Title: Effect of Oral Iron Therapy on Erythrocyte Protoporphyrin Levels in the Erythropoietic Protoporphyrias
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Rare Diseases Clinical Research Network

Porphyrias Consortium

Effect of Oral Iron Therapy on Erythrocyte Protoporphyrin Levels in the Erythropoietic Protoporphyrias

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1. Protocol Synopsis

Interventional Synopsis

Protocol Number:	7209
Protocol Title:	Effect of Oral Iron Therapy on Erythrocyte Protoporphyrin Levels in the Erythropoietic Protoporphyrias
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:	Porphyrias Consortium
Participating Sites:	Icahn School of Medicine at Mount Sinai , New York, NY University of Alabama at Birmingham, Birmingham, AL University of California at San Francisco, San Francisco, CA University of Texas Medical Branch, Galveston, TX University of Utah, Salt Lake City, UT Wake Forest University Health Sciences, Winston-Salem, NC
Activation Date:	
Current Status:	
Sample Size:	20
Target Enrollment Period:	4 years
Study Design:	Interventional
Primary Study Objective:	To assess the potential efficacy of iron therapy for decreasing erythrocyte protoporphyrin levels in patients with EPP and XLP
Secondary Study Objective(s):	1) To assess whether oral iron therapy can improve iron status and decrease plasma porphyrin levels and clinical symptoms 2) To assess if there in an improvement in the quality of life in treated patients
Study Population and Main Eligibility/ Exclusion Criteria:	**Inclusion criteria:** 1. Enrollment in the Longitudinal Study of the Porphyrias (7201) 2. Male or female age ≥18 years 3. Willing to sign informed consent form and follow protocol 4. History of nonblistering cutaneous photosensitivity 5. Biochemical findings- A marked increase in erythrocyte protoporphyrin (total erythrocyte protoporphyrin >400 ug/dL) with a predominance of metal-free protoporphyrin 6. Serum ferritin ≤30 ng/mL at baseline 7. Able to tolerate oral iron **Exclusion criteria:** 1. History of liver or bone marrow transplant or clinically significant liver dysfunction as determined by the investigator 2. Patient reported known or suspected allergy to oral iron 3. Clinical evidence of active and ongoing GI bleeding 4. Use of any other clinical or experimental therapy in the past 3 months which could confound study results 5. Individuals with elevations of porphyrins in plasma or erythrocytes due to other diseases (i.e., secondary porphyrinemia) such as liver and

	bone marrow diseases 6. Pregnancy 7. Patients with any clinically significant comorbid condition, which in the opinion of the investigator, precludes participation
Treatment	
Agent-	Ferrous sulfate USP
Dosage, schedule, route of administration-	325 mg capsule oral administered twice daily (total 650 mg daily) for 12 months
Safety Issues-	Gastrointestinal irritation, constipation, diarrhea, nausea, vomiting
Primary Outcome Measures:	The primary outcome is the relative difference in erythrocyte protoporphyrin levels between baseline and 12 months after treatment
Secondary Outcome Measures:	1) Erythrocyte protoporphyrin over time: Mean values and standard deviations will be computed at each time point and values will be compared descriptively. Additionally, a random effects longitudinal model of erythrocyte protoporphyrin with time as the predictor variable will be fit and used to assess whether subjects' erythrocyte protoporphyrin varied with time. 2) EPP-specific QOL: Mean scores and standard deviations will be computed at each time point and values will be compared descriptively 3) Safety: Rates of Adverse events will be computed and safety variables such as physical and other laboratory findings will be analyzed descriptively.
Statistical Considerations (sample size and analysis plan):	A Bayesian approach based on the posterior distribution that the mean relative difference between baseline and 12-month erythrocyte protoporphyrin levels is greater than 0 will be used to assess the strength of potential efficacy. The posterior probability that the relative difference is greater than 0 will be computed. A probability ≥ 80% (corresponding to an approximate quadrupling of the odds that iron is effective in lowering erythrocyte protoporphyrin) will be considered evidence that additional study of iron is warranted. Assuming the distribution of baseline erythrocyte protoporphyrin is gamma (2.3, 861) and that baseline and 1 year values are correlated with r = 0.7, simulations indicate that a total sample size of 20 patients will allow us to detect an approximate quadrupling of the odds that iron is effective in lowering erythrocyte protoporphyrin if the true effect size is 10%.
Sponsors (federal, state, foundation and industry support):	National Institutes of Health (NIH)

1.1 Overview

Erythropoietic Protoporphyria (EPP) and X-Linked Protoporphyria (XLP) are inborn errors of heme biosynthesis, presenting clinically with severe, acute photosensitivity. Although the clinical presentation is similar, EPP results from autosomal recessive "loss-of-function" mutations in the ferrochelatase (*FECH*) gene, whereas XLP results from "gain-of-function" mutations in the erythroid-specific 5-aminolevulinate synthase (ALAS2) gene (1, 2). Mutations in the *FECH* gene reduce ferrochelatase activity to ~10-30% of normal (3). This enzymatic deficiency leads to the accumulation of predominantly metal-free protoporphyrin in erythroid and hepatic cells. In XLP, gain of function mutations result in increased enzymatic activity of erythroid-specific 5-aminolevulinate synthase 2, leading to accumulation of approximately equal amounts of metal-free and zinc-protoporphyrins (2). EPP is the most common porphyria in children and the second most common porphyria in adults. Patients with EPP and XLP present with painful cutaneous photosensitivity, generally in early childhood, presenting immediately or soon after exposure to sunlight. This pain can be accompanied by some redness and swelling of the sun exposed skin. The pain is severe and not responsive to analgesics (1). Currently there is no FDA approved treatment for EPP or XLP.

In the medical literature there are conflicting case reports on the effect of iron supplementation on EPP symptoms (4, 5, 6). In the case of an inherited deficiency of *FECH* activity, the lack of iron, a cosubstrate of *FECH*, could theoretically limit its capacity and thereby enhance the accumulation of protoporphyrin IX and in turn, aggravate the phototoxic symptoms. Iron supplementation in the case of iron deficiency is therefore hypothesized to improve EPP symptoms.

Therefore, the purpose of this study is to determine the effect of oral iron therapy for EPP and XLP patients.

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 has funded several 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. Specific Aims (Hypothesis and Objectives)

The overall objectives of this study are to determine the efficacy and safety of oral iron administration in the protoporphyrias. Efficacy will be based on erythrocyte protoporphyrin levels, photosensitivity, other clinical symptoms, and quality of life in patients with EPP and XLP who have diminished iron stores (serum ferritin $\leq$30 ng/ml).

