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Clinical, Biochemical, and Genetic Characterization of North American Patients With Erythropoietic Protoporphyria and X-linked Protoporphyria

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IMPORTANCE Autosomal recessive erythropoietic protoporphyria (EPP) and X-linked protoporphyria (XLP) are rare photodermatoses presenting with variable degrees of painful phototoxicity that markedly affects quality of life. The clinical variability, determinants of severity, and genotype/phenotype correlations of these diseases are not well characterized.

OBJECTIVE To describe the baseline clinical characteristics, genotypes, and determinants of disease severity in a large patient cohort with EPP or XLP.

DESIGN, SETTING, AND PARTICIPANTS A prospective observational study was conducted among patients with confirmed diagnoses of EPP or XLP from November 1, 2010, to December 6, 2015, at 6 academic medical centers of the Porphyrias Consortium of the National Institutes of Health Rare Diseases Clinical Research Network. Detailed medical histories, including history of phototoxicity and treatment, were collected on standardized case report forms. Patients underwent baseline laboratory testing, total erythrocyte protoporphyrin (ePPIX) testing, and molecular genetic testing. Data were entered into a centralized database.

MAIN OUTCOMES AND MEASURES Results of biochemical and genetic tests were explored for association with clinical phenotype in patients with EPP or XLP.

RESULTS Of the 226 patients in the study (113 female and 113 male patients; mean [SD] age, 36.7 [17.0] years), 186 (82.3%) had EPP with a *FECH* (OMIM 612386) mutation and the common low-expression *FECH* allele IVS3–48T>C, and only 1 patient had 2 *FECH* mutations. Twenty-two patients had XLP (9.7%; 10 male and 12 female patients), and 9 patients (4.0%) had elevated ePPIX levels and symptoms consistent with protoporphyria but no detectable mutation in the *FECH* or *ALAS2* (OMIM 301300) gene. Samples of DNA could not be obtained from 8 patients. Patients' mean (SD) age at symptom onset was 4.4 (4.4) years. Anemia (107 [47.3%]), history of liver dysfunction (62 [27.4%]), and gallstones (53 [23.5%]) were commonly reported. Higher ePPIX levels were associated with earlier age of symptom onset (median ePPIX levels for those who developed symptoms before vs after 1 year of age, 1744 vs 1567 μg/dL; $P = .02$), less sun tolerance (median ePPIX levels for those reporting symptoms before vs after 10 minutes of sun exposure, 2233 vs 1524 μg/dL; $P \leq .001$), and increased risk of liver dysfunction (median ePPIX levels for those with liver dysfunction vs normal liver function, 2016 vs 1510 μg/dL; $P = .003$). Patients with EPP and *FECH* missense mutations had significantly lower ePPIX levels than those with other mutations (1462 vs 1702 μg/dL; $P = .01$). Male patients with XLP had significantly higher ePPIX levels, on average, than did patients with EPP (3574 vs 1669 μg/dL; $P < .001$). Marked clinical variability was seen in female patients with XLP owing to random X-chromosomal inactivation.

CONCLUSIONS AND RELEVANCE These data suggest that higher ePPIX levels are a major determinant of disease severity and risk of liver dysfunction in patients with EPP or XLP. These findings provide a framework for clinical monitoring and management of these disorders.

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Erythropoietic protoporphyria (EPP) is an autosomal recessive, childhood-onset, rare porphyria with severe phototoxic manifestations.[1-3] On sun exposure, patients experience prodromal symptoms, including tingling, burning, or itching, that serve as a warning signal, as continued exposure to sunlight leads to severe phototoxic pain and erythema and swelling of the exposed skin.[4] The phototoxic pain is not responsive to treatment, including narcotic analgesics, and the severe pain may last for days.[2,4] After experiencing the disabling phototoxic attacks, patients typically develop an ingrained fear of exposure to sunlight, leading to a conditioned behavior of sun avoidance that limits their daily activities and markedly impairs their quality of life.[5,6]

