Original Article

Hepatoerythropoietic Porphyria Caused by a Novel Homoallelic Mutation in Uroporphyrinogen Decarboxylase Gene in Egyptian Patients

(hepatoerythropoietic porphyria / HEP / uroporphyrinogen decarboxylase / UROD / skin photosensitivity / red urine)

M. S. FARRAG[1], I. MIKULA[1], E. RICHARD[2,3], V. SAUDEK[4], H. DE VERNEUIL[2,3], P. MARTÁSEK[1]

[1]Department of Paediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Czech Republic
[2]University of Bordeaux, [3]INSERM U1035, Bordeaux, France
[4]University of Cambridge Metabolic Research Laboratories and MRC Metabolic Diseases Unit, Wellcome-MRC Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK

Abstract. Porphyrias are metabolic disorders resulting from mutations in haem biosynthetic pathway genes. Hepatoerythropoietic porphyria (HEP) is a rare type of porphyria caused by the deficiency of the fifth enzyme (uroporphyrinogen decarboxylase, UROD) in this pathway. The defect in the enzymatic activity is due to biallelic mutations in the *UROD* gene. Currently, 109 UROD mutations are known. The human disease has an early onset, manifesting in infancy or early childhood with red urine, skin photosensitivity in sun-exposed areas, and hypertrichosis. Similar defects and links to photosensitivity and hepatopathy exist in several animal models, including zebrafish and mice. In the present study, we report a new mutation in the *UROD* gene in Egyptian patients with HEP. We show that the homozygous c.T163A missense mutation leads to a substitution of a conserved phenylalanine (amino acid 55) for isoleucine in the enzyme active site, causing a dramatic decrease in the enzyme activity (19 % of activity of wild-type enzyme). Inspection of the UROD crystal structure shows that Phe-55 contacts the substrate and is located in the loop that connects helices 2 and 3. Phe-55 is strictly conserved in both prokaryotic and eukaryotic UROD. The F55I substitution likely interferes with the enzyme-substrate interaction.

Received July 18, 2015. Accepted August 4, 2015.

This work was supported by grants from Charles University in Prague (UNCE 204011, PRVOUK P24/LF1/3).

Corresponding author: Pavel Martásek, Department of Paediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague. Ke Karlovu 2, 121 00 Prague 2, Czech Republic. Phone: (+420) 602 935 339; Fax: (+420) 224967099; e-mail: pavel.martasek@gmail.com

Abbreviations: CEP – congenital erythropoietic porphyria, EDTA – ethylene diamine tetraacetic acid, fPCT – familial porphyria cutanea tarda, HEP – hepatoerythropoietic porphyria, HPLC – high-performance liquid chromatography, IPTG – isopropyl thiogalactoside, PPOX – protoporphyrinogen oxidase, PV – porphyria variegata, UROD – uroporphyrinogen decarboxylase, UROS – uroporphyrinogen synthase, sPCT – sporadic porphyria cutanea tarda.

Introduction

Haem is essential for nearly all forms of life as it plays a pivotal role in oxygen transport, detoxification, respiration, and other vital processes (Padmanaban et al., 1989; Igarashi and Sun, 2006; Puy et al., 2010). It was shown that haem also serves as a signalling molecule with modulation capacity for various cellular processes (Ogawa et al., 2001; Mense and Zhang, 2006), ligand for the orphan nuclear receptors (Raghuram et al., 2007), and as a critical gas sensor (Shimizu at al., 2015; Martínková et al., 2013). Haem is a product of a cascade of eight consecutive enzymatic steps (Puy et al., 2010). To follow the decreased bioavailability of haem and its consequences, severe cases of porphyrias, diseases with inherited defects in the haem synthetic pathway, serve as a natural model for studies of evolutionarily conserved metabolic pathways. Rare severe porphyrias are caused by biallelic genetic defects (Puy et al., 2010). Hepatoerythopoietic porphyria (HEP) represents a prototype of such disease (Pinol-Aguade et al., 1969). HEP is caused by a biallelic defect in the fifth enzyme in the haem biosynthetic pathway, uroporphyrinogen decarboxylase (UROD) (Elder et al., 1981; de Verneuil et al., 1984; To-Figueras et al., 2011).

HEP is characterized by an early disease onset, with prominent skin lesions in childhood and UROD cata-

lytic activity decreased under 30 % of normal values. Uroporphyrinogen decarboxylase (UROD) (UROD; E.C.4.1.1.37) is a cytosolic enzyme that sequentially decarboxylates the acetate radicals of uroporphyrinogen III, producing coproporphyrinogen III in the fifth step of the haem biosynthetic pathway (Jackson et al., 1976; Elder, 1998; Anderson et al., 2001). The enzyme is coded by the *UROD* gene, which is located on chromosome 1 (Dubart, et al., 1986) and spans over 3 kb (Dubart, et al., 1986). The gene is regulated by a single known promoter, has 10 exons, and a polyadenylation signal with a canonical AATAAA element (Romana et al., 1987; Morán-Jiménez et al., 1996). The *UROD* gene codes for a primary polypeptide precursor formed of 367 amino acyl residues (Romeo et al., 1986). The human UROD protein is a homodimer with a molecular mass of approximately 40.8 kDa (Whitby et al., 1998).