Primary Objective: To determine if oral iron therapy can decrease erythrocyte protoporphyrin levels in patients with EPP and XLP.

Secondary Objectives:

1) To assess whether oral iron therapy can improve iron status and decrease plasma porphyrin levels and clinical symptoms
2) To assess if there is an improvement in the quality of life in treated patients

This is an open label pilot study of the safety and efficacy of oral iron therapy in patients with EPP and XLP with diminished iron stores as defined by serum ferritin level.

3. Background

Erythropoietic Protoporphyria (EPP) is characterized by painful, non-blistering, cutaneous photosensitivity, with onset in early childhood. EPP is the most common porphyria in children and the third most common in adults (after porphyria cutanea tarda and acute intermittent porphyria). Reports of prevalence vary between 5 and 15 cases per million population. (2-4)

EPP is most often due to decreased activity of ferrochelatase (*FECH*), the enzyme that catalyzes the incorporation of ferrous iron into PPIX, the final step in the production of heme. The pattern of inheritance is autosomal recessive. Homozygosity for a *FECH* mutation is rare. In most cases, the decreased activity is a consequence of a combination of an inherited inactivating mutation affecting one *FECH* allele and an intronic polymorphism that is common in the population and which alters splicing of the other allele. The alternative splice site, when used, produces a non-functional *FECH* mRNA. The alternative splice site is used approximately 40% of the time. Therefore, the polymorphic allele produces approximately 60% of normal *FECH* activity, and for this reason, is termed hypomorphic. When the hypomorphic *FECH* allele is in *trans* with the non-functional mutant allele, the result is 30% or less of the normal *FECH* enzyme activity. This subnormal *FECH* activity becomes rate-limiting, resulting in accumulation of intracellular protoporphyrin IX (PPIX). Although the defect is presumably expressed in all tissues, the PPIX responsible for photosensitivity derives primarily from marrow reticulocytes. X-linked Protoporphyria is less common than EPP, occurring in ~10% of cases, but has the same phenotype, and results from gain of function mutations in the *ALAS2* gene, leading to the overproduction of protoporphyrin in the bone marrow (2, 4).

Significance

There are no FDA-approved treatments for EPP and XLP other than pharmaceutical grade β carotene (Lumitene), which was FDA approved for EPP and later classified as a nutritional product and is now available without a prescription. Cysteine and Vitamin C have been studied in these patients, but with little convincing evidence of efficacy. Most patients report minimal, if any improvement with any of these products (7). There have been few multicenter trials, which are required for collecting high quality data, on safety and efficacy of new and existing treatments. Afamelanotide (α-melanocyte stimulating hormone), one of the few drugs under investigation for the treatment of EPP and XLP

completed Phase 3 trials in August 2013. Although beneficial, afamelanotide acts by darkening the skin rather than by decreasing circulating protoporphyrin levels (5, 8). No treatments reported to date, other than marrow transplantation and Panhematin® infusions, given in the setting of acute liver failure, decreases erythrocyte protoporphyrin levels, in these patients. A large proportion of patients with the EPP phenotype are mildly iron deficient, but that proportion is not well defined, and its significance and influence on disease severity is not known. We believe that iron therapy in patients with the EPP phenotype and low iron stores has the potential to increase iron availability and thereby increase protoporphyrin utilization for heme synthesis. Additionally, we also believe, that this therapy, may sufficiently decrease protoporphyrin accumulation to be clinically beneficial. If so, this would provide significant and low cost benefit to these patients.

Erythropoietic Protoporphyria and Iron: As noted above, EPP is caused by deficiency in ferrochelatase, the last enzyme in the heme biosynthetic pathway. The role of ferrochelatase is to catalyze the insertion of ferrous iron into protoporphyrin to form heme (1). Defective ferrochelatase activity results in the accumulation of protoporphyrin initially in the marrow and then in erythrocytes and plasma, which circulate to the skin, liver and other tissues leading to the clinical symptoms. Recent reports suggest that the availability of iron modulates ferrochelatase activity by regulating the amount of correctly spliced *FECH* mRNA (9), and thus iron appears to play an important role in disease expression.

Many patients with EPP, also have mild anemia, with evidence of iron deficiency, including microcytosis and low serum ferritin and iron levels (10). In a Swedish cohort, of EPP patients, about 45% had low ferritin levels (11). In our longitudinal study, 52% of EPP and XLP patients had low ferritin at baseline Other than microcytosis, there is little evidence of impaired erythropoiesis or abnormal iron metabolism in these patients. Although inappropriately low iron absorption has been proposed to explain these findings, this has not been substantiated.

The role of iron replacement in EPP and the clinical response to increased iron is controversial, some reports suggest clinical improvement (12, 13, 14, 15) while others describe an exacerbation of photosensitivity (16, 17). Notably, an increase in protoporphyrin levels, has never been documented, in EPP patients treated with iron. Based on these conflicting reports, clinical practice varies among physicians with some using oral iron replacement in EPP patients with low iron stores and others choosing no intervention. No results or follow up on the effectiveness of oral iron therapy have been reported. A recent report from the U.K. showed clinical and biochemical improvement in a single EPP patient that had been treated with low doses of intravenous iron (17). Hemin therapy has also been used in EPP patients with advanced liver disease prior to transplantation and decreases in erythrocyte and plasma protoporphyrin levels have been observed (18). Although the mechanism of action is unclear, a possible hypothesis is that hemin, which contains iron, improves iron stores in these patients by providing iron, as well as bypassing the deficient *FECH* enzyme, leading to a decrease in protoporphyrin levels. Results from previous studies of the natural history of EPP/XLP

suggest that there is little change in erythrocyte protoporphyrin levels over time and there is little intra-individual variation (11).

Interestingly, oral iron replacement in one patient with XLP with diminished iron stores also led to a decrease in erythrocyte protoporphyrin levels (2). We also administered oral iron to a patient with XLP and observed a 20% decrease in erythrocyte protoporphyrin levels. We have now enrolled a large number of patients in an EPP Natural History study, several of whom were treated in the past with oral or intravenous iron therapy and experienced improvements in iron status without adverse consequences before they entered our studies. Unfortunately, serial measurements of protoporphyrin levels were not done prior to enrollment in our longitudinal studies. While these reports and our experience to date suggest that iron replacement can improve iron status and may decrease erythrocyte protoporphyrin levels, the efficacy and safety of iron therapy in EPP and XLP have not been systematically investigated, and whether or not to use iron in these patients remains an unanswered clinical question.

