Erythropoietic (Congenital) Porphyria: A Rare Abnormality of the Normoblasts

By Rudi Schmid, Samuel Schwartz and R. Dorothy Sundberg

Recent studies\(^1\) have emphasized that in human beings, photosensitivity occurs as a result of two fundamentally different disturbances of porphyrin metabolism. In porphyria cutanea tarda, in which the excessive porphyrin formation is believed to take place in the liver, symptoms are seldom manifest before adult life.\(^1\) In porphyria erythropoietica (congenital, photosensitive), where large amounts of porphyrins are formed in the bone marrow, red urine and photosensitivity are either present shortly after birth or develop early in life.\(^1\) In addition, anemia and splenomegaly usually appear sooner or later in these cases, and may be accompanied by hypertrichosis and erythrodermia.

In studying bone marrow sections of a case of congenital porphyria (case Petry), with the fluorescence microscope, Borst and Königsdörffer observed that developing erythropoietic cells contained unusually large amounts of porphyrin.\(^2\) Subsequent studies have shown that in these cells, most of the porphyrin appeared to be concentrated in the nuclei,\(^1\) but at the same time, normoblasts in various stages of maturation were found, which lacked any red fluorescence.\(^1\) In the present study, observations are reported on bone marrow samples from 5 cases of erythropoietic (congenital, photosensitive) porphyria.

Material and Methods

Unstained bone marrow slides, prepared from material aspirated from the sternum were obtained from 5 female children with erythropoietic porphyria. Two of these patients have been hospitalized and extensively studied in Minneapolis\(^4\) (Dept. of Medicine file No. 71 and 76). The other 3 girls were diagnosed and studied in Rochester,\(^4\) New York,\(^5\) Detroit\(^6\) and Chicago\(^7\) respectively.\(^7\) In three of the five instances, bone marrow specimens were available before and at various intervals after splenectomy.\(^8\)

All five patients had a hemolytic anemia with splenomegaly, varying erythrodermia and hypertrichosis, excretion of red urine having been observed either shortly after birth or within the first 3 years of life. In four of the five cases, splenectomy was performed. In all

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instances, the operation resulted in at least temporary improvement in photosensitivity and in decreased urinary porphyrin excretion. In one case splenectomized in 1949, great improvement in photosensitivity has persisted over a 5 year period of observation, the hemolytic anemia has disappeared and urinary porphyrin excretion has remained considerably lower as compared to pre-splenectomy values.4 12

Quantitative data for porphyrin concentration in red blood cells and spleen are available in 3 of the cases, for the liver in 2 cases, and for bone marrow before and after splenectomy in one case.1 3 4 12 All 5 patients excreted large amounts of uroporphyrin in the urine and smaller amounts in the feces, the converse being true for coproporphyrin.

For fluorescence microscopy, an especially constructed microfluorospectrometer, an essential part of which is a Steinheil microanalytical spectrograph, was used. As light source a modified Bol water-cooled high pressure GE AH-6 mercury vapor lamp was employed, with a primary Corning filter No. 5-58 (5113) to isolate the 405 mp line. An orange Corning filter No. 3-67 (3452) was placed between the objective and the eyepiece of the microscope. After studying and photographing the unstained bone marrow slides in the fluorescence microscope, the areas of the smears so examined were marked with a Zeiss slide marker and were subsequently stained with Wright’s stain for detailed morphologic study of the individual cells.

For demonstration of hemoglobin in developing erythropoietic cells of the bone marrow, a staining method employing benzidine as an essential ingredient was used, as indicated by Garcia-Blanco.13 In addition, the unstained slides were studied by near ultraviolet light microscopy, employing monochromatic light of 405 mp, as proposed by Wilkins.14 With this method, cellular structures containing hemoglobin appear dark, because of the strong absorption by the Soret band of hemoglobin, whereas the remainder of the cell is translucent for the 405 mp light. For fluorescence and near ultraviolet microscopy, the same microscope and light source could be used merely by changing the filter systems.

