L237P Substitution in the UROS Gene Causes X-linked Recessive Congenital Erythropoietic Porphyria in Pakistani Consanguineous Family Through Altered Uroporphyrinogen III Binding

Roshana Mukhtar 1, Shaheen Shahzad 1,*, Sajid Rashid 2, Maryam Rozi 2, Madiha Rasheed 3, Imran Afzal 4 and Pakeeza Arzoo Shaiq 5
1 Genomics Research Lab, Department of Biological Sciences, International Islamic University, Islamabad
2 National Centre of Bioinformatics, Quaid-i-Azam University, Islamabad
3 Beijing Key Laboratory for Separation and Analysis in Biomedicine and Pharmaceuticals, School of Life Sciences, Beijing Institute of Technology, Beijing, China.
4 Department of Biology, Lahore Garrison University, Lahore
5 University Institute of Biotechnology and Biochemistry, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi

ABSTRACT

Congenital erythropoietic porphyria (CEP) is an inherited heterogeneous metabolic disorder caused by an abnormal activity of uroporphyrinogen-III synthase (UROS), aminolevulinic acid and GATA1 (GATA binding factor 1) genes. The fundamental genetic cause of CEP has been linked to a sequence variant of UROS on chromosome 10q25.2-q26.3. The enzyme catalyzes the fourth step of heme synthesis pathway. The present study focused on the clinical assessment of CEP affected individuals in a Pakistani consanguineous family by Sanger sequencing of UROS gene to identify potential pathogenic sequence variants. CEP patients were identified using successive clinical tests. Blood samples of patients were collected and processed for genomic DNA extraction followed by Sanger sequencing to identify pathogenic mutations in UROS gene. Sequence analysis revealed a pathogenic missense mutation (c.935T>C [p. L237P]) in exon 10. The sequence was further analysed in-silico to determine the effect of pathogenic mutation on protein structure. In-silico analysis and comparison between UROS WT and UROS*18 3-dimensional structures revealed remarkable changes in the binding site of Urogen (3-[7, 12, 18-tris (2-carboxyethyl)-3, 8, 13, 17-tetraakis (carboxymethyl)5, 10, 15, 21, 22, 23, 24-octahydroporphyrin-2-y]) propanoic acid) due to narrowing of domain-I and domain-II (18.46-12.17Å) of UROS L237P as compared to UROS WT. This suggests that UROS L237P mutation may influence heme biosynthesis mechanism through altered Urogen binding mechanism. Therefore, we propose that the newly identified pathogenic missense variant (c.935T>C [p.L237P]) p.Gly439Ser) of the UROS gene causes CEP in a large consanguineous Pakistani family, possibly by hindering heme biosynthesis mechanism through altered Urogen binding mechanism.

INTRODUCTION

Congenital erythropoietic porphyria (CEP) is an inherited heterogeneous metabolic disorder that occurs due to malfunctioning of UROS enzyme causing excessive accumulation and excretion of porphyrins and their toxic precursors (Szlenk et al., 2016). It is a rare genetic disorder inherited either as autosomal recessive or X-linked trait due to mutations in UROS and GATA 1 genes (Di Pierro et al., 2016).

The main symptoms of CEP include harsh cutaneous photo-sensitivity with damage and sub-epidermal blistering (Poh-Fitzpatrick, 1998), scarring, erythrodermia (Darwich et al., 2011), mild bone loss and expansion of the bone marrow (Berry et al., 2005), eye sight problem (Agarwal et al., 2015), pink to dark red staining of the urine.

Abbreviations

CEP, congenital erythropoietic porphyria; ALAS, aminolevulinic acid synthase; HMB, hydroxy methyl bilane; HGMD, human gene mutation database; PCR, polymerase chain reaction; SIFT, sorting intolerant from tolerant; PolyPhen-2, polymorphism phenotyping v2; PDB, protein data bank.
(Szlenak et al., 2016), thrombocytopenia (Ged et al., 2004), and disturbance in accumulated porphyrin, leading to the development of cutaneous reactions (Thunell, 2000). Besides this, there is rapid development of cutaneous vesicles with increased skin fragility and bullae on the face and hands (Baran et al., 2013) with variable hematological symptoms, which includes asymptomatic microcytic anemia, hemolytic anemia and pancytopenia (Egan et al., 2015). The disease may be hepatic, erythropoietic, organ system, cutaneous and neuropsychiatric, depending upon the biochemical problems at different stages of heme biosynthesis pathway (Scarlett and Brenner, 1998).