Erythropoietic protoporphyria results from reduced activity of ferrochelatase, the final enzyme in the heme biosynthetic pathway, to less than 30% of normal, leading to significantly elevated erythrocyte protoporphyrin (ePPIX) levels.[7,8] X-linked protoporphyria (XLP) is a less common condition with the same phenotype, including increased ePPIX levels resulting from gain-of-function mutations in the erythroid-specific form of 5-aminolevulinate synthase 2 (*ALAS2* [OMIM 301300]).[9,10] Mutation analyses indicate that 2% to 10% of patients with protoporphyria have XLP.[7,11,12] In both disorders, erythroid PPIX released into plasma is taken up by the liver and vascular endothelium. The PPIX accumulated in the superficial skin vessels is activated by exposure to sunlight, generating singlet oxygen radical reactions leading to tissue damage and severe pain.[4] Circulating PPIX taken up by the liver is excreted in the bile. It may crystallize in bile and/or hepatocytes, causing gallstones and cholestatic hepatitis.[13,14] In 2% to 5% of cases, cholestatic injury progresses to liver failure and liver transplantation.[15,16]

The diagnosis of EPP or XLP is established by demonstrating elevated ePPIX levels with a predominance of metal-free PPIX in EPP (typically >90% metal-free PPIX) and XLP (approximately 50%-85% metal-free PPIX), with the remainder of excess PPIX complexed with zinc.[7,17] Sequencing the *FECH* gene (OMIM 612386) and exon 11 of the *ALAS2* gene is required to confirm the diagnosis of EPP or XLP and for subsequent testing and counseling of family members.[8,18] Treatment is limited to sun protection, and there are no currently available therapies approved by the US Food and Drug Administration for these disorders.

The severity of phototoxicity varies among unrelated patients with EPP or XLP, presumably associated in part with their ePPIX levels.[5] Previous studies have described the clinical and biochemical features of EPP but not the determinants of disease severity.[5,19] Erythropoietic protoporphyria has not been characterized in the North American population. Although there is limited information on XLP, reports suggest that male patients with XLP may have a more severe phenotype, including a higher risk for liver disease, but this possibility has not been confirmed in a larger, well-characterized cohort.[9,11] Here, we describe comprehensive clinical, biochemical, and genetic findings from the largest reported EPP and XLP cohorts.

Key Points

Question What are the baseline characteristics and determinants of disease severity in patients with erythropoietic protoporphyria and X-linked protoporphyria?

Findings In this cohort study of 226 patients, higher erythrocyte protoporphyrin levels were correlated with earlier age of symptom onset, decreased sun tolerance, and increased risk of liver dysfunction. Patients with erythropoietic protoporphyria and *FECH* missense mutations had lower erythrocyte protoporphyrin levels and a less severe phenotype, and male patients with X-linked protoporphyria had significantly higher erythrocyte protoporphyrin levels than did patients with erythropoietic protoporphyria.

Meaning Erythrocyte protoporphyrin levels are a significant determinant of disease severity in patients with erythropoietic protoporphyria or X-linked protoporphyria.

Methods

The study (clinicaltrials.gov identifiers, NCT01561157 and NCT01688895) was performed from November 1, 2010, to December 6, 2015, at 6 sites of the Porphyrias Consortium of the National Institutes of Health Rare Diseases Clinical Research Network[20] and conducted in accord with the Declaration of Helsinki.[21] Patients (children and adults) were included if they had a history of photosensitivity and a marked increase in ePPIX (total ePPIX>200 µg/dL [to convert to micromoles per liter, multiply by 0.0178]) or more than a 1.5-fold increase in ePPIX with a predominance of metal-free PPIX and/or confirmatory genetic testing by *FECH* and/or *ALAS2* sequencing as previously described.[8] The institutional review board at each site reviewed and approved this study. Patients provided written informed consent.

Data on medical and family history and history of phototoxicity and treatment were recorded using standardized case report forms. Laboratory studies included a complete blood count, iron indices, hepatic function tests, and vitamin D levels. Erythrocyte PPIX levels (metal-free and zinc PPIX) and plasma porphyrin levels were obtained. All data were entered into a central database at the Rare Diseases Clinical Research Network's Data Management Coordinating Center, University of South Florida, Tampa. Only ePPIX levels determined at the Porphyria Testing Laboratories at the University of Texas Medical Branch and the Mayo Clinic were included in the final analysis owing to limitations of laboratory testing for PPIX.[17]

Statistical Analysis

Demographics, symptoms, photoprotective measures, medical conditions, baseline laboratory test results, and measures of disease severity are reported descriptively using mean (SD) or median values and interquartile ranges (IQRs) for continuous variables and numbers and percentages for categorical variables. Erythrocyte PPIX levels were compared between groups using Wilcoxon tests when 2 groups were compared and Kruskal-Wallis tests when 3 or more were compared. All analyses

were considered exploratory and were conducted using SAS, version 9.4 (SAS Institute Inc), with $P < .05$ considered significant.

