

Erythropoietic Protoporphyrins: Studies of the Natural History, Genotype-Phenotype Correlations, and Psychosocial Impact

PI: Dr. Manisha Balwani
NCT01688895
Document Date: Jun 26, 2015

Rare Diseases Clinical Research Network

Porphyrias Consortium

Erythropoietic Protoporphyrins: Studies of the Natural History, Genotype-Phenotype Correlations, and Psychosocial Impact

This protocol is for research purposes only and should not be copied, redistributed, or used for any other purpose. The procedures in this protocol are intended only for use by the Porphyrias Consortium investigators in carefully controlled settings. The Study Chair of this study should be consulted before using this protocol.

Study Chair:

Manisha Balwani, MD, MS

Department of Genetics & Genomic Sciences
Icahn School of Medicine at Mount Sinai
One Gustave L. Levy Place, Box 1497
New York, NY 10029
Phone: 212-241-0915
Fax: 212-426-9065
Email: manisha.balwani@mssm.edu

Table of Contents

Contents

Participating Institutions/Investigators Table (contact information).....	4
1. Synopsis.....	6
1.A. Protocol Overview.....	8
2. Objective and Research Question	8
3. Background	8
4. Study Design and Methods	10
4.A. Inclusion criteria	11
4.B. Exclusion Criteria.....	11
4.C. Recruitment and Enrollment of Subjects	11
4.D. Retention strategies.....	13
4.E. Data Elements	14
4.E.1. Study Visits Overview:	14
4.E.2. Data Collection, Maintenance, and Storage	15
4.E.3. Data Items	17
4.F. Sample Collection and Storage	18
4.F.1. Sample Collection	18
4.F.2. Sample Identification and Storage	19
4.F.3. Banking of Samples	19
4.G. Longitudinal investigation of natural history, complications, and outcomes	20
4.H.1. Method to determine if there are specific genotypes or other biomarkers that predict outcome	20
4.H.2. Method for determining risk of disease complications and adverse outcomes	21
4.H.3. Method to assess the long-term medical complications of the erythropoietic protoporphyrias and their treatment	21
4.I. Challenges	21
5. Data and Safety Monitoring Plan	22
5.A Definitions	23
5.B Reporting Timeline.....	23
5.C Standard Elements	23
5.D Procedures	24
5.E Study Discontinuation	24
5.F Subject Discontinuation	24
5.G Data Quality and Monitoring Measures.....	24

6. Statistical Considerations.....	25
6.A Analysis Plan	25
6. B Power Calculation.....	26
7. Data Management.....	26
7.A Enrollment.....	27
7.B Data Entry.....	27
7.C Data Quality Control	27
7.D Laboratory data flow	Error! Bookmark not defined.
8. Human Subjects.....	27
8.A. Human subjects involvement and characteristics	27
8.B Informed consent	28
8.C Source of material	29
8.D Potential risks	29
8.E Protection against risk	29
8.F Potential benefits of the proposed research to the participants and others	30
8.G Importance of the knowledge to be gained	30
8.H Confidentiality	30
8.I Financial considerations.....	31
8.J Conflict of interest.....	31
9. References	31

Participating Institutions/Investigators Table (contact information)

Principal Investigator: Manisha Balwani, MD

Contact: Hetanshi Naik, MS, CGC

Institution: Icahn School of Medicine at Mount Sinai

Address: Department of Genetics & Genomic Sciences

One Gustave L. Levy Place, Box 1497

New York, NY 10029

Phone: 212-241-7699

Fax: 212-426-9065

Email: manisha.balwani@mssm.edu, hetanshi.naik@mssm.edu

Sub-Investigators: Robert J. Desnick, MD, PhD; Lawrence Liu, MD

Principal Investigator: Joseph R. Bloomer, MD

Contact: Toni Seay

Institution: University of Alabama at Birmingham

Address: UAB Porphyria Center

Liver Center, Division of Gastroenterology and Hepatology, Department of
Medicine

████████████████████
Birmingham, Alabama 35294-0012

Phone: 205-934-7332

Fax: 205-975-9777

Email: jbloomer@uab.edu

Sub-Investigators: Ashwani Singal, MD; Brendan M. McGuire, MD

Principal Investigator: D. Montgomery Bissell, MD

Contact: Yuvraaj Kapoor

Institution: University of California at San Francisco,

Address: UCSF Porphyria Center

████████████████████
San Francisco, CA 94143-0538

Phone: (415) 476-8405

Fax: (415) 476-0659

Email: montgomery.bissell@ucsf.edu

Sub-Investigator: Bruce Wang, MD; Jennifer Lai, MD, MPH; Almira Correia, PhD

Principal Investigator: Karl E. Anderson, MD

Contact: Csilla Hallberg, MD

Institution: University of Texas Medical Branch

Address: Porphyria Center, 301 University Boulevard, Galveston, TX 77555-1109

Phone: 409-772-4661

Fax: 409-772-6287

Email: kanderso@utmb.edu

Sub-Investigators: Gagan Sood, MD; Eric Gou, MD

Principal Investigator: John Phillips, PhD

Contact: Tiffanie Hales, BS

Institution: University of Utah

Address: Porphyria Center

Department of Internal Medicine, Division of Hematology

████████████████████
Salt Lake City, Utah 84132

Phone: (801) 581-6650
Fax: (801) 585-3432
Email: john.phillips@hsc.utah.edu
Sub-Investigators: Charles Parker, MD

Principal Investigator: Herbert L. Bonkovsky, MD
Contact: Denise Faust
Institution: Wake Forest Baptist Medical Center
Address: Department of Internal Medicine/Gastroenterology
Wake Forest University School of Medicine
Winston-Salem, NC 27157
Phone: 336-713-7301
Fax: 336-713-7322
Email: hbonkovs@wakehealth.edu
Sub-Investigators:

Data Management and Coordinating Center

Principal Investigator: Jeffrey Krischer, Ph.D.
Contact: Carrie Light, Research Compliance Administrator
Institution / Department: Data Management and Coordinating Center (DMCC); Pediatrics
Epidemiology Center; University of South Florida
Address: [REDACTED]; Tampa, FL 33612
Phone: (813) 396- 9248
Fax: (813) 910-5997
Email: Carrie.Light@epi.usf.edu

1. Synopsis

Protocol Number: 7207
Protocol Title: Erythropoietic Protoporphyrins: Studies of the Natural History, Genotype-Phenotype Correlations, and Psychosocial Impact
Study Chair: Manisha Balwani, MD, MS
Statistician: Inga Peter, PhD (Icahn School of Medicine at Mount Sinai), Jessica Overbey, MS (Icahn School of Medicine at Mount Sinai)
Consortium: The Porphyrins Consortium
Participating Sites: <ul style="list-style-type: none"> ★ Icahn School of Medicine at Mount Sinai, New York, NY ★ University of Alabama at Birmingham, Birmingham, AL ★ University of California, San Francisco, San Francisco, CA ★ The University of Texas Medical Branch, Galveston, TX ★ University of Utah, Salt Lake City, UT ★ Wake Forest Baptist Medical Center, Winston-Salem, NC
Activation Date: 23AUG2012
Sample Size: 150
Target Enrollment Period: 5 years
Study Design: Longitudinal, observational
Primary Study Objective: This is an ancillary study of the Longitudinal Study of the Porphyrins (#7201). The initial objective of this protocol is to assemble a well-documented group of patients with confirmed diagnoses of the erythropoietic protoporphyrias, including autosomal recessive Erythropoietic Protoporphyria (EPP) and X-Linked Protoporphyria (XLP) for clinical, biochemical, and genetic studies. The long-term objectives are (1) to conduct a longitudinal investigation of the natural history, complications, and therapeutic outcomes in people with erythropoietic protoporphyria, (2) to systematically investigate the psychological effects of the erythropoietic protoporphyrias on children and adults, and (3) to investigate the correlation between the identified genotypes and the resulting clinical presentation, also determining the possible interaction of other genetic markers.
Study Population and Main Eligibility/ Exclusion Criteria:
Inclusion criteria: <ol style="list-style-type: none"> 1. Enrollment in the Longitudinal Study of the Porphyrins (7201) 2. Willing to sign informed consent form (and provide assent if participant is a minor) 3. Biochemical findings- a marked increase in erythrocyte protoporphyrin [total erythrocyte protoporphyrin >200 ug/dL, or more than 1.5-fold increase (relative to ULN of 80 ug/dL)], with a predominance of free protoporphyrin (85-100% in EPP and 50-85% in XLP). 4. Molecular findings – one of the following: <ol style="list-style-type: none"> a. A disease causing FECH mutation trans to the IVS3-48C>T low expression FECH allele b. Two disease-causing <i>FECH</i> mutations c. A gain-of-function <i>ALAS-2</i> C-terminal deletion/exon 11 mutation (in XLP). <p>If no mutation is found and subjects fulfill criteria 1-3 they are eligible for enrollment</p>
Exclusion Criteria: <ol style="list-style-type: none"> 1. Elevations of porphyrins in urine, plasma or erythrocytes due to other diseases (i.e. secondary porphyrinuria or porphyrinemia), such as liver and bone marrow diseases [13].

2. A diagnosis of porphyria that cannot be documented by review of existing medical records or repeat biochemical or DNA testing.

Primary Objectives:

1. Systematic characterization of the phenotypic, psychosocial, biochemical, and molecular data for EPP and XLP;
2. Longitudinal investigation and analysis of pertinent issues involved in prognosis for the erythropoietic protoporphyrias, including morbidity, development of disease complications, and outcomes of commonly used treatments, as well as more advanced treatments such as liver or bone marrow transplantation, and new photoprotective drugs now under investigation;
3. Identification of biochemical features (biomarkers) that may predict future symptomatic episodes so that interventions can be initiated before symptoms develop; and
4. Provide a resource of well-documented data, samples, and subjects that may be available for other studies, including genetic studies (predictors, biomarkers, modifying genes, etc.), mechanistic investigations, and clinical trials.