There are various enzymes that catalyze different steps of heme synthesis. Aminolevulinic acid synthase (ALAS) is the enzyme which catalyzes the first rate limiting step, whereas UROS enzyme in cytosol is involved in the fourth step of heme pathway (Ponka, 1997). UROS enzyme helps in the formation of uroporphyrinogen III by rearranging and cyclising the linear HMB (hydroxy methyl bilane). URO I and COPRO I are toxic isomers, which are abnormally produced due to defect in the enzyme (Di Pierro et al., 2016).

CEP is a genetically heterogenic state that occurs due to mutations in different genes. Acquired GATA1 and UROS gene mutations have been reported in the CEP patients (Sarkany et al., 2011). About 95% cases of CEP occur due to genetic mutation in the UROS gene and are responsible for the disease manifestations (Di Pierro et al., 2016). UROS III was identified in 1998, being approximately 34 kb long and having 10 exons, out of which 1 and 2A are untranslated (non-coding) exons. The remaining 9 exons (2B to 10) are translated into a protein (Aizencang et al., 2000). UROS gene encodes a protein of 265 amino acids and is chromosomally located at the position “10q25–q263” (Astrin et al., 1991). It is a monomeric enzyme with a molecular weight of 29 kDa, purified and characterized from human erythrocyte (Tsai et al., 1987). The protein structure shows two domains and each domain contains a parallel beta-sheet surrounded by alpha-helices, linked with each other by a two-strand antiparallel beta-ladder (Mathews et al., 2001). Nuclear Magnetic Resonance (NMR) has revealed the interaction of the enzyme with a ligand through chemical shift perturbation. The active location was mapped in the cleft section between structural domains 1 and 2 where conserved residues were clustered (Cunha et al., 2008). Approximately 49 mutations have been reported in UROS gene responsible for CEP, based on literature and “Human Gene Mutation Database (HGMD)”, (http://www.hgmd.cf.ac.uk/ac/index.php). Most of these mutations are dispersed all over the coding regions of the UROS gene and some are in the promoter region (Solis et al., 2001).

Point mutations have been reported mostly in UROS gene. Deletions and insertions cause the rearrangements in genes and only 6 mutations of this category have been reported, with 56% of UROS mutations being missense. Other reported mutation types include one non-sense mutation, five mutations linked to splicing defects, six in regulatory region of the gene, four belonging to the group of deletions, four to insertions groups, and two from indels group (ben Bdira et al., 2014).

This study focused on the clinical assessment of individuals affected with CEP in a Pakistani consanguineous family to determine the possible involvement of UROS gene mutation in the CEP manifestation. The UROS gene sequence was obtained and analyzed in silico to compare the mutant UROS<sup>L237P</sup> structural abnormalities to the wild type UROS<sup>WT</sup> gene structure in order to identify the possible role of the mutation in CEP manifestation.

**MATERIALS AND METHODS**

**Family recruitment and ethical approval**

The study was approved by Ethical Review Committee of the International Islamic University, Islamabad, Pakistan. A written informed consent was provided by all the participants and legal guardians. The consanguineous family presently studied is from Punjab province of Pakistan (Fig. 1). The family pedigree of this large family indicated an X-linked recessive CEP. Affected individuals were examined by a physician for the disease phenotype and detailed clinical history was established (unpublished data). The clinical symptoms are summarized below.

1. Skin abnormalities (harsh cutaneous photosensitivity with blistering, scarring)
2. Erythrodontia (reddish discoloration of the teeth)
3. Defect in the heme synthesis within the red blood cells of bone marrow (which leads to anemia and reddish colour urine)
4. Hypertrichosis (excessive hair growth on face and hands)
5. Liver and spleen problems
6. Hands mutilating deformities of fingers with no finger prints

After clinical examination, the phenotype of patients clearly indicated CEP with typical cutaneous lesions and hypertrichosis.

**Clinical tests**

Clinical tests were performed to confirm the type of porphyria. Wood’s lamp test was performed to confirm the CEP as the test involves the UV light and it is a diagnostic marker test for the CEP (Bhavasar et al., 2011).
Fig. 1. (A) Pedigree of the 5-generation consanguineous Pakistani family, showing features of X-linked recessive Congenital Erythropoietic Porphyria. Consanguineous unions are shown through double lines. Males and females are represented using squares and circles respectively. The affected and unaffected family members are shown using filled and clear symbols respectively, while a deceased individual is represented through a diagonal line. (B) Urine under wood lamp test. (C) Teeth colour under wood lamp test. (D-H) the clinical appearance of the affected persons (D) Erythrodondia, (E) Hypertrichosis, (F) Blisters formation on skin exposed to sunlight, (G) Blister healing with scarring, (H) Hands mutilating deformities of fingers with no finger prints.