Results

Demographics

The study population included 226 patients (mean [SD] age, 36.7 [17.0] years) enrolled at the 6 sites of the Porphyrias Consortium (**Table 1**). Of these, 217 (96.0%) were white, 204 (90.3%) had EPP (mean [SD] age at enrollment, 35.8 [17.0] years), and 22 (9.7%) had XLP (10 male patients with a mean [SD] age of 49.6 [16.1] years and 12 female patients with a mean [SD] age of 41.5 [12.2] years). Among the male patients with XLP, 8 (80.0%) had a known family history of phototoxicity.

Symptoms and Photoprotective Measures

For the overall study population, the mean (SD) age at symptom onset was 4.4 (4.4) years. Patients with EPP reported a mean (SD) age at symptom onset of 4.1 (3.0) years (n = 158). Among patients with XLP, the mean (SD) age at symptom onset was 2.7 (2.4) years for male patients (n = 10) and 11.6 (11.4) years for symptomatic female patients (n = 11). A total of 79 patients with EPP (38.7%) reported phototoxic symptoms in the first year of life, and most patients with EPP (109 [53.4%]) had symptoms in childhood (1-12 years of age). Only 6 patients with EPP reported symptom onset after 13 years of age. All male patients with XLP had symptoms in the first year of life or childhood, while 4 female patients with XLP reported symptoms beginning after 13 years of age, and 1 female patient, whose condition was diagnosed by family testing, reported no symptoms in her 50s (Table 1).

Most patients with EPP (116 [56.8%]) reported symptom onset within 30 minutes of sun exposure, with 52 patients (25.5%) reporting onset in less than 10 minutes. In contrast, 2 male patients with XLP (20.0%) experienced symptoms within 10 minutes of sun exposure, and all had symptoms within 30 minutes of sun exposure. Female patients with XLP showed a variable degree of phototoxicity; 1 female patient had no symptoms, and 6 female patients (50.0%) reported symptoms within 30 minutes of sun exposure (**Table 2**). A total of 186 patients with EPP (91.2%) described the initial phototoxic pain as burning, followed by tingling (160 [78.4%]) and itching (159 [77.9%]). The most common sign reported was swelling (176 [86.3%]), followed by redness (97 [47.5%]). All male patients with XLP reported experiencing tingling and swelling in addition to burning (9 [90.0%]), itching (8 [80.0%]), stinging (8 [80.0%]), redness (4 [40.0%]), and blistering (3 [30.0%]). Sensitivity to fluorescent lights was reported by 48 patients with EPP (23.5%), 4 male patients with XLP (40.0%), and 2 female patients with XLP (16.7%).

Of patients with EPP, 87 of 193 (45%) reported experiencing 3 to 10 phototoxic episodes per year, with most patients (118 [57.8%]) requiring 1 to 3 days to recover from pain. Patients with XLP reported similar numbers of phototoxic episodes and time to recovery.

Table 1. Demographics of Study Participants

Characteristic	EPP (n = 204)	XLP (n = 22)
Demographic		
Age at enrollment, mean (SD), y	35.8 (17.0)	45.2 (14.4)
Age, median (range), y	34.0 (3-77)	49.0 (21-67)
Sex, No. (%)		
Male	103 (50.5)	10 (45.5)
Female	101 (49.5)	12 (54.5)
Race/ethnicity, No. (%)		
White	197 (96.5)	20 (90.9)
African American	0	2 (9.1)
Hispanic	0	0
Asian	3 (1.5)	0
Other	4 (2.0)	0
Clinical		
Age at first symptom, No. (%)[a]		
<1 y	79 (38.7)	5 (50.0)/2 (16.7)
1-12 y	109 (53.4)	5 (50.0)/5 (41.7)
13-17 y	4 (2.0)	0/2 (16.7)
≥18 y	2 (1.0)	0/2 (16.7)
Unsure	1 (0.5)	0/0
No symptoms	0	0/1
No response	9 (4.4)	0/0

Abbreviations: EPP, erythropoietic protoporphyria; XLP, X-linked protoporphyria.

