to answer questions. Consent will be 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 will be sent to the CS responsible for the clinical evaluation and uploaded to the Gateway managed by the CC. The CS will also record the consent version signed by the family member and family member's preferences in the Gateway managed by the CC. Genetic counseling will also be provided by a physician or genetic counselor to all family members. This genetic counseling will include 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. A search for secondary findings will not routinely be performed in family members. However, incidental findings may be discovered by chance during the genetic testing process. During the consent, family members will be given the choice of learning incidental findings if they are identified. Genetic counseling aids were developed to supplement this session.

5. Collection of blood for DNA extraction: Once a signed consent or assent form has been received from the proband and family member(s), a kit will be sent to the proband and family member(s) for obtaining DNA. The kit will include:
 - a. Tubes for blood collection
 - b. An order form
 - c. Directions for payment by the CS (direct costs for the collection and shipping of samples will be covered by the CS)
 - d. An addressed shipping container for the blood to be sent back to the CS.

It is expected that the blood collection will be completed with the assistance of the proband's local healthcare provider or local laboratory.

6. DNA extraction and sequencing: For probands selected for pre-admission sequencing, DNA will be extracted at the CSs and sent to one of the SCs, where the sequencing will be performed and analyzed prior to the evaluation in order to have the analysis available by the time the plan for the proband's admission is finalized. Significant variants will be identified using standard programs for assessing pathogenicity, Mendelian segregation patterns, allele frequencies, and databases of benign variants. Through this process, secondary and incidental findings may be identified. Findings intended to be reported to probands or participating family members for use in clinical decision making will be confirmed by Sanger sequencing in a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory.
7. Return of sequencing results: If possible, results of genetic testing will be 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 clinical evaluation or when the participation of a non-local family member is limited to a blood sample for DNA analysis and an incidental finding has been identified. CLIA-certified results of genetic testing will be 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 will be returned to all probands (or legal guardians) and their referring providers.

If a primary finding is identified, it will be 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 will be discussed during a genetic counseling session with a qualified physician or genetic counselor.

The SCs will report secondary findings, i.e., variants that are medically actionable in the genes recommended for such reporting by the American College of Medical Genetics and Genomics. In addition, both cores will report other secondary findings beyond the currently recommended 56 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 will be applied; reported secondary variants will either be previously reported as pathogenic or expected to be pathogenic based on the usual molecular mechanism associated with the gene. As a further measure to ensure consistency between the SCs, the

SCs will communicate and reach consensus on the reporting of each secondary variant that they propose to report.

During the pre-test genetic counseling/informed consent session, probands (or legal guardians) will be given the option of receiving secondary and incidental genetic results that are unrelated to the indication for testing, including results related to: (1) medical conditions with treatment or management options and (2) carrier status (only applicable for adult probands). The proband's genetic testing results will be shared with family members only if the proband (or legal guardian) provides permission. Analyses to identify secondary and incidental findings will not be performed on family members. However, incidental findings may be discovered by chance during the sequencing process. During the pre-test genetic counseling/informed consent session, family members will be given the choice of learning incidental findings if they are identified. If a secondary or incidental finding is discovered in a proband and a family member is interested in learning if he/she also has this finding, the family member will be referred to a clinical genetics program for genetic counseling and testing. As this counseling and testing would not be done as part of the UDN, cost associated with this clinical follow-up would be billed to insurance.

There will be cases where the results of the sequencing performed prior to the clinical evaluation point to a likely diagnosis and in these cases, the CSs will be strongly encouraged to continue with the complete evaluation of the individual. The evaluation would allow the site to collect phenotypic data about the condition, provide counseling, and make suggestions about management. Exceptions would be 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 would still be appropriate.

III. Planning the evaluation

Once a proband has been enrolled into the UDN and assigned to a CS, the site will work with the proband (or legal guardian) and the local team to create a plan to maximize the efficiency of the evaluation.

1. Information gathered by the CS prior to the evaluation (some of this information may have already been collected as part of the applicant selection process):
 - a. Recent medical records (including consultation reports)
 - b. Previous tests and results
 - c. Pathology data/slides
 - d. Imaging/radiography results
 - e. Review of the medical history and review of systems
 - f. Family history (including name, age, and contact information of family members)
 - g. Medication list (including doses, schedule)
 - h. Contact information for the proband's relevant physicians
 - i. Environmental assessment
 - j. Nutritional assessment
 - k. Proband needs for travel and admission (including ventilators, mobility issues, etc.).
 - l. Optional: administer surveys and perform interviews. For example, probands will be sent an email asking them to complete an environmental exposures questionnaire. The survey will be administered through Qualtrics and data will be stored in Qualtrics and in the UDN database. If participants are unable to complete the survey online,

paper surveys can be requested. Completed paper surveys will be sent to the CC for data entry. Survey responses will be used by the CSs to better inform the clinical evaluation.

2. The CS will create a plan for evaluation, that will include:
 - a. Determining the lead physician.
 - b. Determining clinical tests, procedures, consults, or research studies to be performed, including a determination of whether additional IRB approvals will be required.
 - c. Scheduling dates for the proband evaluation – typically evaluations will occur over several days (expected to be five sequential days in most cases).
 - d. Determining the sequence and schedule of tests, procedures, and consults.
 - e. 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).
 - f. Arranging travel based on medical needs.

IV. Evaluation

1. Schedule recommendations:
 - a. Day 1:
 - i. Informed consent (see Section III.1 Informed consent above for details)– if the proband (or legal guardian) has not already provided consent (see Section III.1 Informed consent above), consent will be 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 will be obtained for all non-decisionally impaired children 7-17 years of age. Following the consent, the CS will record the consent version signed by the proband and proband’s preferences in the database managed by the CC. Proband may also be consented for other research projects at this time.
 - ii. Initial visit with the primary care team including the lead physician:
 1. Review the medical history, review the family history, and perform a physical examination.
 2. Genetic counseling may occur if results of genetic testing are available.
 3. Surveys and interviews for research may be administered.
 4. The goals of the visit and schedule will be reviewed. Changes in the schedule based on this initial visit will be made.
 - b. Days 1-5: All tests, procedures, and consultations will 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 will be 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 will be drawn, DNA will be extracted and sent to a Sequencing Core for sequencing.

- c. Day 5: The team will meet with the proband and family to summarize the evaluation and make plans for follow-up.
- 2. Clinical diagnostic studies: Clinical diagnostic studies will be performed as clinically indicated and within the standards of accepted medical practice.
- 3. Specialized research studies: Specialized research studies may be performed as deemed relevant.
- 4. Biological specimens:
 - a. Clinical specimens: Clinical specimens will be collected as medically indicated and at the discretion of the CS where the proband is being evaluated. Recommendations for certain clinical tests to be sent to specific facilities are presented in Appendix 10: Suggested Sites for Testing.
 - b. Research specimens: Recommendations for research specimen collection are presented in the Biospecimens section of this document (see section XI).
- 5. Environmental studies: Environmental data will primarily be collected for clinical purposes through the use of a comprehensive questionnaire derived from the PhenX toolkit and National Health and Nutrition Examination Survey (NHANES) survey questions. The environmental survey will be completed on line for each proband. In addition, the following may be obtained:
 - a. Food Frequency Questionnaire (FFQ)
 - b. Medications
 - c. Assessment of environmental exposures: Questionnaire, saliva or specific tissue collection for methylome analysis

V. Unanticipated non-genetic medical information

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

VI. Change in clinical stability

If, during the course of the UDN 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, will be 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 will be assumed either by the referring provider or appropriate clinical team members at the CS. Payment for further acute care will be provided by the patient's insurance company.

VII. Terminating subject participation

During the UDN study, if a participant (or legal guardian) does not comply with study procedures or does not follow instructions given by UDN investigators, the participant's involvement in the protocol may be terminated.

VIII. Clinical evaluation wrap-up

- 1. At the conclusion of the evaluation, and prior to discharge, the lead physician and other members of the care team as appropriate will meet with the proband and family to:

- a. Summarize the results of the clinical evaluation (clinical and research tests performed, procedures performed, consultations provided, results of testing received, and pending test results).
- b. Provide genetic counseling as indicated.
- c. Make recommendations for follow-up with the medical home team.
- d. Provide clear instructions about how to contact UDN team members if additional questions or concerns arise.
- e. Answer any questions the proband or family may have.

This wrap-up will be facilitated using a structured wrap-up form (see Appendix 11: Wrap-up Template).

2. The wrap-up form and a short letter highlighting the key findings and follow-up recommendations will accompany the transfer of records to the referring healthcare provider and other providers designated by the proband or family.
3. The wrap-up form and a narrative summary of the evaluation will be uploaded to the Gateway.
4. All consultation and laboratory study reports pending at the time of discharge will be 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 will be issued.

IX. 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.

C. Post-evaluation Activities and Follow-up

I. Transitions of Care

1. Background

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 patients undergoing diagnostic evaluations performed by the UDN due to the potential for poor communication between providers; inadequate patient, family, and/or caregiver understanding of findings and follow-up needs of the patient; 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.

2. Best practices

Best practices at the CSs will include:

1. Providing a written summary of the diagnostic work up to the family upon departure (see section VIII. Clinical evaluation wrap-up).
2. Confirming that probands have made it home safely (via text message, email, or phone call).
3. Being available to families and caregivers in case any clinical issues arise.
4. Maintaining open lines of communication.

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

3. Obtaining participant feedback

The CC and/or other UDN investigators will 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 have been created, vetted by the CC and Genetic Counseling and Testing Working Group, and approved by the CIRB (See Appendix 12: Participant Follow-up Surveys).

Survey Summary

Time point	Survey administered
7-14 days post-visit	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)

a. Immediately after UDN site visit (7-14 days after participant returns home)

The immediate post-visit period is a delicate time for patients 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 will allow the UDN CC to directly assess the participant experience at the CSs.

Patient experience can be thought of as having several components including:

1. **The patient's experience with the medical setting.** There are several elements that influence a patient's assessment of their experience with a medical setting. First is the establishment of a good interpersonal rapport with the clinicians. Second is the existence of efficient workflow so that the patient can move through a visit and have every part of the practice work seamlessly together, including needs that unexpectedly arise over the course of the visit.
2. **Communication.** Communication involves patient understanding of medication lists, tests results, follow-up plans, and upcoming appointments.
3. **Coordination among health settings.**

The UDN will measure aspects of the patient and family experience with the Interpersonal Processes of Care (IPC) Survey ¹⁻³. 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.

The CC will call the participant or the participant's representative (parent if the participant is a child, or spouse or relative designated as representing the participant if the participant is unable to provide information) and administer the IPC survey over the phone. The call will also be recorded and available for transcription, pending funding. If the recording is transcribed, discrepancies between real time data entry and transcribed data will be resolved by the CC.

b. 6 months post-visit

The 6-month post-visit survey is a participant-specific clinical and research outcomes survey. The timing of this survey at 6 months 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 this time, new topics are introduced, such as whether the investigators discussed research options. This survey also addresses the "coordination between health settings" element of the participant experience in more detail.

Data will be entered in real-time by the CC while on the call, and the call will also be recorded and available for transcription, pending funding. If the recording is transcribed, discrepancies between real time data entry and transcribed data will be resolved by the CC.

c. Yearly post-visit

The yearly post-visit survey assesses participant -specific clinical and research outcomes and provides updates on cases that may have been lost to follow-up. The current plan is to administer the yearly surveys by phone. The CC feels that personal contact with participants is important. Due to funding issues, this plan could change.

It should be noted that it will be important for the person conducting the surveys to check, as far as is known by the UDN, if the participant is deceased. Follow-up with the family will still be important, but will need to be handled in a manner that is sensitive to the death of the participant.

d. Evaluation of Survey Measures

- 1) If survey respondents have specific questions or areas that require follow-up, the CC will reach out to the participant's CS.
- 2) The analysis plan will be developed in consultation with the CC survey methodologist. At a minimum, survey results will be compared among sites, by

sex, age, race, and by other demographic information and results will be made available to all CSs.

- 3) The survey methodology (including timing) and questions will be reviewed at least annually or more frequently as needed.

4. Participant Engagement Group (PEG)

Overview

The purpose of the UDN Participant Engagement Group (PEG) is to provide the participant and family perspective on UDN research goals and participant experience. The PEG will engage with UDN investigators in the development and assessment of participant oriented materials and identify best practices for receiving participant input in research. There are many potential benefits of the PEG, 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.

PEG Membership

The PEG will include 6-7 participants and family members who are interested in partnering with UDN investigators to assess and contribute to the UDN research process and participant experience. Members of the PEG 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 will be adult, adolescent, and pediatric participant and family member representation.

All UDN participants or legal guardians will be offered the opportunity to participate in the PEG following the Transitions of Care survey. The application form will be sent to all individuals interested in participating. Following the submission deadline of August 1st, the applications will be sent to the clinical site coordinators for review. Based on the coordinator review, a small group of individuals will be selected for an interview by the CC. Following these interviews, the CC will draft a proposed member list. The proposed member list will then be submitted to the UDN Executive Committee and will be ratified by the UDN Steering Committee.

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

Terms will be one year in duration and renewable. Following the first submission deadline of 8/1/2016, and starting in 2/1/2018, terms will be staggered to avoid all members rotating in/out at the same time and will be August and February. This means that for 3 of the first 6-7 members, the term will be 18 months instead of 12 months. Members can reapply for a second term.

Activities

The PEG will be responsible for their structure and activities. Activities may include:

- 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

Schedule & Compensation

Conference calls will be organized by the CC on a monthly basis or at a frequency determined by the PEG. Annual in-person meetings will be held at alternating locations. The tentative date for the UDN Participant Engagement Group Kick Off Meeting is Monday, October 24th, 2016 and will be held in Boston at the CC at HMS. The PEG chair may be invited to attend one in-person Steering Committee meeting annually.

PEG members will receive \$500 annually for their participation. Travel expenses will be covered for the annual in-person meeting and the Steering Committee meeting as needed.

Evaluation

The CC will develop satisfaction surveys to be completed annually by PEG members. The PEG will be asked to develop an annual report on activities to be shared with the Steering Committee. There will be an annual Steering Committee review of the PEG.

II. Transitions to basic science

In most cases, the transition of participant data and/or sample to basic science will occur through the CS to which the participant was assigned, or through cross-site UDN collaborations. We expect that there will be referrals to the basic science community based on candidate genes or other diagnostic information, and it will be important to track these activities. A research follow-up plan will be developed by the CC (See Appendix 13: Research Inventory Form for an example). The goals of the plan will be to keep track of all research activities that the participant, samples, and data were involved in. In cases where there are no leads to pursue, the case could be reassessed again at two years after evaluation in order to determine if a research plan is indicated.

III. Participant web pages

In the UDN, 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- an amount of time that many of these patients simply do not have. By using web pages to identify second cases, the UDN hopes to shorten the diagnostic process for patients, generate new knowledge of rare conditions, and connect patients and families to one another for support.

All UDN participants from volunteer clinical sites that are undiagnosed or have rare conditions after the evaluation will be asked if they would like to partake in this project. If a participant is interested in participating, the clinical site will contact the CC and the CC will consent the participant. Consent will be obtained over the phone or by videoconferencing (or in person if reasonable) from the enrolled participant, parent, or guardian. Assent will be 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 will be 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 will be available to answer questions and obtain consent. The signed consent and assent forms will be sent to the CC. The CC will provide pre-paid, self-addressed envelopes to mail these forms. The CC will record the consent and assent versions signed by the participant in the database managed by the CC.

A web page about the participant will be created by the CC by gathering information from the database and communicating with the volunteer clinical site and participant. The web pages will include the following:

- Genetic variants (including gene name(s) and mutation)
- Symptoms/signs, both past and present, using both human phenotype ontology (HPO) terms and plain language
- Past medical history (including suspected diagnoses with dates or on a timeline)
- Past and current treatments, procedures, and medications and their outcome and effectiveness (treatments, procedures, and medications with dates or on a timeline) (outcome and effectiveness could be on a scale)
- Gender, race, and ethnicity
- Pictures and/or videos, if participant consents (pictures at multiple time points would be ideal)
- Contact information (phone number, email address) for CC

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

When searching for symptoms or genetic variants, clinicians and families may find a UDN participant web page. The CC will triage and record contacts made regarding these web pages. For possible matches, additional information will be collected, potentially through the application website. The CC will facilitate conversations between the potential match or clinician, the volunteer clinical site, and the participant. Participants will be asked if they would like to communicate with each potential match or clinician before they are connected. If true matches are made, the CC will update the web pages accordingly. The CC will record 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 will collaborate with the UDN patient advisors and participants to further refine this process and foster the creation of rare disease patient communities.

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.

IV. Data Standards

A. Background

The success of the UDN depends on the collection and subsequent sharing of well-described proband data. In order for the UDN data to be comparable and maximally useful, information about probands and their families must be captured in a uniform way. Several well-established standards have already been adopted by the undiagnosed and rare diseases community. These will be adopted by the UDN and are described here.

B. Applicant Data

Contact Information

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Email Address	Applicant or representative	Text
First and Last Name	Applicant or representative	Text
Address	Applicant or representative	Text
Phone Number	Applicant or representative	Text
Parent/Guardian Name	Applicant or representative	Text
Healthcare Provider Email Address	Applicant or representative	Text
Healthcare Provider First and Last Name	Applicant or representative	Text
Healthcare Provider Address	Applicant or representative	Text
Healthcare Provider Phone and Fax Number	Applicant or representative	Text

Demographics

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Date of Birth	Applicant or representative	Date
Gender	Applicant or representative	Structured data
Race	Applicant or representative	Structured data
Ethnicity	Applicant or representative	Structured data
Primary Spoken Language	Applicant or representative	Structured data, text

Medical Information

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Primary Symptom Category	Applicant or representative	Structured data
Date Symptoms First Noticed	Applicant or representative	Date
Similarly Affected Family Members	Applicant or representative	Structured data
Environmental Exposures	Applicant or representative	Structured data, text
Clinical Trial Participation	Applicant or representative	Structured data
Previous Evaluation Sites	Applicant or representative	Structured data
Site Preference	Applicant or representative	Structured data, text
Travel Limitations	Applicant or representative	Structured data, text
Referral Letter	Applicant or representative	PDF, Word document

Application Characteristics

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Date Application Submitted	CC Gateway	Date
Assigned CS	CC Gateway	Structured data
Date Reviewed by CS	CSs	Date
Decision Date	CC Gateway	Date

Application Review

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Name of Primary Reviewer	CSs	Text
Category of Primary Condition	CSs	Structured data
Narrative Summary of Applicant's Condition	CSs	Text
Height, Weight, Head Circumference	CSs	Text
Prior Pertinent Evaluations	CSs	Text
Provisional Diagnosis/Working Plan	CSs	Text
Number of Family Members Affected	CSs	Structured data
Affected Family Members Available for Analysis	CSs	Structured data
Images and Other Files	CSs	PDF, Word document
Decision	CSs	Structured data
Reason for Decision	CSs	Structured data

C. Participant Data

Relatives

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Email Address	CSs	Text
First and Last Name	CSs	Text
Address	CSs	Text
Phone Number	CSs	Text
Date of Birth	CSs	Date
Gender	CSs	Structured data
Race	CSs	Structured data
Ethnicity	CSs	Structured data
Relation to Proband	CSs	Text
Affected Status	CSs	Structured data
Evaluation Status	CSs	Structured data
Parent/Guardian Information (if applicable)	CSs	Text
Consent Data *see Consent section	CSs	Date, Structured data, Text
Sequencing Data *see Sequencing section	CSs	Date, Structured data, Text

Consent/Assent

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Date of Consent/Assent	CSs	Date
Consent/Assent Form Version	CSs	Text
Consent/Assent Document	CSs	PDF, Word document
Remote Consent/Assent Status and Date Received, if applicable	CSs	Structured data, Date
Preferences for Video/Voice Recording, Photographs, Skin Biopsy, Secondary/Incidental Findings	CSs	Structured data

Sequencing

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Prior Sequencing (Laboratory, Date, Reanalysis)	CSs	Structured data, Date
DNA Extraction Laboratory Name, Address, and CLIA Number	CSs	Text
Sequencing Core	CSs	Structured data
Type of Sequencing	CSs	Structured data
Rationale for type of sequencing	CSs	Structured data, Text
Test Requested	CSs	Structured data
Accession number	CSs	Text
DNA Source	CSs	Structured data
Date DNA Extracted	CSs	Date
Date DNA Sent To and Received by SC	CSs, SCs	Date
Tracking Number	CSs	Text
Sample Barcode(s)	CSs	Text
Method of Extraction	CSs	Text
DNA Quantity and Quality	CSs	Text
Notes to SC	CSs	Text
Results Communication	CSs	Date
Date Data Uploaded	CC Gateway	Date
FASTQ File	SCs	FASTQ [text]
BAM File	SCs	BAM [binary]
VCF File	SCs	VCF [text]
Coverage Metrics	SCs	CSV, WIG, Text
Clinical Report	SCs	PDF
Research Variants	CSs	CSV, XLSX

Medical Information

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Date of Death	CSs	Date
Maternal and Paternal Ethnicity	CSs	Structured data, Text
Health Conditions in Family	CSs	Text
Consanguinity in Family	CSs	Structured data
Miscarriages in Family	CSs	Structured data

Global Mode of Inheritance	CSs	Structured data
Pedigree	CSCSs	PED file
Prenatal and Perinatal History, if applicable	CSs	Structured data, Text
Allergies	CSs	Text
Age of Onset	CSs	Structured data
Medications	CSs	Text
Medical Reports	CSs	PDF, Word document
Measurements and Growth Charts	CSs	Structured data
HPO Terms*	CSs	HPO Term, ID
Segregation studies being pursued	CSs	Structured data
Genotype Information	CSs	HGNC Gene Name, Text
Diagnosis	CSs	OMIM Disorder, Text
Case Resolution	CSs	Structured data, HGNC
Photographs	CSs	JPG
Environmental Exposures	Applicant or	Electronic survey

*The Human Phenotype Ontology (HPO) is a resource for connecting genomic data with disease data, and provides links to diseases listed in OMIM and other disease databases [1].

The consistent annotation of UDN data with HPO terms will allow identification of probands who share the same or similar disease phenotypes across all CSs and, ultimately, more broadly with other large-scale efforts, including phenotype comparisons across model organisms [2]. Phenotypic data for UDN participants will be collected using the PhenoTips tool [3], which has fully integrated the HPO.

The HPO defines and organizes thousands of terms and relationships that characterize phenotypic variation and is regularly updated in response to requests from the research community, including the NIH UDP. The ontology has three sub-ontologies that cover 1) the mode of inheritance, 2) the onset and clinical course of the disease, and 3) phenotypic abnormalities, describing a wide-range of abnormalities across all body systems. HPO is cross-referenced to the Unified Medical Language System, the Medical Subject Headings, and other terminologies, including Orphanet's signs and symptoms.

Research

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Webpages Involvement, Start and End Date, Inquiries, and Matches, if applicable	CC	Structured data, Date
Study Involvement	CSs	Text
Publications	CSs	Text
Funding	CSs	Text

Evaluation Tracking

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Date Evaluation Started	CSs	Date
Date Evaluation Ended	CSs	Date

Follow-up

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Follow-up Provider	CS	Text
Dates of PhenoTips Record Completion	CSs	Date
Wrap Up Form/Letters	CSs	PDF, Word Document
Interest in Webpages	CSs	Text
Diagnosis	CSs	Structured data
Survey Communication Log	CC	Text

Other

<i>Term</i>	<i>Source</i>	<i>Data Format</i>
Schedule of Evaluation	CSs	Structured data
No Evaluation	CSs	Structured data
Agreed to Speaking with Media	CSs	Structured data
Comments	CSs	Text

References

1. Köhler S et al. The Human Phenotype Ontology project: linking molecular biology and disease through phenotype data. Nucl. Acids Res. (1 January 2014) 42 (D1): D966-D974. <http://www.human-phenotype-ontology.org/>.
2. Mungall CJ, Gkoutos GV, Smith CL, Haendel MA, Lewis SE, Ashburner M. Integrating phenotype ontologies across multiple species. Genome Biol. 2010 Jan 8;11(1):R2.
3. Girdea M, Dumitriu S, Fiume M, Bowdin S, Boycott KM, Chénier S, Chitayat D, Faghfoury H, Meyn MS, Ray PN, So J, Stavropoulos DJ, Brudno M. PhenoTips: patient phenotyping software for clinical and research use. Hum Mutat. 2013 Aug;34(8):1057-65. <http://phenotips.org/>.

V. Technology and Data Management

A. Privacy, Security Quality, and Compliance

In order to provide prompt and effective data management across a geographically diverse and highly specialized network, it is clear that the UDN data network, systems, and applications will need to store, manage, and protect personally-identifiable information (PII) and personal health information (PHI). This necessitates that primary engineering, policies, and procedures are strongly driven and governed to ensure the necessary security and compliance.

I. 'Above the line' and 'Below the line' Technologies, Processes, and Systems.

'Above the Line' refers to all technologies, processes, and systems that are operated under the responsibility of the CC.

'Below the Line' refers to all technologies, processes, and systems that are operated within each CS and Core. It is fully understood that each CS has preexisting processes, systems and novel technology capabilities, and the CC does not dictate which systems or processes a given CS 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.

A few examples of 'Below the Line' systems include:

1. Electronic Medical Record (EMR) systems
2. Laboratory Information Management Systems (LIMS), such as UDPICS from the NIH UDP
3. Local document and record management systems
4. Local bio-bank and clinical laboratory systems

II. Security Controls at the CC

1. *Physical Controls*: For physical records (paper, photographs, pen drives etc.), the CC will employ appropriate physical access controls (e.g., locked cabinet in a locked room).
2. *Computer Systems Controls*. Electronic security will consist of multiple levels of protections.
 - a. For computer systems containing PII and PHI, security controls that are compliant with HIPAA, National Institute of Standards Technology (NIST) guidelines, and the Federal Information Security Management Act (FISMA) will be utilized and a proper Federal Information Processing Standard Publication (FIPS) 199 assessment will be performed prior to commissioning of these systems.
 - b. For computer systems that contain PII but not PHI, appropriate roles-based access and security controls will be used and a 3rd-party security assessment will be performed and documented.
 - c. For computer systems that contain neither PII nor PHI, roles-based controls will be used and will conform to the information security and compliance standards of Harvard University.

III. UDN Technology Security and Compliance Policies

As proper security privacy and compliance can only be accomplished via an integrated approach of people, policies, processes, and technologies, a comprehensive approach is required. This approach will minimally require:

- a. Selection of regulatory standards, strategy and compliance approach
- b. Information asset and data security training
- c. Uniform roles-based access strategies
- d. Technology monitoring and logging
- e. Independent verification of procedural and technology controls

IV. Technology and Compliance Services and Coordination

The UDN will provide advice and access to information security and privacy expertise via a set of pre-qualified partners and internal resources that can help with security and privacy assessments, vendor qualification, and procedural document control.

V. Auditing

All key 'Above the Line' technology will be assessed via a risk-based approach to determine security, privacy, and compliance requirements. Systems containing high-sensitivity data and technologies (i.e., the Gateway) will be wrapped into FISMA Moderate compliant structures. 'Below the Line' components and technologies will need to take precautions, such as having up-to-date virus scanners, disk encryption, workstation-level authentication, and lockouts on all machines accessing Above-the-Line systems.

IV. Technology Standards

Technology standards will be essential to enable automated communications and rapid transmittal of data as well as for the essential elements of technology resilience, security, and privacy. An ongoing set of technology standards will be developed, managed, and governed by a standards oversight committee or working group.

B. UDN Data Flow Process

Since the UDN will operate as a real-time knowledge network, it is essential that appropriate and essential data flow securely and privately through each required step.

Step 1. Patient application and initial response. The process is initiated when the patient (or her/his representative) applies to the UDN through the secure UDN Gateway. The patient provides contact, medical, and demographic information and is assigned a UUID and a UDN ID. The UUID ensures that there is a universally unique ID. The UDN ID is a simplified ID that humans can use in communication with each other. Once registered, the patient receives a confirmation of application as well as an initial referral to a CS. This referral, based primarily upon geography, may be automated, but may involve some input from a medical manager to better distribute patients to the various clinical sites. As this step involves the capture of PII and, possibly, PHI, the portal is built and administered in a manner that is compliant with HIPAA and FISMA regulations. Any physical data or correspondence that accompanies the application process for the UDN will be managed with compliant physical document management controls.