DNA extraction and amplification

Genomic DNA was extracted from peripheral leucocytes of the affected (IV-1, IV-8, V-2) and unaffected (III-4, IV-4, IV-6, VI) family members using organic DNA extraction protocol (Wood, 1983). DNA quantification was performed using Nanodrop (AUVS. 102/avans). DNA concentration was adjusted to 40-50 ng/L for its amplification. For PCR amplification, primers were designed for UROS gene containing 10 exons by using primer3 software and gene was amplified using Thermal Cycler (BioRad-T100) using standard PCR conditions (Rozen and Skaletsky, 2000).

The UROS gene on chromosome 10q252–q263 (NM_001324036) was sequenced for all the available affected and normal persons of the family. DNA purification was performed with a commercially kit (Axygen Inc., CA, USA) and sequencing was also done commercially using the BigDye Terminator v3.1 Cycle Sequencing Kit, together with an ABI Prism 310 Genetic Analyzer (Applera, Foster City, CA, USA). Mutation was identified using BioEdit sequence alignment editor version 6.0.7.

Bioinformatics analysis

SIFT and polyphen scoring

Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping v2 (PolyPhen-2), were used to characterize missense variation and examine the possible effect of amino acid substitutions on the stability and function of proteins.

Data set

The crystal structure of human UROSWT (PDB ID: 1JR2) was retrieved using protein data bank (PDB) (Berman et al., 2000). The energy minimization procedure was performed through UCSF Chimera 1.5.6 (Pettersen et al., 2004) by means of conjugate gradient method and Amber force field. 3-dimensional structure of UROS127P was predicted by Modeller 9.14 (Sali et al., 1995) using 1JR2 structure as template. The predicted 3-dimensional structure was confirmed by MolProbity (Darwich et al., 2011) analysis, followed by structure optimization through WinCoot (Emsley et al., 2010). 2D structure of UROGEN (PubChem ID: 1179) was retrieved through PubChem database (Hanwell et al., 2012) and converted into PDB format through UCSF Chimera. Avogadro tool was utilized to obtain proper protonation
and stereo-isomerization state of UROGEN using GAFF force field (Kim et al., 2015).

**Molecular docking analysis**

PatchDock was used to perform molecular docking analysis of UROS<sup>WT</sup> and UROS<sup>L237P</sup> structures against UROGEN (Schneidman-Duhovny et al., 2005) and FireDock (Andrusier et al., 2007) servers. PatchDock docking was done in three stages: First, the detection of geometric patches through segmentation method was done; second, surface matching and filtering was performed; and in the final stage, scoring was done. In the process of docking, hundreds of binding poses are produced and best docked structure is considered with minimum energy pose (Huang and Zou, 2010). Through UCSF Chimera ver 1.5.6 and LigPlus the comprehensive interactions were characterized (Laskowski and Swindells, 2011).

**RESULTS**

**Clinical profile**

The result of Wood’s lamp test revealed that the urine sample of the affected members of the family under UV light had red color due to the presence of excess porphyrin compounds. The teeth color appeared pink to red due to the accumulation of porphyrin compounds in teeth tissues during teeth development (Fig. 1).

The characteristic symptoms in the affected members of the family included: Skin abnormalities (harsh cutaneous photo-sensitivity with blistering, scarring), Erythrodontia (reddish discoloration of the teeth), heme synthesis defect within the red blood cells of bone marrow (leading to anemia and reddish colour urine), Hypertrichosis (hair growth increases on face and hands), and hands mutilating deformities of fingers with no fingerprints. The symptoms were present in all affected individuals by birth, but the severity of symptoms was variable among affected patients and become more severe due to sun exposure, which is supposed to be because of reduced enzymatic activity of UROS. One of the members, the oldest among the affected ones, also had liver and spleen problem.

**Mutation screening**

Sanger sequencing of the UROS gene carried out using DNA samples from all available family members (Fig. 1), revealed a reported missense mutation c.935T>C (p. Leu237Pro). The mutation segregated in the family with the disease phenotype (Fig. 2a-2c). Affected individuals were homozygous for the altered allele (CC), the parents were heterozygous carriers (CT), and the unaffected children were either heterozygous (CT) or homozygous for the wild-type allele (TT) (Fig. 2a-2c). The disease-causing mutation (c.935T>C) resides in exon 10 of the UROS gene (NM_001324036) which causes Leucine to Proline substitution at position 237 (p. Leu237Pro) within the amino acid sequence of the translated UROS protein. This mutation occurs in the coding region, predicted to be damaging according to PolyPhen2 (http://genetics.bwh.harvard.edu/pph), SIFT/PROVEAN (http://sift.jcvi.org). After mutation screening, SIFT and Polyphen test was performed to check the type of mutation and also predict the effect of mutation on the structure and function of the protein. The mutation is highly deleterious and has effect on protein structure and function as the scores lies in the damaging region and predicted to have negative effect on the protein function, as predicted through SIFT and Polyphen scores (Tables I and II).