Three metabolic phenotypes result from the decrease in the catalytic activity of the UROD enzyme: sporadic porphyria cutanea tarda (sPCT), familial porphyria cutanea tarda (fPCT), and hepatoerythropoietic porphyria (HEP) (de Verneuil, et al., 1984; Puy et al., 2010). sPCT is the most common subtype of porphyria, which results from a slight decrease in the UROD activity and is limited to the liver. sPCT occurs due to the exposure to a predisposing factor such as smoking, alcohol consumption, viral infection, and drugs including oral contraceptive pills. No mutations in the *UROD* gene are found in sPCT (Rocchi et. al., 1986; Fargion and Fracanzani, 2003), indicating a molecular link to additional metabolic pathways. The decrease in the UROD enzyme activity almost to 50 % of normal values in all tissues leads to fPCT (OMIM: 176100). The diminished catalytic activity of the enzyme is due to a heterozygous mutation in the *UROD* gene (de Verneuil et al., 1978; Elder et al., 1981; Muñoz-Santos et al., 2010). This condition usually appears in adults and is also associated with a predisposing factor similarly as in the case of sPCT (Fargion et al., 1992; Elder, 1998; Morán et al., 1998; Bulaj et al., 2000; Anderson et al., 2001; Cruz-Rojo et al., 2002; Nordmann and Puy, 2002; Méndez et al., 2005; Muñoz-Santos et al., 2010). In fPCT, porphyrin precursors, particularly uroporphyrinogen and heptaporphyrinogen, are accumulated in hepatocytes and subsequently oxidized to their corresponding porphyrins, which pass to the circulation and are excreted by urine (Elder, 1998; Anderson et al., 2001). This results in the clinical manifestations of the disease such as cutaneous photosensitivity of sun-exposed areas, skin fragility, pigmentation, hypertrichosis, and in advanced cases liver cell damage. The resulting fPCT is an autosomal dominant trait with low clinical penetrance in which the heteozygous *UROD* gene mutations lead to variable phenotypes of carriers or manifested patients (Anderson et al., 2001). Strikingly, the phenotype observed in human patients is recapitulated in zebrafish strains carrying a *UROD* mutation (yquem). Fluorescent erythrocytes loaded with uroporphyrin are not only found in these mutant fish, but also in morphants where UROD activity has been suppressed. In the zebrafish community, UROD inactivation serves as an excellent phenotypic marker for method development (Wang et al., 1998; Kong et al., 2015).

In the present study, we report a mutation of a conserved phenylalanine for isoleucine, which dramatically decreases UROD enzymatic activity and causes HEP in an Egyptian family. Molecular analysis of this human genetic defect provides a new insight into the pathogenesis of the disease and possibly leads to individual therapeutic strategy.

Material and Methods

Subjects

The probands, a 13-year-old boy and his 11-year-old sister, were identified as suspected carriers of a mutation in *UROD* and HEP patients in cooperation with Professor Hany Elweshahy from the Cairo University Hospital, Department of Dermatology based on the clinical symptoms presented by red urine and elevated urinary uroporphyrin (data not shown, personal communication).

The ethics committee of the First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague approved the study under No. 112/12. Blood samples were obtained from the probands and the mother after informed consent from the mother.

DNA analysis

Blood samples were collected in tubes containing ethylene diamine tetraacetic acid (EDTA). Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer‘s protocol.

PCR amplification of the coding sequence exons 1–10 of the *UROD* gene and the associated splice donor and acceptor sites was performed by using the primer pairs and the optimized conditions as described by Moran-Jimenez et al. (1996). The PCR products were analysed by direct sequencing in an ABI PRISM 3100-Avant Genetic Analyzer automatic sequencer (Applied Biosystems, Waltham, MA). The mutation was confirmed by sequencing a second amplified fragment. In addition, the coding exons of the *UROS* gene including the exon/intron boundaries and the coding sequence of the *PPOX* gene were amplified using the primer set and the optimized conditions described by Xu et al. (1995) and by Rossetti et al. (2008), respectively.

Expression of uroporphyrinogen decarboxylase proteins

The expression plasmid pGEX-UD, which contains the human *UROD* cDNA, was designed. Mutation identified in the *UROD* gene of the probands was introduced into pGEX-UD using the Quik-Change II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). The mutations were confirmed by sequencing. Bacterial cell pel-

lets containing the normal and the mutated pGEX constructs were grown overnight prior to induction with 0.1 mM isopropyl thiogalactoside (IPTG). Bacterial cell pellets were washed in PBS and resuspended in lysis buffer (250 mM potassium phosphate, pH 6.0, 0.1% Triton-X 100) and the cells were disrupted by sonication on ice. The bacterial lysates were centrifuged (Heraeus Multifuge X 1R, 7000 *g* for 10 min, Thermo Scientific, Waltham, MA) and the UROD activity was measured in the supernatants using pentaporphyrinogen I (Porphyrin Products Inc., Logan, UT) as a substrate, as previously published by de Verneuil et al. (1986). The porphyrins produced were analysed by high-performance liquid chromatography (HPLC) with fluorescence detection in a Shimadzu apparatus (Shimadzu, Kyoto, Japan) according to the method of Lim et al. (1983). The specific activity was calculated as nmoles of coproporphyrinogen I formed per hour per mg of protein.