Statistical Considerations (sample size and analysis plan):

1. Sample size: 150
2. Analysis plan:
 - a. Correlations between QoL scale scores, clinical severity measures and genotype will be computed. The clinical course and severity of patient will be described.
 - b. Relationships between clinical and laboratory values will be evaluated in longitudinal mixed effect models. We will develop models that capture the combined frequency and severity of disease manifestations while accounting for age of onset, protoporphyria subtype (EPP and XLP), and genotype. A sample size of 150 (assuming a minimum of 3 follows ups per patient, a correlation of 0.7 among outcomes over time from the same subject, a variance of outcome equal to 1 and a compound symmetric covariance structure) yields 91% power to detect a moderate interaction ($\beta=0.4$) between time and an associated factor of interest.
 - c. A severity score based on predictive demographic and clinical characteristics will be developed
 - d. After 100 participants are enrolled, the mean score of the Hospital Anxiety and Depression Scale (HADS) will be computed and compared to the general population value. A sample size of 100 yields 90% power to detect a 20% difference in HAD-scale between the general population and our sample at the .05 significance level. The illness Perception Questionnaire and EPP/XLP-specific questionnaire will also be scored.
 - e. During this interim look at $n=100$, we will re-evaluate the estimates used in our initial power calculation that yielded a sample size of 150 and determine whether the study needs to be extended beyond this point to examine correlations of QoL scale scores with clinical severity and genotypes

Sponsors (federal, state, foundation and industry support): National institutes of Health (NIH)

1. Protocol Overview

The porphyrias are a group of rare metabolic diseases that may present in childhood or adult life and are due to deficiencies of enzymes in the heme biosynthetic pathway. The most common manifestations are related to accumulation of intermediates in the pathway and usually occur as acute neurological attacks (as in the acute or hepatic porphyrias), or cutaneous photosensitivity (as in the cutaneous porphyrias, including the erythropoietic protoporphyrias). Multiple mutations have been identified in each of the porphyrias [Anderson, 2001]. The risk of disability or death from these disorders is significant, in part because diagnosis is often delayed due to lack of adoption of diagnostic testing in clinical practice. Moreover, the natural history of these disorders is not well described and it is not known what determines differences in outcomes. New therapies are needed. For existing therapies, high-quality evidence on short and long term efficacy and safety is generally lacking [Sood 2008]. Therefore, the purpose of this study of a large group of participants with EPP and XLP is to provide a better understanding of the natural history of these disorders, as affected by available therapies, and to aid in developing new forms of treatment. Much of the data collected on subjects as participants in the Longitudinal Study of the Porphyrias will be accessed for this study specific to the investigation of the erythropoietic protoporphyrias. To maximize the information that can be informative in our objectives, additional data will be collected, including additional biochemical findings and EPP-specific psychosocial parameters.

The Office of Rare Diseases (ORD) of the National Institutes of Health (NIH) established a Rare Diseases Clinical Research Network (RDCRN) in collaboration with other NIH Institutes and currently has funded 19 rare diseases clinical research consortia and one Data Management and Coordinating Center. The Porphyrias Consortium was created as part of the RDCRN, to study the human porphyrias. The Porphyrias Consortium is a consortium of the academic institutions listed in the participating institutions table. All Centers in the Porphyrias Consortium are participating in this study. Additional centers may be added if funding is available.

2. Objective and Research Question

The objective of this protocol is to conduct a systematic multidisciplinary investigation of the erythropoietic protoporphyrias, including the natural history, morbidity, psychosocial issues, and genotype-phenotype correlations in people with EPP and XLP. The research questions are:

- A. To characterize the natural history and clinical variability (e.g., age of onset, severity, progression) in EPP and XLP participants.
- B. To establish genotype-phenotype correlations and identify markers of disease severity.
- C. To understand the impact on quality of life and psychosocial issues experienced by pediatric and adult EPP/XLP patients
- D. To chronicle the use, and potential benefits and risks of current and emerging therapies for the erythropoietic porphyrias.

3. Background

Porphyrias are metabolic diseases that result from deficiencies of specific enzymes of the heme biosynthetic pathway (Figure 1). Heme is the prosthetic group for a variety of essential hemoproteins, including respiratory cytochromes, catalase, nitric oxide synthase, myoglobin, etc. Heme synthesis occurs in all tissues but is most active in bone marrow and liver. The bone marrow requires large amounts of heme for hemoglobin and the liver utilizes heme in large amounts for cytochrome P450 enzymes (CYPs). The DNA sequences and chromosomal locations are established for the human genes of the enzymes in this pathway, and multiple disease-related mutations have been found for each.

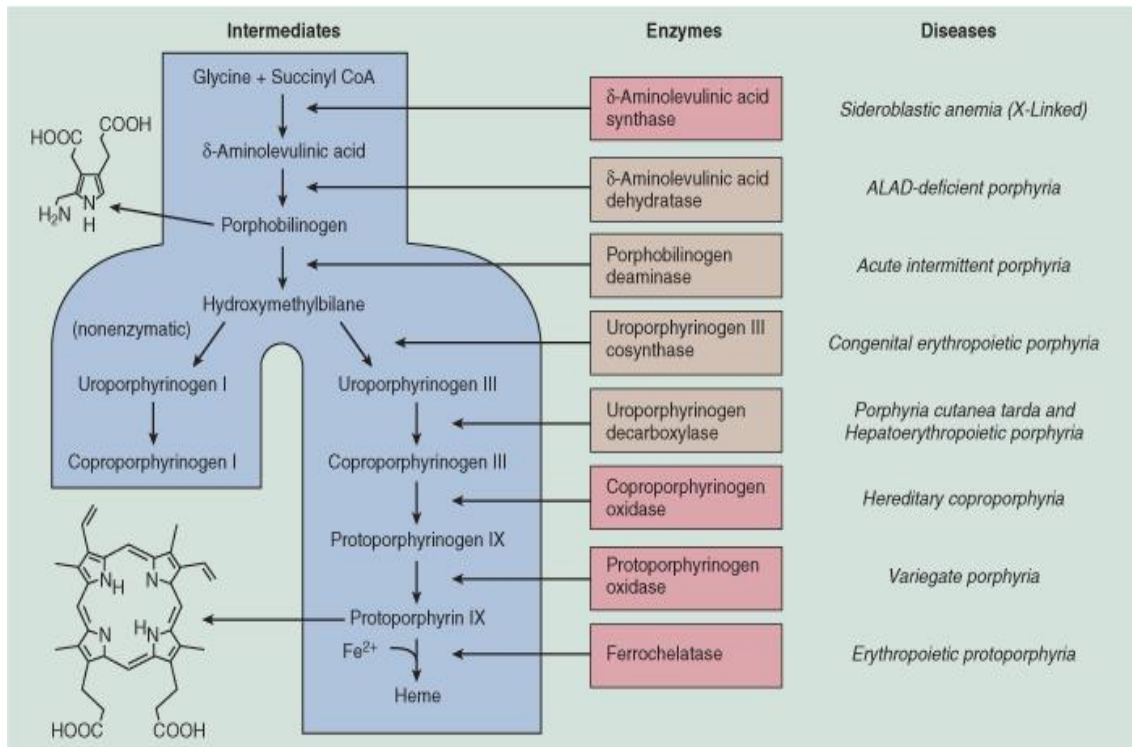


Figure 1. Enzymes and intermediates in the heme biosynthetic pathway and diseases associated with deficiencies of each enzyme. Mitochondrial and cytosolic enzymes are noted in red and tan backgrounds, respectively. Porphobilinogen deaminase (PBG-deaminase or PBGD) is also known as hydroxymethylbilane synthase (HMB-synthase or HMBS)[1].

Erythropoietic protoporphyria (EPP) is the third most common porphyria, with an incidence of 2 to 5 per 1,000,000, and the most common in children. Most cases are due to the markedly reduced activity of the enzyme, ferrochelatase (20-30% of normal). The inheritance of EPP follows an autosomal recessive pattern. In about 95% of EPP cases, the individual has a severe mutation of the ferrochelatase (*FECH*) gene inherited from one parent, and a common genetic variation of the same gene, inherited from the other parent. This common genetic variation causes reduced production of the enzyme, but does not cause disease in the absence of a severe mutation. The frequency of this common genetic variation in the *FECH* gene varies by population, such that it is present in about 10% of Caucasians, 43% of Japanese, 31% of Southeast Asians, and 1 to 3% in Blacks. Alternatively, in about 5% of EPP cases, two severe *FECH* gene mutations, one inherited from each parent, are found in the same patient,

A second type of erythropoietic protoporphyria, X-linked Protoporphyrria (XLP) is an X-linked porphyria caused by a mutation in the last exon of the δ -aminolevulinic acid synthase-2 (*ALAS2*) gene. The *ALAS2* gene mutation results in a “gain of function” and results in increased stability and/or activity of this enzyme. Therefore, more protoporphyrin than is needed for hemoglobin synthesis is produced by the bone marrow. In all cases of EPP, protoporphyrin accumulates in the marrow and is transported to the skin in the plasma and red blood cells, causing photosensitivity. Protoporphyrin is not excreted by the kidneys, but is taken up by the liver and excreted in bile. It can reduce bile flow and damage the liver.

Photosensitivity in both EPP and XLP begins in early childhood, and can be difficult to diagnose, since there is usually no blistering and little scarring. Photosensitivity is typically acute – appearing soon after exposure to sunlight – rather than chronic. It is also painful, and may be accompanied by swelling of the skin. These symptoms greatly impair quality of life and limit employment opportunities and life style. Patients are sensitive to sunlight that passes through window glass (long wave ultraviolet light). Large

amounts of protoporphyrin in bile can form stones rich in this porphyrin. Severe liver complications are difficult to treat and sometimes require liver transplantation.

The diagnosis is established biochemically by finding increased protoporphyrin in red blood cells, with a predominance of free protoporphyrin rather than zinc protoporphyrin in EPP or more similar proportions of free and zinc protoporphyrins in XLP. Plasma porphyrins are also increased in most cases, and fecal porphyrins, consisting mostly of protoporphyrin, may be normal or increased. Urine porphyrins are not increased.

Although initial diagnosis of porphyria by biochemical methods remains essential, it is important to confirm the diagnosis by demonstrating a specific gene mutation(s). This also enables genetic counseling and accurate identification of others in a family who carry the same mutation. More than 125 mutations have been identified in the *FECH* gene, resulting in EPP, and 3 deletion mutations in exon 11 of the *ALAS2* gene, resulting in XLP [Whatley 2010, Whatley 2008].