![Fig. 2. Sequence analysis of the UROS gene revealing the mutation c.935T>C (p.Leu237Pro). (A) shows the nucleotide sequence of an affected individual, (B) a heterozygous carrier, (C) a homozygous normal individual. The arrow represents the missense mutation.](image)

| Table I. SIFT analysis. |
|-------------------------|
| ENSP | Position | Reference | Substitution | Prediction | SIFT score | Median information content |
|------|----------|-----------|--------------|------------|------------|---------------------------|
| ENSP 0000035787 | 237 | L | P | damaging | 0.01 | 1.51 |

| Table II. Polyphen analysis. |
|-----------------------------|
| Mutation | Hum div score | Hum var score |
| L237P | 0.999 Damaging | 0.995 Damaging |

**Bioinformatics analysis**

3-dimensional structure superimposition for UROS<sup>WT</sup> and UROS<sup>L237P</sup> structures showed similar folds adapted by both structures through sharing a well-conserved helix-loop-helix structure, oriented by the association...
Substitution in UROS Gene Leads to CEP

Fig. 3. Structure analysis of UROSWT and UROS237P. (A) Superimposition of UROSWT and UROSL237P structures. UROSWT is indicated in purple color, while UROS237P is indicated in grey color. (B) Topview representation of UROSWT-Urogen complex in grey color. (C) UROS237P-Urogen complex in grey color. Urogen is shown in red colored stick representation. Leu237 and Pro237 residues are indicated by green and blue spheres. (D) Structural comparison. (D) UROSWT is indicated in purple color, (E) UROSL237P model is indicated in grey color. Leu237 and Pro237 residues are indicated by green and blue spheres. Dotted lines indicate distance between adjacent residues. Bond length is indicated in Å. (F) Binding mode analysis of UROSWT and UROSL237P. (G) 2D structure of Urogen (3-[7, 12, 18-tris (2-carboxyethyl)-3, 8, 13, 17 tetrakis (carboxymethyl-yl) propanoic acid]. (H) UROSWT (purple) binding with Urogen (red). (C) UROS L237P (grey) binding with Urogen (red). The interacting residues are labelled in black color with wire representation.

of α-helices at the outer periphery, while the inner core mainly consisted of β-sheets (Fig. 3a). Overall, UROSWT and UROSL237P revealed 12 α-helices and 10 β-strands. Due to a high structural homology between UROSWT and UROSL237P models, the inner cores were measured between two globular regions.

An RMSD value of 0.968 Å indicated significant change in the helical conformation at structural level. The change in helical conformation of UROS237P involving residues LEU6, ASN77, ALA93, ILE110, SER137 and LEU259 led to the elongation of loop region in UROSWT (Fig. 3b and 3c). In comparison to UROSWT-Urogen, the prominent structural differences were observed at the Urogen binding site of UROSL237P-Urogen. The structure comparison exhibited notable changes in the bond length of UROSL237P as compared with UROSWT. In UROSWT, the bond length between C13 and C110 was 18.46 Å, whereas in UROSL237P, bond length was reduced to 12.17 Å (Fig. 3d and 3f).

UROSWT and UROSL237P structures in complex with Urogen were assessed based on the values of their binding energy. PatchDock specific energy value for UROSWT and Urogen complex was -74.56 kcal/mol, while for UROSL237P–Urogen complex, binding energy value was -70.86 kcal/mol. In case of UROSWT-Urogen complex, THR62, SER63, PRO64, ARG65, GLY100, GLY120, ASN121, ALA122, ARG148, GLU149, ILE150, TYR168, THR170 and VAL99 residues were involved in hydrophobic bonding with Urogen (Fig. 3g and 3h). In UROSL237P-Urogen complex, ASP8, ALA9, ILE20, ALA30, THR31, LEU32, PRO34, ASN179, SER182, TYR183, GLN186 and GLN187 residues of UROSL237P were involved in hydrophobic interactions with Urogen (Fig. 3g-3h).

DISCUSSION

The present study was conducted to identify the UROS gene mutation as a causative agent in the inherited CEP and to predict the mutated structure of
the gene with docking analysis. Here, we describe a large consanguineous Pakistani kindred affected with rare CEP caused by a known but pathogenic missense mutation (c.935T>C [p.Leu237Pro]) in the UROS gene that encodes uroporphyrinogen III synthase. This was previously reported as a missense variant (Moghbeli et al., 2012). Presently we report the pathogenic disease-causing mutation of the UROS gene for the first time in the Pakistani family and its association with X-linked recessive CEP. All the affected persons were homozygous for the missense mutation in L237P and their consanguineous parents were shown to be heterozygous for the same mutation.

