

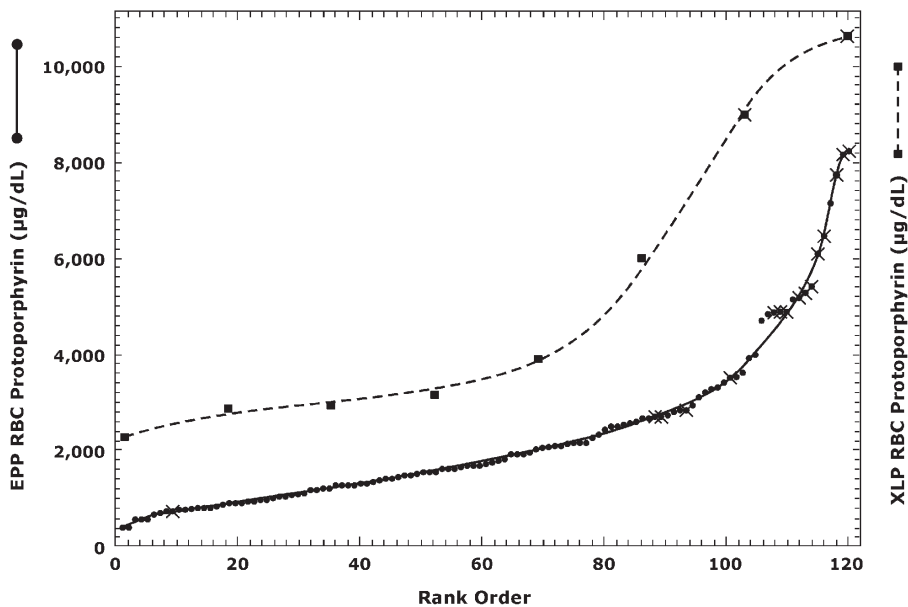
Supplemental Data

Loss-of-Function Ferrochelatase and Gain-of-Function Erythroid-Specific 5-Aminolevulinate Synthase Mutations Causing Erythropoietic Protoporphyrinemia and X-Linked Protoporphyrinemia in North American Patients Reveal Novel Mutations and a High Prevalence of X-Linked Protoporphyrinemia

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Supplementary Figure S1. Distribution of erythrocyte protoporphyrin levels in EPP patients and XLP males. The erythrocyte protoporphyrin levels for 120 EPP patients (closed circles) were plotted left to right in order of increasing concentration. The erythrocyte protoporphyrin levels for 8 XLP males (closed squares), are in order of increasing concentration. The X's mark values for patients who had liver disease. The normal level of erythrocyte protoporphyrin is 20 to 80 µg/dL.

FREQUENCY OF FECH AND ALAS2 MUTATIONS IN EPP

Supplementary Table S1. Oligonucleotide primers for amplification and sequencing of FECH and ALAS2.

| Gene | Exon | Primer ^a | Sequence | Size of PCR Product | Genomic Location ^b |
|-------|-------------|---------------------------|------------------------------|----------------------------------|----------------------------------|
| FECH | Promoter +1 | 1F | AACAGGATCTGCACACTTGTGTTC | 605 | c.1-478 to -455 109 to 127 |
| | | 1R | CTCTGCCACCGCCTTCTCA | | |
| | 2 | 2F | CTGCCTGCAGAGAAATGCTAGG | 390 | 6279 to 6300 6645 to 6668 |
| | | 2R | AGAAAATAGCTCCTTCCACACTGG | | |
| | 3 | 3F | ACGGCAGTAAAAAGAGCCATAG | 370 | 13125 to 13146 13470 to 13494 |
| | | 3R | GCTGTTTAAACCATTAACCAGATACGC | | |
| | 4 | 4F | CCAGGTTTCTCTGCATGGGTG | 553 | 14797 to 14817 15327 to 15350 |
| | | 4R | GAAACACCACAAATTGAGTTGCC | | |
| | 5 | 5F | ACCGTCAGTGCCATAGGAAATTAC | 412 | 19915 to 19938 20304 to 20326 |
| | | 5R | ATGGACTGACCTGAACTCTCGTG | | |
| | 6 | 6F | CATTTGCAGGGTAGTTAAATCTATATGC | 391 | 23464 to 23491 23833 to 23854 |
| | | 6R | GCAAGGGAACAGTAAGGCTCAG | | |
| | 7 | 7F | CCCAGCAAATTCCCCTAGGCC | 414 | 27158 to 27177 27550 to 27571 |
| | | 7R | GGACATAATGGAAGCTGGACCC | | |
| | 8 | 8F | GGAGGGCTTTGTTCCAGGG | 341 | 31566 to 31584 31887 to 31906 |
| | | 8R | TGCCCATGGTGAGCAATCAG | | |
| | 9 | 9F | CAGGTGTAAAGAGTGGCTCTTGC | 487 | 32076 to 32098 32544 to 32562 |
| | | 9R | AGGTTTCCCCCTCCCCTGC | | |
| 10 | 10F | CAGTCTGCGAACAGTTGAAGTCAG | 369 | 35107 to 35130 35450 to 35475 | |
| | 10R | GCTATCTGAAGGAAAAAGACTGAGC | | | |
| 11 | 11F | AAGCCAGAGCGCTGACCTG | 588 | 35678 to 35696 36244 to 36265 | |
| | 11R | GGGACTCTCCGTACCCTTTTCAG | | | |
| ALAS2 | 11 | 11F | GGGGATCAATATCTTGGCTC | 477 | 16565 to 16585 17018 to 17041 |
| | | 11R | CCAACAAGTGACCTATGGTTACCT | | |