Results

Skin phenotype and its appearance from childhood implicated, in the absence of biochemical data for the above-mentioned reasons, three basic diagnostic possibilities – HEP, less aggressive form of congenital erythropoietic porphyria (CEP, uroporphyrinogen synthase / *UROS*/ gene defect) and homozygous porphyria variegata (PV, protoporhyrinogen oxidase /*PPOX*/ gene defect), especially because parents are the first cousins. Simultaneous sequencing of *UROD*, *UROS*, *PPOX* genomic DNA (exonic sequences and exon/intron border sequences) revealed mutation only in the *UROD* gene.

Physical examination revealed hypertrichosis on the face and on the dorsal aspects of the forearms, as shown in Fig. 1 and Fig. 2. The HEP pedigree is outlined in Fig. 3.

UROD gene sequences in the different members of the family

Analysis of the *UROD* gene (Fig. 4) revealed a new homozygous missense mutation in both probands. T→A transition at position 163 in exon 3 was identified (c.163T>A), which leads to the substitution of phenylalanine to isoleucine at codon 55 (p.Phe55Ile or F55I). The mutation was confirmed by sequencing a second amplified fragment. The mother was a heterozygous carrier, as shown in Fig. 4.

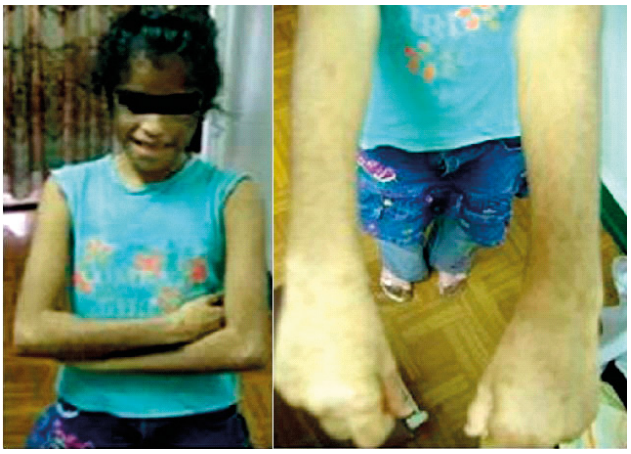

Fig. 1. Hypertrichosis on the cheeks and hair over-growth on the dorsal aspects of the forearms.

Prokaryotic expression of the F55I mutant

The enzymatic activity of recombinant normal and mutant UROD proteins was measured in bacterial lysates using pentacarboxyl porphyrinogen I as a substrate. As shown in Table 1, UROD-F55I had 19 % residual activity as compared to the normal.

Discussion

Enzymes involved in the biosynthesis of haem and haem metabolism are critical for living organisms. Their conservation across biological taxa offers a wealth of data shedding light at the basic mechanisms supporting life functions. UROD is the fifth enzyme of the haem biosynthetic pathway, which catalyses the removal of four carboxymethyls from uroporphyrinogen reversing it to coproporphyrinogen, thus affecting the steric proportions of the substrate and transforming it into a further enzymatically manageable new substrate (Elder et al., 1978; Silva and Ramos, 2005). Interestingly, this enzyme homodimerizes with a low monomer-dimer dissociation constant of 0.1 μmol/l (in case of human

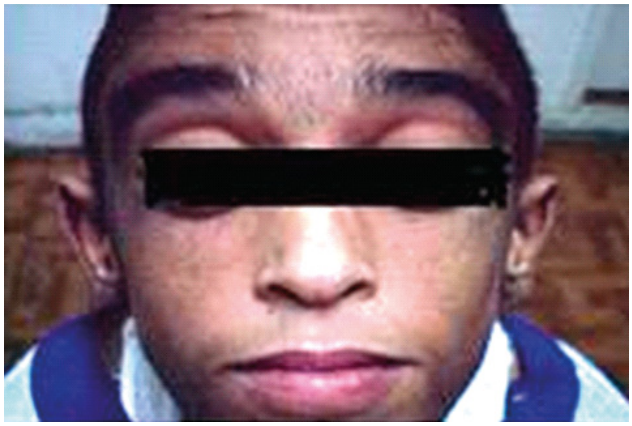

Fig. 2. Hypertrichosis and scars on the face.

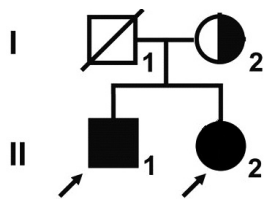

Fig. 3. Pedigree of the fPCT family. The father of the probands I-1 was not available for DNA analysis. The mother I-2 was an obligate carrier. Half-filled symbols denote carriers of HEP. Black-filled symbols denote HEP patients. Completely open symbol denotes the individual who was not tested.

