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"PORPHYRIAS AND ASSOCIATED PATHOLOGIES. BIOCHEMISTRY AND MOLECULAR BIOLOGY"
PART II

To begin with, we wish to thank again Professor Dr. Raymond Wegmann "The Founding President" and Professor Dr. Jean-Michel Maixent, "The President", Editors-in-Chief of ***Cellular and Molecular Biology***[TM] for their generous invitation to become Guest- Editor and Co-Guest Editor of this special theme issue on ***"Porphyrias and associated pathologies. Biochemistry and Molecular Biology",*** which has been published in two Volumes, Part I, appeared in February 16 th, 2009 and now, Part II, scheduled for July 1 st 2009.

Although ***Cellular and Molecular Biology***[TM] has already published three others special issues on this subject, one in 1997, and two in January and December 2002, …*"to have another issue on "Porphyrins and Porphyrias" is not too much, since it belongs to one of the modern problems for which orphan drugs are requested"…* (quoted from Professor Dr. Raymond Wegmann, *Cellular and Molecular Biology*[TM], , 2002, **48**, 823).

We also wish to thank again Mr Mourad Fares, Executive Editor of CMB, for his support, composition and editing of the papers.

The articles put together in Part II of this theme issue, come from the highest experts in the world in this field, biologists, physicians, chemists, physics, all of them leading porphyrinologists, which will assure, to make Part II as well as it was with Part I, a highly important reference issue for the future. Therefore we are also deeply thankful to all the authors for their contributions and most important for their collaboration in making possible the production of this theme CMB issue on ***"Porphyrias and associated pathologies. Biochemistry and Molecular Biology"***

"Porphyrins and Porphyrias", since the middle of the 50's, porphyrinologists from all over the world have been meeting at least once or later more than once a year, mostly in Europe and USA, to discuss about the latest innovative results on the biochemistry, molecular biology, regulation and functional mechanisms of the enzymes involved at each step of the porphyrins biosynthesis, which have been isolated, cloned and even chrystalized, and its related diseases: the Porphyrias.

In 1997, Professor Robert Aquaron, attempted to list a number of the International Porphyrins and Porphyrias Meetings, mostly hold in Europe, since 1955, until then. However we should also recall and include the Gordon Research Conferences, started in 1968, occurring in USA, every two years (about 22) which were just mentioned as well as the Tetrapyrrole Discussion Group Meetings, gathering somewhere in Europe, much more often in UK, every nine months since 1967 (by now there have been about 33 meetings).

Porphyrins and Porphyrias, certainly, one of the most important and endless theme for discussion. Some of us have had the honour and pleasure of having been closely associated, known and being personally involved in Porphyrins and Porphyrias research for nearly 50 years by now, also having had the pionners in this field as our mentors, something to celebrate, so we will, after reading this special issue of CMB.

Porphyrins synthesis is one of the most fundamental attributes of all living cells.

Central to the fundamental processes of photosynthesis and respiration, are chlorophylls and haem, respectively, which are porphyrins, that is the reason why, Lemberg and Ledge in 1949, coined the expression *"porphyrins are the pigments of life"*, could it be anything more important than that?.

We would wish to partially quote one of us (Batlle, CMB [TM], 2002, **48**, 823), when saying that porphyrins are unique and intriguing molecules, historically having a geologic and a biologic medical chapter. The former going back many millions of years, when the formation of porphyrin-like compounds and porphyrins was contemporary with the development of life on earth. The latter started at the beginning of nineteen century when iron-free hematin was obtained by Scherer in 1941, after treating dried

blood with concentrated sulphuric acid. This pigment was later purified by Thudichum in 1867, who named it *"cruentine"* and described for the first time its *"splendid blood-red"* fluorescence. The term *"porphyrin"* was soon after coined by Hoppe Seyler in 1871.

As already stated, porphyrin biosynthesis is one of the most fundamental attributes of all living cells. Classical isotope tracer studies from the laboratories of Shemin, Rimington, Granick and Neuberger, identified the precursors and intermediates in the haem biosynthetic pathway marking the beginning of a whole new field of research in the biogenesis of these pigments. Then, most of the eight enzymatic steps involved were identified. Today all the enzymes have been cloned and sequenced and most of them have also been crystallized.