In CEP, a broad variation has been described regarding the extent and severity of clinical manifestation. The symptoms reported in earlier studies were cutaneous photo-sensitivity, porphyrin overload leading to metabolic disturbance; Erythrodontia and dentine disorders; sub-epidermal blistering; hyperorthokeratosis; sclerosis in the skin layer dermis; disappearance of sweat glands; more fragile and blisters on skin of hands and face upon sun exposure; hypo and hyper-pigmentation on skin, abnormal growth of hairs on hands, face and extremities, etc.; scarring alopecia; significant mutilations; contraction and shortening of the digits and limb and facial disfigurement, i.e., loss of facial features; vitamin D deficiency; shortening of stature and backbone problems; abnormal enlargement of spleen; disturbed liver function; and common mild to severe anemia among the patients (Poh-Fitzpatrick, 1986; Freesemann et al., 1997; Berry et al., 2005; Arunachalam et al., 2013; Baran et al., 2013; Verma et al., 2014; Szlendak et al., 2016). One unique feature that affected individuals of the studied family had no finger prints which may be due to ectopic eczema. The finger prints were not missing by birth. As the affected person grows up and the severity of the disease increases due to regular exposure to sun light, more blistering, scarring and sclerosis problems starts appearing on their finger’s tips.

Several studies have reported molecular heterogeneity in CEP in which UROS gene mutations were identified, including missense, nonsense, frameshift and splice-site mutations (Deybach et al., 1990; Boulechfar et al., 1992; Warner et al., 1992; Tanigawa et al., 1995; Xu et al., 1995; Tezcan et al., 1998; Solis et al., 2001; Shady et al., 2002). The leucin residue at the 237 position is conserved in mouse, rat, zebra fish, and some bacteria. Other amino acids encountered at this position are isoleucin (e.g. in Xenopus laevis or Gallus gallus), glutamine (Drosophila melanogaster) and threonine (Schizosaccharomyces pombe), which are all large and uncharged amino acids. By contrast, the mutation replacing leucine residue with proline (L237P) resulted in secondary amino acid (Yisgedu et al., 2010). The resulting circular structure greatly reduced the structural flexibility and, thus, a leucin to proline substitution at this site may result in an important structural alteration with subsequent disturbance of the encoded protein (Wiederholt et al., 2006). The protein structure of the UROS comprises of two domains that exhibit similarity at sequence level. To check the effect of mutation on protein structure and function, SIFT and Polyphen tools were used, which revealed damaging effect of mutation. UROS protein structural studies demonstrated that Leucine residue at 237 position might have a role as a hydrogen bond donor and acceptor in the UROS protein active site (Mathews et al., 2001). The structural changes at the active site might result in the modulation of enzyme activity (Schubert et al., 2008).

Interestingly, in UROS<sup>237P</sup>-Urogen (uroporphyrinogen III) binding was completely shifted as compared to UROS<sup>WT</sup>. In UROS<sup>WT</sup>-Urogen complex, active site THR62, SER63, PRO64, ARG65, GLY100, GLY120, ASN121, ALA122, ARG148, GLU149, ILE150, TYR168, THR170 and VAL99 residues lying at the cleft between domain-I and II (Mathews et al., 2001) were involved in Urogen binding. In contrast, UROS<sup>237P</sup>-Urogen complex exhibited the involvement of ASP8, ALA9, ILE20, ALA30, THR31, LEU32, ARG34, ASN179, SER182, TYR183, GLN186 and GLN187 residues of UROS<sup>237P</sup> in the interaction. Predicted Urogen binding site for human UROS<sup>WT</sup> is highly similar to the experimentally mapped site of Urogen and T. thermophilus UROS (Schubert et al., 2008). These data clearly demonstrate that L237P point mutation in UROS may influence heme biosynthesis mechanism through altered Urogen binding.

**CONCLUSION**

We have identified a pathogenic missense variant (c.935T>C [p.L237Pro]) of the UROS gene as a causative agent for CEP in a large consanguineous Pakistani kindred. L237P induced a binding shift of Uroporphyrinogen III due to narrowing of domain-I and domain-II (18.46-12.17Å) of UROS<sup>237P</sup> as compared to UROS<sup>WT</sup>. For disease management, mutation analysis of various genes causing CEP is important. Although, presently no specific therapy is available for the treatment of CEP, different options for the treatment of CEP are in progress. Taken together, our findings are expected to strengthen the role of UROS mutation as a cause of CEP, and provide further facts for the lack of genotype-phenotype correlation and clinical variability in patients with UROS mutation and CEP.
ACKNOWLEDGEMENTS

The Authors acknowledge Dr. Nigar Kunwal, Associate Physician Dermatologist facilitated in clinical diagnosis of the disease. Muhammad Mobeen Zafar and Ms. Samra Batool, Research Scholars provided guidance during lab experiments.