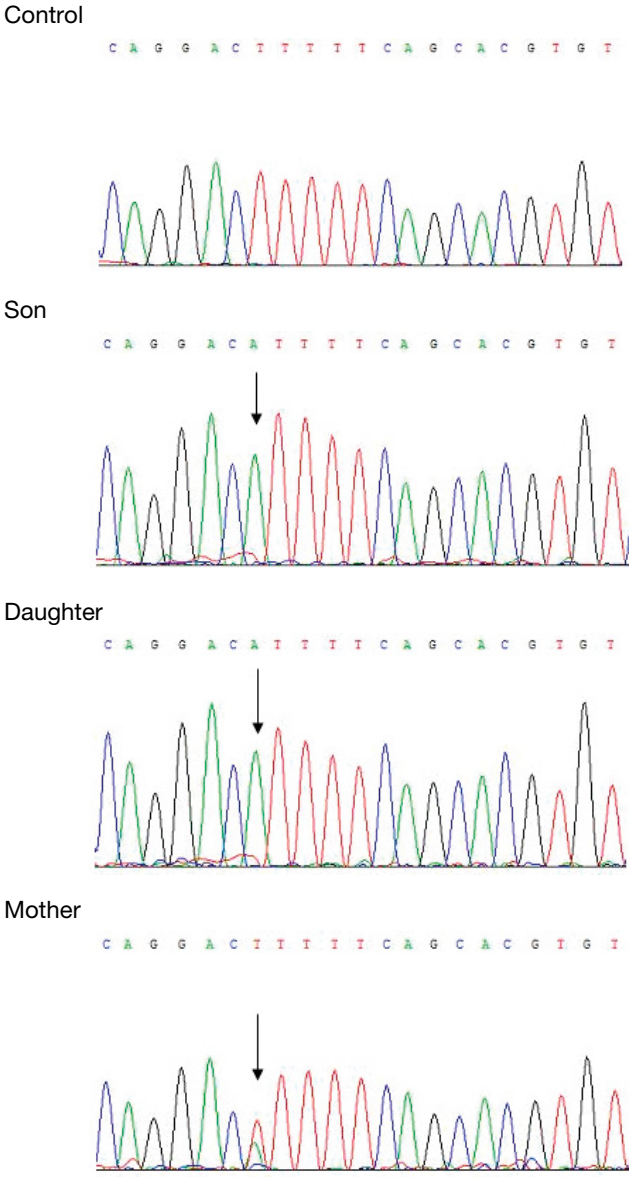

Fig. 4. DNA analysis
Sequencing profiles for the mutation of the *UROD* gene in the affected family members. *TTT* indicates the normal allele (phenylalanine) present in the control sample. *ATT*, the mutant allele (isoleucine) is present at the homozygous state in the probands and heterozygous state (*WTT*) in the mother; W means A and T when both *ATT* and *TTT* alleles are present.

UROD) (Philips et al., 2007). The dimerization is also found in the case of bacterial UROD; nevertheless, it is not clear whether the dimerization is necessary for the UROD enzymatic function. Porphyrin ring biosynthesis shares a common pathway for animals and plants including the UROD enzyme (Heinemann et al., 2008).

HEP is a rare disease: fewer than 100 cases of HEP have been described in the literature (Liu et al., 2013). Table 2 depicts the frequency of the mutations of 21 unrelated families reported in the literature. A genetic homogeneity has been observed in Spain with the predominance of the G281E mutation. By contrast, a great heterogeneity of mutations has been found for other countries in Europe, Africa or America. Because of the heterogeneity of the mutations, the clinical outcome is very variable, with mild or moderate phenotypes difficult to distinguish from familial PCT or a more severe phenotype similar to CEP or homozygous VP. For example, the genotype F46L found in two different families (Ged et al., 2002; Armstrong et al., 2004) is associated with a mild phenotype. By contrast, the genotype G281E is associated with a severe phenotype resembling CEP.

This report describes a new missense mutation of the *UROD* gene at the homoallelic state in young patients in an Egyptian family, characteristic of HEP. The observed mutation in the probands was present at the homozygous state and their mother showed to be a heterozygous carrier. There was no possibility to obtain the blood from the father, but he should be bearing the same mutation as the mother did. This is more evident because father and mother of the affected siblings were cousins.

The mutation in the probands leads to the substitution of phenylalanine to isoleucine at position 55 of UROD. According to the crystal structure (Phillips et al., 2003), this residue participates in the loop between helices 2 and 3 and interacts tightly with the substrate in the active site (Fig. 5A). Moreover, F55 is conserved in both eukaryotes and prokaryotes (Fig. 5B). This offers the possibility to study this new mutation in the UROD active site with a specific human phenotype and enzyme activity using a prokaryotic expression system. The F55I mutant protein expressed in bacteria exhibits 19 % of the wild-type protein activity (Table 1). We do not have the possibility to investigate biological samples of affected individuals to measure the UROD enzyme activity directly. However, the relatively moderate skin problems of the children correlate well with the high residual activity of UROD.

The mutation identification helps us to better understand the disease and provide counselling to the affected families. We report the first cases of HEP in the Egyptian population based on molecular diagnosis of the *UROD* gene. Because of a very low number of HEP patients worldwide, the identification of a novel *UROD* mutation

Table 1. Expression of the normal and mutated GST-UROD fusion proteins

Plasmid	Specific activity[#]		No. of assays	Residual activity (% vs WT)
	Mean (SD)	Range		
pGEX-UROD	3.1 ± 0.9	2.6–3.2	3	100
pGEX-UROD-F551	0.6 ± 0.1	0.5–0.7	3	19

[#] Data are given as picomoles of coproporphyrinogen I formed per hour and per milligram of protein