The porphyrin pathway is very finelly controlled. In most tissues and species, Aminolevulic Acid Synthetase (ALA-S) is the regulatory enzyme. Regulation occurs by feed back inhibition of ALA-S, so haem deficiency, owing to blocking the pathway at some step, as it happens in the Porphyrias, releases this inhibition. The term *"porphyria"*, gradually emerged after Stokvis(1889) reporting the death of an elderly women, excreting dark red urine after having received sulfonal.

Human porphyrias are specific inherited or acquired defects, each representing a partial failure of one of the seven enzymes beyond ALA-S and they are characterised by a typical excretion pattern of porphyrin intermediates.

We would like to recall that porphyrins are the only photosensitizers synthesized in the cells and the best examples of these endogenous sensitizers are the porphyrin intermediates formed and accumulated in the cutaneous porphyrias, producing the characteristic skin photosensitization.

Photodynamic Therapy (PDT) is a promising new modality of cancer treatment, which involves the combination of a photosensitizing agent, which is taken up selectively and retained by tumoural cells, and light of an appropriate wavelength. Separately, each of these factors is harmless by itself, though, when combined, in the presence of oxygen, cytotoxic reactive oxygen species are produced, leading to irreversible cellular damage, causing cell death and tumor destruction.

After either exogenous administration or endogenous synthesis, porphyrins finally accumulate in higher proliferative cells. Light energy absorbed by the photosensitizer (PS) can produce fluorescence. The tumour localizing properties of the PS have been extensively employed for the Photodetection (PD) and diagnosis, as well as for the PDT of tumours.

Photodynamic properties of porphyrins are a well known characteristic of these compounds. As already stated, a clear demonstration of their powerful photosensitizing properties, can be seen in the cutaneous porphyrias, where abnormal quantities of circulating porphyrins result in the typical skin photosensitivity.

One of the main developments in PDT, has been the novel approach using the precursor 5-Aminolevulinic Acid (ALA), leading to the endogenous synthesis of the active photosensitizer (PS) Protoporphyrin IX (PPIX), the so called ALA based PDT or ALA-PDT.

Besides PPIX, other PSs have been synthesized or extracted from natural products, to be used in PD or PDT.

In the paper *"Endogenous and exogenous Porphyrins as Photosensitizers in the HEP-2 Human Carcinoma Cell Line"* by M.G.Alvarez, N.E. Milanesio, V. Rivarola, E. Durantini, A. Battle and H. Fukuda, the photodynamic activity of three PSs: ALA-induced PPIX, the porphyrin derivative 5-(4-trimethylammoniumphenyl)-10, 5, 20-tris (2,4,6-trimethoxyphenyl) porphyrin (CP) and the molecular dyad porphyrin-C60 (P-C60), the last two incorporated into liposomal vesicles, was evaluated on Hep-2 human larynx carcinoma cell line.

When photosensitized with ALA and P-C60, chromatine condensation characteristic of apoptotic cell death was found; instead, 58 % of necrotic cells were observed with CP. The results show that in the Hep-2 cells, of the three PSs analyzed, the molecular dyad P-C60 was more efficient than CP and PPIX, and confirm that PDT can induce different mechanisms of cell death depending on the PS and the irradiation dose.

In the next paper *"ROS production by endogenously generated Protoporphyrin IX in murine leukemia cells"*, by B. Diez, R. Cordo Russo, M.J. Teijo, S. Hajos, A. Batlle and H. Fukuda, they studied the efficiency of PpIX synthetized from ALA on ROS generation, in the Vincristine resistant (LBR-V160), Doxorubicin resistant (LBR-D160) and sensitive (LBR-) murine leukemia cell lines. Cells were incubated with 1 mM ALA and then irradiated during different times with fluorescent light. Then, production of ROS was analyzed by flow cytometry using different fluorescent probes.

It was found that superoxide anion production in the three cell lines increased with irradiation time whereas no peroxide hydrogen was detected. Mitochondrial damage also increased in an irradiation time dependent manner, being higher in the Vincristine resistant line. Because the apoptotic cell death increased with irradiation time, the authors have shown that ROS are critical in ALA-PDT efficiency to kill malignant cells.

Clinical manifestation of porphyrias are often associated with exposure to precipitating agents, including polyhalogenated aromatic hydrocarbons, alcohol abuse, estrogens and steroids ingestion, stress and infection with Hepatitis C virus (HCV), less frequently, Hepatitis B virus (HBV). and association with infection with the Human Immunodeficiency virus (HIV).