[a] Data for XLP given as number (percentage for male/female patients). In the XLP group, 10 were male and 12 were female patients.

Of patients with EPP, 121 (59.3%) had a history of skin changes, in contrast to 4 male patients with XLP (40.0%) and 4 female patients with XLP (33.3%). Skin changes were predominantly on the dorsum of the hands, with scarring and thickened skin being the most common. A total of 54 patients with EPP (26.5%) and 59 patients in the total study group (26.1%) self-reported blistering after sun exposure. Most patients practiced sun protection, with a majority using long-sleeved shirts, caps, gloves, and sunglasses. Other photoprotective measures included sunscreens (with or without zinc oxide or titanium dioxide) and tinted car windows. Beta carotene (Lumitene) was tried by 155 patients with EPP, 8 male patients with XLP, and 5 female patients with XLP. Of these, 93 of 155 patients with EPP (60.0%), 4 of 8 male patients with XLP (50.0%), and 3 of 5 female patients with XLP (60.0%) found it ineffective, while 53 of 155 patients with EPP (34.2%), 3 of 8 male patients with XLP (37.5%), and 2 of 5 female patients with XLP (40.0%) reported partial symptom improvement. Only 2 patients with EPP reported symptom resolution with beta carotene.

Medical History

Abnormal results of liver studies were reported by 54 patients with EPP (26.5%), 3 male patients with XLP (30.0%), and 5 female patients with XLP (41.7%). Of these, 18 patients with EPP, 2 male patients with XLP, and 2 female patients with XLP had undergone liver biopsies. Two patients (1 patient with EPP

Table 2. Erythrocyte Protoporphyrin Levels and Time to First Symptom

Time to First Symptom	No.[a]	Erythrocyte Protoporphyrin Levels, µg/dL		
		Mean (SD)	Median (Range)	Interquartile Range
EPP				
<10 min	46	2354.9 (1213.9)	2233.0 (487.0-6179.0)	1522.0-3056.0
11-30 min	63	1765.0 (935.5)	1687.0 (137.0-5017.0)	1035.0-2161.0
31-59 min	27	1583.4 (610.9)	1536.0 (715.0-3194.0)	1047.0-1995.0
1-3 h	35	1528.7 (766.3)	1457.0 (562.0-3691.0)	873.0-1869.0
>3 h	7	873.9 (393.6)	741.0 (407.0-1513.0)	589.0-1213.0
No symptoms	0	NA	NA	NA
Unsure	2	871.0 (19.8)	871.0 (857.0-885.0)	857.0-885.0
XLP female				
<10 min	2	4982.5 (4079.3)	4982.5 (2098.0-7867.0)	2098.0-7867.0
11-30 min	4	5440.0 (2012.4)	5708.0 (2800.0-7544.0)	3978.0-6902.0
31-59 min	2	2328.5 (30.4)	2328.5 (2307.0-2350.0)	2307.0-2350.0
1-3 h	1	1420.0	NA	NA
>3 h	2	480.5 (395.3)	480.50 (201.0-760.0)	201.0-760.0
No symptoms	1	379.0	NA	NA
Unsure	0	NA	NA	NA
XLP male				
<10 min	2	7525.5 (4418.7)	7525.5 (4401.0-10 650.0)	4401.0-10 650.0
11-30 min	8	3710.4 (2056.1)	3192.5 (1846.0-7801.0)	2143.5-4682.0
31-59 min	0	NA	NA	NA
1-3 h	0	NA	NA	NA
>3 h	0	NA	NA	NA
No symptoms	0	NA	NA	NA
Unsure	0	NA	NA	NA

Abbreviations: EPP, erythropoietic protoporphyria; NA, not available; XLP, X-linked protoporphyria.

SI conversion factor: To convert protoporphyrin to millimoles per liter, multiply values by 0.0178.