A recent pilot study (19) showed that patients with EPP and XLP absorb oral iron with post dose increases in serum iron. In addition, the serum and urine hepcidin were not inappropriately increased in these subjects at baseline and did not increase over time after oral ferrous sulfate. Thus, the basis of iron deficiency in EPP/XLP patients and the effectiveness and safety of iron therapy are not clear and warrant further investigation.

This provides the rationale for a pilot therapeutic study with oral iron in EPP and XLP patients with well characterized phenotypes, genotypes, and iron status, with serial measurements of protoporphyrin levels in erythrocytes and plasma during treatment.

Preliminary data:

Laboratory Studies: To examine the additive effect of iron on ferrochelatase (*FECH*) activity, an in vitro assay was set up using lymphoblastoid cell lines. Blood samples were collected from the EPP patients, who carried the p.C411G mutation (no detectable enzymatic activity (20) in trans to IVS3-48C (genotype M/C) and from healthy donors (genotype T/T). Lymphoblastoid cell lines were established from these samples by transformation of the peripheral B lymphocytes with Epstein-Barr virus following a standard procedure (21). After treatment of cells with ammonium iron (III) citrate for 24 hours, *FECH* activity was determined by fluorometric measurement of zinc-deuteroporphyrin formation (nmol zinc-deuteroporphyrin/hour/mg protein at 37°C) (22) and compared to the value of lymphoblastoid (T/T) without any treatment. When lymphoblastoid cells (M/C) were treated with iron (III), *FECH* activity increased and reached 35.5%, up to 100μM, followed by a decrease at increased iron concentrations (Figure 1). Dissimilarly, treatment of lymphoblastoid cells (T/T) with iron (III) below 100μM only resulted in a slight decrease in *FECH* activity. These results suggest that

treatment of iron (III) at proper concentrations (below 100 μM) may help to increase the *FECH* activity in cells with loss-of-function mutations on *FECH* gene.

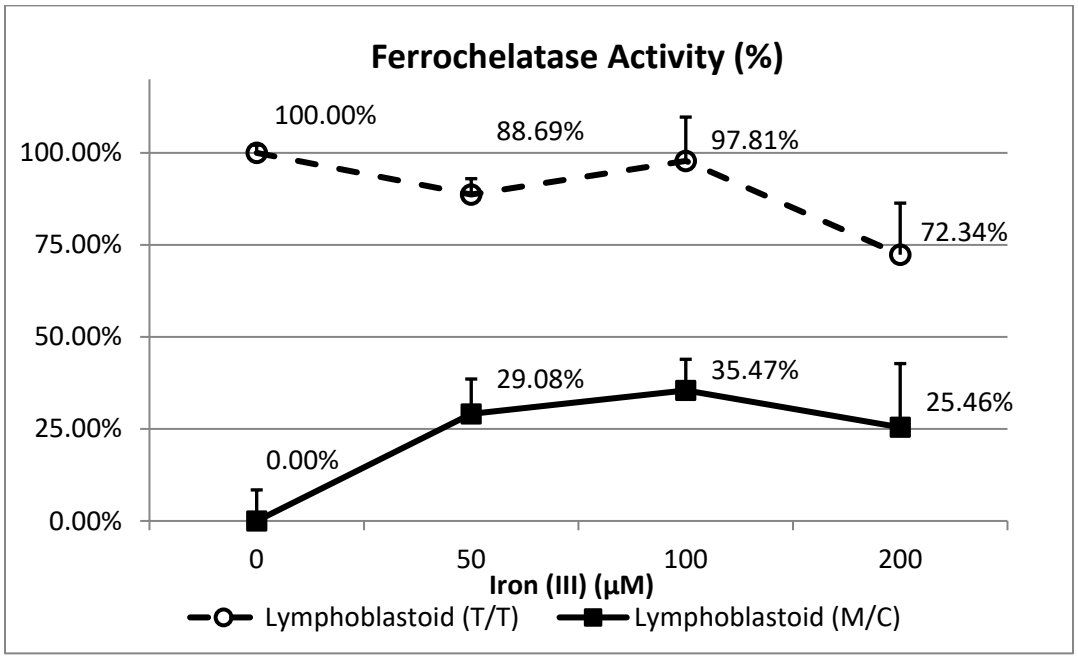

Figure 1. Effect of Iron (III) on the ferrochelatase activity of lymphoblastoid cell lines established from healthy donors (IVS3-48T/T) and patients (C411G/IVS3-48T>C). Data are expressed as mean ± standard deviation of four experiments.

This project derives from observations on the large number of patients with whom we have experience in the PC, and also from isolated experience with iron treatment in some patients. For example, we administered oral iron (325 mg of ferrous sulfate daily) to a 13 year old male with XLP based on clinical indications including a low serum iron level of 29 mcg/dL (normal 50-200 mcg/dL); low ferritin levels 12 ng/ml (50-150 ng/ml); and low transferrin saturation, 9% (normal 15-50%). Erythrocyte protoporphyrin levels at baseline and at one year follow up are shown in Table 1. A 20% decrease in erythrocyte protoporphyrin was seen at one year, and he reported increased tolerance to sunlight.

Table 1: Erythrocyte protoporphyrin levels in an XLP affected male following oral iron supplementation

	Pre-Treatment	Post-Treatment	Normal Range
PLASMA			
Total Porphyrins (mcg/dL)	**10.2**	**9.7**	0-0.9
Fluorescence Max (nm)	**634**	**634**	No peak
ERYTHROCYTES			
Protoporphyrin (mcg/dL)	**6,000**	**4,700**	20-80
Zinc protoporphyrin (%)	**32**	**38**	
Free protoporphyrin (%)	**68**	**62**	
Hematocrit (%)	**35.4**	**36.1**	

Thus, the preliminary data from the laboratory studies as well as anecdotal evidence from patient care provides support for this hypothesis.

This study will test the hypothesis that successful iron replacement in these patients decreases erythrocyte protoporphyrin levels and clinical severity of disease. If effective, this simple treatment may have significant clinical benefit and should decrease symptoms and improve quality of life in these patients. Decreases in circulating porphyrins after iron replacement might also decrease the risk for liver complications. In addition, the cause of iron deficiency in these patients is unknown, and this study may provide insight into the mechanism of iron dysregulation in EPP and XLP.