The role that steroids play in the expression of housekeeping 5-Aminolevulic Acid Synthetase (ALA-S1) has been the subject of scrutiny for a number of years. The very earlier studies from Granick and others, showed that in addition to a female preponderance of acute attacks, numerous steroids of both natural and pharmaceutical origins could be linked to induction of attacks and to changes in the activity of ALA-S. In the paper **"***Functional analysis of the 5' regulatory region of the 5-aminolevulinate synthase (ALAS1) gene in response to estrogen"* BY N. du Plessis, M Kimberg, M G Zaahl, A Sadie, M Venter, L van der Merwe, A Louw & L Warnich, the authors investigated some factors influencing the clinical expression of porphyrias, primarily by altering the rate of heme synthesis. To date, no genotype-phenotype correlation has been made to explain the variable penetrance observed in variegate porphyria (VP) and other acute hepatic porphyrias. As first and rate determining gene in the heme pathway, *ALAS1*, appears to be an ideal candidate modifier. Previous studies established critical mechanisms for *ALAS1* regulation and a direct transcriptional response to drugs by defined drug-responsive enhancer sequences. They evaluated the effects of a possible oestrogen binding receptor at the five-prime end of ALAS1 gene. Its role appears to be in increasing the transcriptional response when oestradiol is present.

It is also known that in some porphyrias, mild to moderate hepatic iron overload plays a key role in its pathogenesis. Hemochromatosis is the commonest cause of primary iron overload and some mutations in the hemochromatosis gene (HFE), associated with hereditary Hemochromatosis, have been found to be more frequent in PCT.

M.V. Rossetti, M. Méndez, S. Afonso, E. Gerez, A. Batlle, A. Muñoz and V. Parera in their paper *"HFE gene mutations in patients with altered iron metabolism in Argentina"* have investigated the prevalence of C282Y, H63D and S65C mutations in 95 individuals bearing iron metabolism alterations to establish an early diagnosis of Hereditary Hemochromatosis (HH). Among this population, 58% carried mutations in the HFE gene. H63D mutation was found in 32.6% of the subjects (29.5% in heterozygocity, 3.15% in homozygocity). S65C mutation was only detected in the heterozygous form (5.3% of the patients), 2 of them carried also H63D mutation. C282Y in heterozygocity was found in 15.8% of the individuals. Their findings were consistent with the Mediterranean origins of the population of Argentina, where the commonest mutation was H63D.

Association of porphyrias with other pathologies, such as diabetes, lupus, leukaemia, Hansen's disease, cancer and autism has been reported. There is also association of porphyrias with the treatments used for other pathologies, such as estrogen-therapy in prostate cancer and hemodialysis in patients with renal failure.

Porphyrias are often multifactorial, therefore, knowledge of all risk or etiological factors in each patient is most important for the management of the disease.

The paper *"Association between Porphyria Cutanea Tarda and Beta Thalassemia Major"* By L. Barbieri, A. Macri, G.L. Palmieri, C. Aurizi and G. Biolcati, describes the first two cases of porphyria cutanea tarda (PCT) associated with beta-thalassemia major. The clinical course of two female patients affected by beta-thalassemia major was complicated by the onset of PCT. Both patients were also suffering from hepatitis C virus infection, iron overload and anaemia. The authors discuss about the role performed by some of these conditions in triggering overt PCT. An improvement of the clinical and biochemical picture of PCT was obtained with chloroquine therapy.

Porphyria cutanea tarda (PCT) is caused by inhibition of uroporphyrinogen decarboxylase (URO-D) activity in hepatocytes. Subnormal URO-D activity results in accumulation and urinary excretion of highly carboxylated porphyrins, uroporphyrin and heptacarboxyl porphyrin. Heterozygosity for mutations in the *URO-D* gene can be found in the familial form of PCT (F-PCT). Over 70 mutations of URO-D have been described so far, but very few have been characterized structurally. In the paper *"Structural*

and kinetic characterization of mutant human uroporphyrinogen decarboxylases", C. A. Warby, J. D. Phillips, H. A. Bergonia, F. G. Whitby, C. P. Hill, and J. P. Kushner, characterized 3 mutations in the *URO-D* gene found in patients with F-PCT. G318R, K297N and D306Y mutations. Expression of the D306Y mutation resulted in an insoluble recombinant protein. G318R and K297N have little effect on the structure or activity of recombinant URO-D, but the proteins displayed reduced stability *in vitro*.