[a] Patients with erythrocyte protoporphyrin testing performed at laboratories other than the University of Texas Medical Branch Porphyria Laborabory or the Mayo Laboratory were excluded.

and 1 male patient with XLP) had received a liver transplant. At the time of the transplant, the patient with EPP was in his 50s, with an ePPIX level of 3050 µg/dL and plasma porphyrin level of 32.7 µg/dL. The patient with XLP (c.1706_1709delAGTG) was also in his 50s, with an ePPIX level of 10 650 µg/dL.

A total of 45 patients with EPP (22.1%) had gallstones, as did 4 male patients with XLP (40.0%) and 4 female patients with XLP (33.3%). The median ePPIX levels for patients with gallstones was 2051.50 µg/dL and 1648 µg/dL for those without gallstones. The mean (SD) age at diagnosis of gallstones was 30.4 (11.3) years for patients with EPP, 43.0 (10.7) years for male patients with XLP, and 24.8 (6.7) for female patients with XLP. Cholecystectomies had been performed for 39 of 45 patients with EPP with gallstones (86.7%) and for all patients with XLP with gallstones. Anemia was reported by 95 patients with EPP (46.6%); among the patients with XLP, anemia was reported in 3 male patients (30.0%) and 9 female patients (75.0%). Of the patients with anemia, use of iron supplements was reported by 73 patients with EPP (76.8%), 2 male patients with XLP (66.7%), and 8 female patients with XLP (88.9%). Seven of 8 female patients with XLP who were taking iron supplementation noted decreased photosensitivity. Vitamin D supplements were used by only 76 patients (33.6%), and only 96 (42.5%) reported immunization against hepatitis B.

Baseline Laboratory Studies

Baseline serum aminotransferase levels were abnormal in 19 of 140 patients with EPP (13.6%), 3 of 8 male patients with XLP (37.5%), and 2 of 9 female patients with XLP (22.2%). Anemia (defined as a hemoglobin level <12.5 g/dL in women and <13.5 g/dL in men [to convert to grams per liter, multiply by 10.0]) was seen in 55 of 147 patients with EPP (37.4%), 3 of 8 male patients with XLP (37.5%), and 4 of 9 female patients with XLP (44.4%).

Genotype/Phenotype Correlations

Erythropoietic Protoporphyria

A total of 186 patients with EPP had a loss-of-function *FECH* mutation in 1 allele and the common low-expression polymorphism IVS3-48T>C in the other. One patient had 2 missense mutations: a previously reported pathogenic mutation P334L and a novel mutation V256L (predicted to be pathogenic); this patient was symptomatic as a teenager. Her plasma porphyrin level was 12.1 µg/dL (normal, <1.0 µg/dL), and she had normal serum aminotransferase levels and no anemia.

The most common *FECH* missense mutation was C411G (28 of 55 [50.9%]), followed by C406Y (7 of 55 [12.7%]). Patients with EPP and *FECH* missense mutations had significantly lower median ePPIX levels than did those with deletions, nonsense, or consensus splice site mutations (1462 µg/dL [IQR, 906-2024 µg/dL] vs 1702 µg/dL [IQR, 1145-2328 µg/dL]; $P = .01$). Median ePPIX levels in patients with EPP (1669 µg/dL [IQR, 1035-2201 µg/dL]) were significantly lower than in male patients with XLP (3574 µg/dL [IQR, 2293-5653 µg/dL]; $P < .001$). Male patients with EPP had significantly higher

Table 3. Erythrocyte Protoporphyrin Level by Mutation Type[a]

FECH Mutation Type in *trans* With IVS3-48T>C	No.	Erythrocyte Protoporphyrin Levels, µg/dL		
		Mean (SD)	Median (Range)	Interquartile Range
Missense	50	1600.0 (1023.9)	1462.5 (137.0-6179.0)	906.0-2024.0
Splice site[b]	51	1893.2 (1054.9)	1718.0 (552.0-4806.0)	1047.0-2399.0
Nonsense	29	1997.9 (987.0)	1697.0 (634.0-4757.0)	1435.0-2608.0
Deletions[c]	44	1863.2 (945.7)	1694.0 (618.0-5017.0)	1129.0-2213.0
Total	174	1818.9 (1009.8)	1678.0 (137.0-6179.0)	1035.0-2178.0

SI conversion factor: to convert protoporphyrin to millimoles per liter, multiply values by 0.0178.