4. Study Design and Methods

This is an open label study of ferrous sulfate USP 325 mg administered twice daily (total 650 mg daily). All patients recruited for the study will be enrolled in the Longitudinal Study of the Porphyrias and have a confirmed diagnosis of EPP or XLP by biochemical testing. The sample size will be 20 patients with EPP or XLP. The study duration will be 12 months.

4.1 Inclusion Criteria

1. Enrollment in the Longitudinal Study of the Porphyrias (7201)
2. Male or female age ≥18 years
3. Willing to sign informed consent form and follow protocol
4. History of nonblistering cutaneous photosensitivity
5. Biochemical findings - A marked increase in erythrocyte protoporphyrin (total erythrocyte protoporphyrin >400 ug/dL) with a predominance of metal-free protoporphyrin
6. Serum ferritin ≤30 ng/mL at baseline
7. Able to tolerate oral iron

4.2 Exclusion Criteria

1. History of liver or bone marrow transplant or clinically significant liver dysfunction as determined by the investigator
2. Known or suspected allergy to oral iron based on patient report
3. Clinical evidence of active and ongoing GI bleeding
4. Use of any other clinical or experimental therapy in the past 3 months
5. Individuals with elevations of porphyrins in plasma or erythrocytes due to other diseases (i.e. secondary porphyrinemia) such as liver and bone marrow diseases
6. Pregnancy
7. Patients with any clinically significant comorbid conditions, which in the opinion of the investigator, precludes participation

4.3 Recruitment of Participants

The methods of recruitment will be:

A. Patients followed by any of the Investigators in the Porphyrias Consortium. Each of the Investigators is a Porphyria specialist in a Porphyria Center, and provides clinical care to individuals suspected of and diagnosed with porphyria, 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.

B. Referrals from the American Porphyria Foundation (APF) to the Porphyrias Consortium centers. 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 research studies available through the Porphyria Consortium. Information about research studies includes contact information for the studies if they are interested in obtaining additional information or participating.

C. Patients contacted through the Rare Diseases Clinical Research Network (RDCRN) Contact Registry. Individuals can register on-line or by faxing or mailing 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. The DMCC can refer them directly to the Porphyria Consortium for contact by their closest clinical site. The DMCC sends information by email

about research studies along with contact information permitting registrants to obtain additional information about participating.

D. Non-study Physician referrals. Physicians providing clinical care to individuals suspected of or diagnosed with EPP who are not investigators in this study may refer patients who may be eligible for and express interest in the study to a Porphyrias Consortium center.
E. Self-referrals, including family members of individuals diagnosed with EPP (proband) and other individuals who may have heard about the study from other subjects or prospective subjects. 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 or to register through the RDCRN-Porphyria Consortium if interested in obtaining additional information or participating.

In the case of patients followed by one of the Consortium Investigators, the physician/Investigator or study coordinator will either: (a) contact the patient by telephone, email or mail informing him/her of the study and requesting that he/she contact the study coordinator if interested in obtaining additional information or participating, or; (b) discuss the study in-person during a clinical visit; informed consent may be obtained at this time. In all other cases, prospective subjects are instructed to contact the study coordinator, either the Project Manager or a site-specific study coordinator, by telephone, email or mail if interested in obtaining additional information or participating.

4.4 Retention Strategies

EPP and XLP are rare diseases. Only a small number of specialists have the expertise to evaluate and treat affected individuals. Clinical relationships established by the investigators with patients who enroll in this pilot study will provide a context for increased communication with patients and their primary physicians that can facilitate retention. Furthermore, this study will provide a number of beneficial services to the participants and their primary physicians that will enhance retention. These include periodic evaluations, repeat laboratory testing, and information on new research developments in the field. Coordinators and other personnel at each site will also work to maintain close contact with participants, including regular clinic visits, newsletters and communication by phone, email and web sites. Counseling services will be provided so that patients and families are made aware of the results of studies and their correct interpretation. There will be no charge for the physician services or laboratory tests done as part of this pilot study.

4.5 Data Elements

The following laboratory tests and procedures will be used as the primary data elements:

- Erythrocyte protoporphyrin
- Plasma total porphyrins
- A complete blood count with differential
- Iron studies (serum iron, TIBC, Transferrin saturation)

- Serum ferritin
- Hepatic function panel (serum ALT, AST, alkaline phosphatase, total and direct bilirubin, albumin, total protein)
- EPP-specific Quality of Life Questionnaire

These will be completed at each study visit, and an adverse event review will be completed bi-weekly.

4.6 Study Procedures

Baseline/Screening Visit:

- Patients will be admitted to the Clinical Research Unit or a comparable facility for ~2 hours. All patients will be enrolled in the Longitudinal Study and the diagnosis of EPP or XLP and inclusion/exclusion criteria will be reviewed prior to baseline visit.
- A complete history and physical exam will be performed
- All concomitant medications will be recorded- if the subject is currently taking an iron supplement they will be instructed to discontinue it for at least 4 weeks and return for re-screening.
- Subjects taking Lumitene at screening will be asked to discontinue it for at least 4 weeks and return for re-screening.
- Patients will be asked to complete an EPP-specific quality of life questionnaire
- Laboratory studies will include:
    - A complete blood count with differential
    - Serum iron
    - TIBC
    - Serum ferritin
    - Transferrin saturation
    - Hepatic function panel
    - Erythrocyte protoporphyrin levels with metal-free and zinc ratio
    - Plasma total porphyrins
- Two yellow top ACD tubes will be collected and banked for future RNA and protein studies in this patient population
- Subjects will be instructed to take ferrous sulfate 325 mg BID with water starting the next day on an empty stomach following discharge.
  Subjects will be discharged with a 3 month supply of medication with some surplus to account for dropped pills.

The first dose of oral ferrous sulfate (two 325 mg tablets) will be administered within 30 days of the screening visit.

For patients with a borderline ferritin level, re-screening will be allowed within 60 days of the original screening date. Only ferritin and erythrocyte protoporphyrin levels should be done at the re-screening visit. If the ferritin level is ≤30 ng/mL on re-screening, the first dose of oral ferrous sulfate will be administered within 30 days of the re-screening visit.

Follow Up Phone Calls:

- Coordinators will call the subject once a month (+/- 7 days) to review adverse events and concomitant medications.