A number of murine models of PCT centered around the administration of iron and compounds that induce transcription of cytochrome P450s have been developed. So, the influence of porphyrins and porphyrinogens themselves on metabolic alterations in hepatocytes has been difficult to distinguish from the effects of the compounds precipitating the porphyric state. The Utah team had previously developed a genetic murine model of PCT by crossing porphyria-susceptible *Urod+/-* mice with mice homozygous for deletion of the hemochromatosis gene (*Hfe*). The *Urod+/-, Hfe-/-* mouse spontaneously develops uroporphyria and allows studying the effects of porphyrinogen and porphyrin accumulation on enzymes and transporters involved in tetrapyrrole disposition without the confounding effects of exogenous factors. In their paper *"Longitudinal Study of a Mouse Model of Familial Porphyria Cutanea Tarda"*, D-D.Arch, H.A. Bergonia, L. Hathaway, J. P. Kushner, J.D. Phillips and M.R. Franklin, describe for the first time, a longitudinal study of *Urod+/-, Hfe-/-* from 8 weeks to 1 year.
The authors have shown that the first sigh of abnormal porphyrin biosynthesis in the *Urod+/-, Hfe-/-* mouse occurs at 8 to 10 weeks of age, a point at which iron accumulation in the liver due to the *Hfe-/-* genotype becomes significant, before total hepatic and urinary levels rise. In spite of the marked reduction of UROD activity, total hepatic heme synthesis remains normal although P450 heme is reduced, indicating that induction of P450 activity is not required for development of the porphyric phenotype. They are also the first to demonstrate that uroporphyrinogen is the dominant compound accumulating in the hepatic cytosol and that oxidation to uroporphyrin is required for transport across the cell membrane. Oxidation may also be required for transport across the lysosomal membrane. High hepatic levels of uroporphyrinogen are also associated with increased glutathione S-transferase activity and elevated mRNA of 2 transporters, *Abcc1* and *Abcc4*. However the nature of the transporters involved remains yet unknown, although they might not be members of the ABC transporter family. This rodent model of familial PCT affords the opportunity of studying changes in porphyrinogen and porphyrin accumulation and transport in the absence of exogenous factors which can alter P450 activity and transmembrane transporters.

Acute intermittent porphyria (AIP), the most common acute hepatic porphyria, is an autosomal dominant disorder with low penetrance that results from a partial deficiency of hydroxymethylbilane synthase (HMBS), the third enzyme in the heme biosynthetic pathway. The disease is clinically characterized by acute neurovisceral attacks that are precipitated by several factors including certain drugs, steroid hormones, alcohol and fasting. Early diagnosis and counselling are essential to prevent attacks, being mutation analysis the most reliable method to identify asymptomatic carriers in AIP families. In the paper "*Identification and characterization of HMBS gene mutations in Spanish patients with Acute Intermittent Porphyria*" , Méndez M., Morán-Jiménez M.J., Gómez-Abecia S., García-Bravo M., Garrido-Astray M.C., Fontanellas A., Poblete-Gutiérrez P., Frank J. and Enríquez de Salamanca R. have investigated the molecular defect in 15 unrelated Spanish AIP patients. Mutation analysis of the *HMBS* gene revealed a total of fourteen mutations including six novel ones, two of them were on the same allele in one patient. The novel mutations were three missense (R26L, R173G and D178H), two frameshift (c.749_765dup and c.874insC) and one intronic deletion (IVS12+3_+11delAGGGCCTGT). RT-PCR and sequencing demonstrated that the intronic mutation caused abnormal splicing and exon 12 skipping. Prokaryotic expression of the novel missense mutations showed that only D178H had significant residual activity. These findings will facilitate the accurate identification of presymptomatic AIP carriers in these families and they further emphasize the molecular heterogeneity of AIP in Spain.

The majority of the known AIP mutations are restricted to one or just a few AIP families, with the exception of the frequent occurring R116W mutation which is found in 19/80 Dutch AIP families. The Dutch R116W families are well documented with extended pedigrees (up to 1750) making possible to study the haplotypes in these families. In paper "*Evidence for an ancestral founder of the common R116W mutation in the hydroxymethylbilane synthase gene in acute*

intermittent porphyria in the Netherlands." By F.W.M. de Rooij, F.G. Kavelaars, H. Koole-Lesuis, J.H.P. Wilson, the authors have investigated haplotype heterogeneity in the Dutch R116W families.