Step 2. Referral and data transfer to a CS. This step is initiated when the patient is contacted by the CS and assembles her/his medical records for admission review at that center. The patient's medical records include current and past reports, laboratory studies, radiographic studies, etc. These data will be stored securely and indefinitely under the oversight and policies of the individual CSs. The records, in this form, will not be stored on the Gateway.

Step 3. Evaluation. All data obtained, created, or managed during the inpatient or outpatient evaluation will be the responsibility of the CSs and will be managed in such a way that ensures the security and privacy according to the guidelines of each institution. These data will serve as the permanent record and be subject to appropriate records retention policies. All UDN-wide agreed-upon "Above-the-Line" data elements will be collected and entered into the UDN Gateway.

Step 4. Post visit reporting and review. Agreed upon UDN-wide "Above-the-Line" data elements from the CS evaluation will be transmitted to the CC via secure electronic transfer and will be archived in a FISMA-compliant repository. These data will be structured data and will be kept in the Gateway's database. An API enabling easier input will be made available. A separate copy of the data/documents, most likely representing a subset of the data including copies of test and procedure results, will be transferred to the home care team.

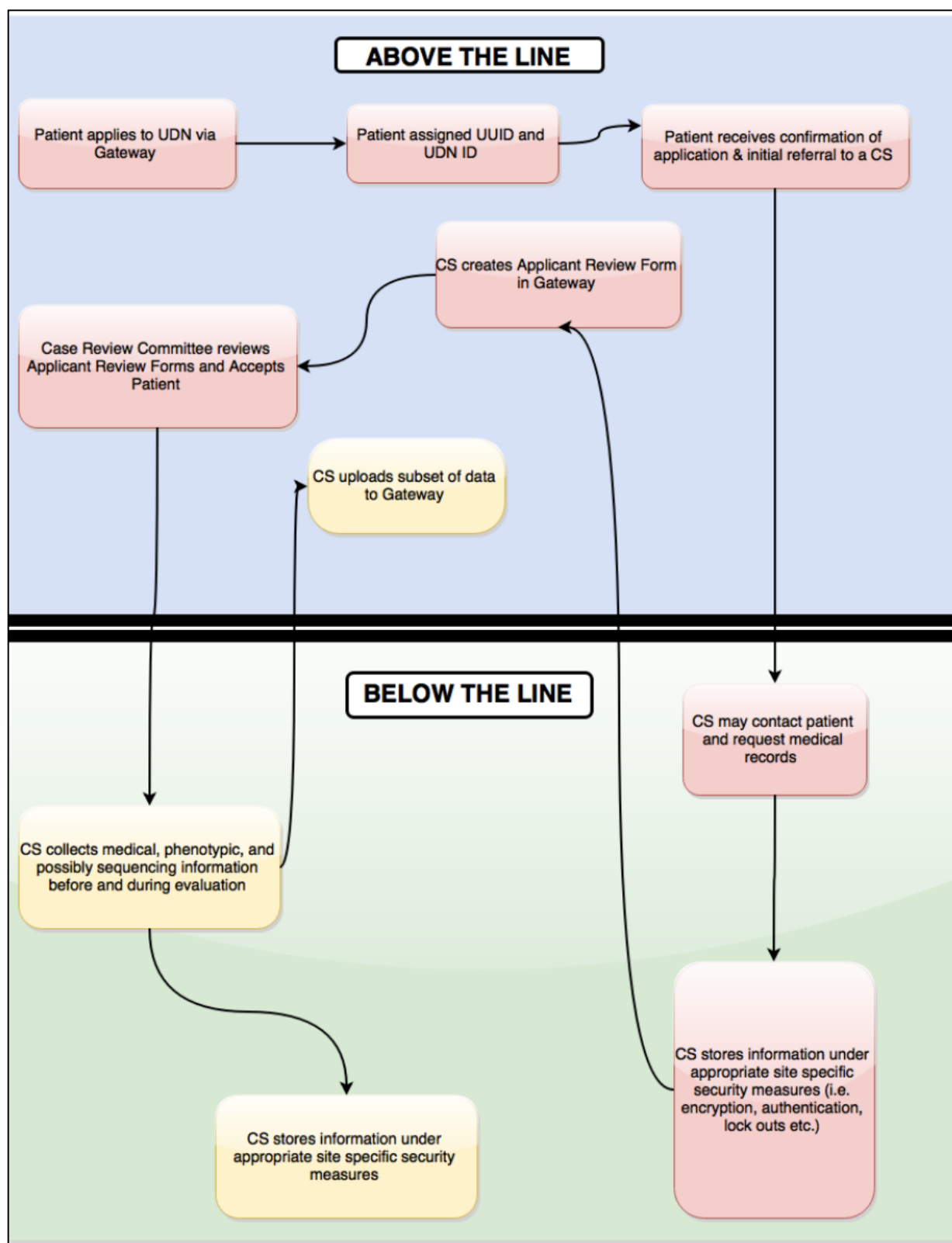


Figure 1. UDN Data Flow Process

C. Sequencing Data

The SCs will ensure the privacy and security of all PII and any PHI collected or generated during the sequencing process. The resulting genetic data will be transferred securely to the UDN Gateway, which will serve as the center of record for these data. The nature and management of the sequencing data provided by the SCs is discussed in detail in the Sequencing section (see Section VI: Sequencing) of this manual.

D. UDN Feature Request Process

The UDN Gateway will evolve over the course of life of the UDN. A major part of this evolution will be driven by requests for additional features. This feature request process described here applies to all UDN Gateway feature requests, both large and small. The CC will begin accepting feature requests upon the public launch of the UDN Gateway.

Definitions:

1. Feature requester: Individual or group who is making the request for the feature.
2. CC project manager: Individual at the CC who communicates with the Site Coordinators, SCs, and CC team members to complete project related activities.
3. CC technology team: Team at the CC that produces and manages the UDN Gateway.

Feature request process:

1. Feature requester completes the feature request form (see Appendix 14: Feature Request Form) and sends the request to the CC project manager.
2. The CC project manager 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 project manager will convey this information to the feature requester and will remove 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.

Note: 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 will always jump the queue and be priority. These sorts of issues aren’t classified as “features” and have a different handling process. They will come through the UDN Help Desk and be issued to the technology team for immediate resolution.

VI. Sequencing

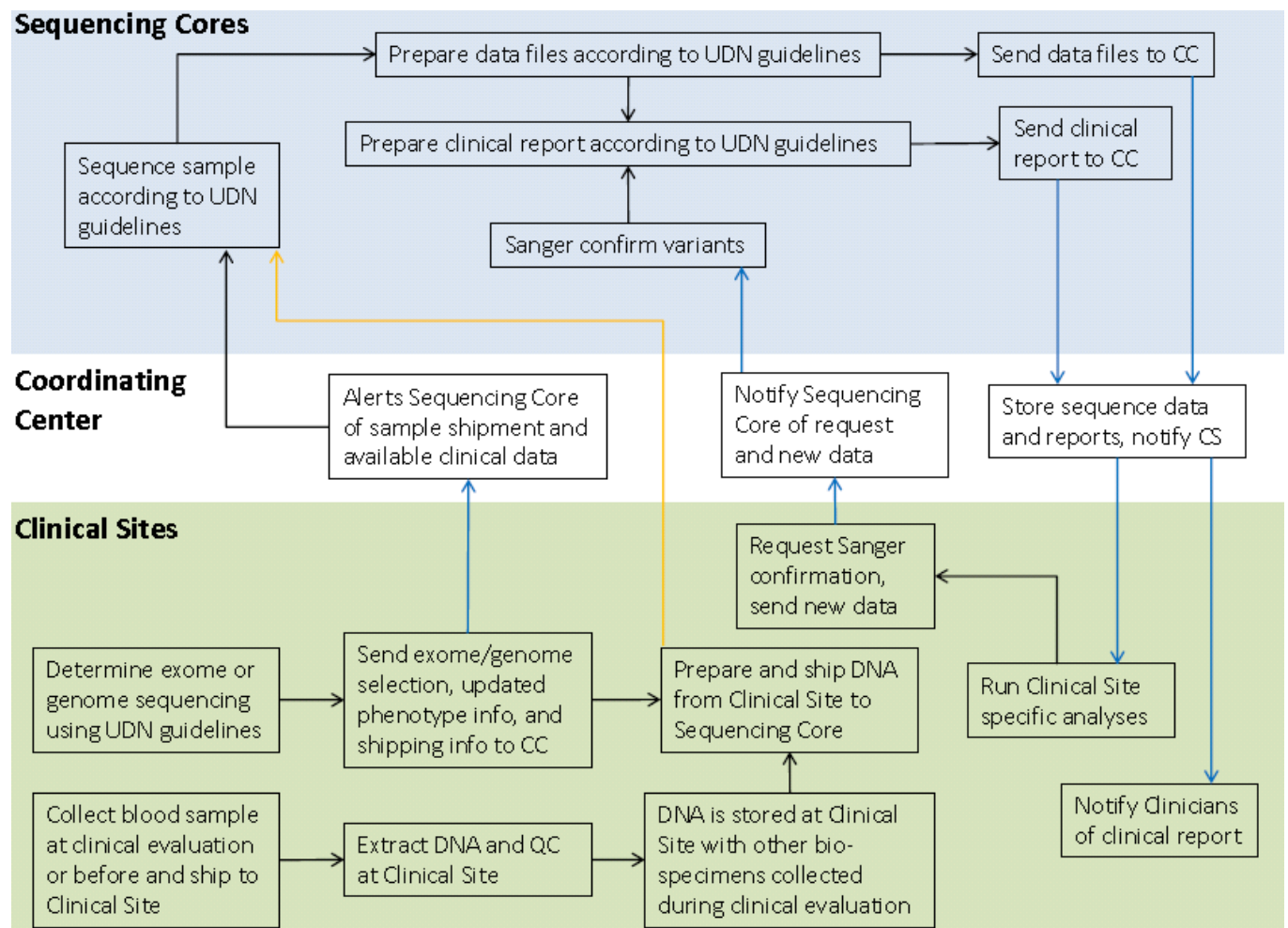


Figure 2. Sequencing Flow Diagram

A. Flow of samples to SC

Sample Collection and DNA Extraction

1. 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 CS.
 - a. Additional sample collection considerations:
 - i. If the individual has had a bone marrow transplant or recent blood transfusion, DNA from fibroblasts is preferred.
 - ii. Samples from deceased individuals should be discussed with the sequencing cores on a case-by-case basis.
2. The CS arranges DNA extraction and quality control (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
3. Additional DNA is stored at the CS with other biospecimens collected during the clinical evaluation.

Shipping After Determination of Exome or Genome Sequencing

1. CS prepares DNA samples for shipment to the appropriate 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 (application review and 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 will contact the CS site directly via phone or email to request a replacement.
8. Sample labeling discrepancies will be addressed on a case-by-case basis at the discretion of each SC.

B. Flow of clinical information to SC

1. CSs will organize the collection of blood specimens and DNA extraction entirely below the line. The CC and SCs will not know about or track the DNA specimens until they are shipped to the SCs. 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.
2. Typically, a CS will send an aliquot of DNA extracted in a CLIA-certified lab and keep the remainder of the DNA for future procedures, developed by the Biorepository working Group.
3. Samples could be sent either before or after the in-person evaluation of the study 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 instance on the Gateway as soon as possible for samples submitted for sequencing. This information will be used by the SCs 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 (ex. John Doe, UDP123).
5. Other required information for sample submission includes:
 - a. Gateway consent form
 - b. Gateway sequencing form
 1. Lab name, address, CLIA number where DNA Extracted
 2. Sequencing core

3. Type of sequencing and rationale
 4. Test requested (proband only, duo, trio, quad, other)
 5. Date DNA extracted
 6. Date DNA sent to sequencing core
 7. Tracking number
 8. Method of extraction
 9. DNA quantity
 10. DNA quality
- c. Affection status of family members

C. Exome-Genome Sequencing

WES - Baylor College of Medicine (BCM)

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

Sample Intake

DNA samples are received at the Whole Genome Laboratory (WGL). A visual inspection of the sample tubes is conducted. 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 will be 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 will notify the referring CS.

Once accepted, samples are accessioned into the WGL 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, 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 will be 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.

Sample QC

DNA samples are then 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:

- ✓ The R-squared value for the standard curve must be $\geq 99.9\%$ DNA concentration
- ✓ 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:

- ✓ 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 (H₂O) 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 is 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 discarded. 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.

- ✓ No more than 9 cycles total can be run for samples
- ✓ If the amount of the post-PCR product is insufficient after a total of 9 cycles, the whole process needs to be repeated.
- ✓ 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 Bionalyzer 2100 DNA 7500 Chip.

- ✓ The library sizes should range from 200 – 750 bp (Majority are 250-550bp) with the peak ranging from 250 - 350 bp.
- ✓ 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. See Figure 3 for an example of a passing pre-capture library.

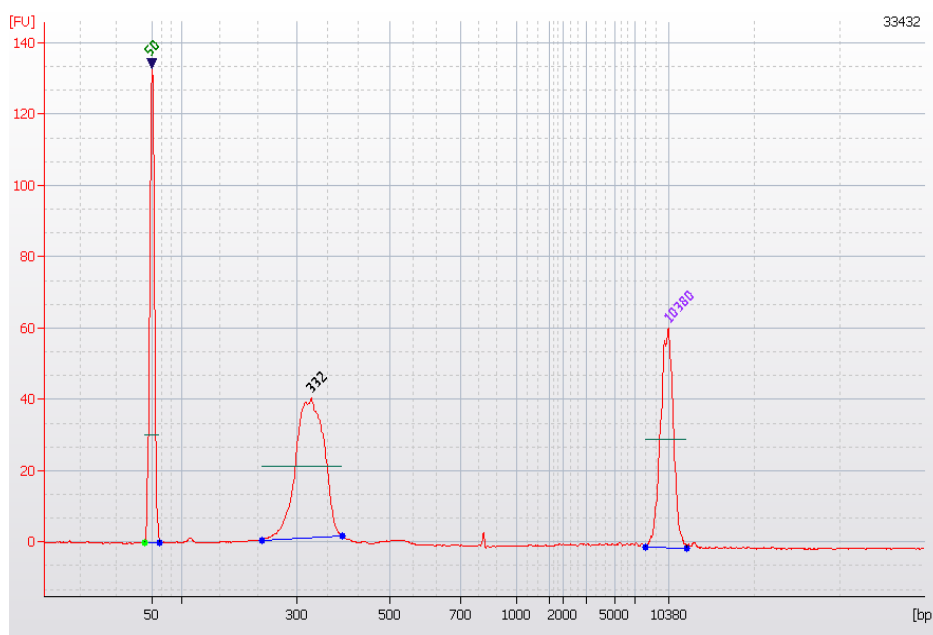


Figure 3: Illumina paired end DNA library on Agilent Bionalyzer 2100

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 4 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.

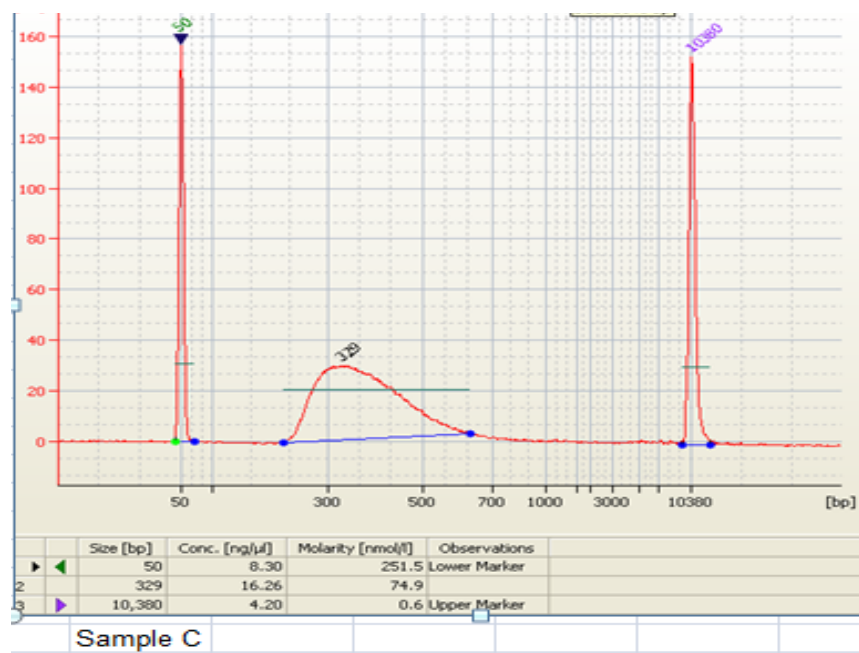


Figure 4: Example of Agilent Bioanalyzer result of final post-capture library

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

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 will stop after imaging is complete for the first cycle and generate a first base report. The first report will be confirmed by manual review and the run will resume 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 Mercury analysis pipeline and provides metrics to gauge the overall quality of the capture process. This pipeline reports:

- Proportion of the aligned reads that map to the targeted region, which is relative to the effective enrichment of the capture
- 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 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%

WGS – HudsonAlpha Institute for Biotechnology (HA)

This section covers sample intake, library preparation, and whole genome sequencing at HudsonAlpha Institute for Biotechnology. This section will describe the UDN sample flow from DNA sample receipt to production of WGS data, including appropriate quality control (QC) and quality assurance procedures.

DNA Samples are received by the Genomic Services Laboratory (GSL) at HudsonAlpha Institute for Biotechnology. A visual inspection of the samples and subsequent accessioning is performed by two GSL employees. Accessioning includes entering the samples into a project in the LIMS of the Clinical Services Lab (CSL) and assigning two identifiers to the samples. The first identifier is the CSL identifier and is formatted as a project number, submitter's initials, and a unique sample number incrementing up from sample 0001 (i.e. C1001-SL-0001). The second identifier is a unique identifier created by the LIMS using the date of accessioning and another digit, which indicates the order in which samples were accessioned. These identifiers deidentify the sample and are used to track the samples through all handling performed by the CSL. This accessioning is performed by the HAIB CSL laboratory manager or a designee.

If the submitted sample is DNA from a CLIA-certified laboratory, it proceeds to QC procedures as described below. If the submitted sample is blood, DNA is isolated in conjunction with its accessioning and another unique sample identifier for the DNA is created by adding a '.1' to the GSL ID (i.e. C1001-SL-0001.1). DNA is extracted from 1ml of whole blood on the QIAasympy instrument using the Blood_1000_V7_DSP protocol. This protocol yields on average of 10-18ug of gDNA from 1 ml of whole blood.

Sample information in the Gateway is reviewed by the HAIB UDN Project Manager to ensure consistency and completeness of the Gateway sequencing and consent forms, proper sample labeling, and sample tube integrity. Coordination between the UDN HAIB Project Manager and the CSL laboratory supervisor ensure that sample labels are cross-checked with information in the Gateway. Sample tubes will be visually compared to information in the Gateway for accuracy prior to accessioning by the CSL laboratory supervisor or an assignee.

Samples will be accepted if no discrepancies are found, sample labels match, and no tube damage is observed. If any of the above criteria is not met, HudsonAlpha will notify the referring CS.

Sample QC

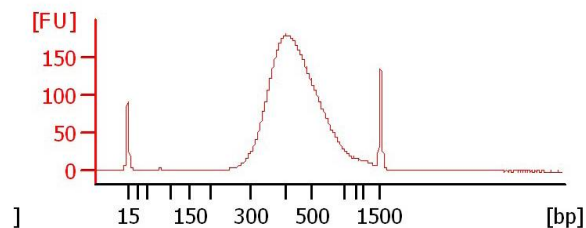
All DNA samples are evaluated for concentration by fluorometric assay (Qubit® or Picogreen®) and for integrity by agarose gel. Ideally, there will be at least 500ng of intact, high quality DNA available to enter the GSL whole genomic sequencing (WGS) library preparation. For fragmented samples or other non-traditional DNA preparations, such as low input samples, QC may be performed with the Agilent Bioanalyzer or Caliper GX. Samples not meeting the minimum requirements for clinical WGS may still proceed into library preparation, but as research samples, not clinical.

Library Preparation

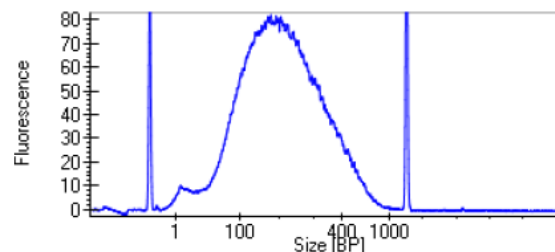
DNA samples are normalized to 1000ng of DNA in 50ul of water. All gDNA samples require fragmentation in a random manner to create the fragments that will become the inserts in the final library. The Covaris L-series and E-series instruments are used to shear the DNA to a final

insert size of ~350bp. This longer insert size improves overall library performance and allows the longer sequencing read lengths on the Illumina HiSeq X 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. Library preparation is then performed using a proprietary GSL methodology with key QC steps performed throughout. QC is performed during preparation, after ligation, to assess yield. 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. Yields are determined with fluorescent measurements (PicoGreen) and fragment sizes and distribution will be determined with either the Agilent Bioanalyzer or the Caliper LabChip GX, depending on batch size. Example traces for sonicated gDNA and final sequencing library are below. The final libraries are diluted to 3 nM stocks for use in clustering and sequencing.

Post sonicated gDNA QC



Post library prep QC



Sequencing

Once a library passes QC, the production sequencing on the HiSeq X will be performed. Clustering and sequencing will be performed as per standard Illumina protocols for HiSeq X sequencing. Each UDN sample is sequenced by itself in one lane, plus also sequenced in a pool of 3 samples across a final lane. One lane of sequencing on the HiSeq X instruments will generate approximately 30X coverage of the human genome when duplicates removed from consideration. The UDN grant specifies a minimum of 40X coverage. Therefore, for a given trio of samples, a pool of the 3 samples is run across a fourth lane to supply an additional ~10X coverage per sample, yielding 40X total. Approximately 360 million paired-end reads, each 150 bp in length, will be generated for each sample, with typical flow-cell runs lasting ~3 days each. Over 105 Gb of sequence per sample is generated per lane and a 40X UDN WGS sample will receive a minimum of 150 Gb of data.

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 will be followed (as defined in this manual and agreed to across all SCs, CSs, and the CC).
- Annual review of current methodologies, with an aim of identifying and potentially incorporating advances of note in analytical approaches supporting interpretation of sequencing data will be performed by the SCs.
 - Any alterations that are considered for inclusion will be shared with the Sequencing Working Group.
 - Those prioritized will be implemented leading to revision of the analysis steps outlined in this document.
- Each CS may conduct analyses on UDN cases sequence data as they see fit but the SCs will undertake 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 will have access to richly annotated data.
 - SCs will share sequencing data with CSs through the Gateway.

Sequencing Files in the UDN Gateway

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

- Standard compliant format FastQ files
- Standard compliant BAM files
- Standard compliant VCF files
- Annotated variant files
 - A tab delimited text file format
 - SCs will work to ensure that the format and data encapsulated in this file is equivalent at both sites
- A spec sheet listing software versions and patches, analysis tools, and annotation repositories will be provided, along with exact parameters used in the analysis.
 - This will allow sites to unambiguously determine the exact steps for reproduction of analysis and, perhaps allow for case based additional optimization of analysis parameters at capable clinical sites. Format of this file TBD; one suggestion is an XML spec sheet for unambiguous representation and downstream automation.
 - In addition, where applicable (for example as the header of the generated VCF file), a human verifiable description of the applications, version, and reference datasets used will be encapsulated in the output files themselves.
- An interpreted clinical report will also be provided by the SCs.

- The format of this report will follow the existing industry standards for clinical sequencing reports.
- Clinical reports will include the following report sections:
 - Lab contact information and general test information
 - Participant name and date of birth
 - Indication for testing
 - Primary findings (pathogenic, likely pathogenic, and variants of unknown clinical significance) in tabular format
 - Secondary and incidental findings in tabular format
 - Interpretation of findings – textual discussion of the relevance of the findings given the clinical presentation of the proband
 - Specific recommendations
 - A description of the methods used
 - Limitations for both the sequencing technology and analytical processes
 - References
- Secondary findings will not be sought or reported for family members, however, incidental findings discovered by chance during the testing process may be returned to family members at the discretion of the SC and CS. Each family member will receive a report containing one of three possible results:
 - Incidental finding identified
 - No incidental finding identified
 - Family member opted out of receiving incidental findings

Primary Analysis

Primary analysis (demultiplexing) will be performed on the HiSeq instrument workstation according to Illumina guidelines. Software used for primary analysis is described in Table 1.

The primary analysis steps at each site will be equivalent, although they may have version differences reflecting the software update timetables in place at the SC. The primary analysis software version used will be listed in the spec sheet provided by the SCs. Changes and updates will be appropriately communicated to the CSs.

Secondary Analysis Background

It is important to note that the secondary analysis steps performed at the SC will not necessarily be identical across all steps. They will, however, be comparable and clinically appropriate as defined by their existing usage in the CLIA and College of American Pathologists (CAP) accredited clinical laboratories at both SCs.

The secondary analysis steps at each SC will be equivalent although they may have version differences reflecting the software update schedules in place at the SCs. Each SC will perform clinically appropriate validation of all datasets and algorithms/software applications in use within its clinically validated analysis pipelines. Significant pipeline component changes will undergo re-validation at the discretion of the SCs.

Secondary Analysis – Read Mapping

Secondary analysis step 1 (mapping and realignment) will be performed at each SC using the methods and tools described Table 2.

GRCh37/hg19 (b37d5) will serve as the alignment template until it is superseded and adopted by leaders in the field. Alignment of reads to GRCh37/hg19 (b37d5) will be performed without truncation of the data. More specifically, duplicates will be marked but not removed from the dataset.

Secondary analysis Step 2 (variant calling) will be performed at each SC using the methods and tools described Table 2.

Tertiary Analysis – Variant Annotation

It is important to note that the variant annotations produced by each SC will not necessarily be identical but they will be equivalent. The reason behind this is that to make them identical would require re-working the clinical pipelines in place at the SCs. This activity was not planned for or funded. The plan is to use the existing clinically validated pipelines at the two sites. The SCs will liaise closely to ensure equivalency and will continue to work towards a unilateral set of variant annotations. The specific variant annotations and the tools used to produce them are outlined in Tables 3 and 4. The SCs will, to the extent possible, ensure that the data is labeled the same in the datasets produced from each CS.

Coverage analysis

In addition to primary, secondary, and tertiary analysis, the SC will also provide a summary of the sequencing coverage for each sample. This report will detail the coverage from the sample run at the gene model, transcript, and exon levels. This will provide the CSs with an indication of regions of likely importance that are not well covered. In cases where a trio or an extended pedigree is submitted for sequencing this coverage report will be provided for both the proband and the other individuals in the pedigree. The CSs and CC will be appraised of any updates to the coverage algorithms made by the SCs.

BCM uses ExCID, an in-house developed tool, to assess capture efficiency and coverage at desired cutoffs. Files from this tool can be viewed in any genome browser to see the actual coverage on genes of interest. This will also report a summary of poorly covered regions.

HA assesses coverage with GapMine v 3.0.1, a software package developed the Medical College of Wisconsin for coverage at the gene, transcript, and exon level. Files can be reviewed in any genome browser. The coverage at these levels and for specific gene lists are provided as a report if requested. Coverage gaps at a defined depth threshold are also reported.

Interpretation

The SCs will also provide an interpreted clinical report. A systematic process will be followed in accordance with ACMG guidelines as published (https://www.acmg.net/docs/Standards_Guidelines_for_the_Interpretation_of_Sequence_Variants.pdf) to determine the clinical significance of each variant considered for reporting.

Analysis output delivery turn around times

Initial analysis (to end of tertiary analysis phase) will be completed within a 2 week turnaround time (TAT) at both SCs.