3 Month, 6 Month, and 9 Month Study Visits (+/- 7 days):

- A review of systems will be performed, a physical exam will only be performed at the 6 Month visit
- Adverse events will be reviewed
- All concomitant medication changes will be recorded
- Patients will be asked to complete an EPP-specific quality of life questionnaire
- Laboratory studies will include:
    - A complete blood count with differential
    - Serum iron
    - TIBC
    - Serum ferritin
    - Transferrin saturation
    - Hepatic function panel
    - Erythrocyte protoporphyrin levels with metal-free and zinc ratio
    - Plasma total porphyrins
- Two yellow top 10mL ACD tubes will be collected and banked for future RNA and protein studies in this patient population (at 6 month visit only)
- Pill counts will be performed and recorded to monitor compliance
- Old medication bottles will be collected
- Subjects will be discharged with a 3 month supply of medication with some surplus to account for dropped pills.

12 Month Study Visit (Or Early Termination Visit) (+/- 7 days):

- Patients will be admitted to the Clinical Research Unit or a comparable facility for ~2 hours
- A review of systems and physical exam will be performed
- Adverse events will be reviewed
- All concomitant medication changes will be recorded
- Patients will be asked to complete an EPP-specific quality of life questionnaire
- Laboratory studies will include:
    - A complete blood count
    - Serum iron
    - TIBC
    - Serum ferritin
    - Transferrin saturation
    - Hepatic function panel
    - Erythrocyte protoporphyrin levels with metal-free and zinc ratio
    - Plasma total porphyrins
- Two yellow top 10mL ACD tubes will be collected and banked for future RNA and protein studies in this patient population

- A single dose of oral ferrous sulfate (two 325 mg tablets) will be administered with water
- Old medication bottles will be collected and pills will be counted for compliance

4.7 Schedule of Events

	Screening	Day 0	1 Month 2 Month (via telephone)	3 Month	4 Month 5 Month (via telephone)	6 Month	7 Month 8 Month (via telephone)	9 Month	10 Month 11 Month (via telephone)	12 Month
	(Day -30 to -1)		(± 7 Days)	(± 7 Days)	(± 7 Days)	(± 7 Days)	(± 7 Days)	(± 7 Days)	(± 7 Days)	(± 7 Days)
Informed Consent	X									
Inclusion/Exclusion Criteria Review	X									
Labs (CBC, Iron, TIBC, Ferritin, Transferrin Sat, HFP)	X			X		X		X		X
Erythrocyte protoporphyrin levels & plasma porphyrins	X			X		X		X		X
2 Yellow top ACD tubes for banked samples	X					X				X
Urine/serum Pregnancy	X					X				X
Medical History review	X									
Review of Systems	X					X				X
Physical Exam	X					X				X
Vital Signs	X			X		X		X		X
EPP QoL Questionnaire	X			X		X		X		X
Provide 3 Month Supply of Medication		X*		X		X		X		
Pill Count				X		X		X		X
Con Med Review	X			X		X		X		X
AE Review			X	X	X	X	X	X	X	X
Review Compliance			X		X		X		X	

4.8 Investigational Product

All study drug will be stored and dispensed from individual centers' research pharmacies. All study drug will be provided to each site Par Pharmaceuticals directly.

Obtaining Study Drug: Each site is provided with an initial years' supply of study drug for five patients.

Dispensing, Dosage, and Accountability: All participants will receive treatment for a total of one year. Study drug will be dispensed with 3 month supplies. It is recommended that 2 weeks of additional drug is provided with each 3 month supply.

The dosage is 325 mg taken twice daily for a total of 650 mg daily. Tablets should not be broken or chewed.

At each study visit the participant will bring all study drug with them. The site coordinator will count and reconcile all study medication at each visit. At each visit (3, 6, 9 and 12 months) the subject will return all previous study drug and empty bottles and receive a new 3 month supply. At the final visit (12 months) a new supply will not be issued.

Study Drug Destruction: After proper documentation, medication returned by subjects can be destroyed onsite per institutional SOP.

If sites require additional study drug contact the PC Project Manager at least 2 months before it is needed on site.

5.0 Data and Safety Monitoring Plan

The study protocol will be reviewed and approved by the National Institutes of Health (NIH) and the Data Safety Monitoring Board (DSMB) before submission to individual center IRBs for approval. Participant enrollment may only begin with IRB approved consent forms.

This is an interventional pilot study that meets the federal definition of moderate risk.

5.1 Study Oversight

The Study Chair has primary oversight responsibility of this clinical trial. The NIH appointed Data Safety Monitoring Board (DSMB) has oversight responsibility of the Data Safety Monitoring Plan (DSMP) for this clinical trial. The DSMB will review accrual, patterns and frequencies of all adverse events, and protocol compliance every 6 months. The DSMB makes recommendations to the NIH regarding the continuation status of the protocol.

Each site's Principal Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) are responsible for identifying adverse events. Aggregate report- detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures – will be available from the DMCC for site review. Adverse events will be reviewed at each study visit by

the research team. A separate report detailing protocol compliance will also be available from the DMCC for site review on a monthly basis. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

5.2 Definitions and Standards

The Rare Diseases Clinical Research Network defines an adverse event as: "…an unfavorable and unintended sign, symptom or disease associated with a participant's participation in a Rare Diseases Clinical Research Network study."

Serious adverse events include 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.

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 by the site investigator where the AE occurred using the current version of the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.

As this study is considered an interventional trial, all adverse events, including those not directly related to study participation, will be reported as specified above.

5.3 Expected/Known Risks/Discomforts/Adverse Events Associated with Study Intervention and Procedures: Definition of Expected Adverse Events

Study Drug/Intervention:
Drug Information for Ferrous sulfate USP

Dosing: Adults- 325 mg capsule administered twice daily (total 650 mg daily)

Human Toxicity/ Adverse Events (adapted from http://www.drugs.com/ferrous_sulfate.html): GI irritation, constipation, diarrhea, nausea, vomiting, and dark stools. In addition, as the effects of oral iron are unknown, patients may develop an increase in erythrocyte or plasma protoporphyrin levels and worsening clinical symptoms.

Study Procedures:
Physical Exam: A complete physical will be conducted every 6 months

Venipuncture: The vein in which the needle has been inserted to draw blood may become sore and red. A temporary bruise may develop, and rarely fainting may occur. The amount of blood, 24mL (~2 tablespoons) required for the tests described in this study is within the guidelines for blood collection for patients with porphyria.

Urine Collection: A sample of urine will be collected. This procedure does not expose the patient to any risk.