They have found that this common R116W haplotype based on 7 single nucleotides polymorphisms strongly suggest that the relatively high frequency of the R116W mutation in Dutch AIP patients is due a founder effect.

The heterogeneity described in the Sweden and Norwegian patients and the homogeneity found in the Dutch R116W carriers is compatible with origin of the mutation in Scandinavia and later introduction into the Netherlands.

Acute Intermittent Porphyria (AIP), alike Hepatic Coproporphyria (HCP) and Variegate Porphyria (VP) are acute hepatic porphyrias, but both HCP and VP could also exhibit cutaneous photosensitivity; that is why they are also known as mixed porphyrias. So, these patients, might show either acute or cutaneous signs or both simultaneously.

In the paper *"Role of multidrug-resistance protein 2 in coproporphyrin transport: results from experimental studies in bile fistula rat models"* by V. Moriondo, S. Marchini, P. Di Gangi, M.C. Ferrari, F. Nascimbeni, E. Rocchi and P. Ventura, from indirect data obtained from animal and human models, the authors have suggested a possible role for Multidrug Resistance–associated Protein 2 (MRP2) and other MRPs in hepatocyte excretion of Coproporphyrin (CP). It has been suggested a key role for the MRP system as a possible regulator of CP traffic and its accumulation in normal and pathological conditions. Alteration in these systems, as those found cholestatic disease, may play an important role in triggering the clinical expression of porphyria in individuals with underlying mutations, leading to CP accumulation and may also help to explain the phenotypic heterogeneity in patients affected by different forms of porphyrias.

Variegate Porphyria (VP), the other mixed Porphyria, very common in South Africa, is an autosomal dominant disorder found worldwide but is rare in Italy. The group from Italy, E. Di Pierro, P. Ventura, V. Brancaleoni, V. Moriondo, S. Marchini, D. Tavazzi, F. Nascimbeni, M.C. Ferrari, E. Rocchi and M.D. Cappellini in their paper *"Clinical, Biochemical and Genetic Characteristics of Variegate Porphyria in Italy"* provided an overview of clinical, biochemical and genetic background of 33 Italian VP patients diagnosed in the last fifteen years. Interestingtly, about 70% of the patients had experienced clinical symptoms: 43.4% had photosensivity, 8.7% acute attacks and 47.8% both. Among these 33 patients, 14 different mutations were identified. Of these only 6 mutations had been previously described in other countries and 8 have been identified for the first time in Italy. In contrast, normal faecal protoporphyrin excretion was highly predictive of silent phenotype. Normal urinary excretion of PBG and ALA, anticipated the absence of neurovisceral symptoms. This paper represents the first compilation of data on genotype-phenotype relation in Italian patients with VP. The experimental design of this paper was to identify genotype-phenotype correlations for VP based on their sample of 33 Italian subjects. While the sample size is relatively small, it is valuable, because it represents the first such study on an Italian population, thus providing value to the porphyria community as a whole.

Deficiency in Protoporphyrinogen oxidase (PPOX), the penultimate enzyme in the haem pathway is the cause of VP. In the paper *"The expression of Protoporphyrinogen Oxidase in human tissues"* by A.V. Corrigal, J.A.C.Campbell, K. Siziba, R.E. Kirsch and P.N. Meissner, the expression of protoporphyrinogen oxidase in a variety of human organs has been documented by immunohistochemical means at the light microscopy level in order to shed light on its inter- and intra-organ distribution. The distribution of haem biosynthetic enzymes in tissues is still poorly understood. This study evaluates the histochemical distribution of PPOX in a variety of human tissues. As might have been expected the strongest expression was found in hepatocytes and kidney tubular cells. The paper provides a very good introduction to PPOX. In addition to the metabolically active tissues, as already indicated, a number of other organs showed region-specific changes in enzyme content, consistent with the tissue energy and the haem demand. The authors correctly indicate that there are some significant sites of haem synthesis in addition to the liver and bone marrow, and that it should be born in mind in studies related to haem porphyrin dynamic and flux.