Preliminary clinical reports are typically available 7-8 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 SCs. The SCs will Sanger confirm up to 8 variants per case. Unused Sanger confirmations may be distributed among each clinical site's case cohort (ex. 25 cases = up to 200 Sanger confirmed variants). The turnaround time for final clinical reports will depend on the timing of CS data analysis.

Requesting Release of Sequencing Data

Each SC will follow its existing institutional policy for fulfilling raw data requests. Raw data will only be released after a final report has been returned to the CS and test results have been communicated to the participant.

Baylor College of Medicine will release WES data to physicians and qualified researchers at the participant's request. See <http://bmgl.com/media/wysiwyg/bmgl/pdfs/ExomeDataReleasePacketv6.pdf> for more information.

HudsonAlpha will release WGS data to participants, physicians, and qualified researchers. Interested participants should contact the Clinical Services Laboratory directly at clinical@hudsonalpha.org or (256) 327-9413.

Table 1. Primary analysis tools used by each SC.

Phase	Step	BCM	HA
Demultiplexing	Bcl2Fastq	bcl2fastq-1.8.3	Dragen executable v01.002.091.0 1.03.17.16114

Table 2. Analysis steps and applications/algorithms/platforms used for secondary analysis at each SC.

Phase	Step	BCM	HA
Read mapping	Alignment	bwa v0.6.2	BWA-mem v0.7.12
	Fixmate, Sort & Index	Picard v1.8.4	SAMBamba v0.5.4
	Mark duplicates	Picard v1.8.4	sambamba_v0.5.4 markdup
	Realignment and recalibration	GATK v2.5.2	GATK v3.3
Variant calling	SNV	Atlas2-SNP v1.4.3	GATK v3.3
	INDEL	Atlas2-Indel v1.4.3	GATK v3.3

Table 3. Tertiary analysis tools used by each SC.

Phase	Step	BCM	HA
Variant annotation	Annotation	Cassandra v15.4.29	CarpeNovo v 6.0.1
Variant prioritization	Trio analysis	Trio Afterburner v2	CarpeNovo v 6.0.1

Table 4. A depiction of the set of variant annotations and tools used by each SC.

Annotation	BCM	HA	Notes
Annovar	yes	no	commercial product
Alamut HT	no	no	commercial product
splice sites	yes	yes	
near splice site	yes	yes	BCM: +/- 5bp , HA +/- 6bp donor & 25bp acceptor
protein coding flag	yes	yes	
syn change flag	yes	yes	
non-syn change flag	yes	yes	
AA change	yes	yes	
Sift prediction	yes	yes	
Polyphen2 HVAR prediction	yes	yes	
Polyphen2 HDIV prediction	yes	yes	
Mutation Taster prediction	yes	yes	stand-alone
Condel prediction	no	no	BCM uses dbNSFP (PMID: 25552646)
MutationAssessor prediction	yes	yes	
AlignGVGD	no	no	web service; not in pipeline
stop gain flag	yes	yes	
stop loss flag	yes	yes	
start loss flag	no	yes	
frameshift flag insertion/deletion/indel	yes	yes	
non-frameshift flag insertion/deletion/indel	yes	yes	
location: intron/exon	yes	yes	
location: 5'UTR/3'UTR/Intergenic/Promotor	yes	yes	
HGNC appropriate Gene Symbol	yes	yes	RefSeq
Transcript ID	yes	yes	
COSMIC	yes	no	BCM: additional HA: scheduled for 2016 inclusion
HGMD ID	yes	yes	
HGMD variant level association	yes	yes	
HGMD gene level association	yes	yes	
OMIM ID	yes	yes	
OMIM variant level association	yes	yes	
OMIM gene level association	yes	yes	
ClinVar ID	yes	yes	
ClinVar metadata (various; to be clarified)	yes	yes	
dbSNP ID	yes	yes	
dbSNP AF	yes	yes	
1000 Genomes AF	yes	yes	
ESP EVS AF	yes	yes	
Mappability score	yes	yes	

VII. Data Sharing

The success of the UDN will depend on the collection and subsequent sharing of well-described data. This UDN Data Sharing Policy is consistent with the goals of the NIH Data Sharing Policy.¹ The NIH states “Data should be made as widely and freely available as possible while safeguarding the privacy of participants, and protecting confidential and proprietary data.”² Data from the UDN are expected to be handled so as to increase the value of the significant public investment in the creation and operation of the UDN. The CC is committed to best practices in data standardization and will develop efficient mechanisms for sharing and dissemination of the data generated by the UDN.

This document outlines the minimum requirements for sharing the data that are collected in the course of participation in the UDN. The document is organized as a set of questions and answers.

Q: What data will be shared within the UDN?

A: All clinical, biospecimen, and sequencing data that are generated by the UDN effort will be shared in a secure and compliant manner within the Network. These identified data will be referred to as “UDN data” throughout this document. UDN data include data generated both in human subjects’ research and in laboratory research.

Q: Who will have access to the UDN data?

A: Any UDN investigator. UDN investigators who acquire UDN data must use the data responsibly and must monitor the use of the data by members of their laboratories. (See Appendix 15: UDN Data Sharing and Use Agreement.)

Q: Will UDN data be shared with investigators who are not currently part of the UDN?

A: Possibly, if there are complementary initiatives with goals that are consistent with the UDN, as for example, would be the case if the NIH awarded grants that are scientifically related to the work of the current UDN. For the diagnosis of individual probands, if there are useful experts outside the UDN, these can/should be involved on an as needed basis.

Q: Will UDN data be shared more broadly in public databases?

A: Yes, in de-identified form. Data resulting from UDN efforts will be deposited in dbGaP, which is maintained by the NCBI at the NIH. De-identified data may also be deposited in other public databases, registries, and repositories, such as PhenomeCentral, the NIH Global Rare Diseases Registry, and be shared with other existing or emerging rare and undiagnosed diseases research efforts.

Q: How will the rights of individual research subjects be protected?

A: Research participants will give consent to have their data shared, according to a UDN agreed upon informed consent process. Each subject in the database will be associated with a UUID that will be used as the primary identifier for all data associated with that participant. Role-based access and physical security controls that are fully aligned with the sensitivity of the data at each point of use and access will be employed. De-identified data shared outside of the UDN will not reveal individual identifying information, consistent with the HIPAA Privacy Rule³.

Q: How will institution-specific intellectual property regulations and restrictions be addressed?

A: The CC will work with the principal investigators at each of the CSs and Cores to develop an approach that is consistent with the data sharing policy described in this document.

Q: What is the publication/authorship policy for UDN collaborative activities?

A: There is a separate Publications section within the manual that describes these policies. If broad data release is required as a condition of publication by the authors or the publisher, the Publications Working Group should be contacted as soon as possible prior to making any commitments to ensure that the data release is feasible.

Q: What is the commitment of each UDN investigator?

A: UDN investigators agree to:

- 1) Further the mission of the UDN: to create new knowledge regarding the biochemistry, physiology, and mechanisms of undiagnosed diseases and improve diagnostic and management options for patients afflicted with them.
- 2) Acknowledge that in pursuit of this mission, common UDN goals may supersede individual goals. Specifically, in the interest of rapid progress, UDN investigators commit to:
 - a. 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.
 - b. Ensure that data generated at individual sites are comparable and additive by adhering to UDN data standards.
 - c. Make data contributions to the UDN in a timely manner.

Q: What is the role of the CC?

A: To facilitate, monitor, and report on the effective and timely sharing of data within the UDN and beyond.

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. HHS - Office for Civil Rights - HIPAA. <http://www.hhs.gov/ocr/hipaa/>
1.

VIII. Publications and Research

One parameter of UDN success will be the number and quality of its publications and presentations. The purpose of this document is to establish a framework, which facilitates and streamlines collaborative manuscript submission, as well as antecedent work, like meeting abstracts and presentations. The UDN Publications and Research Committee will oversee 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 UDN Steering Committee. The UDN 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

- I. To facilitate manuscript submission.
- II. To provide input in abstract submission and scientific presentation (when requested).
- III. To help the CC with content for the UDN website and, if required, social media.
- IV. To maintain an up-to-date list of all UDN presentations, abstracts, publications and proposals. The CC will assist in tracking and coordinating projects.
- V. Notwithstanding anything to the contrary in this document, the scope of UDN Publications Committee activity does not include evaluation of the scientific merit of any publication produced as a result of UDN participation.

B. Manuscript: Authorship Review and Submission

- I. Authors (First, Middle and Senior) will be determined by the type, scope and site of project. First author will take primary responsibility for the manuscript. Given the nature of the UDN's work, shared first or last authors should be remembered as an option.
- II. UDN will be acknowledged at the end of the author list, as "Members of UDN". The UDN member list will include members from the sites nominated by the PIs. A UDN membership list will be provided by the UDN Publications and Research Committee and may be different on a case-to-case basis.
- III. 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.
- IV. All manuscripts will be reviewed and approved by the UDN Publications and Research Committee prior to submission to any journal (see Appendix 16: Publications and Research Reference Sheets for details). Approval shall be for purposes of satisfaction of the points in this section alone and, for purposes of clarity, this means that UDN Publications and Research Committee shall not withhold approval of a manuscript on the basis of its scientific merit.
- V. The UDN Publications and Research Committee will resolve all authorship disagreements.
- VI. All UDN papers (network-wide and local) should include a statement such as: "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) [xxxxx]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health".

C. Manuscript Proposal–Submission and Approval (Concept sheet)

To initiate the process that will lead to a publication, all UDN investigators are invited to submit firm, mature concepts for papers to the Publication Committee. The proposed lead (first) Author submits a completed Manuscript Concept Sheet (see https://hms.az1.qualtrics.com/SE/?SID=SV_3CyZOKOuiEvnCx7), which is reviewed and approved by the Committee before substantial drafting begins. A voting member of the Steering Committee must endorse the concept proposal. If more than one person submits the same or similar topic, the Committee helps decide who will assume the project lead.

The Concept Sheet is submitted to the CC for administrative processing. The CC forwards the proposal to the UDN Publications and Research Committee for review. It is expected that the approval process will not take more than two weeks.

For Special Issue Journals, the journal organizer will submit a Concept Sheet for the entire Issue, listing proposed articles and lead authors along with Concept Sheets for each article. Once the main Concept Sheet and all abbreviated Concept Sheets have been submitted, the CC forwards the proposal as a Special Issue Journal packet to the UDN Publications and Research Committee for review.

D. Abstracts and Presentations:

- I. An abstract submission will not require approval from the UDN Publications and Research Committee.
- II. Abstracts and presentations should mention UDN as “Members of UDN” in the authors list as well as UDN grant number.
- III. All presentations should be sent to the CC so that they may be posted to the UDN web site. All abstract citations should be sent to the CC so that the UDN bibliography can be updated accordingly.
- IV. If there is a NHGRI or Common Fund co-author, final versions of the abstract must be submitted to the PO for review and approval.

E. Database of Publications and Concepts:

The responsibility of the UDN Publications and Research Committee will be to advise the CC in management and population of the database of concepts, presentations, abstracts and publications submitted, finished, and in process.

F. Start of UDN publication:

The UDN Publications and Research Committee will develop a manuscript or multiple manuscripts, which will describe, define, and introduce the network to the medical and scientific community.

G. End of Funding Cycle Publications:

The Publication Committee will be responsible for stimulating the preparation of manuscripts, which describe the UDN experience towards the end of the first four-year funding cycle.

Concept Sheet: The investigator submitting the concept sheet will have sole possession of the idea. Other groups can contribute but the original submitter of the sheet will take the lead. Please note that the concept sheet **has** an **expiration date** of 6 months but can be extended for an additional 6 months if needed upon submission of an updated concept sheet. (See Appendix 16: Publications and Research Reference Sheets and the following *Concept Sheet Link*: https://hms.az1.qualtrics.com/SE/?SID=SV_3CyZOKOuiEvnCx7.)

IX. Website and Social Media

The purpose of this section is to outline the UDN plan for the creation and maintenance of a unified public-facing UDN web and social media presence.

The UDN will have a public-facing website, which will be created and maintained by the CC, with directional input from the UDN Steering Committee. We anticipate that the content will include success stories, descriptions of the CSs and core laboratory sites of the Network, publications, information for potential applicants, and information for researchers, among other content.

In addition, the CC will cultivate a social media presence that will begin with a Twitter account and may expand to include other social media forums, and the CC will proactively solicit information about publications, presentations, abstracts, and scientific publications on a semi-annual basis.

All members of the UDN will be invited to submit content suggestions for the website.

X. Metrics

One of the core functions of 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 measurement 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.

The NIH Program-defined measures are listed below: the NIH Program may update these measures over the course of the network. The CC may calculate a range of other measures to assess the performance of the network. A table of potential metrics is shown in Appendix 17: Proposed UDN Metrics.

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.

Abbreviations: IRP-UDP = NIH Undiagnosed Diseases Program clinical site; ECS (external clinical site)

		Performance Metrics and Milestones
1	NIH	By Oct 1, 2015 - the NIH UDP will identify at least 5 candidate genes annually by working jointly with the UDN through FY2017.
2	NIH	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).
3	NIH	By Sept 1, 2016 – 6 UDN extramural Clinical Sites to accept 35 patients, per site, in order to initiate evaluations.
4	NIH	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.
5	NIH	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.
6	NIH	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.
7	NIH	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.
8	NIH	By Aug 1, 2016 - 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.
9	NIH	By Oct 1, 2015 – Establish collaboration amongst laboratory and clinical

		researchers, and share resulting data and approaches throughout the scientific and clinical communities.
10	NIH	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.
11	NIH	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.
12	NIH	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.
13	NIH	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	NIH	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.
15	NIH	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.
16	NIH	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.

XI. Biospecimens

Recommendations for research specimen collection on all UDN probands

- A) Types of Specimens: The following specimens should be collected on all probands evaluated in person unless doing so would compromise participant safety or if they are refused by the proband (See Section B below regarding blood volume issues in pediatric probands).
1. 3 ml serum in 0.5 ml aliquots
 2. 3 ml plasma in the following aliquots: 6 x 0.5 ml aliquots (for general use) and 3 x 0.05 ml (for metabolomics and lipidomics analyses) (Note: 0.05 ml aliquots will not be sent to the Central Biorepository)
 3. At least 20 micrograms of DNA (with goal of 50 micrograms) at a target concentration of 100-200 ng/ul (with a minimum of 50 ng/ul)
 4. PBMCs stored in 4 aliquots of 2×10^6 cells followed by multiple aliquots of 5×10^6 cells each
 5. 10 ml of urine in the following aliquots: 10 x 1.0 ml aliquots (for general use) and 3 x 0.1 ml (for metabolomics analyses) (Note: 0.1 ml aliquots will not be sent to the Central Biorepository)
 4. Note: In the instructions below, any aliquots that you prepare for the UDN Metabolomics Core need to be placed in 0.5ml Sarstedt Biosphere® SC Micro Tubes (Sarstedt Catalog # 72.730.217), snap frozen, and shipped to the Core lab within 3-6 months for analysis. Collection of these samples is at your own discretion on a patient to patient basis.
- B) Pediatric Probands (and other probands with limited blood collections): For pediatric probands, the volume of blood drawn should be consistent with the allowable blood collection based on subject body weight. In cases where the blood volume that can be obtained is the limiting factor, samples should be obtained in the following order: EDTA tube (3 ml of blood) for DNA; EDTA tube (3 ml of blood) for plasma (if possible consider obtaining plasma and DNA from the same EDTA tube for pediatric patients. Note if you are collecting plasma and DNA from a single tube you need a 6 ml EDTA); serum separator tube for serum (3 ml of blood); and 4 ml Citrate CPT tube for peripheral blood mononuclear cells (PBMCs).
- C) Sample Collection:
- a. General Sample Collection Issues
 - i. Blood samples for serum and plasma (optional for other sample types) 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 clinical center should record whether blood samples were collected as fasting or non-fasting.
 - b. Blood for PBMCs will 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).

- c. Blood for DNA will 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.
- d. Blood for plasma will be collected in one 10 ml purple top EDTA Vacutainer® tube.
- e. Blood for serum will be collected in one 10 ml Red top Vacutainer® Serum Separator Tubes (SST) with clot activator.
- f. 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 clinical center.

D) Blood Sample Processing:

- a. General Sample Processing Issues
 - i. 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.
 - ii. The standard large serum, plasma, urine, DNA, and PBMC aliquots should be stored in screw-cap cryovials appropriate for ultra-cold storage (Example: Nalgene NUNC 1.8 ml Cryovials, Fisher Scientific Catalog #: 12-565-170N). If you also make additional small aliquots of select samples for the Metabolomics Core these need to be stored in Sarstedt Biosphere® SC Micro 0.5ml Tubes, Sarstedt Catalog # 72.730.217
- b. Serum Sample Processing
 - i. After obtaining the SST sample, allow sample to clot 30 minutes in a vertical position.
 - ii. Follow manual instructions for use of local centrifuge, insuring balance of tubes within the centrifuge.
 - iii. Centrifuge at 2500 RPM or 1000 to 1300 g for 10 minutes either at ambient temperature or with refrigeration to 4°C.
 - iv. Remove Rubber Stopper and remove caps from Cryovial Tubes.
 - v. Aliquot 0.5 ml of serum into 6 cryovial storage tubes, and store samples in a -80°C or liquid nitrogen freezer with appropriate labels (UDN ID number (7 digits), sample type, and collection date).
- c. Plasma Sample Processing
 - i. Spin EDTA Vacutainer® tubes at 2500 RPM or 1000 to 1300 g for 10 minutes at 4°C.
 - ii. Transfer plasma to storage tubes, with six 0.5 ml aliquots and three 0.05 ml aliquots. The three 0.05 ml aliquots are for Metabolomics and Lipidomics, and they should be collected in Sarstedt Biosphere® SC Micro Tube 0.5ml, Sarstedt Catalog # 72.730.217
 - iii. If optional targeted Oxy lipid Analysis will be performed (in coordination with the Metabolomics Core), collect 3 aliquots of plasma (1 mL each) in storage vials that contain 10 µl of anti-oxidant stock per 1 mL plasma; the anti-oxidant stock will be prepared and provided by PNNL and can be stored for 1 month at room temperature. Please note that hemolyzed samples CANNOT be used for Oxy lipid analysis.

- iv. Flash freeze in liquid nitrogen or quick freeze in dry ice/ethanol all samples prior to storage in a -80°C or liquid nitrogen freezer with appropriate labels (UDN ID number, sample type, and collection date).
- E) 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 UDNCB should be labeled with UDN ID number, date of birth, sample type, and collection date.
 - c. DNA aliquots sent to the UDN sequencing cores must be labeled with participant name, date of birth, and “UDN” (do NOT include UDN ID number).
 - 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).
- F) PBMC Isolation and Cryopreservation
- a. Isolation of PBMC – 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.
 - i. 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.
 - ii. Use an aspirating pipet to remove the PBMC layer located at the gel interface in the CPT tube.
 - iii. Place the PBMCs in a new 50 ml conical tube.
 - iv. 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.
 - v. Count the PBMCs on a hemocytometer, cellometer, or other standardized cell counting device.
 - vi. 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.
 - b. Preparation of PBMCs for storage in a cryorepository
 - i. 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.
 - ii. 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).
 - iii. 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).
 - iv. Mix cells by gently tapping the tube; do not use a pipette.
 - v. Incubate resuspended cells at 2-8°C for 10 minutes.
 - vi. Pipette gently to minimize shear force and transfer into a labeled cryopreservation vial.
 - vii. 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).

- vii. 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 number, sample type, and collection date).

G) Urine Sample Processing

- a. Urine samples will be collected from all probands as the first morning void sample and used for clinical laboratory studies and research purposes including metabolomics testing.
- b. After collection, centrifuge urine at 1000 x g for 5 min at 4°C to remove any cells and particulates.
- c. Transfer supernatant to storage tubes, with ten 1.0 ml aliquots in 1.8 ml screw-cap cryovials and three 0.1 ml aliquots in Sarstedt Biosphere® SC Micro 0.5ml Tubes (for metabolomics analyses).
- d. Flash freeze in liquid nitrogen or quick freeze in dry ice/ethanol all samples prior to storage in a -80°C or liquid nitrogen freezer with appropriate labels (UDN ID number, sample type, and collection date).

H) Sample Tracking and Storage

- a. Sample Labeling: Barcode labels of samples to be stored at a local or central biorepository should include the UDN ID number, sample type, and date of sample collection.
- b. Sample Storage:
 - i. Serum, Plasma, and Urine aliquots will be stored at -80°C or colder (e.g., liquid nitrogen).
 - ii. DNA samples will be stored at -20°C or colder.
 - iii. PBMC will be cryopreserved in liquid nitrogen.
 - iv. Metabolomics samples should not be subjected to freeze-thawing.

9. Sample Shipment to the Metabolomics Core

- V. A copy of the metabolomics request form should be sent with each sample/family
 - a. If metabolomics analysis will be performed on more than one family member, all samples should be shipped together with a copy of the metabolomics request form provided by the Metabolomics Core
- VI. Clinical Site provides shipping information to Metabolomics Core via email
 - a. Number and type of samples (plasma, urine, CSF)
 - b. Shipping date and tracking information
- VII. Metabolomics Core acknowledges receipt by sending email(s) to Clinical Site contact(s)
- VIII. If a submitted sample does not pass QC at the Metabolomics Core or is otherwise deemed unacceptable, the Metabolomics Core will contact the Clinical site directly via phone or email to request a replacement.
- IX. Sample labeling discrepancies will be addressed on a case-by-case basis at the discretion of the Metabolomics Core.
 - i.

Recommendations for optional research biospecimen collection

A) Cerebrospinal fluid Collection and Processing

Cerebrospinal fluid (CSF) will be collected from neurological cases for clinical laboratory studies and for research use, including metabolomics, lipidomics, glycomics testing and

luminex inflammome studies. All samples will be collected by lumbar puncture in the L3/L4 or the L4/L5 inter-space.

OPTIONAL If neurotransmitters are to be analyzed, they should be collected first and will pre-empt the possibility of obtaining an opening pressure. Neurotransmitters are volatile and have a cephalo-caudal concentration. They can be collected in the following manner:

Tubes

1. Microtubes 1-5 (3.5 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 3-3.5 ml) - the studies offered by each lab varies though the methods are the same.
 - a. 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.
 - b. Fill each tube to the marked line with the following volumes, indicated in the table below:

Tube Number	Required Volume	The total CSF volume required is 3-3.5 ml
1	0.5 ml	
2	0.5 ml	
3	1.0 ml	
4	1.0 ml	
5	0.5 ml	

- 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.
2. 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, glycomics, and lipidomics analyses), and the remainder in a final aliquot (ex. Sarstedt Polypropylene 2ml tube #72.694.107). The Metabolomics Core requires that it's 3 x 0.2 ml aliquots be in Sarstedt Biosphere[®] SC Micro Tube 0.5ml, Sarstedt Catalog # 72.730.217.
 3. 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).
 4. Polystyrene tube 4: for IgG index, Oligoclonal bands, etc. (Note: use 4th tube from lumbar puncture tray here).

Collection

1. After 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.

2. If CSF is bloody, excessive blood may interfere with neurotransmitter and metabolomics testing

3. Flash freeze in liquid nitrogen or quick freeze in dry ice/ethanol all samples prior to storage in the -80°C freezer with appropriate labels (UDN ID number, sample type, and collection date)

B) Optional Blood Collection and Processing

a. Optional Blood Sample Collection

1. As noted above, blood will be 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.
2. Blood for RNA will be collected in PAXgene blood RNA Vacutainer® tubes (VWR 77776-026).
3. Blood for additional PBMC or other buffy coat collection will be collected in additional CPT (citrate) tubes or other site-specific collection tubes.
4. If platelets are to be collected, draw 31.5 ml blood in 7 tubes of light blue top sodium citrate tubes (BLU).
5. If oxylipids are to be analyzed, then blood should be collected in a chilled EDTA tube and placed on ice.
6. Blood spot cards may also be obtained and stored at room temperature.

b. RNA Processing

1. Use PAXgene Blood RNA Kit (Qiagen 762164)
2. Aliquot in 80ul aliquots and store in 500ul sterile, RNase- DNase-free tubes at -20°C until needed

c. RBC isolation

1. After removal of plasma (see procedures above), discard the remaining supernatant above the porous barrier using a plastic Pasteur pipette (wide orifice)
2. Using a glass Pasteur pipette (narrow orifice), transfer the erythrocyte (RBC) pellet to a 50 ml conical tube
3. Fill 50 ml conical tube to 40 ml with Phosphate Buffered Saline (PBS), pH 7.4 and invert several times to mix
4. Centrifuge for 5min at 1811 x g at 4°C
5. Remove saline layer and discard
6. Repeat wash with PBS pH 7.4 until PBS is clear (minimum of 3 times)
7. Aliquot 1 ml of the erythrocyte (RBC) pellet to clean cryovials. Store in -80°C freezer with appropriate labels (UDN ID number, sample type, and collection date). *Note: this fraction also contains granulocytes*

d. Platelet Isolation

1. Add 1 volume HEP buffer + PGE1
2. Mix very gently by inverting the tube slowly

3. Spin at 100 x g for 15-20 min at room temperature (with no brake applied) to pellet contaminating red and white blood cells
 4. Transfer the supernatant into new plastic tube using a transfer pipet (wide orifice)
 5. Pellet platelets by centrifugation at 800 x g for 15-20 min at room temperature (with no brake applied). Discard the supernatant.
 6. 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)
 7. Store the dry platelet pellet at -80°C freezer with appropriate labels (UDN ID number, sample type, and collection date). *Note: Freezing the pellet will disrupt the platelet granules. This pellet is only to be used for determination of glycomics, lipidomics and proteomics that does not include the platelet granules.*
- e. Oxylipid sample processing
1. Centrifuge chilled blood for 10 min at 1200 x g at 4°C
 2. Aliquot 1 mL plasma into storage vials that contain 10 µl of anti-oxidant stock per 1 mL plasma
 3. Gently mix, 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 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.

Note: The anti-oxidant stock will be prepared and provided by PNNL (see below) and can be stored for 1 month at room temperature

C) Skin Biopsy Collection and Processing

Skin biopsies will be used for culturing fibroblasts. These cell lines will 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.

Items needed: DMEM High Glucose (Invitrogen #11965-118); Fetal Bovine Serum (FBS), Certified, Heat-Inactivated, US Origin (Invitrogen #10082-147); 100X Antibiotic-Antimycotic (Invitrogen #15240-062); 0.25% Trypsin-EDTA (Invitrogen #25200-056); 1 x PBS pH 7.4 w/o Calcium or Magnesium (Invitrogen #10010-023); Dimethyl sulfoxide (Sigma #D8418-100ML)

Collection

1. 3-5mm punch full thickness skin biopsy obtained according to standard medical procedure
2. Place biopsy in sterile tissue culture medium (DMEM, 10% FBS, 1% antimycotic, antibiotic) contained in a 15 ml conical tube
3. Store and transport the biopsy at ambient temperature
4. Deliver the biopsy to the laboratory within 24 hours (Up to 96 hours is acceptable if shipped)

Processing- initiation of skin fibroblast culture

1. Spray hood and scalpels with Ethanol and wipe with Kimwipe
2. Clean the biopsy tube by spraying well with Ethanol before placing in the hood
3. Label 6-well tissue culture (TC) plate and place in TC hood
4. Aspirate medium from biopsy sample using a 2 ml aspirating pipet
5. Remove biopsy sample and place in one well of the 6-well dish
6. 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)
7. Add 1 ml of pre-warmed culture medium (DMEM, 10% FBS, 1% antimycotic, antibiotic) to each well, being careful not to dislodge the biopsy
8. Gently swirl the 6-well dish to coat the wells with culture medium
9. Place in the 37°C, 5% CO₂ TC incubator for 4-5 days to allow the biopsy to attach to the well
10. Gently add 2 ml of fresh, pre-warmed culture medium to each well being careful to not dislodge biopsy sample
11. 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
12. 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.
13. After 2 days, aspirate all but 1 ml of medium from flask.
14. 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 Mycoplasma testing below) on this 1 ml aliquot and record results.
15. Add 10 ml of fresh DMEM to flask and return to 37°C tissue culture incubator to allow the remaining cells to proliferate.
16. Allow samples to reach confluency
17. Remove medium and wash cells with 10 ml PBS
18. 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 number, sample type, collection date, and passage number). Transfer to -150°C for permanent storage (Passage 1 cells).