The risk of disability or deformity from these disorders is significant, in part because diagnosis is often delayed due to lack of adoption of diagnostic testing in clinical practice. Moreover, the natural history of these disorders is not well described and it is not known what determines differences in outcomes. New therapies are needed. For existing therapies, high-quality evidence on short and long term efficacy and safety is generally lacking. Therefore, the purpose of this study of a large group of patients with erythropoietic protoporphyria is to provide a better understanding of the natural history of these disorders, as affected by available therapies, to understand the psychosocial effects, and to aid in developing new forms of treatment.

As with the other porphyrias, the major clinical features of these disorders have been described, but there is little information on their variation in symptoms and severity, genotype-phenotype correlations, long term natural histories and outcomes, and their effects on quality of life. For example, it is not known how to predict which patients with EPP will develop life-threatening hepatopathy and require liver transplantation [McGuire 2005]. Although some effective therapies for the erythropoietic protoporphyrias are available, there are few new agents or novel treatment approaches in the pipeline. Moreover, the quality of evidence supporting the use of existing therapies is not high [Sood 2008]. There have been few multicenter trials, which is required for collecting high quality data on safety and efficacy of new and existing treatments. Among the few drugs undergoing clinical trials for treating the photosensitivity of the erythropoietic protoporphyrias, afamelanotide (Clnuvel) has not yet received FDA approval. Purposes of this study include a better understanding of natural history and providing a pool of well characterized patients, both clinically and at the molecular level, for future clinical trials, as well as for studies of disease pathogenesis. Participants may also consider enrollment in other studies including therapeutic trials.

Different types of information will be collected longitudinally for each of the erythropoietic protoporphyrias, reflecting differences in their underlying enzymatic defects, susceptibility factors, established and potential future treatments, complications and prognostic indicators.

4. Study Design and Methods

For this study, a case is defined as an individual with confirmed erythropoietic protoporphyria, either EPP or XLP, or one who is highly likely to have an erythropoietic protoporphyria, based on biochemical criteria that are specific (see below) and, in most cases, by DNA studies. (For technical or other reasons, a mutation may not be found in ~5% of cases of biochemically documented porphyria.)

All subjects will also be enrolled in the Longitudinal Study of the Porphyrias (LS). Data collected from subjects as part of their participation in the LS will be accessed for this study. Active cases will include the following target groups:

- 1) Participants and their family members known to the investigators who can be re-contacted and consented for inclusion, or who have previously consented for the procedures that are part of this study.
- 2) Individuals referred by the American Porphyria Foundation with known or suspected porphyria.

- 3) Individuals referred by physicians and other sources.

4.A. Inclusion criteria

There will be no exclusions based on age, race, ethnicity, or gender for this study. Inclusion criteria are based on 1) clinical features, 2) biochemical findings, as documented by laboratory reports of porphyria-specific testing performed after 1980, and 3) molecular findings documenting the identification of a mutation in a porphyria-related gene. Absolute values are preferred for diagnostic biochemical thresholds. Fold increases in comparison to an upper (or lower) limit of normal (ULN or LLN) are also acceptable, but are complicated by considerable variation between laboratories in normal limits. Equivocal biochemical measurements may require confirmation by a consortium reference laboratory. Provision is made for enrolling relatives who may not have symptoms but have biochemical or molecular documentation of a porphyria.

1. Enrollment in the Longitudinal Study of the Porphyrins (7201)
2. Willing to sign informed consent form (and provide assent if participant is a minor)
3. Biochemical findings- A marked increase in erythrocyte protoporphyrin [total erythrocyte protoporphyrin >200 ug/dL, or more than 1.5-fold increase (relative to ULN of 80 ug/dL)], with a predominance of free protoporphyrin (85-100% in EPP and 50-85% in XLP).
4. Molecular findings – one of the following:
 - a. A disease causing FECH mutation trans to the IVS3-48C>T low expression FECH allele
 - b. Two disease-causing *FECH* mutations
 - c. A gain-of-function *ALAS-2* C-terminal deletion/exon 11 mutation (in XLP)

If no mutation is found and subjects fulfill criteria 1-3 they are eligible for enrollment

We expect to enroll 150 patients with EPP/XLP.

4.B. Exclusion Criteria

We will exclude cases with elevations of porphyrins in urine, plasma or erythrocytes due to other diseases (i.e. secondary porphyrinuria or porphyrinemia), such as liver and bone marrow diseases [Gibson 2000]. We will also exclude patients with a prior diagnosis of porphyria that cannot be documented by review of existing medical records or repeat biochemical or DNA testing.

4.C. Recruitment and Enrollment of Subjects

Subjects will be recruited from the following resources:

1. *Patients followed by one of the Investigators.* Each of the Investigators is a Porphyria specialist in a Porphyria Center, and provides clinical care to individuals suspected of and diagnosed with Porphyria, including the erythropoietic protoporphyrias, providing diagnostic and follow-up evaluations, consultations, and/or treatment. For such patients who are likely to be eligible for the study, the Investigators will either discuss the study with the patient during a clinical visit or will contact the patient by telephone, email, or mail, as appropriate per site-specific IRB approval. Any efforts to contact and recruit patients and families will be with IRB approval and adhere to standards for ethical conduct of research and be fully HIPAA compliant.
2. *The American Porphyria Foundation (APF).* The APF is an advocacy group that provides education about porphyria to patients, their families, healthcare professionals, and the public, and supports porphyria research. Their outreach program includes a website, as well as periodic newsletters and special announcements emailed and/or mailed to individuals who have registered with the APF with information about new developments in treatment, porphyria-related education opportunities, and available research studies. Information about research studies includes contact information for the studies if they are interested in obtaining additional information or participating.
3. *The Rare Diseases Clinical Research Network (RDCRN) Contact Registry.* As is the case for all RDCRN members, the Porphyrins Consortium has a Contact Registry. Individuals can register on-line, over the phone or by faxing or mailing in the registration form expressing an interest in receiving information about research studies for which they might qualify. The Contact Registry is managed by the

Data Management Coordinating Center (DMCC) of the RDCRN. All registration information is submitted directly to the DMCC and is not available to the individual Consortiums, without specific permission by the Registrant. During the on-line registration process the Registrant is given the option of granting permission to the DMCC to share their name and contact information with the Consortium. In such cases where permission is granted, the DMCC emails the information to a point person in the Consortium; in the case of the Porphyrins Consortium, this person is the Project Manager. The Project Manager then forwards this information to the Participating Site closest to the Registrant. The site coordinator can then contact the Registrant directly to discuss this study. As a service to all Registrants, the DMCC also sends information by email and mail about study protocols and who to contact if they are interested in obtaining additional information or participating.

4. *Non-study Physician referrals.* Physicians providing clinical care to individuals suspected of or diagnosed with an erythropoietic protoporphyria who are not investigators in this study may refer patients who may be eligible for and express interest in the study. An information letter (site-specific IRB-approved) announcing the study and providing contact information may be sent to physicians who evaluate and/or treat individuals suspected of or diagnosed with Porphyria.

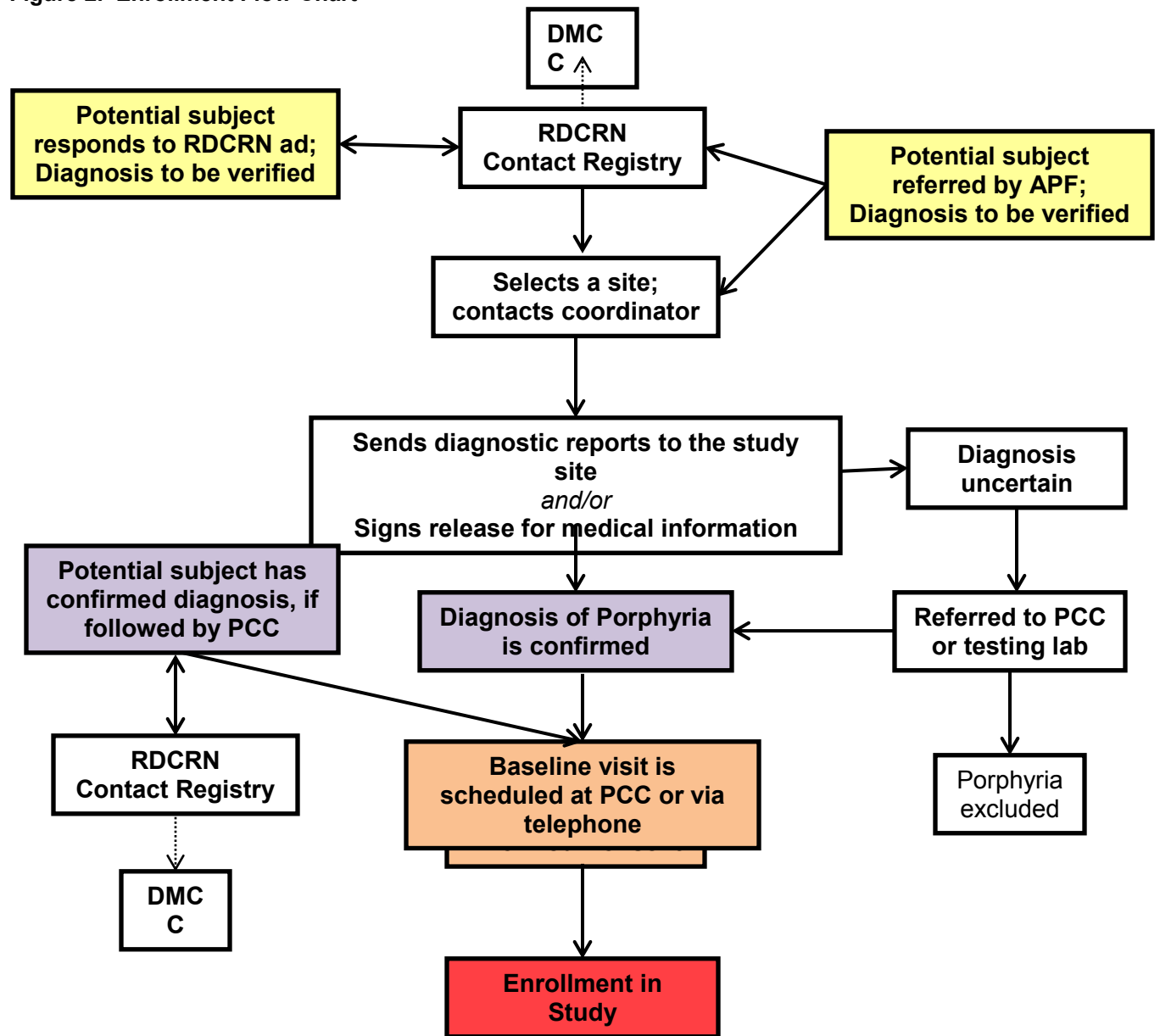
5. *Self-referrals,* including family members of individuals diagnosed with an erythropoietic protoporphyria (proband) and other individuals who may have heard about the study from other participants. In the case of family members, initial contact with the family member will not be made by the study team. The proband will be asked to contact such family members, requesting that they contact the study coordinator if interested in obtaining additional information or participating.