In the paper *"Identification of a recurrent mutation in the protoporphyrinogen oxidase gene in Swiss patients with variegate porphyria: clinical and genetic implications"* by Anne-Moon van Tuyll van Serooskerken, Xiaoye Schneider-Yin, Renske J. Schimmel, Reno Bladergroen, Jasmin Barman, Pamela Poblete-Gutiérrez, Michel van Geel, Jorge

Frank, and Elisabeth I. Minder, the authors have studied thirty unrelated VP index patients and families currently assisted in the Swiss Porphyrin Reference Laboratory in Zürich. In 16 of a total of 24 genetically tested families, they have detected a recurrent mutation in the *PPOX* gene, identified as 1082-1083insC, reflecting a high prevalence of 67%. Haplotype analysis has shown that this mutation arose on a common genetic background, representing a novel founder mutation in the Swiss population. Knowledge on the carrier status within a family does not only allow for adequate genetic counseling but also for prevention of the potentially life-threatening acute porphyric attacks. Therefore, future molecular screening in Swiss VP patients might be facilitated by first seeking for this recurrent mutation 1082-1083insC.

We know that Ferrochelatase (FECH) catalyses the last step in heme biosynthesis and a deficiency of this enzyme results in the hereditary disorder of erythropoietic protopoprhyria (EPP) The third intron of human FECH gene contains according to NCBI, a poly-C (11) and a poly-T (24) tracts which are located approximately 900 bp upstream from the known splice modulating single nucleotide polymorphism (SNP) IVS3-48 c/t. J. Barman, X. Schneider-Yin, R. Mamet, N. Schoenfeld and E.I. Minder in their paper *"Variations in the length of Poly-C and Poly-T tracts in intron 3 of the human Ferrochelatase gene"*, have observed that during the course of mutation analysis in the *FECH* gene among EPP patients, there were variations in the length of the poly-C and poly-T tracts. To study these variations, they have analyzed a total of 54 individuals of Swiss and Israeli origins. Among them, 37 were control subjects (23 individuals with the genotype t/t and 14 with the genotype c/t), 10 were unrelated EPP patients (genotype c/M) and 7 were unrelated asymptomatic mutation carriers (genotype t/M). They have found that the length of poly-C tract varied from 10 to 16, that of poly-T tract from 22 to 24. Statistic analysis showed that the low-expressed FECH allele (IVS3-48c) was associated with poly-C12, C13 and C15 and poly-T22. The segregation of poly-C and poly-T tracts was studied in two Israeli EPP families. Instabilities, as seen by both insertion and deletion of one nucleotide between two generations, were observed only in the poly-T tract. However, the function of the poly-C and poly-T tracts are yet to be explored.

Paper *"Exclusion of ferrochelatase gene mutations in patients with seasonal palmoplantar keratoderma"* by Renske J. Schimmel, Anne-Moon van Tuyll van Serooskerken, Reno S. Bladergroen, Maurice A.M. van Steensel, Michel van Geel, Suzanne G.M.A. Pasmans, and Jorge Frank, is another very interesting paper related to EPP. In fact, it is related to the recently reported occurrence of predominantly seasonal palmar (SPK) and palmoplantar keratoderma (PPK) in patients with homozygous mutations in the *FECH* gene, suggesting that palmoplantar keratoderma might be a clinical sign of EPP. PPKs are a heterogeneous group of genetic skin diseases and include a seasonal variant, erythrokeratolysis hiemalis et estivalis (EH). Because the skin symptoms in EH are similar to those reported for recessive EPP The authors have examined the *FECH* gene in three unrelated Dutch Caucasian patients with a previous diagnosis of EH in whom mutations in several other genes had been excluded. In this paper, sequencing analysis of the entire coding regions and the adjacent splice sites of the *FECH* gene in these patients revealed the absence of mutations. The authors concluded that their data largely exclude the possibility that *FECH* mutations might be responsible for the PPK skin phenotype observed in EH.

In the excellent and very valuable Review on *"The molecular genetics of erythropoietic protoporphyria"*, G.H. Elder, L. Gouya, S.D. Whatley, H. Puy, M.N. Badmington and J-C. Deybach, have reviewed the current knowledge of the molecular genetics of 259 EPP patients from France and UK. They have found that more than 95% of unrelated patients have FECH deficiency while about 2% have X-linked dominant protoporphyria (XLDPP) caused by gain-of-function mutations in the *ALAS2* gene. Most FECH-deficient patients are compound heterozygotes for a hypomorphic allele (*FECH* IVS3-48C) and a deleterious *FECH* mutation, that, together lower FECH activity to around 30% of normal. The frequency of the IVS3-48C allele varies between populations, ranging from less than 1% to 45%. About 4% of unrelated FECH-deficient patients are compound heterozygotes or homozygotes for rare *FECH* mutations and have lower enzyme activities. Acquired somatic mutation of *FECH* secondary to myeloid disease may rarely cause EPP. Interestingly, the risk of liver disease is increased in XLDPP and in FECH-deficient patients who are hetero- or homoallelic for rare *FECH* mutations. Inherited FECH-deficient EPP is an autosomal recessive disorder with some families showing pseudodominant inheritance; the proportion of such families being determined by the population frequency of the IVS3-48C allele.