RESULTS

Essentially similar observations have been made in all 5 patients studied. In the fluorescence microscope, intense red fluorescence was seen in many polychromatophilic and orthochromatophilic normoblasts. Pronormoblasts or erythroblasts showed little fluorescence, basophilic normoblasts somewhat more. In all instances the red fluorescence of the normoblastic nuclei was much more intense than that of the cytoplasm, the latter frequently exhibiting only a barely recognizable pink hue (figs. 1 and 2). Numerous polychromatophilic erythrocytes with slight red fluorescence were also present in the bone marrow (fig. 1). Leukocytes, thrombocytes and mature erythrocytes did not exhibit red fluorescence. In all five cases, it was observed that some normoblasts in all developmental stages failed to exhibit any red fluorescence. There appeared to be a clear separation between fluorescing and nonfluorescing normoblasts, since intermediary forms could not be detected in fresh marrow preparations. After the slides had been exposed for some time to light and air, the fluorescence intensity of most cells faded considerably and the red color often changed to an orange or even yellow-green tinge. Estimation of the percentage of fluorescing normoblasts of the total normoblasts present in the marrow in the different cases gave results ranging from 30 to 70 per cent. It was observed that developing erythropoietic cells of the fluorescing variety tended to cluster together in small groups as did the nonfluorescing variety in separate groups.
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FIG. 1

Unstained bone marrow smear of a patient with erythropoietic porphyria (case No. 76, Minneapolis) photographed in the fluorescence microscope \( \times 800 \). Red fluorescence (seen as white) is particularly intense in the normoblastic nuclei. A polychromatophilic erythrocyte (right upper corner) exhibits a lesser degree of red fluorescence. Reproduced with permission from the A.M.A. Arch. Int. Med. 83: 176, 1954.

FIG. 2

Group of fluorescing normoblasts from a patient with erythropoietic porphyria (Detroit case), photographed in the fluorescence microscope \( \times 2000 \).

FIG. 3

Bone marrow smear from a patient with erythropoietic porphyria (Chicago case) stained with benzidine. Abnormal normoblasts contain dark staining nuclear inclusion bodies. Nuclei of normal immature normoblasts appear light granular; those of mature normal normoblasts white (left lower corner). Hemoglobin-containing erythrocytes stain dark.

FIG. 4

Bone marrow smear from a case of erythropoietic porphyria (case No. 76, Minneapolis) stained with Wright’s stain. Note the central inclusion in the nucleus of the large normoblast. All three normoblasts contain hemosiderin granules (prussian blue staining). Reproduced with permission from the Acta haemat. 10: 153, 1953.

In the peripheral blood, a small percentage of erythrocytes exhibited distinct pink fluorescence, similar in intensity to that seen in polychromatophilic erythrocytes in the bone marrow. Upon staining with Wright’s stain, all of the fluorescing non-nucleated cells in the blood were found to be polychromatophilic erythrocytes. It appeared, however, that both before and after splenectomy, there were additional polychromatophilic red cells in the blood which did not exhibit red fluorescence. A numerical determination of the ratio of fluorescing to nonfluorescing polychromatophilic erythrocytes in the peripheral blood was...
not possible because of the often extremely low fluorescence intensity of some of these cells.

In the three cases where bone marrow specimens were available before and after splenectomy, removal of the spleen did not appear to change the ratio of fluorescing to nonfluorescing normoblasts, and postoperatively, the intensity of red fluorescence in individual normoblasts was comparable to the preoperative one. However, after splenectomy, the total number of normoblasts as compared to all nucleated cells present in the marrow was substantially reduced. Occasional normoblasts found in the circulating blood appeared to be of either the fluorescing or nonfluorescing variety.

Morphologic studies of the fluorescing variety of normoblasts have demonstrated a hitherto unrecognized abnormality. When the benzidine staining method was employed, these cells were observed to contain in the center of the nucleus usually single, but occasionally multiple, dark inclusion bodies. Such nuclei contrasted sharply with the fine granular or white appearance of the nuclei in normal normoblasts stained with this technic (fig. 3). Light staining with Wright's stain sufficed to bring out the nuclear inclusions in some of the cells (fig. 4), but the benzidine method was found to be preferable for demonstration of this structural variation.