Safety Endpoints: The primary safety endpoints will be an increase in reported clinical symptoms or a 35% increase in erythrocyte protoporphyrin levels. If an increase of 35% over baseline erythrocyte protoporphyrin levels is observed this test will be repeated after 2-4 weeks without discontinuing iron. If the level remains elevated ≥35% on repeat testing then the study drug will be discontinued. If clinical symptoms increase to the point where the patient or treating physician is not comfortable continuing then the study drug should be discontinued. The secondary safety endpoints will be any increase in plasma porphyrins and side effects of oral iron therapy such as GI irritation, constipation, diarrhea, nausea, vomiting, and dark stools. If these secondary safety endpoints occur then discontinuing the oral iron will be at the discretion of the treating physician.

5.4 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.

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.

5.5 RDCRN Adverse Event Data Management System (AEDAMS)

Upon entry of a serious adverse event, the DMCC created Adverse Event Data Management System (AEDAMS) will immediately notify the Study Chair, site PIs, the 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 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 expected adverse events that are reported to or observed by the investigator or a member of his/her research team will be submitted to the DMCC in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the MRO and RDCRN DSMB on a bi-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 reported adverse events for site investigators and IRBs.

5.6 Study Discontinuation

The NIH, RDCRN DSMB and local IRBs (at their local site) have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- Early stopping rules have been met (see section 5.3)
- The study objectives have been met
- The Study Chair / Study Investigators believe it is not safe for the study to continue
- The RDCRN DSMB suspends or closes the trial
- The NIH suspends or closes the trial
- The FDA suspends or closes the trial

5.7 Subject Discontinuation

An intent to treat approach will be used. If participants are unable to tolerate the study drug, the study drug will be stopped, but they will be followed at regular visits until the end of the trial unless they withdraw consent. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis. If participants withdraw from the trial, 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. Reasons for withdrawal from the trial may include:

- Withdrawal of consent by the participant
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g. mental status change, large pleural effusion).
-

5.8 Data Quality and Monitoring Measures

As much as possible data quality is assessed at the data entry point using intelligent on-line data entry via visual basic designed screen 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 query to the consortium administrator contact.
- Data Delinquency Tracking: The Data Management and Coordinating Center will monitor data delinquency on an ongoing basis.

5.9 Quality Control: Study Related Procedures

The standard laboratory measures (CBC, CMP, Iron panel, etc) will be performed at each sites' central laboratory. Erythrocyte protoporphyrin measurements will be performed in the CLIA certified laboratory of Dr. Karl Anderson, University of Texas Medical Branch, Galveston, TX.

The investigational pharmacy at each site will be responsible for drug accountability while the investigators/coordinators will be responsible for compliance of the subjects. The Investigational Drug Service at the Icahn School of Medicine at Mount Sinai will serve as the coordinating pharmacy for this study.

6. Statistical Considerations

The primary outcome is the relative difference in erythrocyte protoporphyrin levels between baseline and 12 months after treatment. A Bayesian approach based on the posterior distribution that the mean relative difference between baseline and 12-month erythrocyte protoporphyrin levels is greater than 0 will be used to assess the strength of potential efficacy of oral iron therapy.

The procedure will be carried out as follows. Let X_0 and X_{12} be the observed mean erythrocyte protoporphyrin level at baseline and 12 months. Let X_0 and X_{12} be correlated with a Pearson correlation coefficient of r and let d = $\frac{X_0 - X_{12}}{X_0}$ and Θ = = $\frac{\mu_0 - \mu_{12}}{\mu_0}$. Based on data observed in previous studies, we assume that the distribution of

erythrocyte protoporphyrin follows a gamma distribution (see background data section below). For an individual i, X_{0i} ~ gamma (α_0,β_0) and X_{12i} ~ gamma (α_{12},β_{12}).

$X_0 = \frac{\sum_{i=1}^{n}(X_{0i})}{n}$, therefore $\frac{1}{n}X_0 \sim n * \text{gamma}(\alpha_0, \beta_0) = \text{gamma}(n\alpha_0, \beta_0)$ and likewise $\frac{1}{n}X_{12} \sim \text{gamma}(n\alpha_{12}, \beta_{12})$.

Two correlated gamma distributions can be generated using a single parameter α, the means of each distribution and the standard deviation as detailed in Ronning et al (23). We specify a non-informative normal(n*0, 0.01) prior for α. After data collection the posterior distribution of α|d ~ normal(n*$\frac{X_0}{\sigma_0^2/X_0}$, 0.01). The posterior distribution for Θ|d can be generated by simulating baseline and 12 month mean values using the posterior distribution of α|d, the observed correlation between 12 month and baseline values and the observed standard deviations. After generating the posterior distribution for Θ|d we will be able to compute the probability that Θ|d > 0.

Prior to any data collection, equipoise implies that the probability that iron therapy will improve erythrocyte protoporphyrin levels (i.e. the relative difference between baseline and 12 months will be greater than 0) is 50%. An increase in this probability to a level providing reasonable assurance of an efficacy signal (e.g. 80%) would warrant additional study of iron. A probability of 80% corresponds to an approximate quadrupling of the odds that iron is effective in lowering erythrocyte protoporphyrin (i.e., from 50% probability of irons efficacy, or 1:1 odds, to 80% or 4:1 odds).

Sample size justification:

Assuming the distribution of baseline erythrocyte protoporphyrin is gamma (2.3, 861) and that baseline and 12 month values are correlated with r = 0.7, simulations indicate that a total sample size of 20 patients will allow us to detect an approximate quadrupling of the odds that iron is effective in lowering erythrocyte protoporphyrin if the true effect size is 10%. Table 2 lists simulated estimates (100,000 runs for each) of the probability that Θ|d > 0 across several sample size and effect size scenarios.

Table 2: Simulation results

		Effect Size				
r	Sample Size	0%	5%	10%	15%	20%
.7	10	0. 50	0.63	0.74	0.83	0.90
	15	0. 50	0.65	0.78	0.88	0.94
	20	0. 50	0.67	0.81	0.91	0.96
	25	0. 50	0.69	0.84	0.93	0.97
	30	0. 50	0.71	0.86	0.95	0.98
	35	0. 50	0.72	0.88	0.96	0.99
	40	0. 50	0.74	0.89	0.97	0.99

Background Data

As of March 17, 2015 there were 152 EPP and XLP patients in The Longitudinal Study of the Porphyrias who had an erythrocyte protoporphyrin level >400, were not on Iron therapy and had no reported liver disease (fibrosis, cirrhosis, steatosis) or liver transplant history. The distribution of these patients' baseline erythrocyte protoporphyrin is pictured in figure 2.