There is a number of good animal models for EPP, in particular the Griseofulvin (GRIS) mice, where chronic administration of GRIS to mice, induces pathological changes analogous to those found in patients with EPP-associated liver injury. In paper *"Hepatic damage and oxidative stress induced by Griseofulvin"*, by M. del C. Martínez, S.G. Afonso, R.P. Meiss, A.M. Buzaleh and A. Batlle, the authors aimed to further characterize the GRIS mice model studying the effect of GRIS on different metabolisms in mice feeding GRIS (0-2.5%, 7 and 14 days). PP IX accumulation in liver, blood and feces, induction of ALA-S activity, and a low rate of Holo/Apo tryptophan pyrrolase activity were observed, indicating a reduction in the free heme pool. Progressive liver injury was reflected by the aspect and the enlargement of liver and the induction of hepatic damage. Liver redox balance was altered due to porphyrin high concentrations; as a consequence, the antioxidant defense system was disrupted. Heme oxygenase (HO) was also induced, however, at higher concentrations of GRIS, the free heme pool should have been so depleted that HO would not be necessary. The authors concluded that their GRIS mice model of EPP produced liver alterations similar to those found in EPP patients.

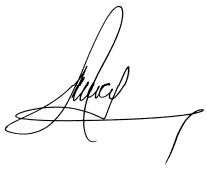
It is well known that several anaesthetics are unsafe drugs for acute hepatic porphyrias. In their paper *"Sevoflurane: Its action on Heme Metabolism and Phase I Drug Metabolizing System"*, R. Sampayo, J.V. Lavandera, A. Batlle and A.M. Buzaleh, have evaluated the effect of the anaesthetic Sevoflurane on heme pathway and the drug metabolizing Phase I system in mice. To this end, animals received different doses of the anaesthetic (1-2 ml/kg) and were sacrificed at different times (5-60 min). Data revealed important alterations in the enzymes involved in AIP, such as an induction in hepatic ALA-S activity and a diminished PBG Deaminase activity in liver and blood 20 minutes after Sevoflurane administration to mice in a dose of 1.5 ml/kg. HO activity was also induced, indicating the onset of oxidative stress. Total CYP levels and CYP2E1 expression were enhanced. As a consequence of these events, heme free pool would be depleted. The authors concluded that their findings in mice would suggest that Sevoflurane should be used with caution and careful control in porphyric patients, as a very likely unsafe drug.

We have already noted above that clinical manifestation of porphyrias are often associated with exposure to precipitating agents, including polyhalogenated aromatic hydrocarbons, alcohol abuse, estrogens and steroids ingestion, stress, infections with several virus, HCV, HBV, HIV and a good number of the so called porphyrinogenic drugs. Nothing more useful than paper *"Safe and Probably Safe Drugs in Acute Hepatic Porphyrias"*, by U. Stölzel, C. Brosche, C. Koszka, T. Stauch, A. Teubner and M. Doss, giving the more recent overview on drugs that are safe and probable safe to be recommended in patients with acute hepatic porphyrias and representing a compilation of the four so far existing lists.

The last paper is an important contribution from Dr Felix de Rooij and Professor Paul Wilson. As it can be read in their Introduction, they felt that it would be a great opportunity, to have the Proceedings of the Porphyrins and Porphyrias International Meeting hold at Rotterdam in 2007, published in Part II of this Theme issue of the scientific Journal Cellular and Molecular Biology™, where you will find the Program, as well as all the Abstracts of the presentations. In this way, it will be possible to use it as a reference for our next papers.

Finally, as we had expected for the papers included in Part I, we also expect that papers here presented proved not only information, but they will also give the sense of their outstanding contribution to our knowledge in this field, and, again, we wish to emphasize that they will constitute another very important and unique reference issue for the future in this theme.

Alcira Batlle
Guest-Editor



María Victoria Rossetti
Co-Guest-Editor