Table 2. Frequency of mutations in 21 independent HEP families

Mutation	Ancestry	N	Allelic status	Allele frequency	References
-M1V	Hungarian	1	Hetero	1/42	Remenyik et al., 2008
-F46L	Spanish	2	Homo	4/42	Ged et al., 2002
	British				Armstrong et al., 2004
-P62L*	Portuguese	1	Homo	2/42	Moran-Jimenez et al., 1996
-Q71X	Northern European	1	Hetero	1/42	Phillips et al., 2007
-V134Q	British/German	1	Hetero	1/42	Meguro et al., 1994
-V166A	Puerto Rican/Dominican	1	Hetero	1/42	Cantatore-Francis et al., 2010
-E167Q*	Italian	1	Homo	2/42	Romana et al., 1987
-G168R	Northern European	1	Hetero	1/42	Phillips et al., 2007
-G170D	African	1	Homo	2/42	To-Figueras et al., 2011
-H220P*	British/German	1	Hetero	1/42	Meguro et al., 1994
-G281E*	Spanish/Tunisian	9	Homo/Hetero**	17/42	Roberts et al., 1995
-R292G*	Dutch	1	Hetero	1/42	de Verneuil et al., 1992
-P235S*	Argentinean	2	Hetero	2/42	Granata et al., 2009
	Hungarian				Remenyik et al., 2008
-Y311C*	Italian	1	Homo	2/42	Moran-Jimenez et al., 1996
-IVS9$^{-1(G>C)}$*	Argentinean	1	Hetero	1/42	Granata et al., 2009
-Del	Dutch	1	Hetero	1/42	de Verneuil et al., 1992
-645del1053* ins10	Puerto Rican/Dominican	1	Hetero	1/42	Cantatore-Francis et al., 2010

*Mutations were identified in both HEP and fPCT

**8 patients (7 Spanish and 1 Tunisian) were homoallelic and one Spanish heteroallelic

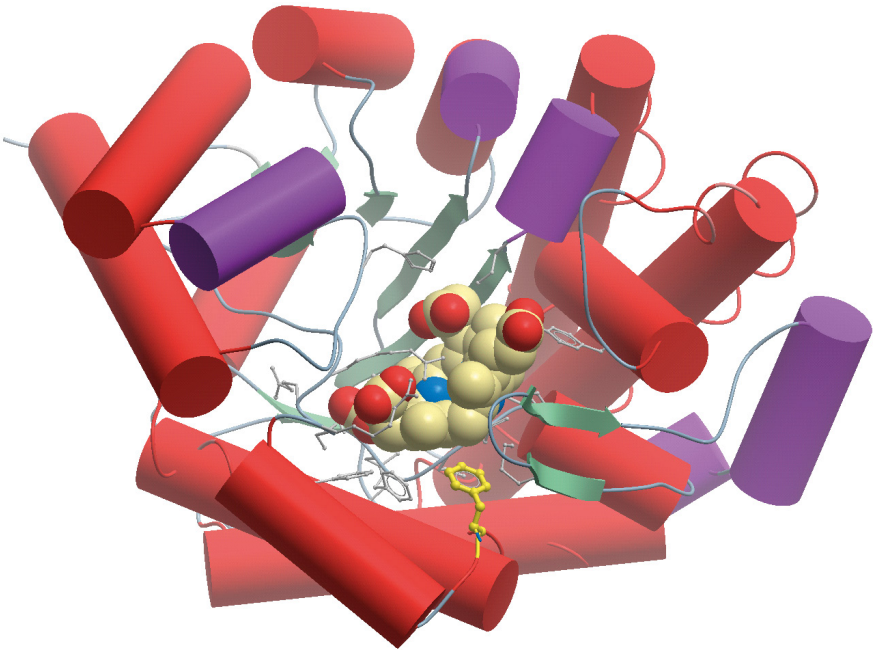

Fig. 5A. Graphical representation of the UROD structure (PDB 1r3y, only one monomeric unit is displayed) with co-proporphyrinogen product in space-filling rendering. Sidechains participating in the direct binding of the product are displayed in a ball and stick model. Phe 55 (mutated in the probands to Ile) is highlighted in bold and yellow.

and its characterization broadens our current knowledge on the molecular heterogeneity of HEP worldwide.

In the mutation described in this paper, an aromatic residue is replaced by a bulky aliphatic one. The stacking interaction of three aromatic residues (Fig. 5A) forming the bottom of the substrate-binding site is thus disrupted and the substrate binding probably becomes much looser. The absolute conservation of the mutated