Statement of all funding sources for this work
None

Statement of conflict of interest
The authors have no conflict of interest to declare.

REFERENCES

Agarwal, S., Majumder, P.D., Srinivasan, B. and Iyer, G., 2015. Scleral necrosis in congenital erythropoietic porphyria: A case report and review of the literature. Oman J. Ophthalmol., 8: 200. https://doi.org/10.4103/0974-620X.169904

Aizencang, G., Solis, C., Bishop, D.F., Warner, C. and Desnick, R.J., 2000. Human uroporphyrinogen-III synthase: genomic organization, alternative promoters, and erythroid-specific expression. Genomics, 70: 223-231. https://doi.org/10.1006/geno.2000.6373

Andrusier, N., Nussinov, R. and Wolfson, H.J., 2007. FireDock: fast interaction refinement in molecular docking. Proteins, 69: 139-159. https://doi.org/10.1002/prot.21495

Arunachalam, M., Bassi, A., Galeone, M., Scarfi, F. and Difonzo, E., 2013. Scleroderma-like hands in a 16 year old boy. JAMA Dermatol., 149: 969-970. https://doi.org/10.1001/jamadermatol.2013.3370a

Astrin, K.H., Warner, C.A., Yoo, H.-W., Goodfellow, P.J., Tsai, S.-F. and Desnick, R.J., 1991. Regional assignment of the human uroporphyrinogen III synthase (UROS) gene to chromosome 10q25- q26.3. Hum. Genet., 87: 18-22. https://doi.org/10.1007/BF01213085

Baran, M., Eliaçık, K., Kurt, İ., Kanik, A., Zengin, N. and Bakiler, A.R., 2013. Bullous skin lesions in a jaundiced infant after phototherapy: a case of congenital erythropoietic porphyria. Turk J. Pediatr., 55: 218. https://doi.org/10.1093/hmp/ddu298

ben Bdira, F., González, E., Pluta, P., Lain, A., Sanz-Parras, A., Falcon-Perez, J.M. and Millet, O., 2014. Tuning intracellular homeostasis of human uroporphyrinogen III synthase by enzyme engineering at a single hotspot of congenital erythropoietic porphyria. Hum. mol. Genet., 23: 5805-5813.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N. and Bourne, P.E., 2000. The protein data bank. Nucl. Acids Res., 28: 235-242.

Berry, A.A., Desnick, R.J., Astrin, K.H., Shabbeer, J., Lucky, A.W. and Lim, H.W., 2005. Two brothers with mild congenital erythropoietic porphyria due to a novel genotype. Arch Dermatol., 141: 1575-1579. https://doi.org/10.1001/archderm.141.12.1575

Blavas, R., Santoshkumar, G. and Prakash, B.R., 2011. Erythrodontia in congenital erythropoietic porphyria. J. Oral Maxillofac. Pathol., 15: 69. https://doi.org/10.4103/0973-029X.80022

Boulechfar, S., Da Silva, V., Deybach, J.-C., Nordmann, Y., Grandchamp, B. and De Verneuil, H., 1992. Heterogeneity of mutations in the uroporphyrinogen III synthase gene in congenital erythropoietic porphyria. Hum. Genet, 88: 320-324. https://doi.org/10.1007/BF00197267

Cunha, L., Kuti, M., Bishop, D.F., Mezei, M., Zeng, L., Zhou, M.M. and Desnick, R.J., 2008. Human uroporphyrinogen III synthase: NMR-based mapping of the active site. Proteins, 71: 855-873. https://doi.org/10.1002/prot.21755

Darwich, E., Guilabert, A., Aceituno, A., Mas, N., To-Figueras, J. and Herrero, C., 2011. Congenital erythropoietic porphyria and Parkinson’s disease: clinical association in a patient with a long-term follow-up. Eur. J. Dermatol., 21: 613-614. https://doi.org/10.1684/ejd.2011.1329

Deybach, J., De Verneuil, H., Boulechfar, S., Grandchamp, B. and Nordmann, Y., 1990. Point mutations in the uroporphyrinogen III synthase gene in Blood, 75: 1763-1765. https://doi.org/10.1182/blood.V75.9.1763.bloodjournal7591763

Di Pierro, E., Brancaloni, V. and Granata, F., 2016. Advances in understanding the pathogenesis of congenital erythropoietic porphyria. Br. J. Haematol., 173: 365-379. https://doi.org/10.1111/bjh.13978

Egan, D.N., Yang, Z., Phillips, J. and Abkowitz, J.L., 2015. Inducing iron deficiency improves erythropoiesis and photosensitivity in congenital erythropoietic porphyria. Blood, 126: 257-261. https://doi.org/10.1182/blood-2014-07-584664