19. 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 number, sample type, collection date, and passage number). Transfer to -150°C for permanent storage (Passage 2 cells).

Processing- freezing fibroblast cells

1. Prepare 3-4 ml of fresh freezing medium (10% DMSO, 90% FBS) per T75 flask and warm to 37°C.
2. Place the 9 ml fibroblast culture into a sterile 15 ml conical tube.
3. 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.
4. Centrifuge culture at 1000 RPM for 5 minutes.
5. Aspirate off supernatant.
6. Add prepared freezing medium and mix by pipetting up and down.
7. Aliquot 1 ml into labeled cryovial.
8. Place cryovial into Cool-Cell that is labeled with your name and date.
9. Place Cool-Cell into -80°C freezer for 3 days (36 hours) with appropriate labels (UDN ID number, sample type, collection date, amount of cells, and passage number) and then transfer into -150°C storage.

Processing- tissue culture for frozen skin fibroblasts

1. Once the tissue culture medium has warmed, remove cell vial from -150°C and immediately place at 37°C.
2. Prepare a T75 tissue culture flask or 10cm petri dish by adding 10 ml of pre-warmed tissue culture medium.
3. Remove cells from vial using a sterile 1 ml pipet tip or 1 ml pipet.
4. Add cells to flask and gently mix.
5. Place inoculated flask in a 37°C, 5% CO₂ tissue culture incubator for 24 hrs to allow cells to attach to the dish.
6. After 24 hrs, remove medium using a 2 ml aspirating pipet and replace with 10 ml of fresh tissue culture medium. Place at 37°C, 5% CO₂ in tissue culture incubator.

7. Feed cells every 2-3 days by aspirating off old tissue culture medium and replacing with fresh, pre-warmed, tissue culture medium.
8. Once cells have reached confluency, cells must be passaged and split into new T75 culture dishes.

Processing- Detaching and Passaging Cells

1. Warm 0.25% Trypsin EDTA to 37°C.
2. Warm DMEM, 10% FBS, and 1% Anti-Anti to 37°C.
3. Once reagents have warmed, aspirate medium from flask containing cells using a 2 ml aspirating pipet.
4. Rinse cells with 10 ml of 1X PBS pH 7.4 without Calcium or Magnesium.
5. Aspirate 1X PBS using 2 ml aspirating pipet.
6. Add 1 ml of pre-warmed 0.25% Trypsin EDTA to TC flask and spread across the attachment area by swirling the flask.
7. Incubate flask at 37°C for ~ 5 minutes (or until cells are rounding), then gently tap the flask to release the cells.
8. Add 8 ml of pre-warmed tissue culture medium and wash cells to the bottom of the flask.
9. 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.

Mycoplasma testing

1. After 2-3 days of cell growth, aspirate all but ~1 ml of medium from flask.
2. Using a cell scraper, scrape the cells from the bottom of the flask only.
3. Using a 1 ml serological pipet, wash cells using the 1 ml of media in the flask.
4. Remove the 1 ml aliquot and place in a sterile 1.5 ml eppendorf tube.
5. Add fresh pre-warmed DMEM to flask and place in 37°C, 5% CO₂ tissue culture incubator for future use.
6. Perform mycoplasma testing using the Universal ATCC Mycoplasma Testing Kit (ATCC #30-1012K) according to the protocol on the 1 ml aliquot.
7. 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.

XII. Central Biorepository

A. CS Web Access

The UDN Central Biorepository (UDNCB) website will be accessed through the Gateway. Here a CS will be able to submit samples, view samples available, request samples, and obtain information to contact the UDNCB lab for assistance.

I. 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. Shipping can be in batches. The UDNCB will accept samples from ~ 4,500 participants during the total grant period.
 - a. Each CS may submit a total of 432 participants (an average of 3.2 participants/family)
 - b. The UDP site may submit a total of 1,920 participants (an average of 3.2 participants/family)
3. CS enters the sample information into the Sample Submission form (must be included with shipment). Until website is available: CS will also email Sample Submission form to the UDNCB at UDNCB@vanderbilt.edu (form includes FedEx tracking number).
4. CS prepares the samples for shipment on dry ice, FedEx priority overnight (Mon-Weds only), include printed copy of sample submission form (see 3). No shipping during a holiday week.
5. Triple pack your samples, select an appropriate dry ice safe shipping box and enough dry ice to last 48 hrs 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 (ex. 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)
6. Ship samples to: Lynette Rives, Vanderbilt Medical Center, DD-2205 MCN, Nashville TN 37232-2578, Phone: 615-875-7198
7. UDNCB will notify CS when samples have safely arrived.
8. Website is still in development. More details on use of website and sample submission will be available once website is established. Remember, until the website is available, the Sample Submission form must be emailed to the UDNCB (in addition to being included with shipped samples).
9. Questions about preparing samples to ship, sample submission form, etc? Email UDNCB@vanderbilt.edu or phone 615-875-7098.

II. Viewing Samples Available

Website is still in development but inventory will be viewable/searchable. Until that time, we will make an excel spreadsheet of all submitted inventory available upon request.

III. Requesting Samples

Prior Approval: Samples stored by the UDNCB are available to UDN investigators and their collaborators. However, quantities are limited. All sample requests will require prior approval from the UDN.

1. CS obtains prior approval from UDN.
2. CS enters sample information into the Sample Request Form (available on website).
3. UDNCB contact investigator to arrange a time to ship samples.
4. CS emails UDNCB that samples have arrived.

B. UDNCB Procedures

I. Storing and archiving of biological specimens

Mailed samples will be opened in a clean “no amplified DNA” laboratory. The frozen samples, pre-aliquoted in screw cap cryotubes and labeled by the sender, will be placed on dry ice while the labels are checked against the Sample Submission Form accompanying the package for confirmation. This form will be available for download on the Gateway and it will be required to be included with all samples shipped to the biorepository. The Sample Submission Form will contain the participant UDN ID #s (also printed on the sample tubes). All tubes will 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 of the Manual of Operations must still be typed legibly on the sample labels.

Storage of Samples: The biological samples will be placed into liquid nitrogen cryotanks (PBMCs and possibly fibroblasts) and -80° 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 will be 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 will be 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 will also be recorded into the Progeny LIMS database. The Sample Submission Forms will be completed by the CS submitting the samples and included with the samples when they are shipped. No patient names or identifying

information is recorded in the Progeny LIMS, the sample intake notebook, or requested on the Sample Submission Forms.

II. Retrieval and Shipping of Biological Specimens

The UDNCB will retrieve biological samples from liquid nitrogen &/or -80° freezers, package samples in dry ice, and ship to UDN investigators and collaborators. Sample information in Progeny LIMS will be used to track quantities and distribution of biological samples.

Locating Biological Specimens in Storage: The Progeny LIMS database will contain 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 will keep track of original and current quantities of the biological samples and record the distribution of samples to investigators.

Sample Retrieval and Transfer: The UDN must approve Sample Requests prior to application to the UDNCB. After UDN approval and receipt of a Sample Request Form the UDNCB will contact the CS or CS designated investigator requesting the samples by email to pre-arrange a date for shipment. The samples will then be 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 will be recorded in the Progeny LIMS database. The Biorepository Sample Inventory on the Gateway will also be updated so that all UDN investigators can log in and 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, will be 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. We will ship frozen samples in EPS foam containers (1.5 inch minimum thickness) with corrugated cartons, 10 lbs dry ice by FedEx priority overnight (Monday–Weds). 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 hrs).

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 will be included with shipment and the CS and CS designated investigator(s) will be notified by email that the sample has shipped, given the FedEx tracking number, and an electronic copy of the sample sheet. The email will request that the UDNCB be notified upon receipt of the shipment and that we be notified of any problems with the samples (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. We have budgeted for a total of 150 shipments over 3 years, as directed by the NIH, with 36 shipments in the first year and 57 in each subsequent 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 (estimated to be \$175/request).

Quality Control: The UDNCB will keep records on the number of samples received, their condition, date shipped/date arrived, etc. We will also keep records on the number of samples we ship out, date shipped/date received, condition upon arrival, any problems reported by the recipient, etc. This information will be compiled into a quality control report, along with total samples received and shipped, and presented to the Steering Committee 3 times per year.

III. Updating UDNCB Website Inventory

Samples collected by the CS and entered into the UDNCB Sample Submission Form prior to shipping are automatically entered into the Sample Inventory and will be able to be viewed in the Gateway. When samples are requested and shipped out through the UDNCB, the repository will edit/update the Sample Inventory on the Gateway.

XIII. Metabolomics

A. Introduction

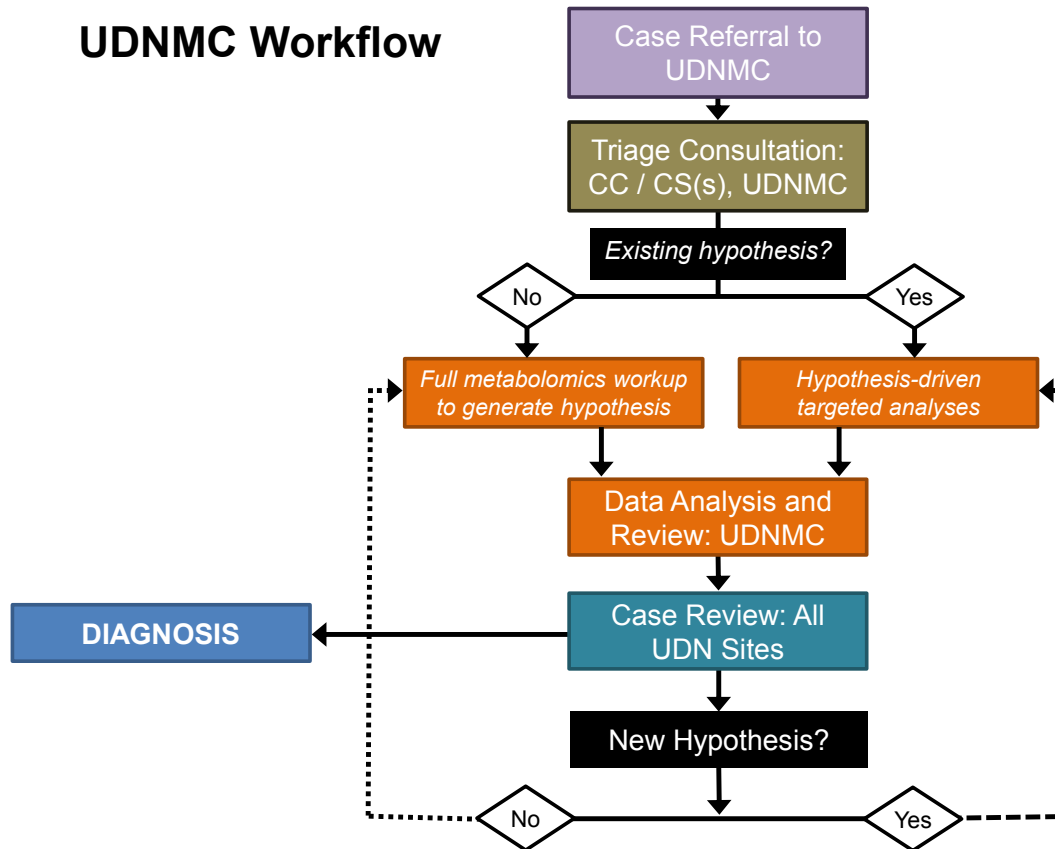
The UDN Metabolomics Core (MC) at Pacific Northwest National Laboratory and Oregon Health & Science University will provide metabolomics and experimental design consultation, laboratory analysis, and bioinformatics/computational expertise to the UDN. More specifically, the MC will support the objectives of the UDN by:

1. Performing comprehensive untargeted and targeted 'omics measurements to identify quantitative and/or qualitative changes in metabolite and lipid abundances.
2. Performing structural characterization of novel metabolites or lipids identified in patients with rare and undiagnosed diseases.
3. Integrating metabolomics and lipidomics and genomics data with patient clinical phenotypes to provide mechanistic insight and aid diagnoses of rare and undiagnosed diseases.

B. General Workflow

If before, during, or after a UDN evaluation, a CS decides that a proband would benefit from one or more of the 'omics analyses available at the MC, then the CS will complete a metabolomics request form (Appendix 22) and submit it to the MC via email. Once a metabolomics request form is submitted, the Metabolomics Case Review Committee will review the case with the referring CS. The Metabolomics Case Review Committee will include representatives from the MC and each CS. The Metabolomics Case Review Committee will generate hypotheses regarding the pathophysiology/etiology of the proband's symptoms and determine the appropriate metabolomics analyses. If no hypothesis is generated, untargeted metabolomics and/or lipidomics analyses will be performed. The resultant data will initially be analyzed and reviewed by the MC, and if necessary, the data will be reviewed by the Metabolomics Case Review Committee and referring 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. The following figure outlines this workflow:

UDNMC Workflow



C. Requesting Metabolomics Analyses

A Metabolomics Request Form (see Appendix 22) will be completed by the requesting CS. The metabolomics request form will serve two purposes, 1) to alert the MC of the request for services, 2) to provide a high-level summary of the clinical phenotype of the proband and other affected family members.

1. CS completes a metabolomics request form for the proband
2. CS completes a metabolomics request form for each additional family member to be evaluated
3. CS submits request form to MC via email

Metabolomics Case Review

1. Requests for metabolomics analysis will initially be reviewed by the MC
2. Request for metabolomics analysis will then be discussed by the Metabolomics Case Review Committee

- a. Primary clinician from referring CS will present a brief summary of the proband's phenotype and genotype information (if available) , and any diagnostic hypotheses
 - b. The Metabolomics Case Review Committee will make recommendations regarding specific analyses to be performed
3. MC and primary clinician from referring CS will agree on analyses to be performed

D. Flow of Samples to the Metabolomics Core

Sample Collection: As outlined in the Biospecimens section of this manual (Section XI), specimens for metabolomics analysis should be collected on all probands evaluated in person, unless doing so would compromise participant safety, or they are refused by the proband.

1. General Sample Collection Guidelines:
 - a. Blood samples for metabolomics analysis 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.
 - b. Processing of blood for plasma, as well as urine, should be performed as quickly as possible and on wet ice or at 4°C, as appropriate, to minimize artifacts in metabolomics data.
2. Plasma Sample Collection and Processing
 - a. Blood for plasma should be collected in a purple top EDTA Vacutainer® tube.
 - b. Spin EDTA Vacutainer® tubes at 2500 RPM (1-1.3 x 10³ g) for 10 minutes at 4°C.
 - c. Aliquot plasma to storage tubes (Sarstedt Biosphere® SC Micro Tube 0.5ml, Sarstedt Catalog # 72.730.217).
 - i. For Metabolomics and Lipidomics analyses: 3 aliquots of plasma (0.05 ml each)
 - ii. For targeted Oxylipid Analysis
 1. 3 aliquots of plasma (1 mL each) in storage vials that contain 10 µl of anti-oxidant stock per 1 mL plasma; the anti-oxidant stock will be prepared and provided by PNNL and can be stored for 1 month at room temperature
 2. Hemolysed samples CANNOT be used for Oxylipid analysis
 - ii. Flash freeze all samples in liquid nitrogen or quick freeze in dry ice/ethanol prior to storage in a -70°C freezer, or in liquid nitrogen, with appropriate labels (UDN ID number, sample type, and collection date)
3. Urine Sample Collection and Processing
 - a. Urine should be the first morning void, collected in a polypropylene container.
 - b. Centrifuge urine at 1,000 x g for 5 min at 4°C to remove any cells and particulates.
 - c. Transfer supernatant to storage tubes (Sarstedt Biosphere® SC Micro Tube 0.5ml, Sarstedt Catalog # 72.730.217)
 - i. For metabolomics analyses: 3 x 0.1 ml aliquots
 - d. Flash freeze urine samples in liquid nitrogen or quick freeze in dry ice/ethanol prior to storage in a -70°C freezer, or in liquid nitrogen, with appropriate labels (UDN ID number, sample type, and collection date)
4. CSF Sample Collection and Processing
 - a. After CSF is collected by lumbar puncture, place the CSF on wet ice immediately and transport to the laboratory
 - b. If CSF is bloody, excessive blood may interfere with metabolomics testing

- c. Transfer CSF to storage tubes (Sarstedt Biosphere® SC Micro Tube 0.5ml, Sarstedt Catalog # 72.730.217)
 - i. For metabolomics analyses and lipidomics analyses: 3 x 0.2 ml aliquots
 - d. Flash freeze in liquid nitrogen or quick freeze in dry ice/ethanol.
- 5. Sample Storage
 - a. Store all samples prior to shipping in a -70°C freezer, or in liquid nitrogen with appropriate labels (UDN ID number, sample type, and collection date)
 - b. Samples should not be subjected to freeze-thawing

Sample Shipping: The CSs will store samples in a -70°C freezer, or in liquid nitrogen prior to shipment.

- 1. A copy of the metabolomics request form should be sent with each sample/family
 - a. If metabolomics analysis will be performed on more than one family member, all samples should be shipped together with a copy of the metabolomics request form provided by the MC
- 2. CS ships samples frozen and on dry ice to MC
 - a. Samples should be shipped Monday – Wednesday and not during or adjacent to holidays.
- 3. CS provides shipping information to MC via email
 - a. Number and type of samples (plasma, urine, CSF)
 - b. Shipping date and tracking information
- 4. MC acknowledges receipt by sending email(s) to CS contact(s)
- 5. If a submitted sample does not pass QC at the MC or is otherwise deemed unacceptable, the MC will contact the CS site directly via phone or email to request a replacement.
- 6. Sample labeling discrepancies will be addressed on a case-by-case basis at the discretion of the MC.

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

Following submission of the metabolomics request form containing a high-level summary of the clinical phenotype of the proband and other affected family members by the CS, the MC will review additional clinical and sequencing information available in the Gateway. The MC will contact the CS if more information is required. The CS will notify the MC via email if new information becomes available after the metabolomics request form is submitted.

F. Metabolomics, Lipidomics, and Oxy lipid Analyses

Metabolomics and Lipidomics – Pacific Northwest National Laboratory

This section covers sample intake, preparation, and analysis at Pacific Northwest National Laboratory (PNNL). This section will describe the sample flow from plasma, urine, or cerebrospinal fluid (CSF) sample receipt to production of metabolomics and lipidomics, including appropriate quality control and assurance procedures.

Sample Intake

Plasma, urine, or CSF samples are received at the Biological Sciences Facility (BSF). A visual inspection of the sample tubes is conducted to ensure that sample tube integrity has not been compromised. Sample tube label is compared with information in the sample submission form to ensure consistency with and completeness of the Gateway data, and proper sample labeling. Samples are then stored at -70°C. 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, PNNL will notify the referring CS.

Once accepted, samples are accessioned into the PNNL Laboratory Information Management System (LIMS), incorporating participant UDN ID and sample type.

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. 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, PNNL staff will notify the referring CS. Any unusual findings are also recorded in the LIMS.

Sample processing

Samples will be batched and processed according to which analysis is requested. For metabolomics or lipidomics analyses in plasma and CSF, a chloroform/methanol extraction will be used to isolate metabolites and lipids. For metabolomics analyses in urine, a methanol extraction will be used to isolate metabolites. Pooled QC samples will be included in all sample batches. Internal reference standards will be added to participant and QC samples. Details of these extraction protocols are given below.

Extraction of metabolites and lipids from plasma and CSF

For plasma, 50 µL is prepared. For CSF, 200 µL is prepared. Samples are transferred to Sorenson low-binding microcentrifuge tubes to which cold (-20°C) chloroform/methanol (2:1, v/v) will be added in a 5-fold excess to the sample volume. Samples are vortexed for 10 s to facilitate mixing of samples and solvent, allowed to sit at 4°C for 5 minutes, and then vortexed again for 10 s. Then, samples are centrifuged to facilitate separation of a hydrophilic layer containing polar metabolites and a hydrophobic layer containing lipids. The two liquid layers are removed and placed into new microcentrifuge tubes and evaporated to dryness in vacuo. Metabolite extracts are stored dry at -20°C until chemical derivatization (see below). Lipid extracts are stored in methanol at -20°C for short term storage or in chloroform/methanol (2:1, v/v) for longer storage.

Extraction of metabolites from urine

For urine, 100 µL is prepared. Samples are transferred to Sorenson low-binding microcentrifuge tubes to which 100 µL of a 1 mg/mL solution of urease prepared in water is added. The samples will be incubated for 30 min at 37°C with mild shaking to deplete urea. Metabolites are then extracted with concomitant protein precipitation by addition of 1 mL of cold (-20°C) methanol with vortexing for 30 s, and precipitated proteins are isolated by centrifugation. The

supernatants will be transferred to glass autosampler vials and then dried in vacuo. Metabolite extracts are stored dry at -20°C until chemical derivatization (see below).

Chemical derivatization of metabolites

Polar metabolites will be chemically derivatized prior to metabolomics analysis. To protect carbonyl groups and reduce the number of tautomeric isomers, 20 µL of methoxyamine in pyridine (30 mg/mL) is added to each sample, followed by vortexing for 30 s and incubation at 37°C with generous shaking for 90 min. At this point, the sample vials are inverted one time to capture any condensation of solvent at the cap surface, followed by a brief centrifugation. To derivatize hydroxyl and amine groups to trimethylsilylated (TMS) forms, 80 µL of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) will be added to each vial, followed by vortexing for 10 s and incubation at 37°C with shaking for 30 min. Again, the sample vials are inverted one time, followed by centrifugation. The samples are allowed to cool to room temperature and are analyzed that day.

Sample analysis

Samples are analyzed using gas chromatography-mass spectrometry (GC-MS) for metabolomics and liquid chromatography-mass spectrometry (LC-MS) for lipidomics.

GC-MS-based metabolomics analyses

An Agilent GC 7890A coupled with a single quadrupole MSD 5975C is used to analyze chemically derivatized metabolites. The samples are batched and analyzed in random order. A HP-5MS column (30 m × 0.25 mm × 0.25 µm; Agilent Technologies, Inc) is used for untargeted metabolomics analyses. The sample injection mode is splitless and 1 µL of each sample is injected. The injection port temperature is held at 250°C throughout the analysis. The GC oven is held at 60°C for 1 min after injection and the temperature is increased to 325°C by 10°C/min, followed by a 5 min hold at 325°C. The helium gas flow rates are determined by the Agilent Retention Time Locking function based on analysis of deuterated myristic acid. Data will be collected over the mass range 50 - 550 *m/z*. A mixture of fatty acid methyl esters (FAMES; C8-C28) are analyzed once per day together with the samples for retention index alignment purposes during subsequent data analysis.

LC-MS-based lipidomics analyses

Lipid extracts are dried in vacuo and reconstituted in methanol if stored in chloroform/methanol, or injected directly on to the LC platform if stored in methanol. A Waters NanoAquity UPLC system interfaced with a Velos-ETD Orbitrap mass spectrometer is used for LC-ESI-MS/MS analyses. A Waters HSS T3 column (1.0 mm x 150 mm x 1.8 µm particle size) is used to separate lipid molecular species over a 90 min gradient (mobile phase A: ACN/H₂O (40:60) containing 10 mM ammonium acetate; mobile phase B: ACN/IPA (10:90) containing 10 mM ammonium acetate) at a flow rate of 30 µL/min. Eluting lipids are introduced to the MS via electrospray ionization in both positive and negative modes, and lipids are fragmented using HCD (higher-energy collision dissociation) and CID (collision-induced dissociation) to obtain high coverage of the lipidome.

Data processing

Metabolomics and lipidomics raw data will be processed using open source, in-house developed, and commercial software tools to align data and identify detected molecules.

GC-MS-based metabolomics data processing

GC-MS raw data files are processed using the Metabolite Detector software, version 2.0.6 beta. Briefly, Agilent .D files are converted to netCDF format using Agilent Chemstation, followed by conversion to binary files using Metabolite Detector. Retention indices (RI) of detected metabolites are calculated based on the analysis of the FAMES mixture, followed by their chromatographic alignment across all analyses after deconvolution. Metabolites are identified by matching experimental spectra to an augmented version of the Agilent Fiehn Metabolomics Retention Time Locked (RTL) Library, containing spectra and validated retention indices for over 700 metabolites, using a Metabolite Detector match probability threshold of 0.6 (combined retention index and spectral probability). All metabolite identifications are manually validated to reduce deconvolution errors during automated data-processing and to eliminate false identifications. The NIST 08 GC-MS library is also used to cross validate the spectral matching scores obtained using the Agilent library and to provide identifications of unmatched metabolites. The three most abundant fragment ions in the spectra of each identified metabolite are automatically determined by Metabolite Detector, and their summed abundances are integrated across the GC elution profile; fragment ions due to trimethylsilylation (i.e. m/z 73 and 147) are excluded from the determination of metabolite abundance. A matrix of identified metabolites, unidentified metabolite features (characterized by mass spectra and retention indices and assigned as 'unknown'), and their abundances are created for statistical analysis. Features resulting from GC column bleeding are removed from the data matrices.

LC-MS-based lipidomics data processing

LC-MS-based lipidomics data will be processed using a series of in-house developed software tools that function to: 1) deisotope raw MS data to give the monoisotopic mass, charge state, and intensity of the major peaks in each mass spectrum; 2) identify groups of mass spectral peaks that are observed in sequential spectra using an algorithm that computes a Euclidean distance in n -dimensional space for combinations of peaks; 3) chromatographically align data from multiple LC-MS analyses, and 4) identify lipid molecular species using a combination of lipid accurate mass and fragmentation information. LC-MS/MS lipidomics data are analyzed using the in-house developed software LIQUID (Lipid Informed Quantitation and Identification). For global data analysis, the software iterates over each MS/MS spectrum to find the best lipid match and presents a graphical display of all observed and theoretical lines of evidence used for identification. The primary evidence shown is a stem plot of the MS/MS spectra including colors and labels for peaks that match to fragments of the identified lipid. A stem plot of the isotopic profile and a line plot of the extracted ion chromatogram are also provided to show the MS-level evidence of the identified lipid. Intensity, mass measurement error, and elution time are also provided. A matrix of identified lipids, unidentified lipid features (characterized by m/z and retention times and assigned as 'unknown'), and their abundances are created for statistical analysis.

Metabolomics and lipidomics

The data matrices from all data types will be \log_{10} transformed and processed similarly to remove metabolites or lipids with inadequate information for statistical analyses and to identify outlier omics analyses. For each analysis, identified and unidentified features are filtered from the dataset if they do not meet the minimum requirements for a quantitative statistical test, such as t-test or Analysis of Variance (ANOVA), and qualitative test (G-test). Extreme behavior in datasets (outliers) are identified using a combination of correlation, principal component analysis (PCA), and an approach based on a robust Mahalanobis distance (rMd) to assess the reproducibility of the distribution of abundance values across biological samples. Strategies for

normalization of the identified and unidentified feature abundances in each data matrix will be evaluated using the Statistical Procedure for the Analyses of Normalization Strategies (SPANS) protocol.

Oxylipid Analysis – Bioanalytical Shared Resource/Pharmacokinetics (BSR/PK) Core, Oregon Health & Science University

This section covers sample intake, preparation, and analysis at the BSR/PK at Oregon Health & Science University (OHSU).

Sample Intake

Plasma samples shipped on dry ice are received by the BSR/PK Core staff. Vials are removed and visually inspected to ensure that sample tube integrity has not been compromised. The sample tube label is compared with information on the metabolomics request form and assigned a BSR/PK Core sample number for the analysis. Samples are then stored at -70°C. 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, the BSR/PK staff will notify the referring CS.

Sample QC

Prior to processing, samples are thawed and inspected for unusual characteristics, such as hemolysis, precipitation, discoloration and appropriate volume. All findings will be documented for each sample and entered into the master sample excel sheet. If there is extensive hemolysis the CS will be asked to confirm they want to proceed with analysis.

Sample processing and data analysis

Due to analyte instability, samples need to be processed within 30 days of the initial sample collection. Where possible, samples will be batched into a single processing run. If that is not possible, they will be prepared as single samples.