In contrast to the light color of the remaining nuclear substance, the inclusion bodies stained dark with benzidine, suggesting that they contained hemoglobin (fig. 3). To test this possibility further, unstained normal and abnormal normoblasts were studied by near ultraviolet absorption spectroscopy (figs. 5 and 6). The results obtained with this optical method were strikingly similar to the findings observed after staining with benzidine. In the normal normoblasts, the nuclei were either entirely translucent or, in more immature cells, exhibited many fine dark granules (fig. 6). In contrast, the central nuclear inclusions of the abnormal cell variety showed very strong absorption in the Soret band which, coupled with the dark staining with benzidine, indicated that they indeed contained hemoglobin.

By studying the same cells, first in the fluorescence microscope, then by near ultraviolet absorption spectroscopy, and ultimately after staining with Wright's

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Fig. 5.—Unstained bone marrow smear from a case with erythropoietic porphyria (case No. 76, Minneapolis), photographed in the near ultraviolet light microscope. Only structures containing hemoglobin appear dark. Note the two normoblasts, both containing nuclear inclusions with hemoglobin.
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Fig. 6.—Unstained bone marrow smear from a patient with erythropoietic porphyria (Chicago case) photographed in the near ultraviolet light microscope X600. Most normoblasts belong to the normal variety, except for the cell in the center, exhibiting a dark nuclear inclusion.

Fig. 7.—Same group of unstained cells as figure 6, now photographed in the fluorescence microscope X1200. Only the normoblast with the nuclear inclusion exhibits red fluorescence. Normal normoblasts are nonfluorescing. The fluorescence to the left of the abnormal normoblast originates from a damaged cell, the nature of which could not be identified after staining.

It was demonstrated that the morphologic abnormality of the nucleus was present only in those normoblasts exhibiting porphyrin fluorescence (figs. 6 and 7). Erythropoietic cells without red fluorescence exhibited the usual nuclear structure. In all cases studied, normoblasts belonging to both the normal or the abnormal variety could be observed in various stages of maturation.

DISCUSSION

The close relationship between hemoglobin and excessive porphyrin formation in erythropoietic porphyria has been discussed previously. The porphyrin content of the red blood cells and particularly of the bone marrow was found to be remarkably high, and in one instance it was possible to isolate crystalline uroporphyrin I from a small sample of bone marrow obtained by needle aspiration of the sternum.

The observation that most of the marrow porphyrin is contained in the nuclei of developing red cells may be of fundamental significance and raises the question as to the relative contribution of the nucleus and the cytoplasm in the formation of porphyrin and heme. Various investigators have shown that hemoglobin appears to be a regular constituent of the normoblastic nucleus. Employing special staining techniques, Macallum, Villa, Knoll, Israélis, and Garcia-Blanco have demonstrated what was believed to be hemoglobin in the parachromatin of the more immature normoblasts. Metcalf and Wilkins made similar observations by absorption microspectroscopy. Stern and coworkers found 19 per cent of the dry weight of fowl red cell nuclei to consist of hemoglobin. The present observations with regard to nuclear porphyrin concentration and inclusion bodies containing hemoglobin appear to support the
concept that the normoblastic nucleus may play a more active role in porphyrin
and heme pigment formation than has hitherto been recognized. On the other
hand, it has been conclusively shown that hemoglobin synthesis from glycine
can take place in immature non-nucleated erythrocytes. It is of great in-
terest in this regard, that Shemin's recent findings point to a close relationship
between pyrrol and nucleic acid metabolism, the biochemical crossroad being
marked by the intermediary δ-amino-levulinic acid.

Borst and Königsdorffer in studying fixed bone marrow sections of the case
Petry, as obtained at autopsy, failed to observe red fluorescence in the nuclei
of erythropoietic cells; in fact, they clearly stated that the red fluorescence was
solely localized in the cytoplasm. It is difficult to explain the apparent contradic-
tion between their earlier observations and the findings presently reported, but
it is perhaps important that they had only fixed tissue sections available, whereas
in the present study, unfixed, usually fresh, marrow smears were at hand, in
which the porphyrin fluorescence and the cellular structures were wholly pre-
served. Gillman also studied the bone marrow of a patient with erythropoietic
porphyria in the fluorescence microscope, but in this instance the orange fluores-
cence indicative of porphyrin was so diffuse that an accurate localization was
not possible.