When contacted by a prospective subject who is not followed clinically by the Investigator, the coordinator will first establish confirmation of a diagnosis of an erythropoietic protoporphyria by requesting that the prospective subject (or parent/guardian) send by fax, email, or mail documentation of the diagnosis (for example, copies of reports of laboratory testing). Limited PHI, including name, telephone number, email address, and mailing address may be collected as part of this initial contact. This information will be maintained separately from study related data either on a paper log or in a database by the site-specific coordinator in order to track follow-up. Prospective subjects sending diagnosis documentation will be instructed to include a notation with the reports that they are being sent for review of diagnosis for the EPP Natural History Study. When this documentation is received, the information will be reviewed by the Investigators to determine confirmation of diagnosis. If the documentation does not provide confirmation of a diagnosis of porphyria, the prospective subject will be informed that he/she is not yet eligible for enrollment in the study, and recommendations will be made for additional testing or evaluations to help establish or rule-out the diagnosis of Porphyria.

Please note: Such a review of records to establish or rule-out a diagnosis of Porphyria is common practice at all Participating Clinical Centers (PCC) as part of standard clinical practice. If the documentation does not establish the diagnosis of an erythropoietic protoporphyria, recommendations are made to the patient or referring physician for additional testing or evaluations to help aid in the diagnosis.

Once a diagnosis of an erythropoietic protoporphyria is confirmed, the site-specific coordinator will contact the prospective subject by telephone. If the prospective subject or parent/guardian is interested in participating in the study, the coordinator will explain the study to the prospective subject, answer all questions, and obtain telephone consent. Eligibility status and consenting process will be documented by the coordinator who will complete the eligibility form. The eligibility form will be reviewed and verified by an Investigator. If consent is obtained by telephone, the Informed Consent Form will be mailed, faxed, or emailed to the subject to read, sign, and return by mail, fax, or email. When the signed consent form is received at the study site, the subject will be contacted by the study coordinator to either (a) schedule an appointment at the Participating Clinical Center for a study visit or (b) arrangements made to have samples and porphyria-related medical records sent to the Participating Clinical Center; the study coordinator will complete all appropriate Case Report Forms, including the Enrollment Form, and Psychosocial Questionnaires with the subject by telephone interview, email, or fax, as appropriate.

Figure 2. Enrollment Flow Chart



4.D. Retention strategies

The porphyrias are a group of complex conditions with only a small number of specialists having the expertise to evaluate and treat affected individuals. Clinical relationships established by the investigators with participants who enroll in this study will provide a context for increased communication with participants and their primary physicians that can facilitate retention. Coordinators and other personnel at each site will also work to maintain close contact with participants, including newsletters and communication by phone, email and web sites. We will additionally provide counseling services so that participants and families are made aware of the results of studies and their correct interpretation. There will be no charge for the physician services provided as part of the research study that are not considered standard of care and covered by insurance.

4.E. Data Elements

Data and samples are collected at the time of *visits*, which will occur at a Porphyrias Consortium Center whenever possible or at a distance such as by a telephone or email interview.

4.E.1. Study Visits Overview:

All Study Visits for this study, including Baseline Visit, annual Study Visits, and Interim Visits, may be coordinated with Study Visits for the Longitudinal Study of the Porphyrias (7201).

Baseline Visit: Once referred for enrollment into the study, a potential participant's diagnostic testing will be reviewed to assure that the correct diagnosis has been made and eligibility requirements are met based on study inclusion and exclusion criteria. Historical and new data will be collected at baseline as summarized below. This initial medical visit may require several hours for complete evaluation and data and sample collection. Methods for obtaining data will include historical review of existing medical records and laboratory data, and/or extraction of data from the RDCRN Porphyrias Consortium Longitudinal Study of the Porphyrias. Careful attention will be paid to accurately noting dates for historical information, including information about previous or ongoing treatment. Other data will be obtained from participants or their families through a standard interview, examination and laboratory testing.. All study-related information, which will include information also collected as standard of care, will be recorded on Case Report Forms (CRFs) prepared specifically for this study. This information will then be entered into a database maintained by the DMCC, using electronic versions of the CRFs with the required data entry fields. The information will be transmitted using encrypted communications links.

As with the Longitudinal Study of the Porphyrias, baseline visits can take place either (1) at a Participating Clinical Center (PCC), (2) arranged locally with the patient's own physician, with data and samples sent to the PCC, as needed, or (3) by telephone or email interview by the site coordinator, with data collected during the interview and arrangements made for sample collection, as needed. For visits where the participant cannot come to a PCC, all medical records must be forwarded to the site coordinator (including lab tests and physician notes). The questionnaires can be completed via phone, email or fax by the participant and sent to the site coordinator. For these visits the site coordinator is permitted to email the questionnaires to the participant and these can be returned by mail, email or fax. Once returned the coordinator should review them and if any issue need clarification contact the participant. The medical history and family history should be done via phone interview for the baseline visit.

If the study visits or sample collections takes place in facilities supported by the local institutions Clinical and Translational Science Award (CTSA), the protocol will be reviewed by any additional Advisory Committee, as appropriate, prior to implementation at the site. Centers in the Porphyrias Consortium may assist each other in data collection and data entry. The subject will be informed of the consortium arrangement and that their protected health information (PHI) will be shared with one or more other Porphyrias Consortium institutions, when approved by the IRB at each site. See Table 1 for the specific CRFs to be completed at Baseline.

Follow-up Assessments: New data as described below will be collected every 1-2 years, or more frequently if the subject experiences any significantly worsening porphyria-specific symptoms (liver disease, worsening sun sensitivity, etc) or has new study-related information to share with the study team. These overall scheduling plans may be modified based on clinical circumstances or if there is reason to anticipate a change in clinical course. Data collected during these follow-up visits will be entered onto the CRFs, see Table 1 for the specific CRFs to be completed for follow ups.

Additional visits will be scheduled for participants who have experienced significant worsening of the disease as determined by the investigators (i.e. liver abnormalities, worsening sun sensitivity, etc). Such visits will be scheduled at the time of these events or shortly after, so that information will be current or recent and more likely to be accurate. Such visits may coincide with visits or hospitalizations for medical treatment. Data collected during these follow-up visits will be entered onto the General Medical History form under the Longitudinal Study of the Porphyrias and any appropriate Lab forms will be completed. Follow-up study visits are expected to take approximately 0.5-3 hours, depending on the testing required.

In order to accommodate participants' schedules, follow-up visits can take place within an acceptable window, defined as within one month of the schedule date. Visits that do not meet this expectation will still be permitted..

As with the Longitudinal Study of the Porphyrrias, follow-up visits can take place either (1) at a Participating Clinical Center (PCC), (2) arranged locally with the patient's own physician, with data and samples sent to the PCC, as needed, or (3) by telephone or email interview by the site coordinator, with data collected during the interview and arrangements made for sample collection, as needed. As with the baseline visit; when the participant cannot come to a PCC for follow up visits all medical records must be forwarded to the coordinator (including lab tests and physician notes). The questionnaires can be completed via phone, email or fax by the participant and sent to the site coordinator. The site coordinator is permitted to email the questionnaires to the participant and these can be returned by mail, email or fax. Once returned the coordinator should review them and if any issue need clarification contact the participant. The medical history and family history update should be done over the phone whenever possible, however if the participant is not able to be reached via phone this can be completed over email.

Data collected during follow-up visits is typically standard of care. Exceptions may include quality of life (Psychosocial) assessments, and collection of samples for the repository.

Interim Events: Participants are likely to experience changes in the clinical course of their disease, or experience other medical events. These will be recorded on the General Medical History form under the Longitudinal Study of the Porphyrrias as they occur. Exacerbations or recurrences of symptoms of the erythropoietic protoporphyria, or development of a complication of erythropoietic protoporphyria, or development of a concurrent condition or progression of such a condition will be defined as Interim Events. If the event was managed elsewhere, medical records will be obtained for study documentation. An Interim Event will include, but not be limited to, any symptom that requires hospitalization, emergency room visit or unscheduled clinic visit.

As necessary for an interim event the Porphyria Specific Lab Tests, Local Lab Tests and Concomitant Medications forms will also be completed under the Longitudinal Study of the Porphyrrias. The investigators may obtain additional information that will enhance interpretation and understanding of the event, such as intercurrent illnesses, exposure to new medications, dietary changes, onset of puberty, pregnancy, or menstruation, etc to be recorded on the source documents for the participant.

Pain Assessment during Acute Symptoms: Efforts will be made to obtain pain assessment information from participants during episodes of phototoxic reactions to better assess the impact of the disease. At the time of their baseline and follow up visits participants/parents will be informed that the study team would like to assess pain levels during acute phototoxic reactions. The study team will give the pain questionnaire and a pre-addressed return envelope to the participant, and instruct him/her to complete them during a phototoxic reaction. Alternatively, the participant will be advised to inform the study team when the participant is experiencing a phototoxic reaction. The site coordinator will then either email or fax the participant the pain questionnaire (labeled with the participants local study ID number only) to be completed while the participant is still experiencing acute symptoms. The participant will be instructed to write the date of completion on the questionnaires and then mail, email or fax them back to the site coordinator. For pediatric participants the parent(s) will be the main point of contact and if the participant is able to complete questionnaires the parents will be instructed to facilitate this. If the pediatric participant is not able to complete the questionnaires during this period of acute symptoms then only the parent proxy questionnaires will be completed. See Table 1 for the specific forms to be completed.

4.E.2. Data Collection, Maintenance, and Storage

Detailed patient information will be collected on Case Report Forms. These data will be entered by the site coordinator, investigator or designee into a database maintained by the DMCC through the RDCRN website, which is responsible for all data management.