[a] The following were excluded from this table: patients with erythrocyte protoporphyrin testing performed at laboratories other than the University of Texas Medical Branch Porphyria Laboratory or the Mayo Laboratory, patients whose disease-causing mutations were not identified in *FECH*, patients who were homozygous for disease-causing mutations in *FECH*, and patients for whom DNA samples were not available.

[b] Splice site includes anything that is consensus splice site (±1 or 2 bases) and alternate splice site (± more than 2 bases).

[c] Deletions include any small insertion or deletions, large deletions, and complex rearrangements.

Table 4. Mutation and Onset in XLP Male and Female Patients

Age, y	Mutation	Time to First Symptom	Age at Onset, y	Baseline ePPIX, µg/dL	Metal-Free/Zinc PPIX, %/%
Male					
20s	c.1737delG	11-30 min	8	1846	60/40
20s	c.1706_1709delAGTG	<10 min	3	4401	64/36
30s	c.1706_1709delAGTG	11-30 min	1.5	2948	66/34
50s	c.1706_1709delAGTG	11-30 min	4	3711	86/14
50s	c.1706_1709delAGTG	11-30 min	5	7801	89/11
50s	c.1706_1709delAGTG	11-30 min	<1	2293	59/41
50s	c.1706_1709delAGTG	11-30 min	<1	1994	NA
60s	c.1706_1709delAGTG	11-30 min	1	3431	85/15
60s	c.1706_1709delAGTG	11-30 min	1	3437	73/27
60s	c.1706_1709delAGTG	<10 min	<1	10 650	78/22
Female					
20s	Q548X	11-30 min	2	5156	89/11
20s	Q548X	11-30 min	11	7544	93/7
20s	c.1706_1709delAGTG	31-60 min	4	2307	69/31
30s	c.1706_1709delAGTG	31-60 min	<1	2350	70/30
30s	c.1706_1709delAGTG	<10 min	9	7867	NA
30s	c.1706_1709delAGTG	>3 h	20	760	NA
40s	c.1737delG	>3 h	17	201	88/12
40s	c.1706_1709delAGTG	11-30 min	<1	6260	86/14
50s	c.1706_1709delAGTG	<10 min	13	2098	67/33
50s	c.1737delG	No symptoms	NA	379	53/47
50s	c.1706_1709delAGTG	1-3 h	40	1420	69/31
50s	c.1706_1709delAGTG	11-30 min	10	2800	79/21

Abbreviations: ePPIX, erythrocyte protoporphyrin; NA, not available; XLP, X-linked protoporphyria.

SI conversion factor: To convert protoporphyrin to µmol/L, multiply values by 0.0178.

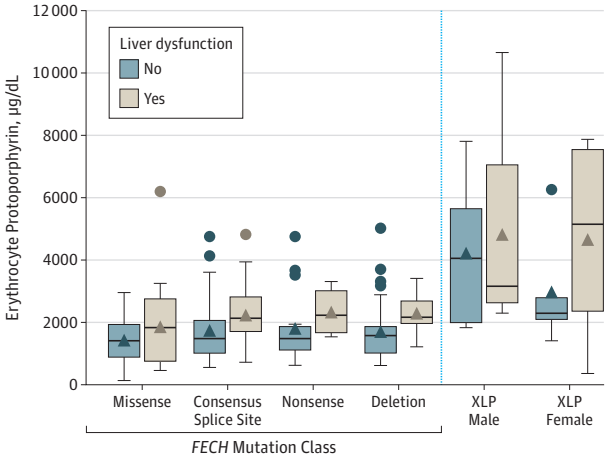
median ePPIX levels than did female patients (1799 µg/dL [IQR, 1035-2201 µg/dL] vs 1489 µg/dL [IQR, 907-2201 µg/dL]; $P = .02$).

Nine patients did not have an identified *FECH* or *ALAS2* mutation but had markedly elevated metal-free ePPIX levels (952-4068 µg/dL). Samples of DNA could not be obtained for 8 patients.