The concept has been discussed elsewhere that during the process of cellular
maturation, uroporphyrin formed in the normoblastic nuclei is released into the
plasma at a relatively rapid rate. It is possible that such a release of porphyrin
occurs at the time of physiological karyolysis or nuclear expulsion. It is interesting
in this regard that in the three splenectomized cases, Howell-Jolly bodies,
present in a great number of circulating erythrocytes consistently failed to
exhibit any red fluorescence.

Borst and Königsdorffer's observation that in Petry's bone marrow sections
some of the normoblasts appeared to be lacking red fluorescence, has been
confirmed in all our cases. The consistent finding of two varieties of normoblasts,
one exhibiting red fluorescence and nuclear alterations, the other nonfluorescing
with normal nuclear morphology, strongly suggests the presence of two differ-
ent lines of erythropoietic cells, best designated as normal and abnormal. It
seems reasonable to assume that cells of the abnormal line in all probability
carry the "inborn error of metabolism."

No consistent relationship was found between the severity of clinical symp-
toms, including uroporphyrin excretion, and the ratio of normal to abnormal
normoblasts in the bone marrow. However, a decrease in compensatory normo-
blastic hyperplasia of the marrow following splenectomy resulted at least temporaril
in decline of porphyrin excretion. There has been some degree of
improvement in photosensitivity in all four cases, although in the
Chicago case it is still too early to evaluate the results of splenectomy and in the
Detroit case our information regarding photosensitivity is fragmentary. De
Marval and Pons, who reported the first case of erythropoietic porphyria in
which splenectomy was performed, observed disappearance of the hemolytic
anemia and partial remission of photosensitivity following removal of the
spleen. It is of interest that Schmidt advocated splenectomy for a case which
he observed, because of the simultaneous presence of hemolytic anemia, but
it is not known whether the operation was actually carried out.
<table>
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<th>Anemia</th>
<th>Splenomegaly</th>
<th>Erythropoiesis</th>
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<td>no statement</td>
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<td>present</td>
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<td>present</td>
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<td>present</td>
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<td>present</td>
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<td>present</td>
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<td>F</td>
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<td>F</td>
<td>English</td>
<td>3</td>
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<td>present</td>
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After submission of this paper, the following recently published case has come to the authors' attention. (Pozzam, Mario: Porfiri congénita con iperemolisi, Acta Paediatrica Matina, 6, 6: 995, 1953). A girl, born of healthy parents, has excreted pink urine since birth. Hemolytic anemia with splenomegaly had first been observed at the age of 6 months. At the age of 2½ years, the patient had extensive photodermatitis with severe scarring of the exposed parts of the body, erythrodontia, hypertrichosis and hemolytic anemia with hepatosplenomegaly. The urine contained large amounts of porphyrins.

* This list is based on a critical review of the cases cited by Turner and Obermayer in 1938 and those subsequently reported. Cases have been included in the table only if the published data clearly established the erythropoietic nature of the disease. Considerable difficulty was encountered due to the frequency with which the same case was reported by several authors even at an interval of many years. The numbers at the right of the author's name refer to the bibliography at the end of the text.

† Hydroa aestivale, red urine, hypertrichosis.
Splenomegaly and hemolytic anemia, or at least increased hemolysis, are, sooner or later, regular features of this disease. In addition to the 5 cases presently considered a hemolytic process has regularly been discovered in those instances where it was looked for. It was stated that Petry, at the time of his death, suffered from pernicious anemia in addition to erythropoietic porphyria. Aldrich and co-workers raised justified doubt as to the correctness of this interpretation of the autopsy findings, and suggested that Petry may have had an unrecognized hemolytic anemia with splenomegaly. In carefully analyzing Borst and Königsdörffer's description of their findings, and from study of the colored illustrations of their bone marrow preparations, one cannot exclude the possibility that their so-called "megaloplasts" were identical with the cells which we have designated as the abnormal normoblasts.