PHI information (including name, address, telephone, cell, and fax numbers, email address, date of birth) will be maintained only at the site of enrollment either (1) in a secure / encrypted database on a secure computer with access limited to the site coordinator and investigators and/or (2) on paper kept in a locked file cabinet in a locked room with access limited to the site coordinator and investigators. This PHI will not be transferred to the DMCC. Each subject will be given a unique code which will be linkable to the subject by a secure and encrypted "linking database" maintained at the site where the subject is enrolled. All data transferred to the DMCC will be identified only by the subject's unique code.

Table 1. CRFs Collected at Baseline and Follow-up Visits

CRFs	Visits			
	Baseline	Annual Follow-up ¹	Interim Event	Acute Symptom Phase
Completed for Longitudinal Study of the Porphyrrias (7201)				
Eligibility	X			
Sociodemographics	X	X ²		
Demographics	X			
General Medical History	X	X ²	PRN	
Concomitant Medications	X	X ²	PRN	
Family History (complete pedigree to be maintained as a source document)	X	X ²		
Baseline Disorder Specific Information: Erythropoietic Protoporphyrria	X			
Disorder Specific Information- Annual Follow-up: Erythropoietic Protoporphyrria		X		
General Physical Exam	X	X	PRN	
Porphyria Specific Lab Tests	X	X	PRN	
Local Lab Tests	X	X	PRN	
Adult PROMIS-57 Questionnaire (≥18 years old) OR Pediatric PROMIS Short Form Questionnaires- Depressive Symptoms, Anger, Anxiety, Fatigue, Pain Interference, Peer Relationships (7-17 years old)	X	X		
Completed for this Study (7207)				
7207 Eligibility	X			
EPP Specific Labs	X	X	PRN	
Hospital Anxiety and Depression Scale (HADS) (≥ 18 years old)	X	X		
EPP Specific Quality of Life Questionnaire (≥ 18 years old)	X	X		PRN
Illness Perception Questionnaire (IPQR) (≥ 18 years old)	X			
PedsQL Quality of Life Inventory (2-18 years old and parent proxy) **NOTE- a pediatric patient in 7207 does NOT need to complete the Pediatric PROMIS Short Forms	X	X		
PROMIS Pain Interference section (≥ 18 years old)				PRN
PedsQL Pediatric Pain Questionnaire (5-18 years old and parent proxy)	X	X		PRN

¹ Follow-up study visits or contacts by site coordinator will be at least on an annual basis. Symptomatic subjects will be followed as needed

² Update as appropriate

PRN= as needed

Medical record review: Local and outside medical records will be obtained, with appropriate consent, for all participants. At the time of enrollment, the participants will be asked to give permission to obtain all past and future medical records. This will provide more complete information on the clinical course of the disease, including retrospective data. To reduce unnecessary heterogeneity of the data, we will initially limit this procedure to the living participants who were diagnosed with porphyria after 1980.

Each participating site will perform a systematic review of its medical record system dating from January 1, 1980, if feasible, to the present in order to identify eligible participants currently living who were diagnosed with porphyria but are not yet enrolled this study. Records of other sources described in section 4.C. will be reviewed, when available and as appropriate. Those persons identified through medical record review who are living and who can be contacted will be offered enrollment in the prospective component of this study. .

The National Death Index (NDI) will be used to supplement identification on mortality of any patient whose vital status cannot be ascertained with certainty based on study follow-up. This will be accomplished by preparing a computerized list for submission to NDI containing the requisite information needed by them to search their files, including the date last known to be alive. This will provide the means to determine survivorship status on any enrolled patient whose status is uncertain owing to follow-up limitations, and primarily those participants whose follow-up was limited by information available from medical record review.

4.E.3. Data Items

Case Report Forms (CRFs) will be completed during Study Visits or by telephone or email interview by the Study Coordinator and/or Investigator to obtain the following data. As per 4.E.1, study visits for this study may be coordinated with LS study visits. This means also that, once a subject is enrolled in this study, data collected and entered into the DMCC data capture system for the LS will be linked to this study Information to be collected for this study which is captured in the CRFs described in detail above includes:.

Baseline:

- Eligibility
- Enrollment/demographic information
- Clinical data to include:
 - Prenatal and neonatal history (for pediatric participants)
 - Medical history
 - For females, pregnancy and menstrual history
 - Lag time for diagnosis (from onset of symptoms to time of diagnosis)
 - Diagnostic laboratory values at presentation- erythrocyte protoporphyrin values (including free and zinc fractionation), and plasma total porphyrins
 - Prior EPP treatments, (names, dosage and efficacy)
 - Concomitant medications
 - Porphyria-related complications
 - Other conditions and their complications
 - Review of past medical records, including growth charts (for children), primary care and specialty evaluations and routine lab testing, and genetic testing (if previously done)
 - Physical examination

- Family history (complete pedigree)
- Laboratory tests (standard of care):
 - Hepatic Function Panel (including at minimum albumin, bilirubin total and direct, alkaline phosphatase, AST, ALT and total protein), CBC with differential, Serum Iron, TIBC, Ferritin, Vitamin D, and any other tests the investigators determines to be necessary for clinical management
 - Biochemical porphyria tests- erythrocyte protoporphyrin values (including free and zinc fractionation), and plasma total porphyrins [preferred lab is UTMB]
 - DNA test of the *FECH* or *ALAS2* genes as appropriate to be determined by the investigator
- Psychosocial questionnaires

Follow Up Visits:

- Clinical evaluation for changes in symptoms, signs, hospitalizations, other conditions, etc.
- Assessment of any ongoing EPP treatments
- Laboratory tests (standard of care):
 - Hepatic Function Panel (including at minimum albumin, bilirubin total and direct, alkaline phosphatase, AST, ALT and total protein), CBC with differential, Serum Iron, TIBC, Ferritin, Vitamin D, and any other tests the investigators determines to be necessary for clinical management
 - Biochemical porphyria tests- erythrocyte protoporphyrin values (including free and zinc fractionation), and plasma total porphyrins [preferred lab is UTMB]
- Psychosocial questionnaires

It is optimal to conduct follow-up visits at least once per year. Participants whose EPP symptoms are stable may be seen at 2 year intervals. More frequent study visits can be scheduled based on Interim Events (as described above) which may include changes in clinical course or treatment.

4.F. Sample Collection and Storage

4.F.1. Sample Collection

Samples (blood and urine) will be collected according to protocol for clinical laboratory testing (consistent with standard of care), porphyria-specific biochemical testing, molecular analysis, and specimen banking.

The following table outlines the specimens that will be requested for research alone. Samples collected for standard of care testing will be processed as normal per the institutions' procedures.

Specimens will be shipped monthly from Porphyrias Consortium sites to The Mount Sinai Porphyrias Repository (Icahn School of Medicine at Mount Sinai, New York, NY).

Table 2: Sample Collection Requirements

Biochemical		Additional DNA ** in addition to samples shipped for standard of care genetic testing
Plasma	Urine	Whole Blood
5 ml blood drawn in Sodium Heparin (green-top) tube. Centrifuge, at 3000 rpm for 10 minutes separate, as follows: Put 1 ml plasma into two plastic (Eppendorf) tubes each, label with ID#, date of birth, and date sample was taken. Wrap immediately in aluminum foil, or place in amber bag, to protect from light, and freeze at -80°C. If a -80°C freezer is not available a -20°C freezer is acceptable.	Put 3 ml urine into two plastic (Eppendorf) tubes each, label with ID#, date of birth, and date sample was taken. Wrap immediately in aluminum foil, or place in amber bag, to protect from light, and freeze at -80°C. If a -80°C freezer is not available a -20°C freezer is acceptable	5-10 ml (or 5 ml on participants <50 lbs) blood in EDTA (lavender-top) tube. Label with ID#, date of birth, and date sample was taken. Ship at room temperature.

Biochemical samples for the Porphyrins Repository should be shipped frozen, and DNA samples should be shipped ambient, via FedEx or UPS priority overnight services to the Icahn School of Medicine at Mount Sinai.

4.F.2. Sample Identification and Storage

Blood, urine, and DNA (from blood) of subjects will be stored only with their consent. These samples will be stored at the facilities of the Participating Clinical Centers. All samples will be given a unique research code. The link between identifying information (subject's full name) and the code is stored on password protected computers in a password protected database at the enrollment site, with access limited to that site's study coordinator and Investigator. Samples will be maintained indefinitely or until the patient provides a written request to withdraw his/her authorization for sample storage. This information is included in the consent form.

4.F.3. Banking of Samples

The subject is asked as part of the Longitudinal Study of the Porphyrins if he/she will permit the PI to bank samples (DNA, blood, and urine) for research studies and must sign the site-specific IRB approved consent form. These samples will be stored at the facilities of the Participating Clinical Centers and/or at the Porphyrins Repository at the Icahn School of Medicine at Mount Sinai, Department of Genetics and Genomic Sciences, New York, NY, under the supervision of the Consortium's PI (Dr. Robert J. Desnick). DNA is given a unique code (as described previously). The link between identifying information (subject's full name) and the code is stored on a password protected computer in a password protected database at the enrollment site.

As part of the consent process for sample storage, the subject is given options in the Longitudinal Study of the Porphyrins consent form, regarding future use of samples:

- 1) Samples can be used by PI in future research projects directly related to Porphyria
- 2) Samples can be used by PI in future research projects unrelated to Porphyria
- 3) Samples can be given to other investigators at MSSM or other institutions for use in future research projects directly related to Porphyria

- 4) Samples can be given to other investigators at MSSM or other institutions for use in future research projects unrelated to Porphyria

Samples will be maintained indefinitely or until the subject provides a written request to withdraw his or her authorization for sample storage.

4.G. Longitudinal investigation of natural history, complications, and outcomes

Method for assessing overall survival: This study on the natural history of the erythropoietic protoporphyrias will evaluate the risk of morbidity in participants. At inception, the study will primarily include known cases and thus will be subject to survivor bias, but later it will be able to identify cases as they are newly diagnosed and therefore provide more representative estimates of morbidity and case-fatality by cause and by risk factor status.

In addition to direct follow-up we will search the National Death Index (NDI) by name to identify deaths among enrollees who are lost to follow-up. Since the NDI provides a virtually complete registry of mortality, we will have a high likelihood of ascertaining all deaths among enrollees.