After thawing on ice, samples are centrifuged to clarify. The samples are then spiked with an internal standard mix that contains 1 ng each of d₈-15 HETE, d₆-20 HETE, d₁₁-14,15 DHET, d₈-14,15 EET, d₄-PGE₂, d₄-9 HODE, d₄-IsoP_{III}, d₄-IsoP_{VI}, d₄-PGF₂α, and d₄-anandamide, as well as 20 μL of anti-oxidant mix (0.2 mg/ml BHT, 0.2 mg/ml EDTA, 2 mg/ml triphenyl phosphine and 2 mg/ml indomethacin prepared in 2:1:1 methanol:ethanol:water, v/v/v). The samples are then diluted with 0.3 mL of methanol and 0.7 mL of water. The samples are gently mixed and then centrifuged at room temperature to remove any particulates. Oasis HLB 60 mg solid phase extraction columns are used for sample purification. The columns are equilibrated with 2 mL of methanol, followed by 3 mL of water. The samples are then loaded and allowed to flow through by gravity. The columns are washed with 3 mL of 10% methanol, and then dried for 15 min under vacuum (~15 mmHg). Oxylipids are then eluted with 2 mL of methanol, followed by 2 mL of ethyl acetate, followed by 2 mL of 50:50 hexane:ethyl acetate, followed finally by 1 mL of hexane, and then completed by a brief application of vacuum. The combined eluates are dried under reduced pressure until just barely dry – approximately 35 min at 35°C. The tubes are then stored at -80°C until analyzed by LC-MS/MS. Immediately before analysis, the tubes are rinsed with 1 mL of hexane and the hexane dried for approximately 7 min under reduced pressure at

35°C. The residue is dissolved in 50 µL of 70:30 water:acetonitrile, filtered through a 0.22 µm PTFE spin filter and 10 µL injected for analysis..

Sample Analysis

Oxylipids are analyzed using a LC coupled with an ABSciex Q5500 hybrid linear ion trap triple quadrupole MS. The LC eluent is introduced into the instrument using electrospray ionization (ESI) in the negative mode with the following settings: source voltage -4000, temperature 450, GS1 40, GS2 40, CUR 40, and EP -10. The LC mobile phase consists of Solvent A: 0.05% acetic acid in water and Solvent B: 0.05% acetic acid in 75% acetonitrile:25% methanol prepared before every run. The column is a Thermo Scientific BetaBasic-18 (100 x 2.1, 3µ). The gradient mobile phase is delivered at a flow rate of 0.5 mL/min. Initial solvent B percentage is 30%, and it increases linearly from 30% to 40% over the first minute, then increases from 40% to 45% over the next 5 minutes. Solvent B increases again from 45% to 60 percent over the next 5 minutes, then from 60% to 62.5% over the next 7 minutes, then increases to 95% over the next 5 minutes. The flow rate is held at 95% for 2 minutes before being dropped to 30% over a half minute and then held at 30% to re-equilibrate for 5.5 minutes. The LC column is kept at 40°C using a column oven. Oxylipids are identified and quantified using isotope dilution and LC-MS/MS in MRM mode. Pure analytes and internal standards were infused into the instrument to obtain optimal instrument parameters including the declustering potential, the collision energy and exit potential. Ions are selected from the product ion scans and fragmentation optimized for each and source parameters optimized for the LC conditions. Standard curves are generated for each oxylipid in PBS and the slopes are identical to extracted curves without the positive intercept observed with spiked plasma. The instrument is controlled and data obtained and analyzed using Analyst 1.5.1 and Multiquant 3.0. Limits of quantification for representative oxylipids are 25 pg/mL for 11,12 EET, 25 pg/mL for 20 HETE, 25 pg/mL for 17,18 EpETE, and 50 pg/mL for 19,20 EpDPE.

G. Analysis

Overview

The steps in the analysis of metabolomics, lipidomics, and oxylipid data can generally be divided into 3 phases: statistical analysis, data integration, and identification of disease-related pathways and signaling networks.

Statistical analysis

There are two approaches to determining statistically relevant features. If the features follow the standard normal distribution, then standard ANOVA methods are used. If the features do not follow the standard normal distribution, a non-parametric version, such as Kruskal-Wallis or sum-rank test, is used. To evaluate the statistical relevance of missing data, for example when the data is absent in the background data sets and present in the participant data, a G-test will be used. However, with multiple comparisons comes the problem of increased likelihood of a significant result by random chance. To counter this problem, the Dunn-Sidak or Bonferroni correction will be utilized to adjust p-values. If the data have a linear relationship, then partial least squares discriminant analysis (PLS-DA) will be used to rank the metabolites and lipids by p-value in a pair-wise comparison to the background data sets.

Data integration

Traditional classification methods work well for individual data streams. However, they do not integrate diverse data because each data stream requires a different classification algorithm for maximum predictive group accuracy. Therefore, statistical classification algorithms coupled with a Bayesian model integration method that identifies the contribution of each data type to overall probabilities of classification accuracy will be used for quantitative integration of MS-based omics data. The approach for integrating clinical phenotyping data with model organism and protein interaction data for prioritizing variants is complementary to the omics data classification. Cross validation approaches are used to select the features that most strongly contribute to the predicted class (e.g. disease phenotype, genotype) for each data type. A combination of features from several different technologies will likely be more predictive of ‘treatment’ effects than any single data type alone using this method. The molecular features (e.g. metabolite, lipid, gene) that contribute to the overall model accuracy are rank ordered, regardless of whether a molecular identity is known. This strategy may be modified in the context of undiagnosed diseases due to the very small sample sizes that will be encountered as a result of evaluating phenotypes restricted to single families. Cluster-based approaches (e.g. hierarchical, k-means) are used on the molecular data types to identify significant groupings in the data. Permutation-based statistics are used to map these clusters to signatures of phenotypes as defined by the Monarch platform.

Identification of disease-related pathways and signaling networks

Metabolites with molecular identifications will be integrated with existing pathway tools, reaction databases, and the integrated corpus of genotype-phenotype data within the Monarch platform for biological interpretation of disease etiology and biomarker signatures. Methods to reconstruct signaling networks describing metabolic changes in the context of protein-protein interactions and integrated genomics data will also be used. In order to determine the key biological pathways that may be perturbed in the presence of measured changes in the metabolome and lipidome the statistically determined sets of identified metabolites will be overlaid onto pathway and reaction databases where functional annotation is known. An integrated corpus of KEGG, Reactome, and HumanCyc databases with EC numbers or SwissProt IDs will be used. Integrated data will be made available for computation in the public Monarch neo4J graph database, SciGraph (<https://github.com/SciCrunch/SciGraph>). SciGraph includes provisions for maximal query utility across the integrated data – such as standard queries for gene, metabolite, ortholog, organisms, and source, but also much more sophisticated queries that leverage subgraph comparisons to identify the most likely biological pathways in which metabolites are involved. Where DNA sequencing data is available to integrate with metabolomics and lipidomics data, a combination of the Monarch Exomiser tool and SciGraph queries described above will be used, or the DAVID bioinformatics web portal - both approaches utilize several different annotation databases simultaneously and perform functional annotation clustering to group annotation categories by membership with statistical metrics of enrichment. Data types will be integrated to identify sets of biomolecules that correspond to the same networks, presenting a weight of evidence approach that said pathways are significantly perturbed by the disease process. Metabolite biomarkers will also be prioritized according to their known mechanism of action in disease pathogenesis, taking advantage of the cross-species data stores available within Monarch.

H. Return of Results of Metabolomics Analyses

The metabolomics data will initially be processed and reviewed by the MC, and subsequently returned to the referring CS via email. If necessary, the data will also be 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 will be undertaken based on new hypotheses generated via discussion between the Metabolomics Case Review Committee and referring CS.

Format of metabolomics results report

1. Summary of key findings
 - a. Metabolomics
 - b. Lipidomics
 - c. Oxylipids
2. Primary diagnostic considerations
3. Spread sheets of output data (see Figure 1)

Metabolite	KEGG	CAS	PubChem	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
1,5-anhydrohexitol*	not found	154-48-5	219984	5098978.4	3317542.6	5143193.1	4500130.2	3757596.4	4936321.3
2,3-dihydroxybutanoic acid*	not found	1518-62-3	192742	1382803.0	583266.8	1137075.3	618951.7	651166.1	797528.7
2-hydroxybutyric acid	C05984	600-15-7	11266	958590.0	644536.0	1062866.6	492711.5	581209.0	604425.9
3,4-dihydroxybutanoic acid*	C10833	1443-76-1	10742	79536.3	4753.2	60380.4	22144.7	14384.8	12307.8
3-hydroxybutyric acid	not found	300-85-6	441	331813.8	112207.3	324314.1	200807.9	173687.1	213907.9
3-phosphoglyceric acid	C00597	3443-58-1	724	104312.8	17735.4	173978.4	126185.4	71361.3	12416.6
alpha ketoglutaric acid	C00026	17091-15-5	51	342294.2	69001.7	539339.0	324638.5	263233.5	387701.0
alpha-hydroxyisovaleric acid*	not found	17407-55-5	99823	440149.0	208393.4	390610.2	563918.6	310641.8	251832.9
capric acid	C01571	111-27-3	2969	549021.8	59215.9	527871.8	201097.5	242959.0	104409.1
caprylic acid	C06423	124-07-2	379	250430.4	256446.9	344759.5	164024.9	275588.6	219791.7
carbonate ion	C01353	144-55-8	22639876	19134541.9	9467804.8	19589625.8	21001927.3	10678799.5	24385863.3
citric acid	C00158	12262-73-6	19782904	1496799.3	1080370.2	2506567.6	1864002.6	887105.6	2155784.2
creatinine	C00791	60-27-5	588	59681.1	6490.0	26029.7	49059.9	4821.0	43795.8
dehydroalanine (possibly from cysteine)	C02218	1948-56-7	123991	328106.6	18550.4	46583.0	64479.3	25931.6	44886.8
D-glucose	C00031	2280-44-6	5793	13203712.3	12382028.9	20419232.9	18225525.2	15478458.6	19564933.1
D-malic acid	C00497	6283-27-8	92824	128226.1	28148.7	255858.1	186997.6	55727.9	124479.4
D-maltose	C01971	133-99-3	6255	73831.3	70902.3	65011.7	104188.2	51359.0	165011.1
D-mannose	C00159	147-74-0	18950	252719.5	91348.1	113996.8	156205.9	197023.6	271538.9
D-ribose	C00121	24259-59-4	5779	340007.7	259022.4	72677.1	185242.7	164739.1	254049.5
D-sedoheptulose	not found	3019-74-7	5459879	7777557.4	6133462.2	15663664.8	12222851.7	15268102.5	16826447.5
D-threitol	C16884	2418-52-2	169019	803449.7	148628.9	252568.7	656278.9	219578.9	328717.9
EDTA	C00284	139-33-3	5232305	51492565.8	35978825.2	41533050.9	25846672.8	17125642.3	84139692.7
formylglycine*	not found	2491-15-8	75606	660903.1	595403.7	910077.0	415284.0	547109.0	385483.0
fructose	C00095	57-48-7	439163	3112797.1	603491.1	3088310.0	4908239.5	525770.9	924281.4
fumaric acid	C00122	110-16-7	21883788	79726.6	95374.2	160754.0	91451.6	88712.4	126839.1
glyceric acid	C00258	473-81-4	439194	365950.4	139873.2	320302.2	219867.0	158642.4	278861.1
glycerol	C00116	25618-55-7	753	16716104.2	12681486.6	15303876.8	16388251.7	16154121.4	21761724.3
glycine	C00037	112898-03-0	5257127	6115626.4	3457832.2	5812929.5	5603209.5	4470356.5	7600203.2
glycolic acid	C00160	1033720-48-7	3698251	52245.2	49312.0	77245.4	38885.7	46207.1	55305.9
hypoxanthine	C00262	146469-94-5	790	25870.0	29055.1	0.0	53058.2	132208.7	0.0
iminodiacetic acid	C19911	142-73-4	8897	1468343.2	1349956.3	1284695.2	1208729.9	1318160.9	728160.6
L-(+) lactic acid	C00186	1715-99-7	107689	30132825.3	26607224.2	40486911.0	43725453.7	45578741.1	42218218.6
L-alanine	C00041	115967-49-2	5950	8016365.7	3876145.8	9932099.8	5823638.1	5007088.7	9924360.9
L-asparagine	C00152	3130-87-8	6267	6160.5	4321.6	6890.6	27653.4	25767.0	40087.9
L-cysteine	C00097	4371-52-2	5862	164875.6	88251.4	106928.4	149788.6	55780.0	84133.2
L-cystine	C00491	24645-67-8	67678	412915.6	283493.5	398942.5	416830.4	138284.5	383301.4
L-glutamic acid	C00025	56-86-0	33032	1036824.0	445926.9	883096.9	1430834.5	681282.1	1036117.1
L-glutamine	C00064	32640-56-5	5961	3145983.9	1820278.9	3983462.2	3273861.1	2285053.3	4753457.4
L-isoleucine	C00407	24629-25-2	6306	1814139.7	509866.5	2502803.8	1488382.5	1454731.3	2585024.7

Example matrix of processed metabolomics data showing metabolite common name, database annotations, and measured abundances.

XIV. Institutional Review Board (IRB) Communications

1. Protocol Development

a. Consent forms

i. Templates

- 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.
- The CC CIRB Liaison will make the template ICFs available to the CS Site Coordinators.
- The CS Site Coordinators will customize only the areas of the ICFs specified in the template, including:
 - Placing the consent form on the institutional letterhead
 - Adding standardized language as required by the CS (due to local policy requirements)
 - 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.
- The CS Site Coordinators will send the completed site specific ICFs to the CC CIRB Liaison.
- The CC CIRB Liaison will review the ICFs and send them to the UDN PI to submit to the CIRB.
- The CIRB will review the site specific ICFs with all of the other submitted site materials provided for site approval.
- The CIRB will communicate the results of the review to the UDN PI, the CC CIRB Liaison, the local PIs, and the Institutional Designees.
- The CC CIRB Liaison will communicate the results of the review to the CS Site Coordinators.
- The CIRB will provide to the CC CIRB Liaison the approved ICFs for each CS. ICFs will have an expiration date as indicated on the last page of each form.
- 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.

b. Investigator documentation

- Investigator documentation includes:
 - A roster of investigators who will be included on the protocol, a roster of non-investigator research staff, 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.
 - Documentation of the local conflict of interest (COI) review for all investigators on the protocol indicating whether there are any unmitigated or existing conflicts.
 - Information about the UDN site's local research context as relevant to the site's role in the protocol.
- The CC CIRB Liaison will send requests for documentation to the CS and Core

Site Coordinators.

- The CS and Core Site Coordinators will send completed documentation to the CC CIRB Liaison.
- The CC CIRB Liaison will review and send the documentation to the UDN PI to submit to the CIRB.

2. Reportable Events

- 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.
- 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.
- 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.
- 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.
- 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.
- The CC CIRB Liaison will be copied on all correspondences between the CC, UDN PI, and the CSs and Cores.

3. Continuing Review

- 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.
- The CC CIRB Liaison will notify each CS and Core Site Coordinator regarding information required for CR, including the CR Local Context Worksheet and other forms provided by the CIRB.
- 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.
- The CC CIRB Liaison will review the UDN site forms for accuracy and completeness.
- The CC CIRB Liaison will provide the individual site-specific CR forms as well as submit a single CR Application to the UDN PI.
- The UDN PI will review the applications and submit all documents to the CIRB.
- The CIRB will conduct CR of all submitted materials.
- The CIRB will communicate the results of the review to the UDN PI, the CC CIRB Liaison, the local PIs, and the Institutional Designees.
- The CC CIRB Liaison will communicate the results of the review to the CS and Core Site Coordinators.
- The CIRB will provide to the CC CIRB Liaison the approved ICFs for each CS, which will include a new expiration date.
- 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.
- **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 at least two months prior to submission to the CIRB**

for CR to permit sufficient time for SRC review. The same submission process used for CR, as referenced above, will be used for the triennial review.

4. Amendments

a. Study-wide amendments

- Study-wide amendments will be approved by the UDN Steering Committee before submission to the UDN CIRB.
- 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.
- The UDN CC CIRB Liaison will finalize study-wide amendments and submit to the UDN PI.
- 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.
- If the study-wide protocol requires changes to the ICF:
 - The CC CIRB Liaison will modify the ICF template.
 - The CC CIRB will send tracked and clean copies of the modified ICF template to the UDN PI to submit for CIRB review.
 - Once the model template ICFs are approved by the CIRB, the CC CIRB Liaison will modify each site-specific document and provide them to the CIRB to update the version of the site specific ICFs.
- The CIRB will communicate the results of the review to the UDN PI, CC IRB Liaison, the local PIs, and the Institutional Designees.
- The CC CIRB Liaison will communicate the results of the review to the CS and Core Site Coordinators.
- The CC CIRB Liaison will make available to the CSs and Cores the approved amendment documents.
- If there are updated ICFs, 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.

b. Site-specific amendments (e.g. study staff changes)

- The CC CIRB Liaison will provide a template for the CSs and Cores to complete for the amendment that fulfills the CIRB requirements.
- 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.
 - For study staff changes, the local PI must sign the completed amendment template indicating conflict of interest review and completed training requirements.
- The CC CIRB Liaison will submit the amendments to the UDN PI.
- The UDN PI will submit the amendments to the CIRB.
- The CIRB will communicate the results of the review to the UDN PI, CC CIRB Liaison, the local PI, and Institutional Designee.
- The CC CIRB Liaison will communicate the results of the review to the CS and Core Site Coordinators.
- The CC IRB Liaison will make available to the CS or Core the approved amendment documents.

5. Affiliated studies

- CSs and Cores may propose studies affiliated with the UDN that are not network-wide.
- These studies will require permission from the UDN Steering Committee and a separate IRB protocol through the site proposing the study.
- The CC CIRB Liaison will keep track of these studies.
- The UDN site carrying out an affiliated study that has an IRB-approved protocol will provide the CC CIRB Liaison with 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.
- 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.

TIMELINE- IRB PROTOCOL YEAR 1

APRIL 2015

UDN Protocol Approved: April 10th

MAY 2015

Site-specific amendments due to CC CIRB Liaison: May 18th

JUNE 2015

Site-specific amendments due to CC CIRB Liaison: June 1st, June 15th

Study-wide amendments due to CC CIRB Liaison: June 1st

JULY 2015

Site-specific amendments due to CC CIRB Liaison: July 6th, July 20th

Study-wide amendments due to CC CIRB Liaison: July 6th

AUGUST 2015

Site-specific amendments due to CC CIRB Liaison: August 3rd, August 17th

Study-wide amendments due to CC CIRB Liaison: August 3rd

SEPTEMBER 2015

Site-specific amendments due to CC CIRB Liaison: September 7th, September 21st

Study-wide amendments due to CC CIRB Liaison: September 7th

OCTOBER 2015

Site-specific amendments due to CC CIRB Liaison: October 5th, October 19th

Study-wide amendments due to CC CIRB Liaison: October 5th

NOVEMBER 2015

Site-specific amendments due to CC CIRB Liaison: November 2nd, November 16th

Study-wide amendments due to CC CIRB Liaison: November 2nd

DECEMBER 2015

Site-specific amendments due to CC CIRB Liaison: December 7th, December 21st

Study-wide amendments due to CC CIRB Liaison: December 7th

JANUARY 2016

Site-specific amendments due to CC CIRB Liaison: January 4th, January 18th

Study-wide amendments due to CC CIRB Liaison: January 4th

Continuing renewal forms sent to sites by CC CIRB Liaison: January 8th

Continuing renewal forms due to CC CIRB Liaison: January 25th

FEBRUARY 2016

Site-specific amendments due to CC CIRB Liaison: February 1st, February 15th

Study-wide amendments due to CC CIRB Liaison: February 1st

MARCH 2016

Site-specific amendments due to CC CIRB Liaison: March 7th, March 21st

Study-wide amendments due to CC CIRB Liaison: March 7th

April 2016

Continuing renewal deadline- April 9th

TIMELINE- IRB PROTOCOL YEAR 2

MARCH 2016

UDN Continuing Review Application Approved: March 31st

APRIL 2016

Site-specific amendments due to CC CIRB Liaison: April 4th, April 18th

Study-wide amendments due to CC CIRB Liaison: April 4th

MAY 2016

Site-specific amendments due to CC CIRB Liaison: May 2nd, May 16th

Study-wide amendments due to CC CIRB Liaison: May 2nd

JUNE 2016

Site-specific amendments due to CC CIRB Liaison: June 6th, June 20th

Study-wide amendments due to CC CIRB Liaison: June 6th

JULY 2016

Site-specific amendments due to CC CIRB Liaison: July 4th, July 18th

Study-wide amendments due to CC CIRB Liaison: July 4th

AUGUST 2016

Site-specific amendments due to CC CIRB Liaison: August 1st, August 15th

Study-wide amendments due to CC CIRB Liaison: August 1st

SEPTEMBER 2016

Site-specific amendments due to CC CIRB Liaison: September 5th, September 19th

Study-wide amendments due to CC CIRB Liaison: September 5th

OCTOBER 2016

Site-specific amendments due to CC CIRB Liaison: October 3rd, October 17th

Study-wide amendments due to CC CIRB Liaison: October 3rd

NOVEMBER 2016

Site-specific amendments due to CC CIRB Liaison: November 7th, November 21st

Study-wide amendments due to CC CIRB Liaison: November 7th

DECEMBER 2016

Site-specific amendments due to CC CIRB Liaison: December 5th, December 19th

Study-wide amendments due to CC CIRB Liaison: December 5th

JANUARY 2017

Site-specific amendments due to CC CIRB Liaison: January 2nd, January 16th

Study-wide amendments due to CC CIRB Liaison: January 2nd

Continuing renewal forms sent to sites by CC CIRB Liaison: January 9th

Continuing renewal forms due to CC CIRB Liaison: January 27th

FEBRUARY 2017

Site-specific amendments due to CC CIRB Liaison: February 6th, February 13th

Study-wide amendments due to CC CIRB Liaison: February 6th

MARCH 2017

Site-specific amendments due to CC CIRB Liaison: March 6th, March 20th

Study-wide amendments due to CC CIRB Liaison: March 6th

April 2017

Continuing renewal deadline- April 9th

XV. Billing Procedures

The UDN RFA stated that the CSs could bill subjects' health insurance for clinically indicated evaluations, procedures and tests, and use grant funds for underinsured or uninsured subjects. The CSs were also required to provide subject transportation and lodging/meals during the one-week stay at the CS. These practices would ensure that subjects 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 subjects 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 subject 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 subjects 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 subject evaluations, made feasible by institutional discounts (~80%) for subject care performed as part of NIH-funded studies. The second option would bill the subjects' insurance companies for the clinical evaluations and cover underinsured/uninsured subjects 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 subject assistance fund with contributions from the Running for Rare Diseases team and a total of \$212,000 has been allocated to the UDN for year 2. The Steering Committee will be allocated ~10% of these funds to spend at its discretion. All the CSs, including the NIH-UDP would receive ~20% of the NORD fund (total \$35,000 in year 2: \$5000 per site) to pay for tests needed for subjects before being accepted into the UDN. This amount will be utilized at the discretion of the CSs. The remaining ~70% of the NORD fund (\$138,600 in year 2) will be utilized by the three CSs that will bill insurance to reimburse co-pays and deductibles for financially stressed patients (defined as those with an income below 300% of federal poverty guidelines). This plan would allow for seven patients at each of the 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)

The NORD funds (\$212,000) for Y2 will be distributed, as detailed below:

	Discretionary	Pre-Clinical Evaluation	Clinical Care
Amount Allocated	\$38,400	\$35,000	\$138,600
Sites Eligible	ALL	ALL	Baylor, Harvard, Stanford
Maximum – Per Site	n/a	\$5,000	\$46,200
Maximum – Per Patient	n/a	\$5,000	\$6,600

Similarly, a collaboration with Mercy Medical Angels (<http://mercymedical.org/>) would allow for provision of commercial air travel expenses for subjects 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 subject and one care taker 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 MMA 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 subjects and their family member. Thus, the network is still able to offer evaluations to patients irrespective of their health insurance status.

The Billing Working Group will continue to review issues (see Appendix 18: Billing Surveys) that arise during the beginning of the patient enrollment period, and significant changes to the billing structures and/or NORD fund distributions will be presented before the Steering Committee for consideration.

XVI. Surveys

Note: This document applies to all UDN surveys. However, there is a distinction in the procedures for survey research using and not using the Central IRB (CIRB). These distinctions are noted below.

A. Background

Surveys will be an important component of UDN research. Surveys offer another opportunity to learn from the unique UDN population of patients with rare and undiagnosed diseases. The purpose of this section is to outline an overall approach to the implementation of surveys in the UDN, keeping in mind standard steps of survey research that need to be followed.

B. Survey Committee

Purpose: The Survey Committee will play a critical role in assuring that surveys are consistent with the UDN mission and that participants are not overburdened. The Survey Committee will be under the umbrella of the Publications and Research Committee (PRC), and will coordinate with the Clinical Protocols Working Group.

Members: The Survey Committee will have expertise and interest in survey development and will include:

1. One voting member from each CS (7 members) and from the CC.
2. At least 1 survey methodologist from a UDN site. The survey methodologist could also be the voting member from their UDN site.
3. Optional: One voting member from each of the UDN Cores.
4. One member will be a liaison with the Clinical Protocols Working Group.

UDN members with expertise in an area covered by a survey under review may be asked to join the committee on an ad hoc basis.

Tasks:

New surveys: The full Survey Committee will review all surveys proposed after the initiation of the Survey Committee.

1. For survey research using the CIRB the Survey Committee will make a recommendation regarding whether or not the survey should be integrated into the UDN.
2. For survey research not using the CIRB the Survey Committee will review the survey in order to have a complete picture of surveys being administered to UDN participants but will not issue any recommendations.

Approved surveys: Each UDN survey using the CIRB, including those approved prior to the initiation of the Survey Committee, will be reviewed once a year, starting one year after IRB approval was obtained. A subgroup of the Survey Committee (2-3 members) will be tasked with reviewing the survey performance characteristics and will make a recommendation to the Survey Committee regarding whether or not the survey should be continued in the UDN. The Survey Committee will decide who the reviewers will be for each survey, with a goal of dividing the surveys up among the Committee members.

Surveys not using the CIRB will not be reviewed yearly.

C. Survey Development

1. Creation of Survey Plan

The UDN group proposing a survey must formulate a survey 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

2. Plan Proposal and Approval

The Survey Committee, which is under the umbrella of the PRC, will coordinate with the PRC in review of survey research proposals.

Investigators proposing research that involves surveys will complete and submit a research concept sheet. Additional questions were added to this sheet to collect information specific to survey research. Research concept sheets will be submitted to the CC and sent directly to the Survey Committee for review.

For surveys using the CIRB, the Survey Committee will make recommendations and send the research concept sheets to the PRC to review. For surveys that do not use the CIRB, the Survey Committee will review the research concept sheets in order to have a complete picture of surveys being administered to UDN participants, but will not issue any recommendations.

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

The Survey Committee will consider each survey in the context of participant burden, value of the data collected, and resources. They will consider ways to optimize survey data collection, e.g., for surveys collected at the same time point. The Committee will vote on a recommended outcome, with a majority vote needed to decide on an outcome. Potential outcomes:

1. Survey Committee makes recommendations to the PRC:
 - a. Recommendation to approve survey
 - b. Recommendation to reject survey
2. Survey Committee sends the research concept sheet back to the submitting group for revision before making a final recommendation.

Surveys recommended for approval or rejection by the Survey Committee will be sent to the PRC for review. Following PRC review, the CC will send the outcome of the review to the chair of the Survey Committee and members of the submitting group. Newly proposed surveys will then be presented to the Steering Committee by the submitting group for final consideration.

The Steering Committee may:

1. Approve the survey research
2. Raise concerns – Steering Committee will send the research concept sheet back to the group for revision
3. Reject the survey research

3. IRB Amendment Submission

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

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

4. Tracking Surveys

The Survey Committee will keep a record of all UDN surveys. For those using the CIRB, the table will include the date when the survey was approved by the CIRB, and the date when the yearly review is due.