X-linked Protoporphyria

Of the 7 unrelated families, 5 had the common *ALAS2* exon 11 deletion c.1706_1709delAGTG, 1 had the exon 11 c.1737delG deletion, and 1 had the Q548X nonsense mutation. The median ePPIX level was 3574 µg/dL (IQR, 2293-5653 µg/dL) for male patients with XLP and 2328 µg/dL (IQR, 1090-5708 µg/dL) for female patients with XLP. Female patients with XLP had higher variability in clinical symptoms, and their ePPIX levels ranged from 201 to 7867 µg/dL. Patients with the lowest ePPIX levels had no symptoms or had symptoms only after prolonged sun exposure (>3 hours). The proportion of metal-free ePPIX ranged from 53% to 93% in patients with XLP, with 11 patients (50.0%) having metal-free ePPIX levels of 70% or more (**Table 3** and **Table 4**).

Figure. Erythrocyte Protoporphyrin Levels in Patients by Mutation Type and Presence or Absence of Liver Dysfunction



Liver dysfunction is based on patient-reported history of abnormal liver enzymes or abnormal serum aminotransferase levels at enrollment. XLP indicates X-linked protoporphyria. The middle line indicates the median; the triangle indicates the mean; the top box line indicates quartile 3; the bottom box line indicates quartile 1; whiskers represent the inner fence or range when outliers are not indicated; and circles indicate outliers.

Disease Severity

Patients with EPP with more severe phototoxicity (symptoms within 10 minutes of exposure) had significantly higher median ePPIX levels compared with those reporting symptoms after 10 minutes of sun exposure (2233 µg/dL [IQR, 1522-3056 µg/dL] vs 1524 µg/dL [IQR, 1003-2002 µg/dL]; $P < .001$). Patients with EPP with onset of symptoms within the first year of life had significantly higher median ePPIX levels than did those who developed symptoms after 1 year of age (1744 µg/dL [IQR, 1132-2778 µg/dL] vs 1567 µg/dL [IQR, 950-2018 µg/dL]; $P = .02$). Liver involvement (reported as medical history or determined by abnormal serum aminotransferase levels at baseline visit) was seen in 66 of 200 patients with EPP (33.0%) and 4 male patients with XLP (40.0%). In patients with EPP, those with liver dysfunction had significantly higher median ePPIX levels compared with those with normal liver function (2016 µg/dL [IQR, 1277-2747 µg/dL] vs 1510 µg/dL [IQR, 971-1953 µg/dL]; $P = .003$).

Discussion

To our knowledge, this study provides the first detailed clinical findings, ePPIX levels, and *FECH* and *ALAS2* mutation analysis of patients with EPP and XLP in the North American population. This is the largest reported cohort of clinically, biochemically, and genetically characterized cases of EPP and XLP, which provides novel insights into important clinical features of these disorders, such as the variability in the age at onset of symptoms, and the association of severity of disease with ePPIX levels. It highlights the clinical variability of this disorder, with 22.5% of patients with EPP experiencing phototoxic reactions to sun or light within a few minutes, while 3.4% could tolerate more than 3 hours of sun exposure (Table 2). Variability is likely associated not only with levels of ePPIX but also with innate differences in skin complexion.

Consistent with previous reports, the median age of presentation was 4.0 years in our EPP cohort and 3.4 years for male patients with XLP.[5,19] Although patients are symptomatic in early childhood, there was a lag in diagnosis, with many patients remaining undiagnosed or misdiagnosed for several years or even decades. This study highlights the need for increased awareness of this disorder among pediatric health care professionals.

The incidence of gallstones was higher in our cohort than previously reported.[5,19] It is postulated that an increased concentration of PPIX in bile predisposes to crystallization and formation of pigmented gallstones.[22] Gallstones can present at an earlier age in these patients; in our cohort, the mean ages at presentation ranged from 24 to 43 years. The higher number of patients with biliary stones in this series may be associated in part with more frequent imaging for stones and protoporphyric liver disease than in the past, but data that could be used to assess this possible effect of ascertainment are not available. Our data suggest that patients with XLP also have an increased risk of gallstones, which is likely associated with their higher ePPIX levels.