^a 'F' denotes Forward primer; 'R' denotes Reverse primer.

^b For genomic locations, the A of the initiation ATG was designated as base 1

Supplementary Table S2. Amino acid sequence conservation of novel FECH missense mutations in 49 species.

| Organism ^a | Number of Species | Amino Acids Involved in Missense Mutations | | | | | | |
|-----------------------|-------------------|--|------|------|------|------|------|------|
| | | T116 | S151 | I206 | R215 | M219 | L265 | C411 |
| Conservation (%) | | | | | | | | |
| Bacteria to Fungi | 23 | 30 | 9 | 4 | 0 | 0 | 70 | 17 |
| Insects to Humans | 26 | 100 | 96 | 100 | 27 | 81 | 100 | 100 |

^a The FECH sequences aligned for conservation analysis using Clustal W are listed by genus and species, followed by the GenBank accession number in parentheses.

Bacteria: *Anaplasma marginale* str. St. Maries (YP_154087), *Bradyrhizobium* sp. BTAi1 (YP_001237498), *Cyanothece* sp. PCC 7425 (YP_002485188), *Cyanothece* sp. PCC 8802 (YP_003137494), *Ehrlichia chaffeensis* str. Arkansas (YP_507215), *Geobacter bemidjiensis* Bem (YP_002136866), *Geobacter lovleyi* SZ (YP_001953157), *Geobacter sulfurreducens* PCA (NP_954352), *Helicobacter pylori* J99 (NP_223722), *Microcystis aeruginosa* NIES-843 (YP_001655979), *Parvibaculum lavamentivorans* DS-1 (YP_001412535), *Prochlorococcus marinus* str. MIT 9312 (YP_397022), *Rickettsia conorii* str. Malish 7 (NP_361010), *Rickettsia rickettsii* 'Sheila Smith' (YP_001495448), *Shewanella baltica* OS185 (YP_001367230), *Synechococcus* sp. WH 8102 (NP_897838).

Protozoan: *Tetrahymena thermophila* (XP_001017797).

Fungi: *Ashbya gossypii* ATCC 10895 (NP_986938), *Kluyveromyces lactis* NRRL Y-1140 (XP_455984), *Magnaporthe oryzae* 70-15 (XP_363587), *Neurospora crassa* OR74A (XP_962504), *Saccharomyces cerevisiae* S288c (NP_014819), *Schizosaccharomyces pombe* 972h- (NP_587720).
Insects: *Acyrtosiphon pisum* (NP_001232972), *Anopheles gambiae* str. PEST (XP_310249), *Drosophila ananassae* (XP_001952891), *Drosophila melanogaster* (NP_524613).

Animals: *Alluopoda melanoleuca* (XP_002920306), *Anolis carolinensis* (XP_003223698), *Bos taurus* (NP_776479), *Branchiostoma floridae* (XP_002591270), *Canis lupus* (XP_852936), *Cavia porcellus* (XP_003474213), *Cricetulus griseus* (XP_003499503), *Danio rerio* (NP_571706), *Equus caballus* (XP_001488965), *Gallus gallus* (NP_989527), *Homo sapiens* (NP_000131), *Hydra magnipapillata* (XP_002159643), *Monodelphis domestica* (XP_001374172), *Mus musculus* (NP_032024), *Oreochromis niloticus* (XP_003440239), *Ornithorhynchus anatinus* (XP_001510557), *Oryctolagus cuniculus* (XP_002713693), *Pan troglodytes* (NP_001033101), *Pongo abelii* (NP_001126366), *Rattus norvegicus* (NP_001101904), *Sus scrofa* (NP_001163994), *Xenopus laevis* (NP_001081718).