5. Gateway Integration

If the survey needs to be integrated with the Gateway, the UDN group must:

1. Enter the survey into Qualtrics. The UDN CC will facilitate the use of Qualtrics.
2. Complete a Feature Request Form (Appendix 14). This form must include step-by-step instructions for how users will interact with the Gateway feature. The form must also include descriptions of where the data will be stored and accessed in the Gateway and any automatic emails that will be generated.

Once these steps are complete, the UDN group must submit the Feature Request Form to the CC to review. For more information on the Feature Request Process, see Section V. Technology and Data Management.

6. Ongoing Review

Surveys will be reviewed once a year by a subgroup of the Survey Committee to determine if the survey should continue to be administered. Those who developed the survey plan will be responsible for completing the Annual UDN Survey Review Worksheet (Appendix 23).

Continuation of the survey will be considered in the context of the other surveys being administered and the burden on participants.

The Survey Committee will contact the group conducting the survey 3 months prior to when the ongoing review is due. The Annual UDN Survey Review Worksheet should be submitted to the chair of the Survey Committee on or before the review date.

Based on their review, the Survey Committee subgroup will make a recommendation to the full Survey Committee regarding continuation of the survey. The Survey Committee will vote on the outcome, with a majority vote needed to decide on an outcome. Following the review, the chair of the Survey Committee will send the outcome of the review to the Executive Director of the CC and members of the group conducting the survey. Potential recommendations to be made to the Steering Committee:

1. Approve continuation of survey – recommend that the Survey Committee send to the Steering Committee for approval.
2. Concerns raised – The subgroup recommends the full Survey Committee review the survey. Potential outcomes:
 - a. Approve continuation of survey
 - b. Send back to the group to address concerns
 - c. Reject continuation of the survey

Surveys recommended by the Survey Committee for approval or rejection will be sent by the Executive Director of the CC to the Steering Committee for final consideration. Potential outcomes of the Steering Committee review:

1. Approve continuation of survey
2. Concerns raised – Send back to the group to address concerns
3. Reject continuation of the survey

XVII. APPENDICES

APPENDIX 1: The NIH UDP Protocol

A) 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 (301-496-1465). 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. See Appendix 2: NIH UDP Patient Flow and Appendix 3: NIH-UDP Pre-CRC Admission for a detailed flow of patients prior to CRC admission.

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 referral 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 referral 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 patients who fail to provide the necessary phenotyping data, or for whom there is no physician referral 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).

B) Creation and Careful Review of a CRC Medical Record (10 patients each week)

The next step in the recruitment process is to carry out a detailed review of each candidate's medical record, including the referral letter from the current personal physician or physician-

extender summarizing the salient features of the person's disorder, 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 cerebrospinal fluid findings, 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 patients, 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 patients for UDP evaluation, there are other potential results. Some patients may be more suitable for referral to other open NIH research protocols. If, in the judgment of the review panel, there has been incomplete patient evaluation, the panel may choose to return the patient 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 (Dr. Gahl and Dr. Tliff) after detailed discussion with consultants and other members of the UDP team. The goal of this review process is to insure, to the extent possible, that the problems posed by invited patients will be appropriate and match the resources of the UDP. A criterion for acceptance to the UDP is that the patient is safe to travel. The pediatric patients in particular are often medically fragile, medical clearance for commercial travel must be documented by the referring physician before patients can be accepted and scheduled for evaluation. Pediatric patients must have clearance from their physician one week prior to making the trip. In some cases, visits need to be rescheduled if the patient 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.

C) 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 patients 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. Patient-specific time constraints must also be considered.

D) Overall approach to phenotyping and specific data gathering in common subgroups

More than half of the patients accepted into the UDP have a neurological phenotype, and in children in particular this leads to a common phenotyping framework that includes intracranial imaging (MRI and MRS), neurologic consultation, EEG, EMG/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, physiatry consultation, and neurocognitive testing. In adult patients CSF is also obtained for immunologic studies.

E) UDN Metrics of success

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 patient medical records reviewed
 - 450 patients admitted to the NIH-CRC (Clinical Research Center)
- b) Weekly metrics:
 - 30 new inquiries
 - 10 patients with completed referral letters and results from prior diagnostic efforts evaluated
 - 3 patients/families admitted for work-up at the CRC.
- c) Diagnostic metrics:
 - Approximately 100 patients (20-25%) were diagnosed with rare to extremely rare diseases
 - Two patients were found with diseases unknown to medicine.
 - 15 genes not previously associated with human disease were discovered and tentatively related to disease phenotypes.

F) Summary

The current NIH-UDP initial approach to identifying and evaluating patients 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.

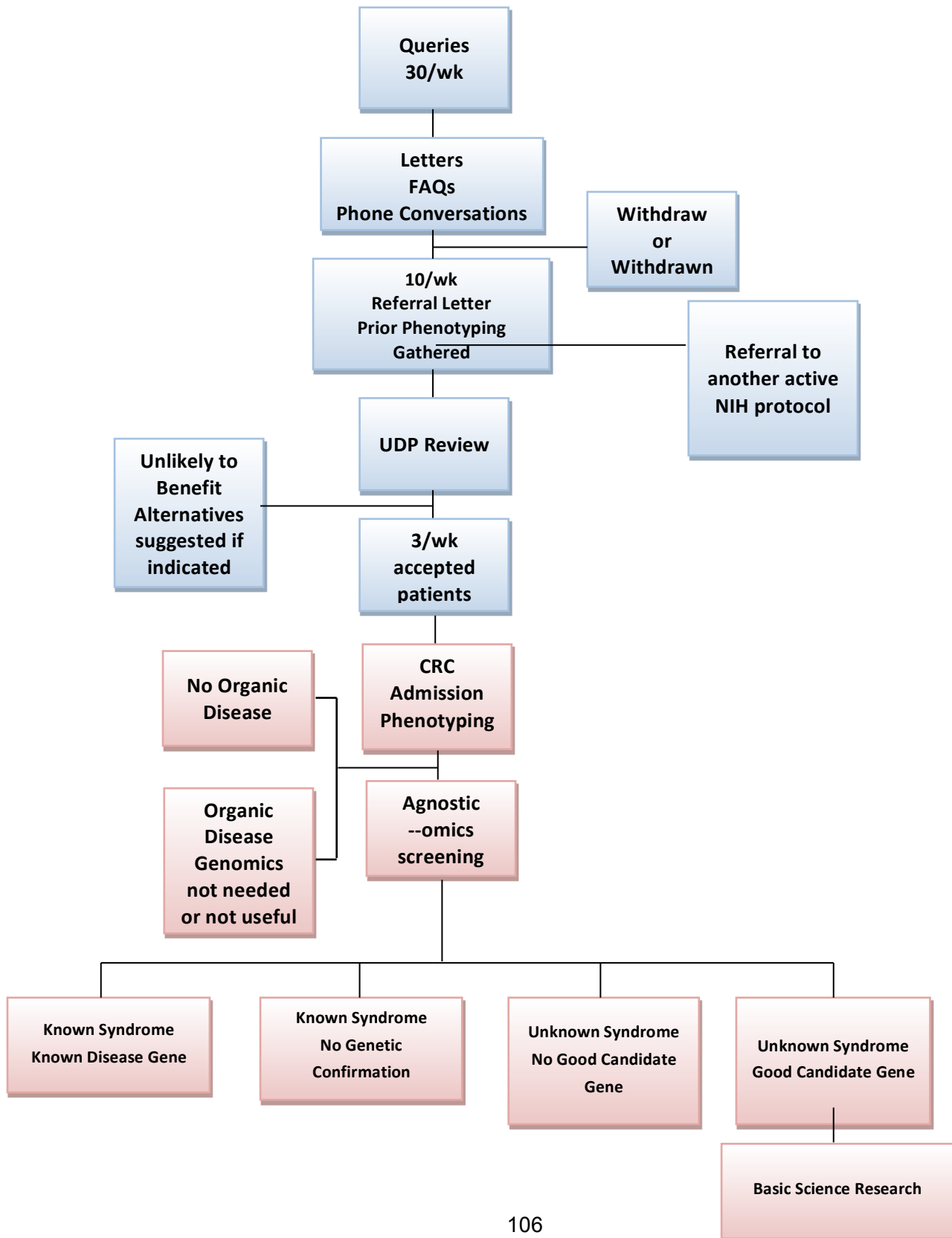
The task for Network investigators, including the NIH-UDP, is to devise a UDN protocol that insures effective and efficient new site performance and retains a common approach to patient recruitment. Most importantly, uniform data collection with submission to the UDN coordinating center is critical to the success of the Network. Another important goal for the Network is the creation of a cooperative and collaborative team of Network investigators that recognizes and celebrates the diversity in new site talent and strengths, particularly in subspecialties. Additional work will be required to determine whether sites, in addition to evaluating all patients referred for evaluation, have a particular sub-specialty that might serve as a Network resource. Regularly scheduled discussions of difficult diagnostic cases among Network experts in the disorder suspected is likely to be yet another advantage of Network operations.

References (UDP background)

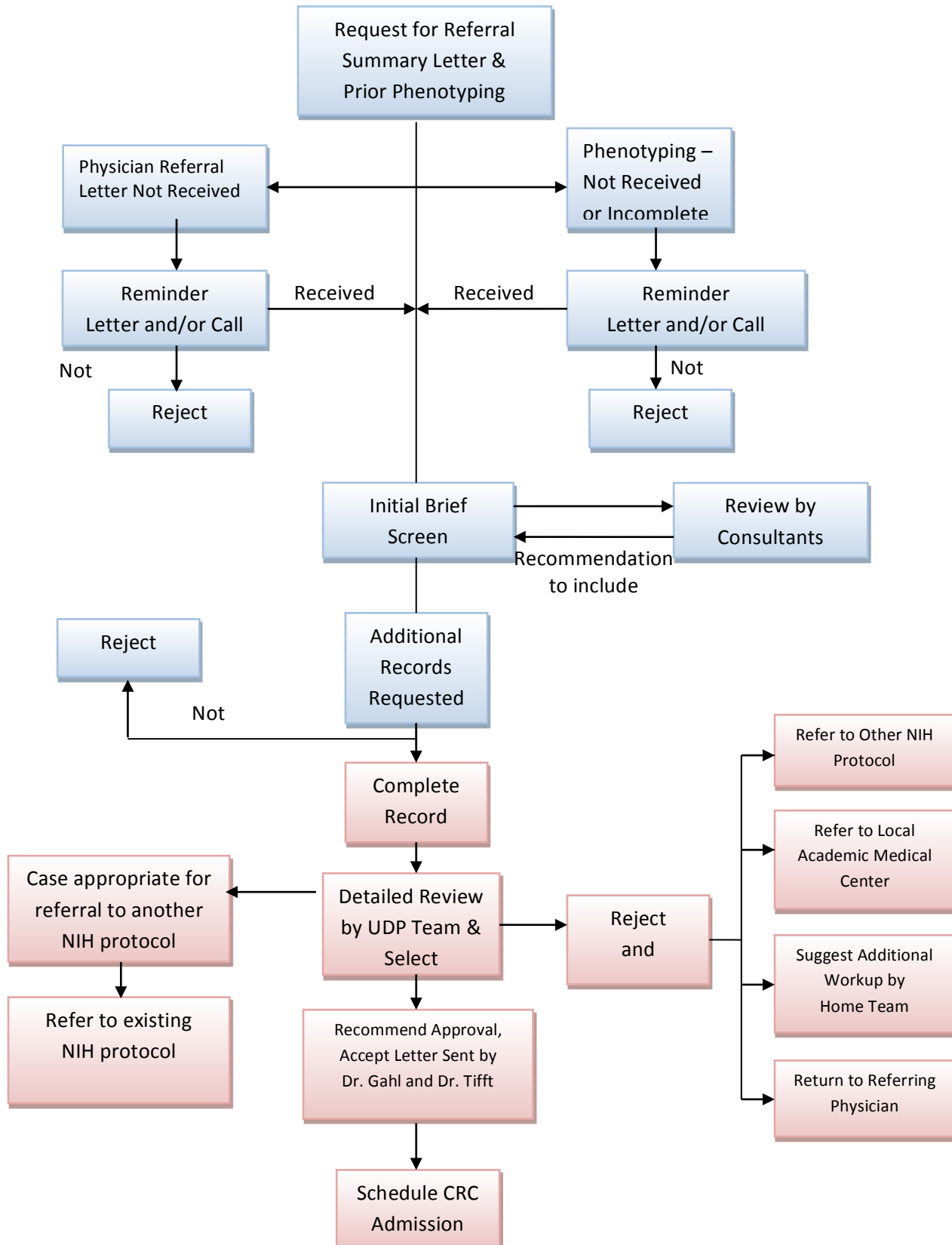
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2. Gahl WA, Markello TC, Toro C, Fuentes Fajardo K, Sincan M, Gill F, Carlson-Donohoe H, Gropman A, Pierson TM, Golas G, Wolfe L, Groden C, Godfrey R, Nehrebecky M, Wahl C, Yang S, Madeo A, Boerkoel CF, Tifft CJ, Adams D. The NIH Undiagnosed Diseases Program: Insights into rare diseases. **Genet Medicine** 14:51-9, 2012.
3. Gahl WA, Boerkoel CF, Boehm M. The NIH Undiagnosed Diseases Program: Bonding scientists to clinicians. **Dis Model Mech** 5:3-5, 2012.
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APPENDIX 2: NIH UDP Patient Flow



APPENDIX 3: NIH-UDP Pre-CRC Admission



APPENDIX 4: Case Review Committee of the UDN

Purpose: The Case Review Committee meeting is a forum for the CSs to concisely present patients to the UDN clinicians for review and input. The patients will fall into two general categories, 1) those that the CS has vetted and intends to invite for evaluation and 2) those for whom the CS is uncertain, has questions about, or thinks may be better served at another CS.

Format: The format of the meetings will be the presentation of 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 should last no more than five minutes. If two CSs need prolonged discussion about a patient, this can be taken off line after the meeting. Cases will be rotated among the seven CSs in each meeting. The meetings will terminate after an hour. A timer will move the meeting forward or take discussions off line if necessary.

Decisions: The decision to invite a patient for evaluation will be made primarily by individual CSs. The meeting will exist to add value to the intended evaluation, to allow the UDN to be informed of the composition of the patient study population, and to ensure that accepted cases fall within the inclusion and exclusion criteria specified in the Clinical Protocols section and the CIRB protocol. A decision to not accept an applicant will be based upon deviation from the inclusion or exclusion criteria, as determined by a majority vote of the Case Review Committee. Each eligible CS will submit a vote to the Committee co-chairs. Each CS will have one vote; the CC, NIH program, and Cores will not have votes. Should the vote result in non-acceptance, the proposing 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.

It is expected that about half of cases will be 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 healthcare provider from any of the institutions that are on that CS's award. A patient recommended by an outside provider will not be considered "known to a CS" even if s/he has been seen previously at an institution on that CS's award.

Structure: Each CS and SC should designate two Case Review Committee members and at least one alternate. Each CS should have at least one clinician present at any Case Review Committee meeting. Ordinarily, a pediatrician and an adult internist from each CS will be on each conference call.

The co-chairs of the Committee will rotate among the CSs every 3 months. One of them should attend at each meeting. If both are conflicted for a meeting, then a member of the Committee will be asked to chair.

APPENDIX 5: ClinicalTrials.gov Record

The UDN protocol “Clinical and Genetic Evaluation of Individuals With Undiagnosed Disorders Through the Undiagnosed Diseases Network” is shown below and **updates** are listed on ClinicalTrials.gov at the following URL: <https://clinicaltrials.gov/ct2/show/NCT02450851>.

ClinicalTrials.gov

A service of the U.S. National Institutes of Health

Clinical and Genetic Evaluation of Individuals With Undiagnosed Disorders Through the Undiagnosed Diseases Network

This study is currently recruiting participants. (see [Contacts and Locations](#))

Verified April 2016 by National Institutes of Health Clinical Center (CC)

Sponsor: National Human Genome Research Institute (NHGRI)

Information provided by (Responsible Party): National Institutes of Health Clinical Center (CC) (National Human Genome Research Institute (NHGRI))

ClinicalTrials.gov Identifier: NCT02450851



Purpose

Background:

- Without an explanation for severe and sometimes life-threatening symptoms, patients and their families are left in a state of unknown. The NIH helped create a network of medical research centers, called the Undiagnosed Diseases Network (UDN), to provide care and answers for these individuals.

Objectives:

- To improve diagnosis and care for people with undiagnosed diseases.

Eligibility:

- People with undiagnosed diseases, and their relatives.

Design:

- Participants will travel to one of the UDN medical centers for a 5-day clinical and research visit.
 - As part of the visit, UDN healthcare providers may ask participants to have:
 - Clinically indicated tests and procedures performed including:
 - A physical exam
 - Blood and urine tests
 - A review of health and family history
 - X-rays and body scans
 - Surveys
 - Photographs of the face and body
 - A special diet to see if the body can handle the food without having a reaction, like vomiting
 - Video or voice recordings
 - Other tests and procedures to help reach a diagnosis
 - Research tests and procedures performed including:
 - A skin biopsy. For this, a small piece of skin will be taken.

- Surveys
- Other tests and procedures for research that may not be related to a diagnosis or treatment.
- Most participants will be asked to give samples for genetic testing.
- Participants may be contacted after their visit to discuss test results. They may also be contacted in the future for interviews and surveys.
- Relatives of participants may be asked to give samples for genetic testing. They may be asked to have parts of their visit recorded and to have additional tests. They may also be contacted in the future for interviews and surveys.
- Clinical and research information collected will be stored in a database.
- Information and samples collected will be shared with others for research purposes.

<u>Condition</u>
Undiagnosed Disease

Study Type: Observational
 Study Design: Time Perspective: Cross-Sectional

Official Title: Clinical and Genetic Evaluation of Patients With Undiagnosed Disorders Through the Undiagnosed Diseases Network

Further study details as provided by National Institutes of Health Clinical Center (CC):

Primary Outcome Measures:

- Making a diagnosis [Time Frame: Admission and ad hoc after that]
 [Designated as safety issue: No]

Estimated Enrollment: 8000
 Study Start Date: May 2015
 Estimated Study Completion Date: January 2021
 Estimated Primary Completion Date: January 2021 (Final data collection date for primary outcome measure)

Detailed Description:

Without an explanation for severe and sometimes life-threatening symptoms, patients and their families are left in a state of unknown. Many individuals find themselves being passed from physician to physician, undergoing countless and often repetitive tests in the hopes of finding answers and insight about what the future may hold. This long and arduous journey to find a diagnosis does not end for many patients- the Office of Rare Diseases Research (ORDR) notes that 6% of individuals seeking their assistance have an undiagnosed disorder. In 2008, the National Institutes of Health (NIH) Undiagnosed Diseases Program (UDP) was established with the goal of providing care and answers for these individuals with mysterious conditions who have long eluded diagnosis. The NIH UDP is 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 NIH UDP are 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; and (4) identify potential therapeutic targets, if possible. To date, the UDP has evaluated 3300 medical records and admitted 750 individuals with rare and undiagnosed conditions to the NIH Clinical Center. The NIH UDP has identified more than 70 rare disease diagnoses and several new conditions. The success of the NIH UDP prompted the NIH Common Fund to support the establishment of a network of medical research centers,

the Undiagnosed Diseases Network (UDN), for fiscal years 2013-2020. The clinical sites will perform extensive phenotyping, genetic analyses, and functional studies of potential disease-causing variants. The testing performed on patients involves medically indicated studies intended to help reach a diagnosis, as well as research investigations that include a skin biopsy, blood draws, and DNA analysis. In addition, the UDN will further the goals of the UDP by permitting the sharing of personally identifiable phenotypic and genotypic information within the network. By sharing participant information and encouraging collaboration, the UDN hopes to improve the understanding of rare conditions and advance the diagnostic process and care for individuals with undiagnosed diseases.

Eligibility

Ages Eligible for Study: 1 Month to 100 Years
Genders Eligible for Study: Both
Accepts Healthy Volunteers: No

Criteria

INCLUSION CRITERIA:

- The applicant does not have a diagnosis that explains the objective findings.
- The applicant (or legal guardian) agrees to the storage and sharing of information and biomaterials in an identified fashion amongst the UDN centers, and in a de-identified fashion to research sites beyond the network.

EXCLUSION CRITERIA:

- The applicant has a diagnosis that explains the objective findings.
- Review of the records suggests a diagnosis and further evaluation is deemed unnecessary.
- The applicant is too seriously ill to travel safely to the UDN site.

Contacts and Locations

Choosing to participate in a study is an important personal decision. Talk with your doctor and family members or friends about deciding to join a study. To learn more about this study, you or your doctor may contact the study research staff using the Contacts provided below. For general information, see [Learn About Clinical Studies](#).

Please refer to this study by its ClinicalTrials.gov identifier: NCT02450851

Contacts

Contact: Paul Mazur (844) 746-4836 udn@hms.harvard.edu

Locations

United States, California

University of California, Los Angeles
Los Angeles, California, United States, 90095

Recruiting

Stanford Medical Center
Stanford, California, United States, 94305

Recruiting

United States, Maryland

National Institutes of Health Clinical Center, 9000 Rockville Pike

Recruiting

Bethesda, Maryland, United States, 20892
Contact: Paul Mazur 844-746-4836 udn@hms.harvard.edu

United States, Massachusetts

Brigham and Women's Hospital Boston, Massachusetts, United States, 02115	Recruiting
Boston Children's Hospital Boston, Massachusetts, United States	Recruiting
Massachusetts General Hospital Boston, Massachusetts, United States, 02114	Recruiting

United States, North Carolina

Duke Univ Health System Durham, North Carolina, United States	Recruiting
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United States, Tennessee

Vanderbilt University Nashville, Tennessee, United States, 37232	Recruiting
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United States, Texas

Baylor College of Medicine Houston, Texas, United States, 77030	Recruiting
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Sponsors and Collaborators

National Human Genome Research Institute (NHGRI)



Investigators

Principal Investigator:	William A Gahl, M.D.	National Human Genome Research Institute (NHGRI)
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More Information

Additional Information:

[NIH Clinical Center Detailed Web Page](#) 
<https://undiagnosed.hms.harvard.edu> 

Publications:

[Gahl WA, Boerkoel CF, Boehm M. The NIH Undiagnosed Diseases Program: bonding scientists and clinicians. Dis Model Mech. 2012 Jan;5\(1\):3-5. doi: 10.1242/dmm.009258.](#)

[Gahl WA, Markello TC, Toro C, Fajardo KF, Sincan M, Gill F, Carlson-Donohoe H, Gropman A, Pierson TM, Golas G, Wolfe L, Groden C, Godfrey R, Nehrebecky M, Wahl C, Landis DM, Yang S, Madeo A, Mullikin JC, Boerkoel CF, Tift CJ, Adams D. The National Institutes of Health Undiagnosed Diseases Program: insights into rare diseases. Genet Med. 2012 Jan;14\(1\):51-9. doi: 10.1038/gim.0b013e318232a005. Epub 2011 Sep 26.](#)

[Gahl WA, Tift CJ. The NIH Undiagnosed Diseases Program: lessons learned. JAMA. 2011 May 11;305\(18\):1904-5. doi: 10.1001/jama.2011.613.](#)

Responsible Party:	National Institutes of Health Clinical Center (CC) (National Human Genome Research Institute (NHGRI))
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ClinicalTrials.gov Identifier: [NCT02450851](#) [History of Changes](#)
Other Study ID Numbers: 150130 15-HG-0130
Study First Received: May 19, 2015
Last Updated: May 11, 2016
Health Authority: United States: Federal Government

Keywords provided by National Institutes of Health Clinical Center (CC):
Rare Diseases
Undiagnosed Disease

ClinicalTrials.gov processed this record on May 19, 2016

APPENDIX 6: Example Referral Letters

PEDIATRIC REFERRAL LETTERS

Example Letter #1:

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 work-up 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,

[Referring provider]

Example Letter #2:

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

Chromosome microarray [years]

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

Bioppterin

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,

[Referring provider]

Example Letter #3:

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]. Etc.

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

Sincerely,

[referring provider]

ADULT REFERRAL LETTER

Example Letter

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 businessman 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:

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/His 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. I enclose the information your program requires including case summaries, laboratory reports, and reports from consults.

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,

[Referring provider]

APPENDIX 7: Suggested Triage Methods

1. Once the applicant has been assigned to a clinical site, the site will contact the applicant and request that he/she send all information they have related to the reason for their application to the UDN. These may include: medical records, reports, laboratory studies, radiographic studies, photographs or videos, and pathology slides and reports.
2. Records will be reviewed by the CS for completeness. The CS will request any missing components (e.g., images, biopsy slides). With appropriate release of information from the applicant, the site may request medical records directly from any medical centers where the patient has been seen.
3. Once the assigned site receives the information, the site will collate the information collected into folders and will scan the files to facilitate distribution for review.
4. The site director or his/her designee will assign the records for review to consultants based upon the specialty involved.
5. If during the review it becomes clear that more information is needed, the staff at the CS will contact the applicant to request more information.

APPENDIX 8: 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 illness
- Date symptoms first noted
- Past medical history
- Previous diagnoses/comorbidities (using ICD terms if possible)
- Prior procedures and surgeries.

Height:

Weight:

Head circumference:

Please indicate the applicant's pertinent prior evaluations. If applicable, please include:

- Prior positive or negative test results
- Prior genetic testing (especially whole exome sequencing)

Provisional diagnosis/working plan:

Other family members affected: Yes/No

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

Patient 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 Patients
- ☐ 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

Recommend for Acceptance- At Clinical Site	Questionable Case- Send to Case Review	Not Accepted with Recommendations- Seek expert care	Not Accepted- Diagnosis Identified
Recommend for Acceptance- At Different Site	Not accepted/Reconsider	Not Accepted with Recommendations- Specific testing	Not Accepted- UDN Would Likely Not be Able to Help Find a Diagnosis
			Not Accepted- Insufficient records made available to UDN site

APPENDIX 9: UDN Generic 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. The study will be conducted at Baylor College of Medicine, Boston Children's Hospital/Brigham and Women's Hospital/Massachusetts General Hospital, Duke University, the National Institutes of Health, Stanford University, University of California-Los Angeles, and Vanderbilt University Medical Center.

Please discuss your participation in this program with your primary healthcare provider.

Important considerations include:

- Many cases accepted will NOT result in a diagnosis.
- A referral by a healthcare 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 referring healthcare provider.
- If your case is accepted for UDN evaluation, the UDN will provide information from the evaluation to you and to your healthcare provider.
- Your healthcare provider will be responsible for your medical care after you have been evaluated in the UDN.

Details about the information needed from your referring provider are on the attached letter.

Please insert information where requested and give the *Information Sheet for Referring Healthcare Providers* 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 new program is on line at <https://undiagnosed.hms.harvard.edu>.

The Undiagnosed Diseases Network Team

Description: *Information for healthcare providers*

Information for healthcare providers

Your patient has contacted the Undiagnosed Diseases Network (UDN) about participating in this research study. Patient participants will be evaluated using the 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, he/she 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
- Primary licensed healthcare provider information (name, address, phone, FAX, email)
- Brief medical history questions
- Previous evaluations
- Travel limitations

A referral letter from a primary licensed healthcare provider is also necessary in order to submit the online application to the UDN. The patient will be asked to upload this referral letter to the online application. The referral 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
- Healthcare provider's diagnostic impressions
- For pediatric patients, prenatal and birth history should also be provided.

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, medical records should not 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 make a decision 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.

The Undiagnosed Diseases Network Team

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 healthcare 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].

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 healthcare 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].



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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 healthcare 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].



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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 Healthcare provider*

Date:

Address:

Dear [healthcare provider]:

Your patient _____ [DOB] has applied to the Undiagnosed Diseases Network (UDN). After a stringent review process, your patient's case has been accepted for evaluation at [your institutional name; city, state]. 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]



Your institutional logo
here

Description: *Acceptance Letter (Adult) to Healthcare provider*

Date:

Address:

Dear [healthcare provider]:

Your patient _____ [DOB] has applied to the Undiagnosed Diseases Network (UDN). After a stringent review process, your patient's case has been accepted for evaluation at [your institutional name; city, state]. 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: *Not Accept Letter to Healthcare provider*

*Note: required sections are in **bold** below.*

Date:

Address:

Dear [healthcare provider]:

Your patient [name] [DOB] has applied to the Undiagnosed Diseases Network (UDN). After a stringent review process, your patient was not accepted for evaluation by the UDN. The Network's goals are to provide answers to patients with mysterious conditions that have long eluded diagnosis and to advance medical knowledge about rare and common diseases. The medical team bases its decisions on whether or not there is a reasonable chance to achieve these goals. Review of each application considers the Network's resources as a whole.