In the light of the recently acquired better understanding of erythropoietic (congenital) porphyria, we have undertaken a critical review of the original protocols of all the cases reported in the literature. In 1936 Turner and Obermayer had collected from the literature a total of 86 cases believed to have had "congenital" porphyria and a number of cases have since been added. In studying these reports, particular attention was given to the following points:

1. Onset of photosensitivity in early life.
2. Absence of abdominal or neurological symptoms.
3. Excretion of red or dark urine containing excessive amounts of porphyrin, at birth or during the first years of life.
4. Erythrodoncia.
5. Hypertrichosis or hirsutism.
6. Evidence of increased hemolysis and/or splenomegaly.

A combination of two or more of the following reported findings were regarded as suggestive of a hemolytic process or hemolytic anemia: splenomegaly, normochromic-normocytic anemia, increased reticulocyte counts or outspoken polychromatophilia of circulating red cells, circulating normoblasts, normoblastic hyperplasia of the bone marrow, increased fecal urobilinogen excretion.

Considering the above listed criteria, it was believed that the reported data permitted the diagnosis of erythropoietic (congenital) porphyria beyond reasonable doubt in a total of 34 cases, namely in 18 out of the 86 cases listed by Turner and Obermayer and in 16 cases reported since 1936. These cases and some of their main features are summarized in table 1. It should be pointed out, that in several instances, splenomegaly was either not present at the time of the first examination or a statement as to the size of the spleen was omitted from the first report; enlargement of the spleen, however, became a conspicuous feature as the patients grew older, and it was mentioned in subsequent reports. On the other hand, in one patient splenomegaly was present in early childhood, but could no longer be demonstrated ten years later.

From table 1 it is seen that 19 of the patients reported were female and 15 male. This is contrary to previous belief that the disease had a substantially higher incidence in the male sex. In several instances more than one case was observed in the same generation of a family, but no evidence was found to support the belief that the disease occurs in subsequent generations. In this regard the report by Kench et al. is of considerable interest. Their patient gave birth to two apparently normal children, one of whom is alive, whereas the other died shortly after a premature delivery. During their first few days of life both babies excreted large amounts of porphyrins, which were believed to have been passively transferred through the placenta. Fraenkel related that the wife of the case Petry had two spontaneous abortions, each in the fourth month of pregnancy. The fetuses were carefully studied, but no abnormalities of pigment metabolism could be detected. Günther had suspected that the disease was recessively inherited, and most textbooks have subsequently adopted this version. However, since there are no reports con-
cerning reproduction in patients with erythropoietic porphyria, with the exception of the two above mentioned instances.\textsuperscript{20-14} it appears to be impossible to evaluate fully the role of genetic factors in this disease. The data presently available permit only the conclusion of occasional familial occurrence.

In addition to these 34 cases in which the diagnosis could be established beyond reasonable doubt, certain other cases have been reported in which some features suggested erythropoietic porphyria.\textsuperscript{16-14} The data given, however, were inadequate to establish an unequivocal diagnosis. It is possible that some of these patients had a hepatic cutaneous porphyria (porphyria cutanea tarda), which, in rare instances, has been observed to become manifest in childhood or during adolescence.\textsuperscript{11,12}

Some additional comment is needed with regard to the patient originally reported by Ashby\textsuperscript{19} and later by Garrod.\textsuperscript{30} At the age of 25, during her first pregnancy, this woman still exhibited moderate photosensitivity and outspoken hemolytic anemia with splenomegaly and normoblastic hyperplasia of the bone marrow.\textsuperscript{31} Large amounts of porphyrins were isolated and some were crystallized from the urine, erythrocytes, plasma and feces. However, two years later, in the course of another pregnancy, she developed signs of hepatic insufficiency, including icterus, and she expired shortly after delivery. At autopsy, the liver showed a coarse nodular cirrhosis, and the bone marrow was reported as exhibiting “extensive myeloid hyperplasia and no significant erythroid hemopoiesis,” but it is not clear whether this meant virtual absence of normoblasts from the bone marrow. No red fluorescence was observed in the liver, although uroporphyrin I was obtained by extracting hepatic tissue. Unfortunately, fluorescence microscopic studies of the bone marrow and peripheral blood were not attempted. From the earlier reports and from the study in 1947, there is no doubt that this patient had erythropoietic porphyria, but the cirrhosis and apparent bone marrow failure observed at autopsy are difficult to understand. It is conceivable that an intercurrent hepatitis or some toxic process was responsible for these terminal changes. The reported findings in the liver, i.e., the absence of red fluorescence in spite of the presence of increased amounts of uro-type porphyrins, are fundamentally different from those observed in hepatic photosensitive porphyria,\textsuperscript{1} but they are compatible with our observations in three cases of erythropoietic porphyria, where liver biopsy specimens were available.