As the study progresses, it will provide opportunities to explore the data on morbidity as related to treatment interventions. Participants who were treated before enrollment will likely include affected individuals who have survived, and who will constitute a biased sample of the original cohort, because those who died prior to the study's inception will not be included in the cohort. Analysis of data only on participants enrolled around the time of diagnosis will allow for improved and more detailed assessment of disease severity before the decision was made to begin treatment. This avoids the problem of survivor bias because the entire cohort can be followed when we assess and compare morbidity and mortality in those who undergo a particular intervention (e.g. liver or bone marrow transplantation). Access to certain other databases, such as national transplantation databases, will provide more complete records on those who undergo such interventions. For those participants who die during their participation in the longitudinal study we will obtain the death certificate to ascertain cause of death. Provision for obtaining autopsy data will also be explored.

Numerous sources of bias are inevitable in this type of longitudinal study. Biases relate to differences in severity of disease and to access to diagnosis and medical care. The study directs considerable effort to assessing severity at baseline and at regular intervals of follow-up, and to recording key longitudinal data such as age of onset of symptoms and diagnosis, hospitalizations, genotypic data, biochemical information as related to disease expression, etc. Information on economic circumstances (family resources, level of education and socioeconomic status) and access to medical care, including specialized and expert consultation, will also be recorded. As the study progresses, the difference between the ages at onset of symptoms and diagnosis and the age at enrollment in the longitudinal study will narrow, and make the collected data more useful for studying effects of interventions on clinical outcomes. Nevertheless, we realize that the level of control of differences in severity and access in this longitudinal observational natural history study will not equal that possible in a randomized clinical trial (RCT). The study will provide information on some interventions where an RCT is unlikely to be performed, and may point to questions where a RCT would be of value and might also be feasible.

4.H. Determination methods

4.H.1. Method to determine if there are specific genotypes or other biomarkers that predict outcome

For the erythropoietic porphyrias, disease severity is determined in part by genotype, although outcomes are also influenced by many other factors. This study will provide opportunities to explore genotype-phenotype correlations in more detail, across different racial and ethnic groups and geographic locations. Investigators will also be able to focus on other factors that modify disease severity and effects of treatment.

4.H.2. Method for determining risk of disease complications and adverse outcomes

We will follow disease and quality of life outcomes in our patient cohorts. This will help define specific long term effects of these diseases as related to genotype, porphyrin precursors, porphyrin levels, other biomarkers and treatment. Especially for diseases with diverse manifestations we will explore the development of scores that combine disease symptoms and other characteristics in a manner that may predict outcomes and complications. Cohorts of participants will be followed after treatments such as liver transplantation and photosensitivity protection drugs and treatments, new and future. The observations will better define the degrees of metabolic correction that follows such treatments and their duration. The methodology involves the performance of tests indicated in 4.E.2 at the described intervals.

For example, we will study or determine:

1. Genotype-phenotype correlations
2. Whether porphyrin levels remain stable over time
3. Frequency of development of liver and biliary tract complications in EPP
4. Effects of treatments, such as bone marrow transplantation and liver transplantation in EPP

4.H.3. Method to assess the long-term medical complications of the erythropoietic protoporphyrias and their treatment

This longitudinal study will enhance our understanding of the long term complications of the erythropoietic protoporphyrias. The first step is to describe the prevalence of these complications for each type of porphyria and generate hypothesis to relate their occurrence to clinical and other risk factors in relatively large groups of participants. Detailed longitudinal data from large numbers of participants will be a resource for analyses that would not otherwise be possible. Some examples include: a) long term complications of the erythropoietic protoporphyrias; c) development of hepatobiliary disease in EPP and XLP, including possible differences in risk.

4.I. Challenges

Our primary goals in this natural history study are to characterize the manifestations, complications, and treatment outcomes for the erythropoietic protoporphyrias, determine psychosocial effects of EPP/XLP, and to evaluate the utility of potential biomarkers for predicting and monitoring the disease severity. We have considered a number of challenges inherent in the design and conduct of this study, such as the following.

1. Identification of a representative, nonbiased sample of participants with EPP and XLP. Our goal is to build cohorts of participants that will be representative of each type of erythropoietic protoporphyria. In any study of this type, the initial enrollment will likely favor longer term cases and under-represent those with very severe disease. We will monitor for disease-severity bias but believe that this bias will not be problematic or in any case will lessen with time. If necessary, we may stratify data analysis by features such as severity and age of onset, and thereby derive estimates of effects even within under-represented segments of the population. Under these circumstances and for some conditions, these stratified estimates of effects will be more valuable than the overall estimates.

We will also characterize enrollees at baseline, in terms of severity of the genotype, biochemical findings and clinical features, and record disease sequelae that have occurred from onset of symptoms to enrollment. Even if we cannot enroll persons at the time of diagnosis, we can try to properly characterize their status at time of enrollment, including both the severity of the underlying defect as well as the consequences or sequelae of the condition that have exerted effects from onset to enrollment. Thus, data collection at enrollment will include information about severity of the genetic and clinical features such as age of onset and at diagnosis. We will ascertain number and severity of disease sequelae and frequency of medical care and requirement for treatment. Thus, a number of such variables will be included as either main effects or as covariates in our analysis to account for disease status at enrollment so that we can focus on changes that have occurred during follow-up.

2. *Providing uniform follow-up to permit relatively complete and nonbiased ascertainment of outcomes and assessment of biomarker status.* We plan to follow enrolled participants and assess their health and disease status at regular intervals. This will be accomplished by direct evaluation at the time of scheduled outpatient visits or by abstracting outpatient and inpatient records of visits to the participant's usual care provider or during hospitalization. Results of interim histories, physical examinations, and laboratory results will be recorded. We also have provisions for obtaining information from medical records and from searching the NDI to obtain more uniform mortality data.

These assessments will allow us to characterize the status of participants and identify important changes in their condition. These variables will frequently be treated as time dependent co-variables or binary indicators of change points during analysis. This will allow us to focus on certain events/changes and to control for other changes happening in the same timeframe.

Follow up with participants may be difficult, especially if their disease remains stable over time. Various contact methods will be used to keep in touch with participants and the importance of follow up information will be stressed. In addition participants will be instructed to inform the study team if any of their contact information changes.

Ascertainment of other confounding factors that might interfere with the interpretation of relationships. We propose also to ascertain other factors, including non-biological factors that may play a role in the natural history and outcome of the erythropoietic protoporphyrias. These include collecting information on socioeconomic status (occupation, education, income, etc.), family support, access to health care, distance from a center with expertise in porphyria. These interacting factors may predict access or resources to select or implement different treatment modalities. These variables will usually serve as confounders, and will usually be included as covariates. Therefore when we evaluate our research questions we will be developing estimates within strata based on condition and severity. And when we develop models to estimate effects arising during follow-up we will control for baseline status, hold constant other confounding including time-dependent effects.

We do not anticipate issues with confounding factors but are aware of the possibility.

5. Data and Safety Monitoring Plan

This is an observational, not interventional, study and therefore adverse events related to study participation are not anticipated.

Accepted principles of data and safety monitoring will be observed throughout the conduct of this study. The study protocol will be reviewed and approved by the National Institutes of Health (NIH) before submission to individual center IRB's for approval. Additionally, this study and its analysis will be performed under Institutional Review Board (IRB) oversight. Prior to the initiation of the study, IRB approval for study of human subjects under this study protocol will be obtained from each of the study center IRBs. Revisions to the study protocol and changes in the study design will also be submitted to the individual IRBs for approval prior to implementation. Participants will be enrolled in this longitudinal follow-up study only after full informed consent which will include the gathering of privileged health information (PHI), the collection of blood and tissue specimens necessary to confirm porphyria diagnoses and for clinically indicated follow-up care, and the collection of medical and quality of life information at defined intervals.

This is an observational study that meets the federal definition of minimal risk.

Although this study is considered to be of minimal risk to study participants, the NIH will nonetheless appoint an independent Data and Safety/Observational Study Monitoring Board (D/OSMB) that will also provide study oversight. The study protocol will be reviewed and approved by the National Institutes of Health (NIH) before submission to individual site Institutional Review Boards (IRBs) for approval. The D/OSMB will review accrual, patterns, and frequencies of all adverse events, protocol compliance every 12 months. This D/OSMB will also approve and monitor all subsequent clinical research protocols based upon the study subjects enrolled in this longitudinal follow-up study. Principal investigators at each study center will be responsible for monitoring the enrollment of subjects and submission of data to the Rare Diseases Clinical Research Network DMCC. Both NIH Program Staff

and the DMCC will be responsible for assuring and monitoring the effective conduct of this protocol and the accurate, timely submission of data, and both the D/OSMB and the study center IRBs will be provided feedback on a regular basis.

5.A Definitions

The Rare Diseases Clinical Research Network defines an adverse event as: "...an unfavorable and unintended sign, symptom or disease associated with a subject's participation in a Rare Diseases Clinical Research Network study." As this is an observational study, reportable adverse events are only those directly related to an individual's participation in this study.

A serious adverse event includes those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."

An unexpected adverse event is defined as any adverse experience...the specificity or severity of which is not consistent with the risks of information described in the protocol.

Therefore, expected adverse events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study.

All reported adverse events will be classified using the current version of the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.

Events such as exacerbations or recurrent symptoms, occurrence of known complications of porphyria, or development of unrelated medical conditions are considered "expected" in many participants as part of the underlying disease of study or other diseases and therefore not reportable to the RDCRN or the IRB as adverse events. However, events such as disease recurrences and other health-related events will be captured by completing the General Medical History case report form.

5.B Reporting Timeline

All unexpected adverse events will be reported to the Rare Diseases Clinical Research Network using the on-line adverse events reporting system provided by the DMCC and to the local IRB per IRB requirements.

RDCRN Reporting Timeline:

- Within 24 hours (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that:
 - Is considered life-threatening/disabling or results in death of subject –OR
 - Is Unexpected/Unanticipated
- Investigators must report all other reportable SAEs within 5 working days (of learning of the event).
- All other (suspected) reportable AEs must be reported to the RDCRN within 20 working days of the notification of the event or of the site becoming aware of the event.
- Expected/anticipated SAEs and AEs will not be reported as adverse events (as defined)

Local institutional reporting requirements to IRBs, any GCRC oversight committee and the FDA, if appropriate, remain the responsibility of the treating physician and the Study Chair.

5.C Standard Elements

A set of standard elements for adverse event data is collected across all studies in the Rare Diseases Clinical Research Network (RDCRN). These elements include: subject ID, reporter name & location, dates for event/event reported/date resolved, the event itself, event severity, whether it was expected and/or serious (as defined above), patient status, place of AE treatment (to determine serious events further), causality, and subsequent changes to protocol or consent form. Additionally, there is ample room for the reporter to write a description of the event and any other pertinent information.