Emsley, P., Lohkamp, B. and Scott, W.G., Cowtan, K., 2010. Features and development of Coot. Acta Crystallogr: D: Biol. Crystallogr., 66: 486-501. https://doi.org/10.1107/S0907444910007493

Freesemann, A., Bhutani, L., Jacob, K. and Doss,
M., 1997. Interdependence between degree of porphyrin excess and disease severity in congenital erythropoietic porphyria (Günther’s disease). *Arch Dermatol. Res.*, **289**: 272-276. https://doi.org/10.1007/s004030050192

Ged, C., Mégarbané, H., Chouery, E., Lalanne, M., Megarbane, A. and de Verneuil, H., 2004. Congenital erythropoietic porphyria: Report of a novel mutation with absence of clinical manifestations in a homozygous mutant sibling. *J. Invest. Dermatol.*, **123**: 589-591. https://doi.org/10.1111/j.0022-202X.2004.23401.x

Hanwell, M.D., Curtis, D.E., Lonie, D.C., Ged, C., Mégarbané, H., Chouery, E., Lalanne, M., Moghbeli, M., Maleknejad, M., Arabi, A., Mathews, M.A., Schubert, H.L., Whitby, F.G., Poh-Fitzpatrick, M.B., 1986. The erythropoietic porphyrias. *Dermatol. Clin.*, **4**: 291-296. https://doi.org/10.1016/S0733-8635(18)30833-7

Poh-Fitzpatrick, M.B., 1998. Clinical features of the porphyrias. *Clin. Dermatol.*, **16**: 251-264. https://doi.org/10.1016/S0738-081X(97)00205-8

Ponka, P., 1997. Tissue-specific regulation of iron metabolism and heme synthesis: distinct control mechanisms in erythroid cells. *Blood*, **89**: 1-25. https://doi.org/10.1182/blood.V89.1.1

Rozen, S. and Skaltsky, H., 2000. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol. Biol.*, **132**: 365-386. https://doi.org/10.1385/1-59259-192-2:365

Šali, A., Potterton, L., Yuan, F., van Vlijmen, H. and Kaplus, M., 1995. Evaluation of comparative protein modeling by MODELLER. *Proteins*, **23**: 318-326. https://doi.org/10.1002/pro.340230306

Sarkany, R.P., Ibbotson, S.H., Whatley, S.D., Lawrence, C.M., Gover, P., Mufti, G.J., Murphy, G.M., Masters, G.S., Badminton, M.N. and Elder, G.H., 2011. Erythropoietic porphyria associated with myeloid malignancy is likely distinct from autosomal recessive congenital erythropoietic porphyria. *J. Invest. Dermatol.*, **131**: 1172-1175. https://doi.org/10.1038/jid.2011.5

Scarlett, Y.V. and Brenner, D.A., 1998. Porphyrias. *J. Clin. Gastroenterol.*, **27**: 192-198. https://doi.org/10.1097/00004836-199810000-00003

Schneidman-Duhovny, D., Inbar, Y., Nussinov, R. and Wolfson, H.J., 2005. PatchDock and SymmDock: Servers for rigid and symmetric docking. *Nucl. Acids Res.*, **33**: W363-W367. https://doi.org/10.1038/nkar481

Schubert, H.L., Phillips, J.D., Heroux, A. and Hill, C.P., 2008. Structure and mechanistic implications of a uroporphyrinogen III synthase product complex. *Biochemistry*, **47**: 8648-8655. https://doi.org/10.1021/bi800635y

Shady, A.A., Colby, B.R., Cunha, L.F., Astrin, K.H., Bishop, D.F. and Desnick, R.J., 2002. Congenital erythropoietic porphyria: Identification and expression of eight novel mutations in the uroporphyrinogen III synthase gene. *Br. J. Haematol.*, **117**: 980-987. https://doi.org/10.1046/j.1365-2141.2002.03558.x

Solis, C., Aizencang, G.I., Astrin, K.H., Bishop, D.F. and Desnick, R.J., 2001. Uroporphyrinogen III synthase erythroid promoter mutations in adjacent GATA1 and CP2 elements cause congenital erythropoietic porphyria. *J. clin. Invest.*, **107**: 753-762. https://doi.org/10.1172/JCI10642

Szlendak, U., Bykowska, K. and Lipniacka, A., 2016. Clinical, biochemical and molecular characteristics of the main types of porphyria. *Adv. clin. exp. Med.*, **25**: 1605-1612. https://doi.org/10.1076/jbm.20084

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C. and Ferrin, T.E., 2004. UCSF Chimera a visualization system for exploratory research and analysis. *J. Comput. Chem.*, **25**: 1605-1612. https://doi.org/10.1002/jcc.20084