Anemia was reported by 95 of 204 patients with EPP (46.6%) and 12 patients with XLP (54.5%), which are higher rates than reported previously.[5,19] Of those with anemia, 73 of 95 patients with EPP (76.8%) and 10 of 12 patients with XLP (83.3%) were prescribed iron supplementation. Seven of 8 female patients with XLP who were receiving iron supplementation reported an improvement in photosensitivity symptoms. Although this was a small subset of patients, this finding supports the use of iron supplementation for these patients. Serum aminotransferase levels were abnormal at baseline or as reported by medical history in a subset of patients, consistent with previous reports. Male patients with XLP have significantly higher ePPIX levels than do patients with EPP, on average, and have an increased risk for liver dysfunction (**Figure**). The level of use of vitamin D supplementation and hepatitis B vaccination was low in our cohort, suggesting that greater attention to such guidelines for management is needed.

Patients with XLP accounted for about 10% of patients with the protoporphyria phenotype in our cohort, which is significantly higher than that reported in Europe.[11] Metal-free ePPIX levels varied significantly among patients with XLP, ranging from 53% to 93% of the total, overlapping the range for patients with EPP. Therefore, genetic testing for *FECH* and *ALAS2* mutations is essential for confirmation of the diagnosis of EPP and XLP. In contrast to male patients with XLP who reported early-onset symptoms, the median age of symptom onset for female patients with XLP was 11 years, with an age range from 2 to 40 years. Among the female patients with XLP, clinical features ranged from symptoms developing within 10 minutes of sun exposure to symptoms developing after prolonged sun exposure or no symptoms. Female patients with XLP with lower ePPIX levels had greater sun tolerance. This clinical

variability presumably is associated with random X-chromosomal inactivation in female patients with XLP, as previously reported.[23]

Patients with missense mutations in the *FECH* gene had lower ePPIX levels than did patients with more severe mutations, including nonsense, consensus splice site, and deletions, which usually obliterate the activity of the encoded enzyme subunit. These findings reinforce the benefit of genotyping patients in addition to performing biochemical testing and suggest that patients with missense mutations may have a somewhat milder disease course.

The primary method of symptom prevention was protection, mainly by use of sun-protective clothing or remaining indoors and avoiding exposure to the sun or light. Sunscreens and beta carotene were reported as minimally or not effective. Other therapies have been reviewed and were found to be ineffective.[24] Recently, high-dose cimetidine was reported to benefit children with EPP,[25] but there is no clear clinical or mechanistic evidence supporting this therapy.[26] Overall, patients lack effective options for preventing phototoxic symptoms or for pain relief, with almost all patients reporting phototoxic episodes despite various sun-protective measures. In addition, patients experiencing phototoxic episodes required several days to recover, often necessitating time lost from work, school, and productivity. Recent clinical trials of afamelanotide, an α-melanocyte-stimulating hormone analogue, reported increased photoprotection and a significant increase in pain-free sun exposure compared with placebo, as well as improved quality of life for patients with EPP.[27] These findings were confirmed in a long-term extension study that showed clinical effectiveness and safety over an 8-year follow-up period.[28] Afamelanotide was approved for use in the European Union by the European Medicines Agency in December 2014 and is currently being evaluated by the US Food and Drug Administration. There are no therapeutics currently available or in clinical trials designed to increase *FECH* expression, decrease *ALAS2* expression, and/or decrease ePPIX production or accumulation. This goal has been accomplished by hematopoietic stem cell transplant in a few patients, usually following liver transplantation for liver failure[16,29-31]; however, this approach has significant morbidity, mortality, and risk of recurrence.[32] Future therapeutic approaches should include other strategies to increase *FECH* activity and reduce *ALAS2* overexpression.

Limitations

Limitations of this study include incomplete data sets, as results of all laboratory studies and responses to all questions were not available for all patients. In addition, DNA was unavailable for a small number of patients.

Conclusions

Our study documents that increased ePPIX levels are a significant determinant of disease severity in a large cohort of patients with protoporphyria. Elevated ePPIX levels correlated with earlier age at onset, decreased sun tolerance, and increased risk of liver dysfunction among patients with EPP or XLP (Figure). Patients with higher ePPIX levels (>2000 µg/dL) should be monitored more closely for evidence of liver disease. Long-term follow-up studies are needed to establish baseline levels of ePPIX to evaluate how they are associated with the development of hepatopathy. This information will be useful to counsel family members about disease severity and risk of liver disease.

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