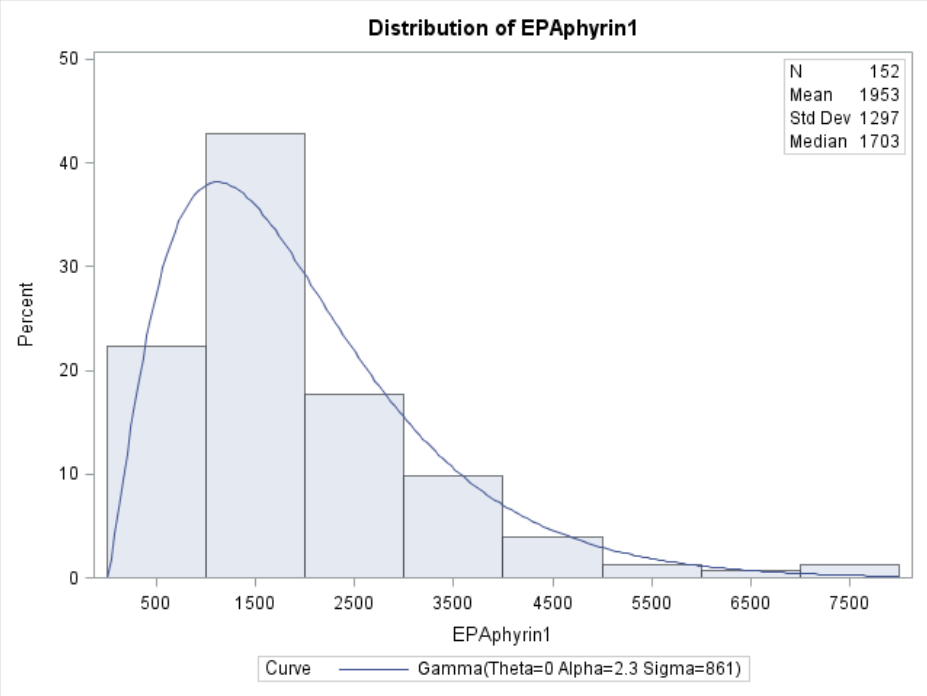

Figure 2: Distribution of 152

Data from The Longitudinal Study of the Porphyrias demonstrated that the correlation between erythrocyte protoporphyrin levels taken approximately 1 year apart was 0.74.

Secondary Analyses:

In addition to the primary outcome, the following secondary endpoints will be analyzed:

1) Erythrocyte protoporphyrin over time: Mean values and standard deviations will be computed at each time point and values will be compared descriptively. Additionally, a random effects longitudinal model of erythrocyte protoporphyrin with time as the predictor variable will be fit. Time will be treated as a continuous measure unless diagnostic plots suggest that a linear mean function for time is not the correct specification. If this is the case, time (ie visit timepoint) will be treated as a categorical variable. Using the fitted model, we will test the null hypothesis that the beta coefficient(s) for time = 0. There have been no previous studies on the effect of iron therapy on erythrocyte protoporphyrin over time. As such, no estimates of between-subject variability or effect size are availble to pre-specify the power of this analysis.

2) EPP-specific QOL overtime: To assess whether the subjects' EPP-specific QOL changed during the duration of the study, the mean score and standard deviation will be computed at each time point and compared descriptively.

3) Safety Endpoints: Rates of Adverse events will be computed. Safety variables such as physical and other laboratory findings will be analyzed descriptively.

7. Data Management

As much as possible, data quality is assessed at the data entry point using intelligent on-line forms via the DMCC. 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.

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.

7.1 Registration

Registration of participants on this protocol will employ an interactive data system in which the clinical site will attest to the participant's eligibility as per protocol criteria and obtain appropriate informed consent. IRB approval for the protocol must be on file at the DMCC before accrual can occur from the clinical site.

The DMCC will use a system of coded identifiers to protect participant confidentiality and safety. Each participant 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 participant is registered to participate in the study, using the DMCC provided web-based registration system; the system will assign a participant ID number. Thus each participant will have two codes: the local one that can be used by the registering site to obtain personal identifiers and a second code assigned by the DMCC. For all data transfers to the DMCC both numbers will be required 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 participant for data entry since the numbers should match to properly identify the participant. In this fashion, no personal identifiers would be accessible to the DMCC.

7.2 Data Entry

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

8. Human Subjects

8.1. GCP Statement

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.

8.2. Risks

The potential risks of this study are:

1) Side effects of Oral ferrous sulfate: GI irritation, constipation, diarrhea, nausea, vomiting, and dark stools. In addition, as the effects of oral iron are unknown, patients may develop an increase in erythrocyte or plasma protoporphyrin levels and worsening clinical symptoms.

2) Blood Draw: Needle insertion can cause pain and bruising at the site of insertion. Rarely fainting caused by a vasovagal response to the phlebotomy procedure may occur. Because the skin is penetrated by the needle, there is a low risk for infection at the venipuncture site. The amount of blood needed for each study visit (8mL tubes) is within approved safety limits. For any participant who has a medical condition that restricts their capacity to provide adequate sample volume, the number of tubes of blood obtained will be modified on an individual basis.

3) Loss of confidentiality: The risk of loss of confidentiality is low because as there are many safe guards in place. Conceivably, data including clinical and diagnostic information could be accidentally divulged. If such an event occurred, subjects might be at risk for discrimination by life or health insurance companies, by employers, and by adoption agencies. The Genetic Information Non-Discrimination Act (GINA) is a federal law that protects Americans from being treated unfairly because of differences in their DNA that may affect their health. This law prohibits genetic discrimination in the workplace and by health insurers. However, it provides no such protection for life insurance and disability insurance.

8.3. Benefits

Participants may derive no direct benefit from being in this study. The potential benefits of this study are lowering the levels of erythrocyte protoporphyrins. Lower protoporphyrin levels may result in some improvement of sun sensitivity symptoms.

8.4. Recruitment

Participants with EPP or XLP 18 years of age or older, including both genders and all ethnic groups can be enrolled in this interventional study should they meet inclusion/exclusion criteria. Participants potentially eligible will include 1) those known to the investigators who can be contacted and informed of the study; 2) participants 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) participants who contact the Data Management and Coordinating Center (DMCC) of the Rare Disease Clinical Research Network (RDCRN) and join the Contract 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.