5.D Procedures

Upon entry of a serious adverse event (SAE), the DMCC created Adverse Event Data Management System (AEDAMS) will immediately notify the Study Chair, site PIs, the RDCRN D/OSMB medical review officer, and any additional agencies (if applicable- industry sponsor) of any reported adverse events via email.

Serious adverse events: The NIH appointed Medical Review Officer (MRO) reviews the site investigator's report and determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The MRO may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the MRO beyond a specified period of time are forwarded to a secondary reviewer. Any follow up reports or requested additional participant data will be entered into the AEDAMS system by the reporting site and reviewed by the MRO. Completed AE reviews by the MRO will sent to Study Chair, site PIs, and the appointed NIH officers.

If warranted, the MRO may request an ad hoc call with the DSMB to review the adverse event. All reported AE's will be reviewed during the regularly scheduled DSMB call.

The Adverse Event Data Management System (AEDAMS) maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious adverse events that are reported to or observed by the investigator or a member of his research team will be submitted to the system in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the MRO and RDCRN D/OSMB on an annual basis. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DMCC will post aggregate reports of all adverse events (serious/not serious and expected, unexpected) for the site investigators and IRBs.

5.E Study Discontinuation

This study will not have study discontinuation rules as it is an observational study. The NIH and local IRB's (at their local site) have the authority to stop or suspend this trial at any time.

5.F Subject Discontinuation

All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless the patient withdraws consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

A participant may be withdrawn from the study under one or more of the following circumstances:

- Withdrawal of consent by participant or parent / guardian
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease

5.G Data Quality and Monitoring Measures

As much as possible data quality is assessed at the data entry point using intelligent online forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency.

- Data Monitoring: The RDCRN DMCC identifies missing or unclear data and generates a data compliance report for the consortium investigators to access on the RDCRN Member's Website.

- Data Delinquency Tracking: The Data Management and Coordinating Center will monitor data delinquency on an ongoing basis.

6. Statistical Considerations

6.A Analysis Plan

General Statistical Considerations

Natural history study and genotype analyses: This is an observational study aimed to provide a systematic description of the severity and clinical course of each patient with the EPP phenotype, develop a severity score and correlate genotype with phenotype for EPP and XLP. Continuous variables representing patient demographic and clinical characteristics will be summarized using means and standard deviations or medians and quartiles as appropriate, while categorical variables will be summarized by frequencies with 95% confidence intervals.

A major objective will be to identify the FECH and ALAS2 mutations in each patient with the EPP-phenotype. In patients with the phenotype and no detectable FECH or ALAS2 mutations identified, a second independent blood sample will be obtained to confirm the absence of FECH and ALAS2 mutations, followed by whole exome sequencing. Exome analysis will be performed at the Mount Sinai Institute for Genomics and Multiscale Biology as described above in more detail.

Clinical and Laboratory Analyses: Clinical and laboratory measurements will be collected/described at each visit. We will develop models that capture the combined frequency and severity of disease manifestations while accounting for age of onset, protoporphyria subtype (EPP and XLP), protoporphyrin levels and genotype. To account for multiple observations obtained for each patient during the follow-up, we will develop linear mixed effects models that account for the main effect of interest and its change over time, correlated data associated with repeat measurements on the same persons, random effects of clinical center and patient, as well as the effects of confounding variables and any modifiers of the main effect. To assess the statistical significance of the effects of biochemical levels alone and these levels by age of patient, we will use a t-test to evaluate whether the coefficient of the interaction between biochemical level alone and biochemical levels by age differs from zero.

Disease Severity Score: Clinical and laboratory values assessed above will be considered potential variables to be included in an overall disease severity score. The distributions of the proposed variables to be included in the severity score will be assessed for utility to the severity score calculation. Considerations for variable utility include sufficient variability in the disease population and interrelationships with other proposed variables (if two variables are highly correlated one will be removed from the score calculation). Variables chosen to be included in the severity score will be assigned categorical ranks based on individual severity and then summed to get an overall disease severity score. The utility of the severity score will be assessed by correlating the score with clinical outcomes.

Psychosocial Impact: The following questionnaires will be administered: a) the Hospital Anxiety and Depression Scale will be used to determine the levels of anxiety and depression in these patients. These data will be scored as per the established protocol and compared to the published age-matched norms; b) the Illness Perception Questionnaire will determine the disease impact and understand how patients think and feel about their disorder. This questionnaire will determine if certain groups, such as younger adults, are more significantly impacted by this disease. The questions are scored on a Likert scale and higher scores indicate greater impairment; c) a recently validated EPP/XLP-specific questionnaire

developed in Europe will more specifically evaluate disease impact on daily functioning. After the target enrollment of n=100 is reached, we will begin the analysis.

Our primary outcome is verifying that EPP and XLP patients have higher than normal anxiety and depression. We will compare the mean Hospital Anxiety and Depression Scale (HADS) of our sample with the general population Scale (mean = 9.82, SD=5.98) (16). The null hypothesis is that there is no difference in HADS score between the general population and EPP/XLP patients.

With a sample size of 100, using a two-tailed 0.05 level one-sample t-test we will be able to detect a 20% difference in scale between the general population and our sample with 90% power.

Secondary analyses include:

- 1) Characterizing EPP and XLP Illness Perception via the Illness Perception Questionnaire, dermatology-specific QOL via the Dermatology Life Quality Index and EPP-specific QOL via the EPP/XLP specific questionnaire. The mean and standard deviation of each scale in our sample will be assessed.
- 2) Scores for all four QOL assessments will be analyzed; FECH and ALAS 2 mutations correlations will be sought with clinical parameters, disease severity score and genotypes.

Secondary analyses will inform whether a study extending beyond 100 patients will be needed to examine correlations of QOL scale scores with clinical severity and genotypes.

6. B Power Calculation

For the proposed analyses, the statistical powers are of two sided tests, with 0.05 level of significance.

Linear mixed effects model: We may assume, for example, that 150 participants will have at least 3 follow up visits per subject. Taking into account the correlation among outcomes over time from the same subject, the main interest is in determining whether the regression coefficient of the interaction between time and covariate of interest is zero or not. Using GPower 3.0, Table 6-1 shows the results when the variance of outcome is equal to 1, where ρ and β denote correlation among repeated outcome measures and effect size of the interaction, respectively. In this calculation, we assume compound symmetry for the covariance structure of repeated outcome measures from the same subject and the same number of subjects in each group with/without a potential factor of interest. The study is certain to detect moderate ($B \geq 0.4$) differences in the analysis.

Table 6-1. Power calculation for linear mixed effects model based on the most common types of porphyria (AIP and PCT)

Sample size	ρ	B for time x factor of interest	Power
150	0.5	0.3	0.45
		0.4	0.72
		0.6	>0.99
	0.7	0.3	0.67
		0.4	0.91
		0.6	>0.99

7. Data Management

All study data will be collected via systems created in collaboration with the RDCRN Data Management and Coordinating Center and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

As much as possible data quality is assessed at the data entry point using intelligent on-line forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency. In addition to those described above, we propose to build these checks into the initial tables and cross tabulations that should reveal any remaining data quality issues.

7.A Enrollment

Enrollment of subjects on this protocol will employ an interactive data system in which the clinical site will attest to the subject's eligibility as per protocol criteria and that appropriate informed consent has been obtained. IRB approval for the protocol at the clinical site must be on file at the DMCC before accrual can occur from that site.

The DMCC will use a system of coded identifiers to protect subject confidentiality and safety. Each subject enrolled will be assigned a local identifier by the enrollment site. This number can be a combination of the site identifier (location code) and a serial accession number. Only the registering site will have access to the linkage between this number and the personal identifier of the subject.

When the subject is enrolled to participate in the study using the DMCC provided web-based registration system, the system will assign a registration number. Thus each subject will have two codes; the local one that can be used by the enrollment site to obtain personal identifiers and a second code assigned by the DMCC. For all data transfers to the DMCC both numbers should be entered to uniquely identify the subject. In this fashion, it is possible to protect against data keying errors, digit transposition or other mistakes when identifying a subject for data entry since the numbers match to properly identify the subject. In this fashion, no personal identifiers would be accessible to the DMCC.

7.B Data Entry

Data collection for this study will be accomplished with online electronic case report forms. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields.

7.C Data Quality Control

As much as possible data quality is assessed at the data entry point. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. The more important of the quality assurance measures are the internal validity checks for reasonableness and consistency. In addition to those described above, we propose to build these checks into the initial tables and cross tabulations that should reveal any remaining data quality issues.

8. Human Subjects

8.A. Human subjects involvement and characteristics

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice and all applicable regulatory requirements.

Patients with an erythropoietic protoporphyria of any age, both genders, and all ethnic groups are eligible to be enrolled in this longitudinal study, as long as they meet all inclusion and exclusion criteria. These criteria will assure that a diagnosis is established prior to entry. Patients potentially eligible will include 1) those known to the investigators who can be contacted and informed of the study; 2) patients who hear

about the study through the American Porphyria Foundation, their physicians or other sources and contact one of the centers to express interest in the study; and 3) patients who contact the Data Management and Coordinating Center (DMCC) of the Rare Disease Clinical Research Network (RDCRN) and enter the Contact Registry at the DMCC website.

Because XLP is an X-linked disorder, it does not affect both genders equally, but overall we expect to enroll an equal number of males and females. The cutaneous porphyrias, including the erythropoietic protoporphyrias, may be less manifest in African Americans due to protective skin pigmentation. Furthermore, the erythropoietic protoporphyrias are uncommon in Africa and in African-Americans because the low-expression allele associated with the disorder in >90% of EPP is rare in these groups. But overall, it is expected the both genders and the various ethnic groups will be represented in proportional numbers to their populations.

Inclusion of vulnerable groups: Children included as subjects of the study, since especially the erythropoietic porphyrias are manifest at birth or in early childhood. Few participants will have intellectual disabilities, as this is rarely considered a manifestation of porphyria. Pregnant women will be included in this study, since the study will not involve an intervention to place them or the fetus at increased risk, and so that we can gain a better understanding of the effects of pregnancy on the management of erythropoietic protoporphyria. As this is a longitudinal protocol and not an intervention protocol, risk to participants will be minor (as discussed in the *Potential risks* section). Information gained from this study may be valuable to this patient population.