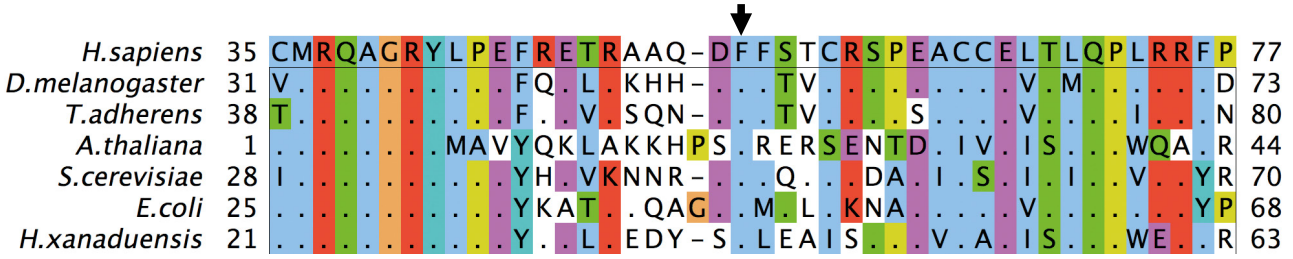

Fig. 5B. Alignment of a segment surrounding F55 (arrow) in human UROD with corresponding orthologous sequences identified in selected metazoans, plants, fungi, bacteria and archaea. Species and UniProt sequence identifiers from top to bottom: *Homo sapiens*, P06132; *Drosophila melanogaster*, Q9V595; *Trichoplax adhaerens,* B3S2H7: *Arabidopsis thaliana* Q93ZB6; *Saccharomyces cerevisiae*, P32347; *Escherichia coli,* P29680; *Halopiger xanaduensis*, F8DD35. Only non-conserved amino acids are shown and their functionality is coloured according to ClustalW scheme (www.jalview.com). F55 has been found conserved in all sequences inspected (over 1100).

residue indicates that the optimal arrangement in the vicinity of the substrate had been reached early in the evolution and no further diversion in this region was possible.

Studies of mutations leading to phenotypic manifestation such as a disease lead directly to discoveries of functionally important primary structures of enzymes. In this respect, disease is a consequence of direct disturbance of the protein function, and biological medicine is becoming an important contributor to general biology, in this case, the biology of haem and its availability.

There are 109 known mutations in the *UROD* gene in humans. Their detailed molecular analysis is likely to contribute to discoveries of new biological roles and functions of this evolutionarily conserved enzyme.

Acknowledgments

We are especially grateful for the interest and cooperation of the patients and their mother to carry out this study. Our thanks go to late Professor Hany Elweshahy from the Cairo University Hospital, Department of Dermatology, for his cooperation.

References

Anderson, K. E., Sassa, S., Bishop, D. F., Desnick, R. J. (2001) Disorders of heme biosynthesis: X-linked sideroblastic anemia and the porphyrias. In: *The Metabolic and Molecular Bases of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., pp. 2961-3062. McGraw-Hill, New York, NY.

Armstrong, D. K. B., Sharpe, P. C., Chambers, C. R., Whatley, S. D., Roberts, A. G., Elder, G. H. (2004) Hepatoerythropoietic porphyria: a missense mutation in the UROD gene is associated with mild disease and an unusual porphyrin excretion pattern. *Br. J. Dermatol.* **151**, 920-923.

Bulaj, Z. J., Phillips, J. D., Ajioka, R. S., Franklin, M. R., Griffen, L. M., Guinee, D. J., Edwards, C. Q., Kushner, J. P. (2000) Hemochromatosis genes and other factors contributing to the pathogenesis of porphyria cutanea tarda. *Blood* **95**, 1565-1571.

Cantore-Francis, J. L., Cohen, J., Balwani, M., Kahn, P., Lazarus, H. M., Desnick, R. J., Schaffer, J. V. (2010) Hepatoerythropoietic porphyria misdiagnosed as child abuse: cutaneous, arthritic, and hematologic manifestations in sibling with a novel UROD mutation. *Arch. Dermatol.* **146**, 529-533.

Cruz-Rojo, J., Fontanellas, A., Morán-Jiménez, M. J., Navarro-Ordoñez, S., García-Bravo, M., Méndez, M., Muñoz-Rivero, M. C., Enríquez de Salamanca, R. (2002) Precipitating/aggravating factors of porphyria cutanea tarda in Spanish patients. *Cell. Mol. Biol*. **48**, 845-852.

de Verneuil, H., Aitken, G., Nordmann, Y. (1978) Familial and sporadic porphyria cutanea tarda, two different diseases. *Hum. Genet*. **44**, 145-51.

de Verneuil, H., Beaumont, C., Deybach, J. C., Nordmann, Y., Sfar, Z., Kastally, R. (1984) Enzymatic and immunological studies of uroporphyrinogen decarboxylase in familial porphyria cutanea tarda and hepatoerythropoietic porphyria. *Am. J. Hum. Genet*. **36**, 613-622.

de Verneuil, H., Grandchamp, B., Beaumont, C., Picat, C., Nordmann, Y. (1986) Uroporphyrinogen decarboxylase structural mutant (Gly281—Glu) in a case of porphyria. *Science* **234**, 732-734.

de Verneuil, H., Bourgeois, F., de Rooij, F., Siersema, P. D., Wilson, J. H. P., Grandchamp, B., Nordmann, Y. (1992) Characterisation of a new mutation (R292G) and a deletion at the human uroporphyrinogen decarboxylase locus in two patients with hepatoerythropoietic porphyria. *Hum. Genet.* **89**, 548-552.

Dubart, A., Mattei, M. G., Raich, N., Beaupain, D., Romeo, P. H., Mattei, J. F., Goossens, M. (1986) Assignment of human uroporphyrinogen decarboxylase (URO-D) to the p34 band of chromosome 1. *Hum. Genet.* **73,** 277-279.

Elder, G. H., Lee, G. B., Tovey, J. A. (1978). Decreased activity of hepatic uroporphyrinogen decarboxylase in sporadic porphyria cutanea tarda. *N. Engl. J. Med*. **299**, 274-278.