Masters, G.S., Badminton, M.N. and Elder, G.H., 2004. Evaluation of comparative protein modeling by MODELLER. *Proteins*, **23**: 318-326. https://doi.org/10.1002/pro.340230306

Shady, A.A., Colby, B.R., Cunha, L.F., Astrin, K.H., Bishop, D.F. and Desnick, R.J., 2002. Congenital erythropoietic porphyria: Identification and expression of eight novel mutations in the uroporphyrinogen III synthase gene. *Br. J. Haematol.*, **117**: 980-987. https://doi.org/10.1046/j.1365-2141.2002.03558.x

Solis, C., Aizencang, G.I., Astrin, K.H., Bishop, D.F. and Desnick, R.J., 2001. Uroporphyrinogen III synthase erythroid promoter mutations in adjacent GATA1 and CP2 elements cause congenital erythropoietic porphyria. *J. clin. Invest.*, **107**: 753-762. https://doi.org/10.1172/JCI10642

Szlendak, U., Bykowska, K. and Lipniacka, A., 2016. Clinical, biochemical and molecular characteristics of the main types of porphyria. *Adv. clin. exp. Med.*, **25**: 1605-1612. https://doi.org/10.1002/jcc.20084
Tanigawa, K., Takamura, N. and Yamashita, S., 1995. Congenital erythropoietic porphyria. *Nihon Rinsho.*, **53**: 1422-1426.

Tezcan, I., Xu, W., Gurgey, A., Tuncer, M., Cetin, M., Öner, C., Yetgin, S., Ersoy, F., Aizencang, G. and Astrin, K., 1998. Congenital erythropoietic porphyria successfully treated by allogeneic bone marrow transplantation. *Blood,*** **92**: 4053-4058. https://doi.org/10.1182/blood.V92.11.4053.4058

Thunell, S., 2000. Porphyrins, porphyrin metabolism and porphyrias. I. Update. *Scand J. clin. Lab. Invest.*, **60**: 509-540. https://doi.org/10.1080/003655100448310

Tsai, S.-F., Bishop, D. and Desnick, R., 1987. Purification and properties of uroporphyrinogen III synthase from human erythrocytes. *J. biol. Chem.*, **262**: 1268-1273.

Urquiza, P., Lain, A., Sanz-Parra, A., Moreno, J., Bernardo-Seisdedos, G., Dubus, P., González, E., Gutiérrez-de-Juan, V., García, S., Eraña, H., 2018. Repurposing ciclopirox as a pharmacological chaperone in a model of congenital erythropoietic porphyria. *Sci. Transl. Med.*, **10**: 7467. https://doi.org/10.1126/scitranslmed.aat7467

Verma, A., Rashidghamat, E., Martinez, A., Fassihi, H. and Sarkany, R., 2014. Congenital erythropoietic porphyria: A case in which symptoms were precipitated by an unrelated anaemia. *Br. J. Dermatol.*, **171**: 422-423. https://doi.org/10.1111/bjd.12945

Warner, C.A., Yoo, H.-W., Roberts, A.G. and Desnick, R.J., 1992. Congenital erythropoietic porphyria: identification and expression of exonic mutations in the uroporphyrinogen III synthase gene. *J. clin. Invest.*, **89**: 693-700. https://doi.org/10.1172/JCI11567

Wiederholt, T., Poblete-Gutierrez, P., Gardlo, K., Goerz, G., Bolsen, K., Merk, H. and Frank, J., 2006. Identification of mutations in the uroporphyrinogen III cosynthase gene in German patients with congenital erythropoietic porphyria. *Physiol. Res.*, **55**: S85-92.

Wood, E.J., 1983. Molecular cloning. A laboratory manual by T Maniatis, E F Fritsch and J Sambrook. pp 545. Cold Spring Harbor Laboratory, New York. 1982. $48 ISBN 0-87969-136-0. *Biochem. Educ.*, **11**: 82-82.

Xu, W., Kozak, C.A. and Desnick, R.J., 1995. Uroporphyrinogen-III synthase: molecular cloning, nucleotide sequence, expression of a mouse full-length cDNA, and its localization on mouse chromosome 7. *Genomics*, **26**: 556-562. https://doi.org/10.1016/0888-7543(95)80175-L

Yisgedu, T.B., Chen, X., Lingam, H.K., Huang, Z., Meyers, E.A., Shore, S.G. and Zhao, J.-C., 2010. Intermolecular dihydrogen-and hydrogen-bonding interactions in diammonium closo-decahydrodecaborate sesquihydrate. *Acta Crystallographr. C.*, **66**: m1-m3. https://doi.org/10.1107/S0108270109040815