8.B Informed consent

The mechanisms described in section 4.C will be used to recruit participants for this protocol. Any contact to recruit families will adhere to standards for ethical conduct of research and be fully HIPAA compliant. Informed consent will be obtained after the diagnosis of porphyria has been established by results of biochemical testing and/or identification of a mutation in a porphyria-related gene and the prospective subject has expressed an interest in participating in the study.

Informed consent will be obtained as follows:

1. If the prospective subject is a patient of an Investigator, and is eligible and willing to participate in the study, informed consent may be obtained in-person during a clinical visit. In a private consultation room, the investigator or delegate will explain the study and answer all questions. If the patient is willing to participate, informed consent will be obtained at this time. Consent can also be obtained over the phone. The coordinator will explain the study to the prospective subject, answer all questions, and obtain telephone consent. The consenting process will be documented by the coordinator or investigator, and the investigator will complete the eligibility form. If consent is obtained by telephone, the Informed Consent Form will be mailed, faxed, or emailed to the subject to read, sign, and return by mail, email, or fax. No study procedures will be completed until the signed consent form is received at the study site.
2. For all other prospective subjects (see 4.C. Recruitment of Subjects), once a diagnosis of erythropoietic protoporphyria is confirmed, the site-specific coordinator will contact the prospective subject by telephone. If the prospective subject or parent/guardian is interested in participating in the study, the coordinator will explain the study to the prospective subject, answer all questions, and obtain telephone consent. Eligibility status and consenting process will be documented by the coordinator or investigator who will complete the eligibility form. If consent is obtained by telephone, the Informed Consent Form will be mailed, faxed, or emailed to the subject to read, sign, and return by mail, email, or fax. When the signed consent form is received at the study site, the subject will be contacted by the study coordinator to enroll the subject into the study. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

If the participant's mental age is the same as their actual age, we will follow the usual consent procedures with those 18 and over signing consent forms, and 7-17 year olds signing assent forms in addition to their

legal guardian signing consent forms. In the case of subjects who are cognitively impaired, for those 18 years old or older, informed consent will be obtained from the subject's legal guardian; for those between the ages of 7 and 17, informed consent will be obtained from the parent or legal guardian assent will be waived.

Medical Record Review – Consent will not be required for medical record review, except as may be required for obtaining outside medical records. Data collection will consist of review of medical records only. Participants identified through medical record review will be offered participation in the study.

8.C Source of material

The source of information obtained for the longitudinal study is from a patient's history, physical examination and medical records, which may include serial examinations and laboratory tests. Most of the data are from clinically indicated examinations and testing. Some data collection, such as the detailed questionnaires and the quality of life questionnaires could be considered research procedures, which although clinically useful might be more extensive than the accepted standard of care.

8.D Potential risks

This study represents not more than minimal risk, has the potential to provide a direct and long-lasting benefit for the study participants and is permissible under the provisions contained in the United States Code of Federal Regulations: 45 CFR 46.404. Most of the testing to be conducted is standard of care. The detailed data collection on clinical features and quality of life do not pose an increased risk, though the quality of life questionnaires may cause some psychological stress. There is risk of loss of confidentiality of medical and personal information, which is no more than minimal given the safeguards in place.

Participation in the study will increase the time spent during the clinical visits from approximately one hour to three hours. Visits are scheduled once yearly, or more often depending on specific clinical needs, such as frequent exacerbations or treatments.

There may be risks and discomforts associated with the blood draw: when the needle is inserted it can hurt. There is a small chance of fainting and/or bruising. Since the skin is broken, an infection at the puncture site can occur, although this is very rare. The amount of blood requested is within approved safety limits and will be modified if the subject has a medical condition that limits his/her ability to provide the requested volume of blood.

The examination of biological samples (tissue, blood and other body fluids) in general represents no direct physical harm to subjects (inflicts no physical pain or suffering). However, genetic testing carries with it the very real possibility of emotional and social risks to the subjects (the risk of harm from learning genetic information about oneself, social labeling, prejudice, unfair treatment, and potential loss of, or difficulty in, obtaining employment or insurance).

Additionally, if the subject (or parent/guardian approves, the subject's DNA and/or cells may be used in other genetic studies which may give results. However, this information will not be given to the subject until such time as the genetic test is validated as reliable, is performed in a New York State Department of Health-approved laboratory, and appropriate counseling can be provided to the subject. Additionally, no results will be provided to the subject unless he/she consents specifically for the additional testing.

Participation may result in currently unforeseeable risks to the subject.

8.E Protection against risk

The study will be conducted whenever possible within the Participant Clinical Interactions Resources of the CTSA at each of the Porphyria Consortium sites. Study visits may be conducted at alternative sites, such as a clinic facility where a porphyria patient might customarily be seen for clinical evaluation and/or treatment. The study implementers will be nursing staff/coordinators of the Centers, who will be familiar with the protocol requirements and trained in its conduct. If any unexpected and untoward event occurs during the testing procedure, such as blood drawing, emergency caregivers would be available on an immediate basis to provide necessary emergent management.

Confidentiality will be protected as required by law and as described below under *Confidentiality*.

8.F Potential benefits of the proposed research to the participants and others

Participants will have access to routine follow up that may improve their medical care and decrease risk of exacerbations, recurrences and long term complications or permit their early detection. They may also increase their understanding of their disease and their local physicians may have better access to information and advice about their management. Participants may also gain access to other studies that may involve therapy for their condition.

Genetic testing to identify the mutation in the subjects' porphyria-related gene is part of this protocol if this testing has not previously been done at an approved clinical laboratory. This testing will be performed at the Mount Sinai Genetic Testing Laboratory which is CLIA- and New York State approved to perform the molecular testing of the porphyria-related genes. The subject will be provided with the results of the molecular testing, with appropriate genetic counseling by a Board-certified genetic counselor; the results of this testing may be beneficial in that the testing is considered standard clinical information, confirms (or rules out) the diagnosis of Porphyria, and may be informative to other at-risk family members. At-risk family members, however, will not be contacted by the study team.

8.G Importance of the knowledge to be gained

This natural history study is longitudinal and should provide important information about the clinical course of the erythropoietic protoporphyrias as well as about biomarkers and correlates of outcome. The information to be learned could be useful in optimizing therapy and clinical management.

8.H Confidentiality

Patient confidentiality is protected in the fullest extent required by law and applicable local, State and Federal regulations and guidelines including HIPAA. All patient records, including case report forms, are kept in locked files accessible only to the health professionals involved in the clinical research or responsible for the patient's care, governmental agencies (e.g., FDA, NIH) and local convening authorities (e.g., IRB, D/OSMB) for the purpose of audit regarding scientific validity and/or aspects pertaining to the ethical conduct of human clinical investigation. Patient identifier data are not released without the patient's or parent's knowledge and consent or assent. Electronic databases with patient identifier data are user-access protected. Studies are conducted in the Participant Clinical Interactions Resources or clinical sites, which are also attentive to protecting confidentiality of clinical and research data. The results of the study will be shared with the participant of the study as soon as they become available.

To help protect participant privacy, a Certificate of Confidentiality has been obtained from the National Institutes of Health (NIH) for the Longitudinal Study of the Porphyrrias, which also applies to this study. With this Certificate, the researchers cannot be forced to disclose information that may identify a study participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify a participant, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

Even with the Certificate of Confidentiality, the investigators continue to have ethical and legal obligations to report child abuse or neglect and to prevent an individual from carrying out any threats to do serious harm to themselves or others. If keeping information private would immediately put the study participant or someone else in danger, the investigators would release information to protect the participant or another person.

Department of Health and Human Services (DHHS) personnel may request identifying information for purposes of performing audits, carrying out investigations of DHHS grant recipients, or evaluating DHHS funded research projects.

8.I Financial considerations

Subjects will not be paid to participate in the study. Costs incurred due to participation in the study may be reimbursed at the discretion of the study site and the local IRB, and will depend on the site's budget.

Participants will be provided emergency treatment if needed. The participant's insurance provider or Medicaid will be billed for standard of care testing and treatment. The grant will pay for testing that is added for study purposes. Participants will not incur the cost of testing if there is a difference between testing billed for and insurance/Medicaid coverage. The grant will cover these charges.

8.J Conflict of interest

None of the investigators involved with this protocol has a conflict of interest. This is a longitudinal study, not an intervention study. This study is funded by a grant from NIH.

9. References

Anderson KE, Sassa S, Bishop DF, Desnick RJ: Disorders of heme biosynthesis: X-linked sideroblastic anemias and the porphyrias; in Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Vogelstein B (eds): The metabolic and molecular basis of inherited disease, 8th edition. New York, McGraw-Hill, 2001, vol II, Chapter 124, pp 2991-3062.

Whatley SD, Mason NG, Holme SA, Anstey AV, Elder GH, Badminton MN: Molecular epidemiology of erythropoietic protoporphyria in the united kingdom. *Br J Dermatol*, 2010 Mar;162(3):642-6

Whatley SD, Ducamp S, Gouya L, Grandchamp B, Beaumont C, Badminton MN, Elder GH, Holme SA, Anstey AV, Parker M, Corrigall AV, Meissner PN, Hift RJ, Marsden JT, Ma Y, Mieli-Vergani G, Deybach JC, Puy H: C-terminal deletions in the *alas2* gene lead to gain of function and cause x-linked dominant protoporphyria without anemia or iron overload. *Am J Hum Genet* 2008;83:408-414.

Sood G, Anderson KE: Porphyrias; in Crowther MA, Ginsberg J, Schunemann H, Meyer RM, Lottenberg R (eds): Evidence-based hematology. Hoboken, Wiley, 2008

McGuire BM, Bonkovsky HL, Carithers RL, Jr., Chung RT, Goldstein LI, Lake JR, Lok AS, Potter CJ, Rand E, Voigt MD, Davis PR, Bloomer JR: Liver transplantation for erythropoietic protoporphyria liver disease. *Liver Transpl* 2005;11:1590-1596.

Gibson PR, Grant J, Cronin V, Blake D, Ratnaik S: Effect of hepatobiliary disease, chronic hepatitis c and hepatitis b virus infections and interferon-alpha on porphyrin profiles in plasma, urine and faeces. *J Gastroenterol Hepatol* 2000;15:192-201.