Elder, G. H., Smith, S. G., Herrero, C., Mascaro, J. M, Lecha, M., Muniesa, A. M., Czarnecki, D. B., Brenan, J., Poulos, V., de Salamanca, R. E. (1981) Hepatoerythropoietic porphyria: a new uroporphyrinogen decarboxylase defect or homozygous porphyria cutanea tarda? *Lancet* **317**, 916-919.

Elder, G. H. (1998) Porphyria cutanea tarda. *Semin. Liver Dis.* **18**, 67-75.

Fargion, S., Piperno, A., Cappellini, M., Sampietro, M., Fracanzani, A., Romano, R., Caldarelli, R., Marcelli, R., Vecchi, L., Fiorelli, G., (1992) Hepatitis virus and porphyr-

ia cutanea tarda: evidence of a strong association. *Hepatology* **16**, 1322-1326.

Fargion, S., Fracanzani, A. L. (2003) Prevalence of hepatitis C virus infection in porphyria cutanea tarda. *J. Hepatol.* **39**, 635-638.

Ged, C., Ozalla, D., Herrero, C., Lecha, M., Mendez, M., De Verneuil, H., Mascaro, J. M. (2002) Description of a new mutation in hepatoerythropoietic porphyria and prenatal exclusion of a homozygous fetus. *Arch. Dermatol.* **138**, 957-960.

Granata, B. X., Parea, V. E., Melito, V. A., Teijo, M. J., Battle A. M., Rossetti, M. V. (2009) The very first description of a patient with hepatoerythropoietic porphyria in Argentina. Biochemical and molecular studies. *Cell. Mol. Biol. (Noisy-le-Grand)* **55**, 61-65.

Heinemann, I. U., Jahn, M., Jahn, D. (2008) The biochemistry of heme biosynthesis. *Arch. Biochem. Biophys.* **474**, 238-251.

Igarashi, K., Sun, J. (2006) The heme-Bach1 pathway in the regulation of oxidative stress response and erythroid differentiation. *Antioxid. Redox Signal.* **8**, 107-118.

Jackson, A. H., Sancovich, H. A., Ferramola, A. M., Evans, N., Games, D. E., Matlin, S. A., Elder, G. H., Smith, S. G. (1976) Macrocyclic intermediates in the biosynthesis of porphyrins. *Philos. Trans. R. Soc. Lond. B Biol. Sci*. **273**, 191-206.

Kong, J. H., Yang, L., Dessaud, E., Chuang, K., Moore, D. M., Rohatgi, R., Briscoe, J., Novit, B. G. (2015) Notch activity modulates the responsiveness of neural progenitors to sonic hedgehog signaling. *Dev. Cell* **33**, 373-387.

Lim, C. K., Rideout, J. M., Wright, D. J. (1983) Separation of porphyrin isomers by high-performance liquid chromatography. *Biochem. J.* **211**, 435-438.

Liu, L., Philipps, J., Bonkovski, H., Porphyrias Consortium of the Rare Diseases Clinical Research Network (2013) Hepatoerythropoietic porphyria. In: *GeneReviews*, eds. Pagon, R. A., Adam, M. P., Ardinger, H. H., pp. 1993-2015. University of Washington, Seattle, WA

Martínková, M., Kitanishi, K., Shimizu, T. (2013) Heme-based globin-coupled oxygen sensors: Linking oxygen binding to functional regulation of diguanylate cyclase, histidine kinase, and methyl-accepting chemotaxis. *J. Biol. Chem.* **288**, 27702-27711.

Meguro, K., Fujita, H., Ishida, N., Akagi, R., Kurihara, T., Galbraith, R. A., Kappas, A., Zabriskie, J. B., Toback, A. C., Harber, L. C., et al. (1994) Molecular defects of uroporphyrinogen decarboxylase in a patient with mild hepatoerythropoietic porphyria. *J. Invest. Dermatol*. **102**, 681-685.

Méndez, M., Rossetti, M. V., Batlle, A., Parera, V. (2005) The role of inherited and acquired factors in the development of porphyria cutanea tarda in the Argentinean population. *J. Am. Acad. Dermatol.* **52,** 417-424.

Mense, S., Zhang, L. (2006) Heme: a versatile signaling molecule controlling the activities of diverse regulators ranging from transcription factors to MAP kinases. *Cell Res*. **16**, 681-692.

Morán-Jiménez, M. J., Ged, C., Romana, M., Enríquez de Salamanca, R., Taiëb, A., Topi, G., D'Alessandro, L., de Verneuil, H., (1996) Uroporphyrinogen decarboxylase: complete human gene sequence and molecular study of three families with hepatoerythropoietic porphyria. *Am. J. Hum. Genet.* **58,** 712-721.

Morán, M. J., Fontanellas, A., Brudieux, E., Hombrados, I., de Ledinghen, V., Couzigou, P., de Verneuil, H., De Salamanca, R. (1998) Hepatic uroporphyrinogen decarboxylase activity in porphyria cutanea tarda patients: the influence of virus C infection. *Hepatology* **27,** 584-589.

Muñoz-Santos, C., Guilabert, A., Moreno, N., To-Figueras, J., Badenas, C., Darwich, E., Herrero, C. (2010) Familial and sporadic porphyria cutanea tarda: clinical and biochemical features and risk factors in 152 patients. *Medicine (Baltimore)* **89**, 69-74.