Inclusion of vulnerable groups: Children and pregnant women are excluded from participating in this interventional study. In addition subjects who are not able to give informed consent will be excluded.

8.5. Written Informed Consent:

Written informed consent will be obtained from each participant before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The participant's willingness to participate in the study will be documented in writing in a consent form, which will be signed by the participant with the date of that signature indicated. The investigator will keep the original consent forms and signed copies will be given to the participants. 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.

8.6. Process of Consent:

At each of the Porphryias Consortium sites the principal investigator and/or study coordinator will identify subjects with EPP/XLP who are enrolled in the Longitudinal Study of the Porphyrias and eligible for this study. The PI and/or coordinator will then approach the subject, either via a phone call or at a clinic visit, to fully describe the intent of the study and what the potential risks and benefits of participation in this study will be. The subject will have an opportunity to review the consent form and ask any questions of the study team, as well as discuss the study with their primary care physician or others of their choosing, prior to signing the consent form.

8.7. Certificate of Confidentiality

To help protect participant privacy, a Letter of Confidentiality has been obtained from the National Institutes of Health (NIH) for the Longitudinal Study of the Porphyrias. 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.

9. References

1. 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.
2. 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.
3. Whatley SD, Mason NG, Holme SA, Anstey AV, Elder GH, Badminton MN. Molecular epidemiology of erythropoietic protoporphyria in the U.K. Br J Dermatol. 2010 Mar;162(3):642-6.
4. Balwani M, Doheny D, Bishop DF, Nazarenko I, Yasuda M, Dailey HA, Anderson KE, Bissell DM, Bloomer J, Bonkovsky HL, Phillips JD, Liu L, Desnick RJ. Loss-of-Function Ferrochelatase and Gain-of-Function Erythroid 5-Aminolevulinate Synthase Mutations Causing Erythropoietic Protoporphyria and X-Linked Protoporphyria in North American Patients Reveal Novel Mutations and a High Prevalence of X-Linked Protoporphyria. Mol Med. Epub Jan 2013. PMID 23364466
5. Harms JH, Lautenschlager S, Minder CE, Minder EI. Mitigating photosensitivity of erythropoietic protoporphyria patients by an agonistic analog of alpha-melanocyte stimulating hormone. Photochem Photobiol. 2009 Nov-Dec;85(6):1434-9.
6. Hwang SI, Lee YY, Park JO, Norton HJ, Clemens E, Schrum LW, Bonkovsky HL. Effects of a single dose of oral iron on hepcidin concentrations in human urine and serum analyzed by a robust LC-MS/MS method. Clin Chim Acta 412: 2241-2247, 2011, [PMID 21867695] [PMCID: PMC3207492]
7. Minder EI, Schneider-Yin X, Steurer J, Bachmann LM. A systematic review of treatment options for dermal photosensitivity in erythropoietic protoporphyria. Cell Mol Biol (Noisy-le-grand). 2009 Feb 16;55(1):84-97.
8. Minder EI. Afamelanotide, an agonistic analog of α-melanocyte-stimulating hormone, in dermal phototoxicity of erythropoietic protoporphyria. Expert Opin Investig Drugs. 2010 Dec;19(12):1591-602
9. Barman-Aksözen J, Béguin C, Dogar AM, Schneider-Yin X, Minder EI. Iron availability modulates aberrant splicing of ferrochelatase through the iron- and 2-oxoglutarate dependent dioxygenase Jmjd6 and U2AF(65.). Blood Cells Mol Dis. 2013 Oct;51(3):151-61
10. Holme SA, Worwood M, Anstey AV, Elder GH, Badminton MN. Erythropoiesis and iron metabolism in dominant erythropoietic protoporphyria Blood. 2007 Dec 1;110(12):4108-10. Epub 2007 Sep 5
11. Wahlin S, Floderus Y, Stål P, Harper P. Erythropoietic protoporphyria in Sweden: demographic, clinical, biochemical and genetic characteristics. J Intern Med. 2011;269(3):278-88.
12. Gordeuk VR, Brittenham GM, Hawkins CW, Mukhtar H, Bickers DR. Iron therapy for hepatic dysfunction in erythropoietic proto-porphyria. Ann of Int. Med 1968. 105; 27-21
13. Holme SA, Thomas CL, Whatley SD, Bentley DP, Anstey AV, Badminton MN. Symptomatic response of erythropoietic protoporphyria to iron supplementation. J Am Acad Dermatol. 2007;56(6):1070-2.
14. Kniffen J. Protoporphyrin removal in intrahepatic porphyrastasis. Gastroenterology 1970;58:1027

15. Landefeld C, Kentouche K, Gruhn B, Stauch T, Rößler S, Schuppan D, Whatley SD, Beck JF, Stölzel U. Iron supplementation improves protoporphyrin overload, liver damage and anaemia. Br J Haematol. Epub 2015 Jul 20
16. Milligan A, Graham-Brown RA, Sarkany I, Baker H. Erythropoietic protoporphyria exacerbated by oral iron therapy. Br J Dermatol, 1988;119:63–66
17. McClements B, Bingham A, Callender ME, Trimble ER. Erythropoietic protoporphyria and iron therapy. Br J Dermatol 1990;122:423–426
18. 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.
19. Bossi K, Lee J, Schmeltzer, Holburton E, Groseclose G, Besur S, Hwang S, Bonkovsky HL. Homeostasis of iron and hepcidin in erythropoietic protoporphyria and X-linked protoporphyria. [Abstract # 621, presented at Annual Meeting of American College of Gastroenterology, Oct 17-22, 2014, Philadelphia, PA]. Amer J Gastroenterology 109 [Suppl 2]: S180, 2014.
20. Schneider-Yin X, Gouya L, Dorsey M, Rüfenacht U, Deybach JC, Ferreira GC. Mutations in the iron-sulfur cluster ligands of the human ferrochelatase lead to erythropoietic protoporphyria. Blood 2000;96:1545-1549.
21. Ling PD, Huls HM. Current Protocols in Molecular Biology. 2001 John Wiley & Sons, Inc.
22. Bloomer J, Bruzzone C, Zhu L, Scarlett Y, Magness S, Brenner D. Molecular defects in ferrochelatase in patients with protoporphyria requiring liver transplantation. Jour Clin Investigation 1998;102:107-114.
23. Ronning, G. (1977). "A Simple Scheme for Generating Multivariate Gamma Distribution with Non-Negative Gamma Distribution with Non-Negative Covariance Matrix". Technometrics, 19, No.2, 179-183.