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

[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/>.

[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.

Again, 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]

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 healthcare 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]



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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 10: Suggested Sites for Testing

Condition/Test	Laboratory	Information	Website
Congenital disorders of glycosylation	Emory Genetics Lab and Mayo Clinic	-Analyze both N-glycosylation and O-glycosylation	http://genetics.emory.edu/egl/tests/?testid=1022 http://genetics.emory.edu/egl/tests/?testid=1341
Mucopolysaccharidoses and oligosaccharidoses	University of Alabama Metabolic Disease Laboratory	-Urine screen	https://www.uab.edu/medicine/genetics/clinical-laboratories/metabolic-dise
Lysosomal storage diseases	Emory Genetics Lab	-Blood -New comprehensive enzyme panel (soon to be released)	
Sanfilippo syndrome	Greenwood Genetic Lab	-Blood -Most comprehensive enzyme panel for Sanfilippo syndrome	http://www.ggc.org/diagnostic/tests-costs/test-finder/test-finder.html?id=
Peroxisomal disorders	Kennedy Krieger Lab		http://www.kennedykrieger.org/patient-care/patient-care-laboratories/genet
Cerebrospinal fluid neurotransmitters	Medical Neurogenetics	-Customer service is modest and website is challenging to navigate	https://www.medicalneurogenetics.com/
Urine purines and pyrimidines	Baylor College of Medicine Medical Genetics Lab		https://www.bcm.edu/cancergeneticslab/test_detail.cfm?testcode=4220&show=

APPENDIX 11: 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/>

Participant Webpages Project:

UDN participants have the option of having a page created on the UDN website to try to find and connect with others with the same or similar condition.

The participant pages include the following information: genetic changes, symptoms, medical history, treatments, procedures, medications, gender, race, ethnicity, and photographs/videos (optional).

Example pages can be found here: <https://undiagnosed.hms.harvard.edu/updates/participant-pages/>



Please indicate if this participant would be interesting in participating in this project:

- ☐ Yes
- ☐ No

APPENDIX 12: 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:

9. 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?
10. What part/s of the UDN experience worked particularly well for you?
11. What part/s of the UDN experience did not work well?
12. What could we do to make the UDN experience better?
13. Did you feel the UDN visit was burdensome? If so, please explain.
14. 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?

15. 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 (ex. 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</i>	Is there anything that you would change	Please explain what you would

<i>comments</i>	about your UDN experience?	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 13: Research Inventory Form

Sample ID:

Candidate discovery progress:

1. Candidate gene(s) or causative element(s) found for phenotype? (enter name of gene or indicate "no")
 - a. Has the result been published or presented at national meeting?
 - i. Citation and collaborators (if any)
 - ii. If not published yet, do you expect to publish these data?

Omics and Models:

1. Please indicate any work done on the following:
 - a. Glycomics
 - b. Lipidomics
 - c. Metabolomics
 - d. Energetics
 - e. Drosophila model
 - f. Mouse model
 - g. Yeast model
 - h. Zebrafish model
 - i. Additional collaboration

Financial data:

1. Has this data been used in any grant applications?
 - a. If grants have been awarded, please name the grant, recipient, award date, and the amount.
2. How else do you plan to use this data? (eg. In-house database)

Other research involvement:

APPENDIX 14: 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 15: UDN Data Sharing and Use Agreement

UDN DATA SHARING AND USE AGREEMENT

This Undiagnosed Diseases Network (“UDN”) Data Sharing and Use Agreement (the “**Agreement**”), effective on _____ (the “**Effective Date**”) is entered into by and among the UDN Participating Institutions listed on Exhibit 1 to this Agreement, each of which will be known as a “**Party**” and collectively as “**Parties**” to this Agreement.

RECITALS

WHEREAS, each non-NHGRI Party has received an award from the NIH (or is the recipient of a subaward) to participate in the UDN and to assist in the conduct of the UDN Study;

WHEREAS, the Parties recognize that to achieve the objectives of the UDN, the Parties will need to share Study Data with one another and to maintain the Study Data in a centralized data repository; and

WHEREAS, the Parties recognize that there must be mutual agreement as to the permitted uses and disclosures of the Study Data.

Now therefore, the Parties agree as follows:

I. Definitions

A. Capitalized Terms. Capitalized terms shall have the meaning as set forth herein or in this Section I.A.

1. Applicable Law shall mean the applicable legal and regulatory requirements, principles, and standards set forth and espoused in: (i) HIPAA; (ii) the Federal Policy for the Protection of Human Subjects, also known as the Common Rule, as applicable; (iii) the U.S. Food and Drug Administration’s research laws, as applicable, including without limitation the regulations contained in 21 C.F.R. Parts 50, 54 and 56, 312, 314, 601, 812 and 814 as amended or augmented from time to time; (iv) Department of Health and Human Services Guidance on “Financial Relationships and Interests in Research Involving Human Subjects: Guidance for Human Subject Protection,” Federal Register, Vol. 69, No. 92, p. 26393 (May 12, 2004); (v) state and federal fraud and abuse laws, including but not limited to Stark and the Anti-kickback Act; (vi) the Federal Privacy Act of 1974; (vii) rules and regulations governing the application for, receipt of, and use of federal research Grants and contracts; and (viii) all other federal, state, or local laws, regulations, guidances, or other requirements governing the conduct of the UDN Study and/or the data sharing activities as contemplated herein.

2. Central Database shall have the meaning set forth in Section I.A.4.

3. Confidential Business Information shall have the meaning set forth in Section V.C.

4. Coordinating Center shall mean the UDN Participating Institution that has principal responsibility within the UDN for establishing and maintaining a central database for the Study Data required by the UDN Protocol to be forwarded to the Coordinating Center (the “**Central**

Database”) and coordinating the sharing of UDN Study Data as part of the UDN Study. The functions of the Coordinating Center will be performed by Harvard Medical School and those additional Parties listed on Exhibit 1 that are recipients of a subaward from Harvard Medical School. With respect to its activities as Coordinating Center for the UDN Study, Harvard Medical School is not a Covered Entity or a Business Associate under HIPAA.

5. De-Identified Study Data shall have the meaning set forth in Section III.B.3.
6. Evaluation Site shall have the meaning set forth in Section II.C.2.
7. Health Care Provider shall mean a health care provider that is not a UDN Participating Institution and that discloses Protected Health Information about a Participating Human Subject, as permitted by the UDN Tier 1 ICA Form or the UDN Tier 2 ICA Form, to the UDN.
8. HIPAA shall mean the Administrative Simplification section of the Health Insurance Portability and Accountability Act of 1996 (Public Law 104-191), the amendments thereto by Title XIII of the American Recovery and Reinvestment Act of 2009 also known as the Health Information Technology for Economic Clinical Health Act, and their respective implementing regulations as amended from time to time.
9. Intake Site shall have the meaning set forth in Section II.C.1.
10. Participating Human Subject shall mean each individual who is enrolled in the UDN Study. For the avoidance of doubt, individuals who only participate in the Tier 1 Phase of the UDN Study are Participating Human Subjects.
11. Principal Investigator shall mean each UDN Investigator listed on Exhibit 2 to this Agreement.
12. Protected Health Information (“**PHI**”) shall have the meaning set forth in HIPAA.
13. Publication shall have the meaning set forth in Section VI.A.
14. Recipient shall mean a Party, other than the Coordinating Center, that receives UDN Study Data from another Party under this Agreement.
15. Required Data Sharing ICA Form Elements shall have the meaning set forth in Section III.B.
16. Required Tier 1 Data Sharing ICA Form Elements shall have the meaning set forth in Section III.A.
17. Sequencing Core shall mean each of the UDN Participating Institutions listed on Exhibit 1 that has principal responsibility for generating genetic sequence data for Participating Human Subjects as part of the UDN Study.
18. Study Data shall have the meaning set forth in Section II.C.2.
19. Tier 1 Phase shall have the meaning set forth in Section II.C.1.
20. Tier 1 Study Data shall have the meaning set forth in Section II.C.1.

21. Tier 2 Phase shall have the meaning set forth in Section II.C.2.
22. Tier 2 Study Data shall have the meaning set forth in Section II.C.2.
23. UDN Central IRB shall mean the IRB of the National Human Genome Research Institute (“**NHGRI**”), which is responsible for the review, approval and on-going oversight of the UDN Study. NHGRI is not a HIPAA Covered Entity.
24. UDN Clinical Site shall mean any clinical site where Participating Human Subjects are evaluated as part of the UDN Study.
25. UDN Tier 1 ICA Form shall mean the combined informed consent and HIPAA Authorization Form that each Participating Human Subject in the UDN Study must sign prior to participating in the Tier 1 Phase of the UDN Study. The UDN Tier 1 ICA Form is incorporated into the UDN Protocol.
26. UDN Tier 2 ICA Form shall mean the combined informed consent and HIPAA Authorization form that each Participating Human Subject in the UDN Study must sign prior to participating in the Tier 2 Phase of the UDN Study. The UDN Tier 2 ICA Form is incorporated into the UDN Protocol.
27. UDN Investigator shall mean a researcher at a UDN Participating Institution that assists in the conduct of the UDN Study.
28. UDN Participating Institution shall mean the Coordinating Center, each Sequencing Core, each of the UDN Clinical Sites, and any other institution in the United States engaged in the conduct of the UDN Study. Each UDN Participating Institution, other than NHGRI, has received an award from the NIH (or a subaward from another UDN Participating Institution) to participate in the UDN.
29. UDN Protocol shall mean the research protocol, attached as Exhibit 3 to this Agreement, for the UDN Study, and any amendments thereto that may be made and approved by the UDN Central IRB during the course of the UDN Study.
30. UDN Study shall mean the research study described in the UDN Protocol.
31. Variant Results shall have the meaning set forth in Section VI.B.1.

II. Background and Purpose

A. **Background.** The National Institutes of Health (“**NIH**”) Common Fund’s UDN is a program established by NIH to promote cross-disciplinary approaches to identifying, diagnosing, and treating rare non-diagnosed/differentiated diseases by academic centers located in the United States. The objectives of the UDN are to: (1) improve the level of diagnosis and care for patients with undiagnosed diseases through the development of common protocols designed by an enlarged 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 clinical sites 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. To achieve these objectives, the Parties wish to share certain Study Data and work collaboratively to enable the identification of eligible individuals to participate in the Tier 2 Phase in an effort to diagnose their conditions and to conduct subsequent research on undiagnosed diseases.

B. UDN Participating Institutions. The UDN is comprised of the UDN Participating Institutions set forth on Exhibit 1, as may be amended from time to time in accordance with this Agreement. In order to be considered a UDN Participating Institution, an Institution must have (a) received an NIH award (or a subaward from another UDN Participating Institution) to participate in the UDN Study (except for NHGRI), and (b) caused an authorized representative to execute this Agreement and provide a copy of the signature page to each Party. The Coordinating Center is authorized to amend Exhibit 1 to reflect the addition of a new UDN Participating Institution upon the Coordinating Center's receipt of notice from the NIH of the addition of the institution to the UDN and the Coordinating Center's receipt of a copy of this Agreement signed by an authorized representative of the new UDN Participating Institution. The grant reference numbers for each non-NHGRI UDN Participating Institution are set forth in Exhibit 1.

Should there be any change in the designation of any Principal Investigator for a Party, including additions or replacements, Exhibit 2 shall be deemed amended upon receipt of a letter signed by the authorized representative of said Party when a copy has been sent to each other Party.

C. UDN Study Tiers. The UDN Study is differentiated into two tiers, as further described in the UDN Protocol.

1. During the first tier phase of the UDN Study (the “**Tier 1 Phase**”) and only after the Participating Human Subject has completed a UDN Tier 1 ICA Form, (1) the Participating Human Subject will submit to the Coordinating Center demographic and medical information; (2) the Participating Human Subject's Health Care Providers will submit to the Coordinating Center additional medical information as well as a Health Care Provider referral letter summarizing medical history and other pertinent clinical information and (3) the Coordinating Center will assign such information to a UDN Clinical Site (the “**Intake Site**”) that will be responsible for evaluating whether the Participating Human Subject is eligible to participate in the Tier 2 Phase (as defined below) of the UDN Study. In connection with assessing eligibility for enrollment in the Tier 2 Phase, the applicable Intake Site will evaluate the information submitted to the Coordinating Center and will also collect additional medical records, laboratory results, radiographic and pathology reports and any other information deemed pertinent by the Intake Site consistent with the UDN Protocol and the UDN Tier 1 ICA Form (collectively, the “**Tier 1 Study Data**”).

2. During the second phase of the UDN Study (the “**Tier 2 Phase**”), and only after the Participating Human Subject has signed a UDN Tier 2 ICA Form, the applicable Intake Site or other UDN Clinical Site to which a Participating Human Subject is assigned (“**Evaluation Site**”) will perform a clinical evaluation of the applicable Participating Human Subject, pursuant to the UDN Protocol, may collect data from the evaluation and from post-evaluation surveys of the Participating Human Subject, and may request other UDN Participating Institutions, including

without limitation, a Sequencing Core, to perform additional tests or analysis pertaining to the Participating Human Subject (all resulting data, collectively, the “**Tier 2 Study Data**” and together with the Tier 1 Study Data, the “**Study Data**”).

III. ICA Forms

A. **Required Content of the UDN Tier 1 ICA Form.** The UDN Tier 1 ICA Form, once approved by the UDN Central IRB, will be attached and incorporated into this Agreement as Exhibit 4. The UDN Tier 1 ICA Form may be amended, subject to UDN Central IRB approval, provided that the elements set forth in Sections III.A.1-3 are included (the “**Required Tier 1 Data Sharing ICA Form Elements**”). Specifically, the Required Tier 1 Data Sharing UDN ICA Form Elements inform Participating Human Subjects that:

1. Tier 1 Study Data pertaining to them, including Tier 1 Study Data containing PHI, may be disclosed to, and maintained by, the Coordinating Center.
2. Tier 1 Study Data pertaining to them, including Study Data containing PHI, will be disclosed to one or more UDN Clinical Sites in connection with efforts to determine whether such Participating Human Subject is eligible to participate in the Tier 2 Phase.
3. Tier 1 Study Data pertaining to them may be maintained by the Coordinating Center and by one or more UDN Clinical Sites.

B. **Required Content of the UDN Tier 2 ICA Form.** The UDN Tier 2 ICA Form, once approved by the UDN Central IRB, will be attached and incorporated into this Agreement as Exhibit 5. The UDN Tier 2 ICA Form may be amended, subject to UDN Central IRB approval, provided that the elements set forth in Sections III.B.1-3 are included (the “**Required Data Sharing ICA Form Elements**”). Specifically, the Required Data Sharing ICA Form Elements inform Participating Human Subjects that:

1. Study Data pertaining to them, including Study Data containing PHI, may be disclosed to, and maintained by, the Coordinating Center.
2. Study Data pertaining to them, including Study Data containing PHI, (a) will be disclosed by the Coordinating Center to UDN Participating Institutions and (b) may be disclosed by one UDN Participating Institution to another UDN Participating Institution, in connection with efforts to diagnose that Participating Human Subject or to identify commonalities with other Participating Human Subjects that might ultimately assist with the diagnosis or treatment of the Participating Human Subject or other Participating Human Subjects.
3. Study Data pertaining to them that has been de-identified in accordance with a methodology set forth in HIPAA (“**De-Identified Study Data**”) may be shared with researchers at UDN Participating Institutions that are not specifically engaged on the UDN Study and with non-UDN third parties so that these recipients can conduct research, which may or may not be related to the objectives of the UDN Study.

C. **Revocation.** In the event that a Participating Human Subject submits a revocation of his/her UDN Tier 1 ICA Form and/or his/her UDN Tier 2 ICA Form to a UDN Participating Institution other than the Coordinating Center, such UDN Participating Institution will

immediately notify the Coordinating Center so that the Coordinating Center can take all necessary steps to effectuate revocation. The Coordinating Center will notify all other UDN Participating Institutions of the Participating Human Subject's revocation. Each UDN Participating Institution shall comply with any required return or destruction of Study Data in its possession containing PHI per the instructions from the Coordinating Center and the Coordinating Center will take the necessary steps to delete and destroy Study Data containing PHI from the Central Database consistent with the Participating Human Subject's revocation.

IV. Permitted Uses and Disclosures of Study Data

A. **Uses and Disclosures of Study Data Within the UDN.** The Study Data may be used and disclosed by a UDN Participating Institution solely for the purposes permitted by, and in accordance with, the UDN Protocol and the UDN Tier 1 and Tier 2 ICA Forms.

1. The Intake Site will forward its summary of findings, along with any additional information relevant to the decision regarding a Participating Human Subject's eligibility for Tier 2 Phase participation, to the Coordinating Center following completion of the Tier 1 Phase for the applicable Participating Human Subject.

2. Each UDN Participating Institution will be responsible to provide all elements of Tier 2 Study Data that it collects in the course of the UDN Study to the Coordinating Center for inclusion in the Central Database. In general, it is expected that each Evaluation Site will make reasonable efforts to transfer Tier 2 Study Data pertaining to a Participating Human Subject to the Coordinating Center within thirty (30) days following completion of the Participating Human Subject's clinical evaluation during the Tier 2 Phase, and to update such information promptly upon receipt of related test results or additional Tier 2 Study Data. Subject to the foregoing, a Participating Human Subject's Evaluation Site may transfer directly such portions of his/her Study Data to another UDN Participating Institution (such as a Sequencing Core) as are necessary for the performance of testing or analysis of such Study Data requested by the Evaluation Site during the Tier 2 Phase of the UDN Study. The results of such testing or analysis shall also constitute Study Data, and the UDN Participating Institution performing such testing or analysis shall transfer the resulting Study Data to the Evaluation Site, which will then transfer them to the Coordinating Center.

3. Subject to the terms of this Agreement, the Coordinating Center will provide the UDN Participating Institutions with access to the Tier 2 Study Data it maintains to enable them to (a) assist in the diagnosis of a Participating Human Subject; (b) identify distinguishing, unique or common clinical or biological themes across Participating Human Subjects that might ultimately assist with diagnosis or treatment of one or more Participating Human Subjects and (c) conduct research on the etiology of undiagnosed diseases, consistent with the procedures and processes set forth in the UDN Protocol and the terms of this Agreement. The Coordinating Center may also use Study Data to perform analyses in support of the UDN Study, including quality control measurement and providing NIH and other institutions with process and outcome measures of the functioning of the UDN Study.

4. UDN Participating Institutions other than the Coordinating Center may also generate and transfer to each other Study Data that, pursuant to the UDN Protocol, does not need to be submitted to the Coordinating Center; provided that each UDN Participating Institution

transferring the Study Data or receiving the Study Data shall be obligated to treat such Study Data in a manner that complies with the terms of this Agreement. The Coordinating Center shall not be responsible for Study Data maintained by or transferred by or among other UDN Participating Institutions.

5. All transfers of Study Data, whether to or from the Coordinating Center, or among UDN Participating Institutions other than the Coordinating Center, will be done via encrypted and authenticated data transfer. Each UDN Participating Institution agrees to employ technical, physical and other safeguards to maintain the Study Data in a secure and confidential manner that prevents uses or disclosures of the Study Data not permitted by this Agreement. For paper records, safeguards include, but are not limited to, locked file cabinets or continual physical presence in a room that locks. For electronic records, safeguards include authentication of each Study Data access, explicit authorization of each Study Data access, end-to-end encryption at-rest and in-transit, and an audit trail of such access. Without limiting the foregoing, each UDN Participating Institution agrees that it shall protect, store and secure all Study Data made available to it as part of the UDN Study, whether received directly or through the Central Database, using HIPAA-compliant systems and controls. No UDN Participating Institution shall be responsible for the security of Study Data held or maintained by another UDN Participating Institution. Access to the Central Database, and transfer of Study Data by one UDN Participating Institution to another Recipient, will be limited only to those authorized named individuals identified by each UDN Participating Institution in a written notice sent to the Coordinating Center by a designated official of the UDN Participating Institution. The Coordinating Center, as administrator of the Central Database, may take such actions as it believes necessary or appropriate for proper administration of the Central Database, at its sole reasonable determination, including without limitation, determining and administering processes for issuance of accounts and passwords for authorized access to the Central Database; determining and issuing standard operating procedures for security incident response within the UDN; and restricting or suspending any Recipient's or UDN Investigator's or other individual's access to Study Data in the Central Database. Failure of a UDN Participating Institution or authorized user of the Central Database to comply with the information security plan and the standard operating procedures regarding incident response as may be issued and modified from time to time by the Coordinating Center, and with the terms of this Agreement, may give rise to restriction or suspension of access to the Central Database.

B. Uses and Disclosures of Study Data Outside of the UDN. The Coordinating Center may provide De-Identified Study Data in the database of Genotypes and Phenotypes (dbGaP) of the National Center for Biotechnology Information or other research data repository for further sharing with researchers from UDN Participating Institutions who are not themselves personally engaged in the UDN Study and with non-UDN third parties, under specific rules established by the NIH and consistent with Applicable Law.

C. Cloud Service or Third Party Provider. The Coordinating Center may engage one or more cloud service or other third party providers to host the Central Database, and may provide third party service providers access to the Central Database, as may be necessary from time to time for maintenance, quality control, and other administration and management of the Central Database. The Coordinating Center may take such measures as it deems necessary from time to

time for proper management and security of the Central Database: this may include downtime for servicing or maintenance purposes, and may include imposition of access restrictions.

D. **Not Expressly Permitted; Prohibited.** For the avoidance of doubt, notwithstanding anything to the contrary in this Article IV, a use or disclosure of Study Data is not permitted unless it is expressly permitted by the UDN Protocol and, as applicable, the UDN Tier 1 and/or Tier 2 ICA Forms.

E. **Principal Investigator Obligations.** Study Data provided to a UDN Participating Institution containing PHI will be used only by the Recipient's Principal Investigator and those individuals under his/her direct supervision in accordance with this Agreement, the UDN Protocol, and, as applicable, the UDN Tier 1 and/or Tier 2 ICA Forms. The Principal Investigator is responsible for informing the individuals under his/her supervision of the provisions and restrictions contained herein and to secure documentation of their agreement to abide by such provisions and restrictions before providing access to the Study Data.

F. **No Contact.** Each Recipient agrees that it will not use the Study Data to contact or attempt to contact Participating Human Subjects about whom the Study Data pertains, except as expressly permitted by the UDN Protocol and, as applicable, the UDN Tier 1 and/or Tier 2 ICA Forms. In the event that a Participating Human Subject must be contacted, such contact will be by the Coordinating Center or applicable Evaluation Site. Each Recipient further agrees that it will not seek to re-identify any individual whose information is included within De-identified Study Data.

G. **Study Data Retention.** Each UDN Participating Institution will maintain all Study Data it collects, at a minimum, for such time as is required by the UDN Protocol.

V. Compliance

A. **Generally.** Each Party agrees to comply with Applicable Law that is pertinent to such Party.

B. **IRB.** The Parties agree to comply with all directives from the UDN Central IRB.

C. **Confidentiality.** In connection with the UDN Study, a UDN Participating Institution may provide another UDN Participating Institution with certain of the disclosing Party's non-public business, technical, financial or strategic information, other than Study Data, that it marks as confidential or indicates is confidential by written notice given to the receiving Party within fifteen (15) days following disclosure ("**Confidential Business Information**"). Each UDN Participating Institution shall maintain the confidentiality of all Confidential Business Information provided to it by another UDN Participating Institution, and shall not disclose such information to any third party, for a period of five (5) years after its receipt of such Confidential Business Information. The receiving Party shall not be bound by confidentiality obligations hereunder with respect to Confidential Business Information if (i) such Confidential Business Information is or becomes part of the public domain, except through breach of this Agreement by the receiving Party; (ii) such Confidential Business Information was in the receiving Party's possession prior to the time of disclosure by or on behalf of the disclosing UDN Participating Institution; (iii) such Confidential Business Information becomes available to the receiving Party from a third party who is not legally prohibited from disclosing such Confidential Business

Information; (v) the receiving Party can demonstrate by clear and convincing written evidence such information was developed by or for the receiving Party independently of the disclosure of the Confidential Business Information to the receiving Party; or (vi) disclosure is required by Applicable Law and the receiving Party, to the extent practicable, provides prior written notice to the disclosing Party of such legal requirement so that the disclosing Party may seek a protective order or similar remedy.

VI. Intellectual Property

A. **Publication.** In all oral presentations or written publications (each, a “**Publication**”) involving the analysis of Study Data, UDN Investigators will acknowledge the UDN in a form of acknowledgement specified in the UDN Publication Policy. The Parties will abide by the UDN Publication and Presentation Policy and Procedures as defined by the UDN Publications Working Group and ratified by the UDN Steering Committee, attached as Exhibit 6 to this Agreement. The UDN Publications Working Group may from time to time propose, and the UDN Steering Committee may from time to time ratify, modifications to UDN Publication and Presentation Policy and Procedures, with material changes to be emailed to each Party’s email address as provided on the signature pages to this Agreement. No Publication will contain PHI.

B. **Commercial Purposes; Prohibited.** The Parties agree that Study Data will not be used for commercial purposes, including selling, advertising, commercial screening, or transferring the Study Data to a third party for commercial purposes.

1. Notwithstanding anything to the contrary in Section VI.B, De-identified Study Data involving the use of variant information, including allele frequency and other derived characteristics (collectively “**Variant Results**”) generated during the term of this Agreement by a Sequencing Core for any previously diagnosed, subsequently diagnosed, and still undiagnosed diseases, may be used by any such Sequencing Core as part of commercial sequencing services it offers to third parties; provided, however that no Party providing said services shall secure, nor attempt to secure or apply, a patent or other intellectual property right, including any trade secret protection, to the use of the Variant Results. Each Sequencing Core agrees to the foregoing proviso for itself only.

C. **Intellectual Property.** Subject to the rights of the Parties to use and share Study Data pursuant to this Agreement and the UDN Protocol, each Party shall own the portion of original Study Data it collects under, and in accordance with the terms of, its NIH award (or subaward). Inventorship of any invention that is either (i) conceived or (ii) first actually reduced to practice in the performance of the UDN Study will be determined according to U.S. patent laws, and ownership shall follow inventorship. Joint inventions will be owned jointly. Other results obtained from the uses permitted by this Agreement will be governed by the NIH Grants Policy Statement, section 8.2 “Availability of Research Results: Publications, Intellectual Property Rights, and Sharing Research Resources” (see http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch8.htm).

VII. Certification

The Parties certify and affirm that the contents of any statements made herein are truthful and accurate and that they are authorized by their institution to agree to adhere to the principles and policies specified outlined in this document.

VIII. Term and Termination

A. **Term.** The term of this Agreement shall commence on the Effective Date and continue in full force and effect until terminated in accordance with Section VIII.B.

B. **Termination of the Agreement.**

1. This Agreement may be terminated upon the mutual written agreement of all Parties.
2. This Agreement shall terminate automatically upon completion of the UDN Study.
3. In the event that the UDN Central IRB requires that the UDN Study terminate early for any reason, this Agreement will terminate immediately; provided that the Parties will follow any UDN Central IRB requirements regarding the orderly termination for the protection of Participating Human Subject safety.

C. **Termination of a Party.**

1. In the event of the termination or expiration of any Party's UDN award from NIH (or subaward, if applicable), such Party shall be automatically terminated from this Agreement and Exhibit 1 shall be deemed modified to remove such Party's name from the list of UDN Participating Institutions.
2. Upon notice of termination of any Party to this Agreement, the UDN Principal Investigator of the terminating Party shall transfer Study Data for inclusion in the Central Database to the Coordinating Center in accordance with Section IV.A.2.