Nordmann, Y., Puy, H. (2002) Human hereditary hepatic porphyrias. *Clin. Chim. Acta* **325,** 17-37.

Ogawa, K., Sun, J., Taketani, S., Nakajima, O., Nishitani, C., Sassa, S., Hayashi, N., Yamamoto, M., Shibahara, S., Fujita, H., Igarashi, K. (2001) Heme mediates derepression of Maf recognition element through direct binding to transcription repressor Bach1. *EMBO J.* **20**, 2835-2843.

Padmanaban, G., Venkateswar, V., Rangarajan, P. N. (1989) Haem as a multifunctional regulator. *Trends Biochem. Sci*. **14**, 492-496.

Pinol-Aguade, J., Castells, A., Indacochea, A., Rodes, J. (1969) A case of biochemically unclassifiable hepatic porphyria. *Br. J. Dermatol.* **81**, 270-275.

Philips, J. D., Whitby, F. G., Kushner, J. P., Hill, C. P. (2003) Structural basis for tetrapyrrole coordination by uroporphyrinogen decarboxylase. *EMBO J.* **22**, 6225-6233.

Philips, J. D., Whitby, F. G., Stadtmueller, B. M., Edwards, C. Q., Hill, C. P. Kushner, J. P. (2007) Two novel uroporphyrinogen decarboxylase (URO-D) mutations causing hepatoerythropoietic porphyria (HEP). *Transl. Res.* **149**, 85-91.

Puy, H., Gouya, L., Deybach, J. C. (2010) Porphyrias. *Lancet* **375**, 924-937.

Raghuram, S., Stayrook, K. R., Huang, P., Rogers, P. M., Nosie, A. K., McClure, D. B., Burris, L. L., Khorasanizedeh, S., Burris, T. P., Rastinejad, F. (2007) Identification of heme as the ligand for the orphan nuclear receptors REV-ERBα and REV-ERBβ. *Nat. Struct. Mol. Biol.* **14**, 1207-1213.

Remenyik, E., Lecha, M., Badenas, C., Koszo, F., Vass, V., Herrero, C., Varga, V., Emri, G., Balogh, A., Horkay, I. (2008) Childhood-onset mild cutaneous porphyria with compound heterozygotic mutations in the uroporphyrinogen decarboxylase gene. *Clin. Exp. Dermatol.* **33**, 602-605.

Roberts, A. G., Elder, G. H., De Salamanca, R. E., Herrero, C., Lecha, M., Mascaro, J. M. (1995) A mutation (G281E) of the human uroporphyrinogen decarboxylase gene causes both hepatoerythropoietic porphyria and overt familial porphyria cutanea tarda: biochemical and genetic studies on Spanish patients. *J. Invest. Dermatol.* **104**, 500-502.

Rocchi, E., Gibertini, P., Cassanelli, M., Pietrangelo, A., Jensen, J., Ventura, E. (1986) Hepatitis B virus infection in porphyria cutanea tarda. *Liver* **6**,153-157.

Romana, M., Dubart, A., Beaupain, D., Chabret, C., Goossens, M., Romeo, P. H., (1987) Structure of the gene for human uroporphyrinogen decarboxylase. *Nucleic Acids Res.* **15**, 7343-7356.

Romeo, P. H., Raich, N., Dubart, A., Beaupain, D., Pryor, M., Kushner, J. P., Cohen-Solal, M., Goossens, M. (1986)

Molecular cloning and nucleotide sequence of a complete human uroporphyrinogen decarboxylase cDNA. *J. Biol. Chem.* **261,** 9825-9831.

Rossetti, M. V., Granata, B. X., Giudice, J., Parera, V. E., Batlle, A. (2008) Genetic and biochemical studies in Argentinean patients with variegate porphyria. *BMC Med. Genet.* **9**, 54.

Shimizu, T., Huang, D., Yan, F., Stranava, M., Bartosova, M., Fojtíková, V., Martínková, M. (2015) Gaseous O_2, NO, and CO in signal transduction: structure and function relationships of heme-based gas sensors and heme-redox sensors. *Chem. Rev.* **115**, 6491-6533.

Silva, P. J., Ramos, M. J. (2005) Density-functional study of mechanisms for the cofactor-free decarboxylation performed by uroporphyrinogen III decarboxylase. *J. Phys. Chem. B*. **109**, 18195-18200.

To-Figueras, J., Phillips, J., Gonzales-López, Badenas, C., Madrigal, I., Gonzáles-Romarís, E. M., Ramos, C., Aguirre, J. M., Herrero C. (2011) Hepatoerythropoietic porphyria due to a novel mutation in the uroporphyrinogen decarboxylase gene. *Br. J. Dermatol.* **165**, 499-505.

Wang, H., Long, Q., Marty, S. D., Sassa, S., Lin, S. (1998) A zebrafish model for hepatoerythropoietic porphyria. *Nat. Genet.* **20**, 239-243.

Whitby, F. G., Phillips, J. D., Kushner, J. P., Hill, C. P. (1998). Crystal structure of human uroporphyrinogen decarboxylase. *EMBO J.* **17**, 2463-2471.

Xu, W., Warner, C. A., Desnick, R. J. (1995) Congenital erythropoietic porphyria: identification and expression of 10 mutations in the uroporphyrinogen III synthase gene. *J. Clin. Invest.* **95**, 905-912.