**SUMMARY AND CONCLUSIONS**

1. In 5 patients with erythropoietic (congenital) porphyria, unstained bone marrow preparations were studied and photographed by fluorescence and absorption microscopy. The same marrow slides were also studied after ordinary staining.

2. Two morphologically different varieties of normoblasts were observed, which were designated as normal and abnormal cells. Normoblasts of the abnormal variety exhibited nuclear inclusion bodies containing hemoglobin.

3. Red fluorescence, indicative of porphyrin, was found only in normoblasts belonging to the abnormal cell variety. Normal normoblasts failed to exhibit red fluorescence.

4. The red fluorescence originated predominantly from the normoblastic nucleus. The fluorescence intensity of the cytoplasm was usually very low. Polychromatophilic erythrocytes also exhibited only weak fluorescence.

5. These findings indicate the coexistence of two different lines of normoblasts. The nuclei of cells belonging to the abnormal line probably form excessive amounts of porphyrins and may release them into the plasma in the course of cellular maturation. It is believed that these cells carry the abnormal trait representing the “inborn error of metabolism.”

6. A critical review of all cases of porphyria, reported as “congenital,” has
shown that on the basis of the presented data, it was possible to establish the diagnosis of erythropoietic (congenital) porphyria beyond reasonable doubt in but 34 instances. Nineteen of the patients were female, fourteen were male. In a few families, more than one case was observed in the same generation, but in no instance was the disease recognized in subsequent generations.

7. Erythropoietic porphyria is a congenital disease, entirely distinct from hepatic photosensitive ("cutanea tarda") porphyria. No evidence has been found of a genetic mixture of these two forms of porphyria.

**Summario e Conclusiones in Interlingua**

1. In 5 patientes con (congenite) porphyria erythropoietic, non-colorate preparatos de medulla ossee esseva studiate e photographate per medio del microscopio a fluorescentia e del microscopio absorptional. Le mesme preparatos eseva etiam studiate post coloration secundo le methodos ordinari.

2. Esseva observate duo varietates de normoblastos con differentias morphology. Le duo varietates esseva distinguite como normal e anormal. Normoblastos del varietate anormal exhibiva inclusiones nuclear que contineva hemoglobina.

3. Fluorescentia rubie, que indica le presentia de porphyrina, esseva observate solo in normoblastos del varietate de cellulas anormal. Normoblastos normal non exhibiva ulle fluorescentia rubie.

4. Le fluorescentia rubie emanava predominantemente ab le nucleos del normoblastos. Le intensitate del fluorescentia del cytoplasma esseva generalmente multo level. Etiam le erythrocytos polychromatophile exhibiva solo un level fluorescentia.

5. Iste constatationes indica le coesistentia de duo differente lineages de normoblastos. Le nucleos del cellulas a lineage anormal pare formar excessive quantitates de porphyrinas que illos libera, in le curso de lor maturatiom, a in le plasma. Nos crede que iste cellulas es portatores del tracto que representa le "innate error metabolic."

6. Un revista critic de omne le casos de porphyria que es reportate in le litteratura como "congenite" monstrava que le datos usate per le autores permitte un undubitabile diagnose de (congenite) porphyria erythropoietic in solo 34 casos. Decenove del 34 patientes involvit esseva feminas, dece-cinque masculos. In alcin familias plus que un caso esseva observate in un sol generation, sed il ha nulle exemplo de un constatation del morbo in generationes posterior.

7. Porphyria erythropoietic es un morbo congenite. Illo es integremente distincte de hepatic porphyria photosensitive (porphyria cutanea tarda). Nos ha trovate nulle datos que indicarea le occurrentia de un mixtura genetic de iste duo formas de porphyria.

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Erythropoietic (Congenital) Porphyria: A Rare Abnormality of the Normoblasts

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