IX. Liabilities

A. **Liabilities.** Each Party shall be responsible for its negligent acts or omissions and the negligent acts or omissions of its employees, officers, or directors, to the extent allowed by law; provided, however, that any Party that is an agency of the United States Government, may be liable only to the extent as provided under the Federal Tort Claims Act (28 U.S.C. Chapter 171). No indemnification for any loss, claim, damage, or liability is intended or provided by any Party under this Agreement.

B. **Limitation.** The Study Data are provided as a service to the research community. THEY ARE BEING SUPPLIED TO RECIPIENT WITH NO WARRANTIES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. NO WARRANTY WITH RESPECT TO THE CENTRAL DATABASE IS PROVIDED, INCLUDING WITHOUT LIMITATION, ANY UPTIME WARRANTY. The Parties make no representations that the use of the Study Data will not infringe any patent or proprietary rights of third parties.

X. Miscellaneous

- A. **Amendments and Modification.** This Agreement may only be amended, modified or supplemented by an agreement in writing signed by each Party, except as specified in Sections II.B., VI.A. and VIII.C. above. Amendments to the UDN Protocol will be deemed incorporated as modifications to Exhibit 3 (and Exhibits 4 and 5, as applicable) upon approval by the UDN Central IRB.
- B. **Assignment.** No Party will assign or transfer any rights or obligations under this Agreement without the prior written consent of each of the other Parties, and only if permitted under the UDN Protocol, the UDN Tier 1 and Tier 2 ICA Forms, the applicable NIH award (or subaward), and Applicable Law.
- C. **Entire Agreement.** This Agreement and any Exhibits attached hereto constitute the entire agreement among the Parties and supersede all prior communications, representations, or agreements, either verbal or written among the Parties with respect to the subject matter hereof. Notwithstanding the foregoing, this Agreement shall not supersede the terms of any NIH notice of grant award. Each Party confirms that it is not relying on any representations or warranties of any other Party except as specifically set forth herein.
- D. **Independent Contractors; Relationship of the Parties.** This Agreement shall not be deemed to create any partnership, joint venture, or agency relationship between or among the Parties. Each Party shall act hereunder as an independent contractor and its agents and employees shall have no right or authority under this Agreement to assume or create any obligation on behalf of or in the name of, the other Parties. All persons employed by a Party shall be employees of such Party and not of the other Parties, and all costs and obligations incurred by reason of any such employment shall be for the account and expense of such Party.
- E. **Notice.** All notices and other communications required or permitted to be given pursuant to this Agreement shall be in writing and shall be deemed sufficiently given if personally delivered or sent by mail, recognized delivery service, electronic mail, postage prepaid, or by facsimile transmission with mail confirmation. Such communications shall be given to each Party at the addresses listed on each signature page.
- F. **Severability.** All agreements and covenants contained herein are severable, and in the event any of them shall be held to be invalid by any competent court, this Agreement shall be interpreted as if such invalid agreements or covenants were not contained herein.
- G. **Survivability.** All causes of action accruing to any Party under this Agreement shall survive termination. Each provision of this Agreement that would by its nature or terms survive any termination of the Agreement shall survive, including without limitation Articles I, IV.D.-G., V, VI.B., VI.C, VIII.B.3, VIII.C.2, IX and X.
- H. **Waiver.** Failure by any Party to insist upon strict performance of any provision herein by any Party shall not be deemed a waiver by such Party of its rights or remedies, or a waiver by it of any subsequent default by such other Party, and no waiver shall be effective unless it is in writing and duly executed by the Party entitled to enforce the provision being waived.
- I. **Signatures.** The Parties individually and collectively have caused this Agreement to be executed by their duly authorized representatives as of the dates below on their respective signature page. This Agreement may be executed in one or more counterparts, all of which shall

be considered one and the same. The Parties agree that execution of this Agreement by exchanging facsimile, PDF, or e-Signature signatures shall have the same legal force and effect as the exchange of original signatures.

E-signature, for purposes of this Section XI.I. shall mean signature that consists of one or more letters, characters, numbers or other symbols in digital form incorporated in, attached to or associated with the electronic document, that (i) is unique to the person making the signature; (ii) the technology or process used to make the signature is under the sole control of the person making the signature; (iii) the technology or process can be used to identify the person using the technology or process; and (iv) the electronic signature can be linked with an electronic document in such a way that it can be used to determine whether the electronic document has been changed since the electronic signature was incorporated in, attached to or associated with the electronic document.

APPENDIX 16: Publications and Research Reference Sheets

TITLE: Grant Applications (which utilize UDN data and resources)

1. RESPONSIBLE PERSONNEL:

- 1.1. Investigators: Investigators at the UDN Clinical Sites, Cores, Coordinating Center, and NIH.
- 1.2. Coordinating Center Project Manager: Individual at the Coordinating Center who communicates with the Clinical Sites, Cores, Coordinating Center, and NIH team members to complete project-related activities.
- 1.3. Coordinating Center Executive Director: Individual at the Coordinating Center who directs project-related activities of the UDN.
- 1.4. Steering Committee: Committee made up of UDN Investigators that decides on the priorities and order of business of the UDN.

2. PROCEDURE:

- 2.1. Investigators submit a concept form (link: https://hms.az1.qualtrics.com/SE/?SID=SV_3CyZOKOuiEvnCx7) for a grant application that will rely on UDN data to the Coordinating Center Project Manager.
- 2.2. The Coordinating Center Project Manager updates the grant application log in Box.com.
- 2.3. The Coordinating Center Project Manager submits the grant application request to the Coordinating Center Executive Director.
- 2.4. The Coordinating Center Executive Director notifies the Coordinating Center Project Manager of the grant application Steering Committee presentation date.
- 2.5. The Coordinating Center Project Manager notifies the Investigator of the grant application presentation date.
- 2.6. The Investigators present at the Steering Committee meeting.
- 2.7. The Steering Committee votes on the application.
- 2.8. The Coordinating Center Project Manager updates the grant application log with the decision.
 - 2.8.1. If the grant application is accepted, the Investigators notify the Coordinating Center Project Manager and the Coordinating Center Project manager updates the grant application log.

Version: 1
Effective Date: May 1, 2015
Last Reviewed Date: June 9, 2015

TITLE: Internal UDN Research Concept Sheets
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1. RESPONSIBLE PERSONNEL:

- 1.1. Investigators: Investigators at the UDN Clinical Sites, Cores, Coordinating Center, and NIH.
- 1.2. Coordinating Center Project Manager: Individual at the Coordinating Center who communicates with the Clinical Sites, Cores, Coordinating Center, and NIH team members to complete project-related activities.
- 1.3. Publication and Research Committee Co-Chairs: Individuals who lead the Publications and Research Committee.
- 1.4. Publication and Research Committee Members: Individuals who participate in the Publications and Research Committee.
- 1.5. Coordinating Center Administrator: Individual at the Coordinating Center who handles the administrative tasks for the Coordinating Center.

2. PROCEDURE:

- 2.1. Investigators submit a research concept sheet (link: https://hms.az1.qualtrics.com/SE/?SID=SV_3CyZOKOuiEvnCx7) to the Coordinating Center Project Manager.
- 2.2. The Coordinating Center Project Manager updates the research concept sheet log in Box.com.
- 2.3. The Coordinating Center Project Manager sends the research concept sheet to the Publication and Research Committee Co-Chairs or to the Survey Committee chair if the proposed research involves a survey (see section XVI. Surveys for survey research approval workflow).
 - 2.3.1. The co-chairs identify any duplication of effort or other concerns before circulation to the full committee.
- 2.4. The Publication and Research Committee Co-Chairs send the research concept sheet to the Publication and Research Committee Members for comments.
- 2.5. The Publication and Research Committee Members vote on the research concept sheet by email. For example, one basis of rejection would include if there is already another study/s underway with overlapping aims.
 - 2.5.1. If a Publication and Research Committee Member wants discussion, the Publications and Research Committee co-chairs contact the Coordinating Center Administrator to schedule a meeting.
- 2.6. The Publication and Research Committee Co-Chairs notify the Investigator and Coordinating Center Project Manager with the decision.
- 2.7. The Coordinating Center Project Manager updates the research concept sheet log with the decision.
 - 2.7.1. If the research concept sheet is accepted, the Coordinating Center Project Manager uploads the research concept sheet to Box.com.

Version: 1
Effective Date: May 1, 2015
Last Reviewed Date: June 09, 2015

TITLE: Research Projects Led by External Investigators

1. RESPONSIBLE PERSONNEL:

- 1.1. Investigators: Investigators outside of the UDN.
- 1.2. Coordinating Center Project Manager: Individual at the Coordinating Center who communicates with the Clinical Sites, Cores, Coordinating Center, and NIH team members to complete project-related activities.
- 1.3. Coordinating Center Executive Director: Individual at the Coordinating Center who directs project-related activities of the UDN.
- 1.4. Steering Committee: Committee made up of UDN Investigators that decides on the priorities and order of business of the UDN.

2. PROCEDURE:

- 2.1. Investigators submit a concept form (link: https://hms.az1.qualtrics.com/SE/?SID=SV_3CyZOKOuiEvnCx7) for an external project that will rely on UDN data to the Coordinating Center Project Manager.
- 2.2. The Coordinating Center Project Manager updates the external project log in Box.com.
- 2.3. The Coordinating Center Project Manager submits the external project request to the Coordinating Center Executive Director.
- 2.4. The Executive Director notifies the Coordinating Center Project Manager of the external project Steering Committee presentation date.
 - 2.4.1. The Executive Director or the Steering Committee may form an ad hoc review committee to vet the initial proposal.
- 2.5. The Coordinating Center Project Manager notifies the Investigator of the presentation date.
- 2.6. The Investigator presents at the Steering Committee meeting.
- 2.7. The ad hoc review committee gives its recommendations to the Steering Committee.
- 2.8. The Steering Committee votes on the external project.
- 2.9. The Coordinating Center Project Manager updates the external project log with the decision.

Version: 1
Effective Date: May 1, 2015
Last Reviewed Date: June 9, 2015

1. RESPONSIBLE PERSONNEL:

- 1.1. Investigators: Investigators at the UDN Clinical Sites, Cores, Coordinating Center, and NIH.
- 1.2. Coordinating Center Project Manager: Individual at the Coordinating Center who communicates with the Clinical Sites, Cores, Coordinating Center, and NIH team members to complete project-related activities.
- 1.3. Publication and Research Committee Co-Chairs: Individuals who lead the Publications and Research Committee.
- 1.4. Publication and Research Committee Members: Individuals who participate in the Publications and Research Committee.
- 1.5. Coordinating Center Administrator: Individual at the Coordinating Center who handles the administrative tasks for the Coordinating Center.

2. PROCEDURE:

- 2.1. Investigators submit a manuscript to the Coordinating Center Project Manager.
- 2.2. The Coordinating Center Project Manager updates the manuscript log in Box.com.
- 2.3. The Coordinating Center Project Manager sends the manuscript to the Publication and Research Committee Co-Chairs.
- 2.4. The Publication and Research Committee Co-Chairs send the manuscript to the Publication and Research Committee Members for comments (initial review of appropriate authorship, acknowledgements and broad science).
- 2.5. The Publication and Research Committee Members vote on the manuscript by email.
 - 2.5.1. If a Publication and Research Committee Member wants discussion, the group contacts the Coordinating Center Administrator to schedule a meeting.
- 2.6. The Publication and Research Committee Co-Chairs notify the Investigator and Coordinating Center Project Manager with the decision (ideally within 2 weeks).
- 2.7. The Coordinating Center Project Manager updates the manuscript log with the decision.
 - 2.7.1. If the manuscript is accepted for publication, the Coordinating Center Project Manager uploads the manuscript to Box.com.

Version: 1
Effective Date: May 1, 2015
Last Reviewed Date: June 9, 2015

APPENDIX 17: Proposed UDN Metrics

Note: NIH indicates NIH Program metrics, which will be calculated over the course of the network. UDN indicates UDN-nominated metrics, which may be calculated over the course of the network.

	Source	Performance Metrics and Milestones
1	NIH	By Oct 1, 2014 IRP-UDP will identify at least 5 candidate genes.
2	NIH	IRP-UDP will analyze 400 SNPs and 400 WES or WGSs per year through FY2017.
3	NIH	By Oct 1, 2015 Extramural Clinical Sites (ECSs) to see 25 patients per year per site to initiate phenotyping.
4	NIH	By Oct 1, 2015 ECSs to identify candidate genes by analyzing 200 SNPs and 200 exomes/genomes per year, increasing to 800 SNPs and exomes/genomes per year in years 3 and 4.
5	NIH	Define the mechanism of at least 1 candidate gene in the pathophysiology of a rare or yet-to-be described disease.
6	NIH	By Oct 1, 2016 all ECSs to see 50 patients per year per site.
7	NIH	By Jan 2016—Identify 10 unidentified diseases; by Jan 2018, identify 20 unidentified diseases.
8	UDN	Number of inquiries from potential patients and doctors through the UDN portal (coordinating center).
9	UDN	Number and percentage of inquiries that result in admission reviews performed (each site).
10	UDN	Number and percentage of admission reviews that result in acceptance (each site).
11	UDN	Number and percentage of people accepted for admission who are actually admitted (each site).
12	UDN	Number and percentage of people admitted who get a provisional diagnosis (each site).
13	UDN	Number and percentage of people admitted who get a validated diagnosis (each site).
14	UDN	Number and percentage of people admitted who have a novel diagnosis (overall).
15	UDN	Number and percentage of people admitted who die or become ineligible before admission (each site).
16	UDN	Number and types of adverse events and deaths while traveling to/from site or during hospital stay (each site).

	Source	Performance Metrics and Milestones
17	UDN	Number and percentage of people admitted who experience an adverse event while traveling to site or during hospital stay (each site).
18	UDN	Time from inquiry to chart completion (each site).
19	UDN	Time from chart completion to admission review/decision (each site).
20	UDN	Time from decision to admit to admission (each site).
21	UDN	Time from completion of admission stay to data upload to UDN data repository (each site).
22	UDN	Time from completion of admission stay to first provisional or validated diagnosis communicated to participant (each site).
23	UDN	Number and percentage of participants with complete data in the UDN data repository (each site).
24	UDN	Time from when sample is gathered to when extracted DNA is received by the sequencing center (each site).
25	UDN	Time from receipt of extracted DNA to providing raw sequencing data (sequencing center).
26	UDN	Time from completion of sequencing to network sequencing analysis completion (each site).
27	UDN	Number and percentage of isolated individual sequences that result in identification of a candidate gene (network-wide).
28	UDN	Number and percentage of trio (or larger) sequences that result in identification of a candidate gene (network-wide).
29	UDN	Number and percentage of sequences that result in the identification of a candidate gene (network-wide).
30	UDN	Number of people who receive a non-genetic diagnosis.
31	UDN	Time from sequence generation and completion of phenotyping to upload to dbGaP (clinical site + coordinating center?).
32	UDN	Number and percentage of candidate variants that go on to be matched with a lab for functional testing (network-wide).
33	UDN	Quality of Human Phenotype Ontology phenotyping by site (each site).
34	UDN	Participant and family satisfaction (each site and network-level).
35	UDN	Ability to integrate or link data with Phenome Central (coordinating center).
36	UDN	Geographic distribution (zip code) of patient.
37	UDN	Geographic distribution (zip code) of referring doctor.
38	UDN	Number and percentage of admissions referred by site (e.g., Site A refers patient who is seen at Site A) (each site).
39	UDN	Number and percentage of admission reviews, admissions, and diagnoses

	Source	Performance Metrics and Milestones
		by race, ethnicity, and age-range (network-wide).
40	UDN	Predominant phenotype class/subspecialty admitted (each site).
41	UDN	Proportion of participants for whom insurance was billed (each site).
42	UDN	Average, minimum, and maximum direct cost of evaluation tests per participant.
43	UDN	Publications and presentations (each site and coordinating center).
44	UDN	Time to complete phenotype from date of admission (each site).
45	UDN	Number and proportion of participants with an HPO phenotype (by site).

APPENDIX 18: Billing Surveys

Billing Survey- Institutions using Clinical Billing (to be completed for an individual participant)

1. List the primary, secondary, and tertiary insurers that were billed for the participant (**if participant is self-pay and if all expenses were thus paid for out of the grant, please fill out the individual participant grant billing survey rather than this one**).
2. List all clinical consultations/procedures/tests/imaging ordered for this participant

Please list those that were:

- a) Rejected:
- b) Reimbursed:
- c) Appealed (outcome of the appeal):
- d) Paid for out of grant funds:
- e) Paid for out of Supplemental Funds (please specify the fund e.g CTSA):
- f) Written-off by medical center:

If available, please list the total co-pay/deductible payment that this participant incurred:

\$

List total reimbursed costs for this participant \$_____

List total unreimbursed costs for this participant \$_____

3. Please list services (if any) that were directly charged to the grant (never submitted for reimbursement) and why.
4. Was pre-authorization or a billing estimate performed for the participant? Yes/No

Was it for the entire UDN evaluation? Yes/No

If it was for a specific test/s, please list:

Did study personnel perform this task, or did you have assistance from the medical center?

5. Was the billing processed “specially” for this UDN participant at your medical center (for example manually handled rather than automated)? Yes/No

Please provide details regarding the special handling.

Who was responsible for the special handling (study personnel, medical center staff, other)?

6. Were there any unanticipated costs for the participant? Please list the reasons and the costs (These would not include co-pays/deductibles, but would be expenses such as needing acute care for adverse events related to the UDN procedures and/or unrelated acute illnesses that needed hospitalization etc).

How were such costs paid for? Insurance billed _____ Grant funds _____
Self-pay _____ Other (specify) _____

7. Did your participant incur any other out-of pocket expenses (do not include co-pays/deductibles)? If so state the reasons and the amount.
8. What was the total cost of travel for this participant and family member and what did this include?

How were the travel costs reimbursed? Grant _____ NORD central fund _____

9. Please describe your or your participant's experience with being reimbursed for travel by the NORD fund.
10. Any lessons learned from this participant's billing experience? Please elaborate.

Billing Survey- Institutions using Grant Billing (to be completed for an individual participant)

1. List all clinical consultations/procedures/tests/imaging ordered for this participant

Please list those that were:

- a) Paid for out of grant funds:
- b) Paid for out of Supplemental funds. Please specify type/source of funds:
- c) Written-off by medical center:

Total costs paid for out of the grant \$ _____

2. List the primary, secondary and tertiary insurers (if available) for the participant that could have been billed for the UDN evaluations at your site. Please specify if the participant is self-pay.
3. Was a billing estimate performed for the participant prior to or at the UDN visit? Yes/No
If so, did study personnel perform this task, or did you have assistance from the medical center?
4. Was the billing processed "specially" for this UDN participant at your medical center (for example manually handled rather than automated)? Yes/No

Please provide details regarding the special handling.

5. Were there any unanticipated costs for the participant? Please list the reasons and the costs (These would not include co-pays/deductibles, but would be expenses such as needing acute care for adverse events related to the UDN procedures and/or unrelated acute illnesses that needed hospitalization etc).

How were such costs paid for? Insurance billed _____ Grant funds _____
Other (specify) _____

6. Did your participant incur any other out-of pocket expenses? If so state the reasons and the amount.
7. What was the total cost of travel for the participant and family member and what did this include?

How were the travel costs reimbursed? Grant _____ NORD central fund _____

8. Any lessons learned from this participant's billing experience?

Billing Survey- Institutions using Clinical Billing (summary of first 5 participants)

1. What is the average reimbursed expense for the first five participants (sum of all reimbursed expenses/total sum of all expenses submitted for reimbursement across the 5 participants)?
\$ _____
(Please include all costs that were submitted to insurers: clinical consultations, tests, procedures etc. Do not include co-pays/deductibles and travel costs or expenses that were paid for out of grant funds)
2. What is the average unreimbursed expense for the first five participants (sum of unreimbursed expenses/total sum of all expenses submitted for reimbursement across the 5 participants)? \$ _____
3. Please detail the insurance carriers that you have billed so far (n= number of participants):
 - a. Private Carriers (n=)
 - b. Medicaid (n=)
 - c. Medicare (n=)
 - d. Other (institutional funds etc.) (n=)
4. How many of your participants have been uninsured thus far and thus expenses were paid for out of the grant?
5. Any lessons learned from your clinical billing experience thus far?

APPENDIX 19: International Collaborative Clinical Site Application



International Collaborative Clinical Site Application

Date of Application: _____

Organization: _____

Main Contact Name: _____

Main Contact E-mail: _____

The UDN is open to International Collaborative Clinical Sites that agree to the criteria for participation described below.

Criteria for Participation in the UDN are:

- Each organization will inform the UDN NIH PO and the UDN Steering Committee about their group's plans for an affiliate UDN site.
- Each organization will specify the sequencing, laboratory, and clinical evaluation plans for their proposed affiliate site.
- Each organization is expected to contribute significantly to the project, bringing their particular expertise to bear on accomplishing the goals of the UDN in a timely manner. Participation in the UDN should include substantial intellectual contributions to the Network.
- Each organization will adhere to UDN publications policies, guidelines and agreements.
- Each organization will have a data-sharing plan.
- Each organization will take part in group activities, including attending some UDN Steering Committee meetings and working group calls and contributing to the products of these groups.
- Each organization will agree that they will not disclose confidential information obtained from other members of the UDN.
- Additional criteria may be added upon recommendations of the UDN Steering Committee, External Scientific Advisors, and the NIH UDN Working Group.

Applications will be reviewed by the UDN Steering Committee, UDN program staff, and the UDN External Scientific Advisors to determine whether a International Collaborative Clinical Site will be accepted. **A limited number of International Collaborative Clinical Sites may be approved and acceptance may be limited to one-year after which an assessment will be conducted for continuation.**

Please return your application to: UDN@hms.harvard.edu

The specific goals of the UDN are to:

- (1) Improve the level of diagnosis and care for patients with undiagnosed diseases through the development of common protocols designed by a large community of investigators.
- (2) Facilitate research into the etiology of undiagnosed diseases, by collecting and sharing standardized, high-quality clinical and laboratory data data, including genotyping, phenotyping, and documentation of environmental exposures.
- (3) Create an integrated and collaborative community across multiple clinical sites and among laboratory and clinical investigators prepared to investigate the pathophysiology of these newly recognized and rare diseases.

-
1. Please provide a concise description of your DNA sequencing, other laboratory, and clinical evaluation plans and a rationale for how your proposed International Collaborative Clinical Site addresses the goals of the UDN. *(maximum length 3 pages, font 11, single spacing)*

2. Please provide evidence that the proposed International Collaborative Clinical Site's research has received appropriate IRB approvals and is consistent with participants' informed consent.

3. Please provide evidence of funding to conduct the proposed research.

--

4. Please describe your data-sharing plan.

--

5. _____ (organization name) agrees to participate fully in UDN activities, including attending some UDN Steering Committee meetings and working group calls and contributing to the products of these groups. Initials of main organization contact: _____

6. _____ (organization name) agrees to abide by the UDN publications policies.

7. _____ (organization name) agrees to not disclose confidential information obtained from other members of the UDN.

APPENDIX 20: UDN Sequencing – Secondary and Incidental Findings

Purpose: This appendix is meant to provide clarification to UDN members about secondary and incidental findings that may be returned to participants during the UDN study. The appendix was developed by the UDN Genetic Counseling and Testing Working Group

“Secondary findings” are findings that the laboratory will look specifically for. “Incidental findings” are findings discovered by chance during the genetic testing process.

The UDN sequencing cores will report medically actionable secondary and incidental findings in the genes recommended for such reporting by the American College of Medical Genetics and Genomics (PMID: 23788249). In addition, both cores will report other secondary and incidental findings beyond the currently recommended 56 genes, provided these additional findings meet the threshold of having defined medical treatment or specific management guidelines. Stringent criteria for interpretation of variants in these medically actionable genes will be applied; reported variants will either be 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	TRPM4
Long QT type 4	ANK2
Familial Gastric Cancer	CDH1
Von Willebrand disease	VWF

Findings without medical treatment or specific management guidelines will not be reported. Examples of these types of disorders include Alzheimers disease and late onset skeletal dysplasias (ex. spondyloepiphyseal dysplasia - TRAPPC2 gene).

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.

It is important to note that the lack of reportable secondary and incidental findings does not rule out any disease-causing genetic changes in these or other genes. If a medical concern is raised regarding a specific condition, further genetic testing may be warranted.

Version: January 12, 2016

APPENDIX 21: UDNCB Sample Submission Form

UDN Central Biorepository

Sample Submission Form

Today's Date:

FedEx Tracking Number:

PI's Name: _____	Institution: _____
Phone: _____	Email: _____

Contact Person's Name (if different from PI): _____
Phone: _____ Email: _____

Comments: Add information regarding samples in table below that do not meet standard protocol (ex. Plasma tubes with < 500µl, PBMCs < 5x10⁶ cells)

Samples submitted

(1ST line is an example of a possible participant sample for the UDNCB). Add rows to table as needed.

Participant UDN ID#	Date of Birth	Date of Collection	Site	DNA (µg)	Serum (tube #)	Plasma (tube #)	Urine (tube #)	PBMCs (tube #)	Other (specify)
UDN12345 6	4/12/1981	9/18/2015	Stanford	1/3 total	3	3	5	1	3 fibro

**Ship samples Priority Overnight FedEx
(Mon – Weds, Holidays excluded) to:**

Lynette Rives

Vanderbilt Medical Center

DD-2205 MCN


Nashville, TN 37232-2578

Phone: 615-875-7198

Alt Phone: 615-343-0796

- Email copy of form prior to shipping to UDNCB@vanderbilt.edu
- Also enclose completed form with shipment
- Problems: Contact Lynette Rives
Phone: 615-875-7198 or email
UDNCB@vanderbilt.edu

APPENDIX 22: UDN Metabolomics Core Request Form

 UDN Metabolomics Request Form				
UDN Number				
Status	<i>proband</i>	<i>affected relative</i>		
UDN Clinical Site				
Submitter Information	<i>Name</i>	<i>Email</i>	<i>Phone</i>	<i>FAX</i>
Primary Contact	<i>Name</i>	<i>Email</i>	<i>Phone</i>	<i>FAX</i>
Sample Information				
Sample type				
Sample quantity				
Physiologic State at Time of Sample Collection				
Fasting	<i>yes</i>	<i>no</i>		
	<i>duration of fasting</i>			
Symptomatic	<i>yes</i>	<i>no</i>		
	<i>type of symptom</i>			
Medications				
Supplements / Unusual diet / Other comments				
Primary Diagnostic Hypotheses				
1				
2				
3				
4				
Analyses Requested				
Metabolomics (polar metabolites ≤500 Da)				
Lipidomics (glycerophospholipids, glycerolipids, sphingolipids, etc)				
Oxy-Lipids (prostaglandins, leukotrienes, etc)				
Shipping Information				
Ship Date				
Tracking Number				

APPENDIX 23: Annual UDN 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 24: UDN Patient Engagement Group Application



Overview of the UDN Participant Engagement Group (PEG)

Purpose: The purpose of the UDN Participant Engagement Group (PEG) is to provide the participant and family perspective on UDN research goals and participant experience. The PEG 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 PEG 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 PEG will include 6-7 participants and family members. Members of the PEG 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 will typically be one year in duration and renewable. However, in order to create staggered terms, for 3 of the first 6-7 members, the term will be 18 months instead of 12 months.

Meetings: Conference calls will be organized on a monthly basis or at a frequency determined by the PEG. Annual in-person meetings will be held at alternating locations. The tentative date for the first in-person meeting is Monday, October 24th, 2016, which will be held in Boston.

Compensation: PEG members will receive \$500 annually for their participation. Travel expenses will be covered for the annual in-person meeting and the Steering Committee meeting as needed.



Application Form: UDN Participant Engagement Group (PEG)

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

Application Deadline: August 1, 2016

Contact Information:

Last Name: First Name: UDN ID:

Street Address:

City: State: Zip Code:

Phone Number:

E-mail Address:

I am:

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

Areas of Interest:

I 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:

Availability:

Please list times when you are able to attend meetings:

☐ Daytime:

☐ Evening:

I am available to attend the first PEG in person meeting on October 24, 2016.

Yes ☐

No ☐