Erythropoietic protoporphyria patients in Slovenia

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ABSTRACT

Background: There are only scarce epidemiological data on the prevalence of erythropoietic protoporphyria (EPP) in a given population. The aim of this study was to assess the prevalence of EPP within the Slovenian population.

Materials and methods: The patients were selected by routine examination of photosensitive patients and by studying hospital records. A quantitative spectrophotometric method was used to assess protoporphyrin, with values larger than 530 nm/l considered elevated.

Results: 32 EPP patients were detected, which allows us to estimate the prevalence of EPP in Slovenia at 1.75 per 100,000 inhabitants.

KEY WORDS

erythropoietic protoporphyria, patients, Slovenia, prevalence

Introduction

In the literature only scarce data are available on the prevalence of erythropoietic protoporphyria (EPP) in different populations. The prevalence in the United Kingdom is estimated at one in 130,000, and in Northern Ireland at one in 79,000 (1). There are rather ample data on EPP cases from departments and laboratories involved in research on various aspects of porphyrias, but these data cannot be used to ascertain the prevalence in a given population. In view of this, we decided to assess the prevalence of EPP in Slovenia, which, with its barely 2 million inhabitants and a relatively well-established health service, offers a good opportunity to carry out such a study.

The first unequivocal clinical case of erythropoietic protoporphyria (EPP, OMIM 1777000) was published in 1953 by Kosenow and Treibs (2). Two subsequent reports followed in 1961: Langhof et al. (3) described multiple patients in one family, whereas the report by Magnus (4) has received the most publicity and also introduced the term *erythropoietic protoporphyria,* which is now accepted worldwide.

Photosensitivity, which is a key symptom of *erythropoietic protoporphyria*, results from accumulation of protoporphyrin (PP) in the red blood cells due to inherited deficiency of the enzyme *ferrochelatase (FeCH)* (5). The mode of inheritance is mostly autosomal dominant with a reduced penetrance (6), but autosomal recessive inheritance has also been reported (7). One of the reasons for incomplete penetrance is an intronic single nucleotide polymorphism (SNP) IVS3-48T/C,

Table 1. Protoporphyrin values, UV sensitivity grade (+ mild, ++ medium, +++ severe) and chronic symptoms (OP = orange peel skin, LH = lichenification, F = furrows on the lips, VK = verruciform keratoses on the knuckles) in patients with erythropoietic protoporphyria.

Patient	Erythrocyte protoporphyrin (nmol/l)	UV sensitivity (acute photodermatosis)	Chronic symptoms
P1	938	+	OP LH F
P2	1,292	+	OP LH F
P3	690	+	OP LH F
P4	12,478	++	OP LH F VK
P5	6,814	++	OP LH F VK
P6	9,593	++	OP LH F VK
P7	26,900	+++	OP LH F VK
P8	23,150	+++	OP LH F VK
P9	20,286	+++	OP LH F VK
P10	774	+	OP LH F
P11	1,413	+	OP LH F VK
P12	14,980	+++	OP LH F VK
P13	21,200	+++	OP LH F VK
P14	7,280	+	OP LH F VK
P15	18,700	+	OP LH F VK
P16	540	++	OP LH VK
P17	732	+	OP LH
P18	761	+	LH VK
P19	11,434	++	OP LH F VK
P20	9,640	+++	OP LH F VK
P21	8,640	++	OP LH
P22	12,868	+++	OP LH F VK
P23	22,266	+++	OP LH F VK
P24	46,286	+++	OP LH F VK
P25	5,130	++	OP LH F VK
P26	550	+	OP LH VK
P27	923	+	LH VK
P28	1,052	+	OP
P29	21,400	+++	OP LH F VK
P30	9,913	++	OP LH F VK
P31	12,800	++	OP LH F VK
P32	1,784	+	OP LH F VK

which modulates the use of a constitutive aberrant acceptor site (8) whose mechanism is operative in addition to the established mutation.

Larger series of patients started to appear soon after 1961: Haeger Aronsen and Krook in Sweden (9), Suurmond in Holland (10), Schmidt in Denmark (11), Heilmeyer and Clotten in Germany (12), and Mirzoeva in Russia (13). In the United States, cases were published by Peterka (14), Harber (15), Redeker and Bronow (16), Reed (17), De Leo (18), and others. The first reports on EPP in Slovenia were published in 1969 (19) and 1972 (20), followed by a more extensive report in 1981 (21). During the last 10 years or so, larger series of EPP patients have been reported worldwide by clinical departments and laboratories where molecular biologic investigations were also being performed. However, these and similar reports do not meet the criteria required for epidemiological studies.

Materials and methods

The diagnosis was based on patients' histories of photosensitivity, clinical findings, and family backgrounds, and on the quantitative assays of protoporphyrin (PP) in the peripheral red blood cells (RBCs). The spectrophotometric tests were performed as proposed by Rimington (22) in the porphyrin laboratory of the Department of Dermatology in Ljubljana. The investigation was temporarily suspended because the principal investigator was absent from Slovenia for 12 years, and it was resumed in the second half of the 1990s. Since then, the biochemical tests have been carried out at the Biochemical Laboratory of the General Hospital in Maribor.

Detecting patients. After the spectrophotometric assay for porphyrins was introduced at the Department of Dermatology's laboratory in Ljubljana, patients with expressed photosensitivity were investigated. Relatives of patients with elevated values of PP in the RBCs were also invited for checkups. A number of Slovenian dermatologists cooperated in detecting photosensitive patients. Patients' charts at Slovenian departments of dermatology were also studied. We believe that we have included all the manifest EPP patients in Slovenia and thus fulfill the criteria for calculating the prevalence.

Control group. A control group of 32 volunteers, without a personal or family history of EPP or other

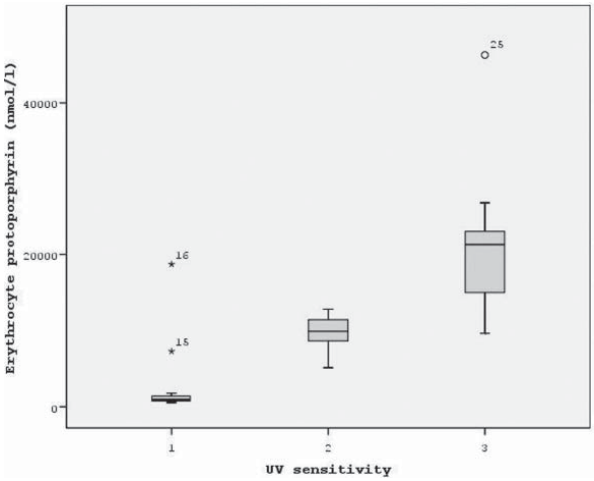

Figure 1. The range of erythrocyte protoporphyrin (nmol/l) by different UV sensitivities (1 mild, 2 medium, 3 severe).

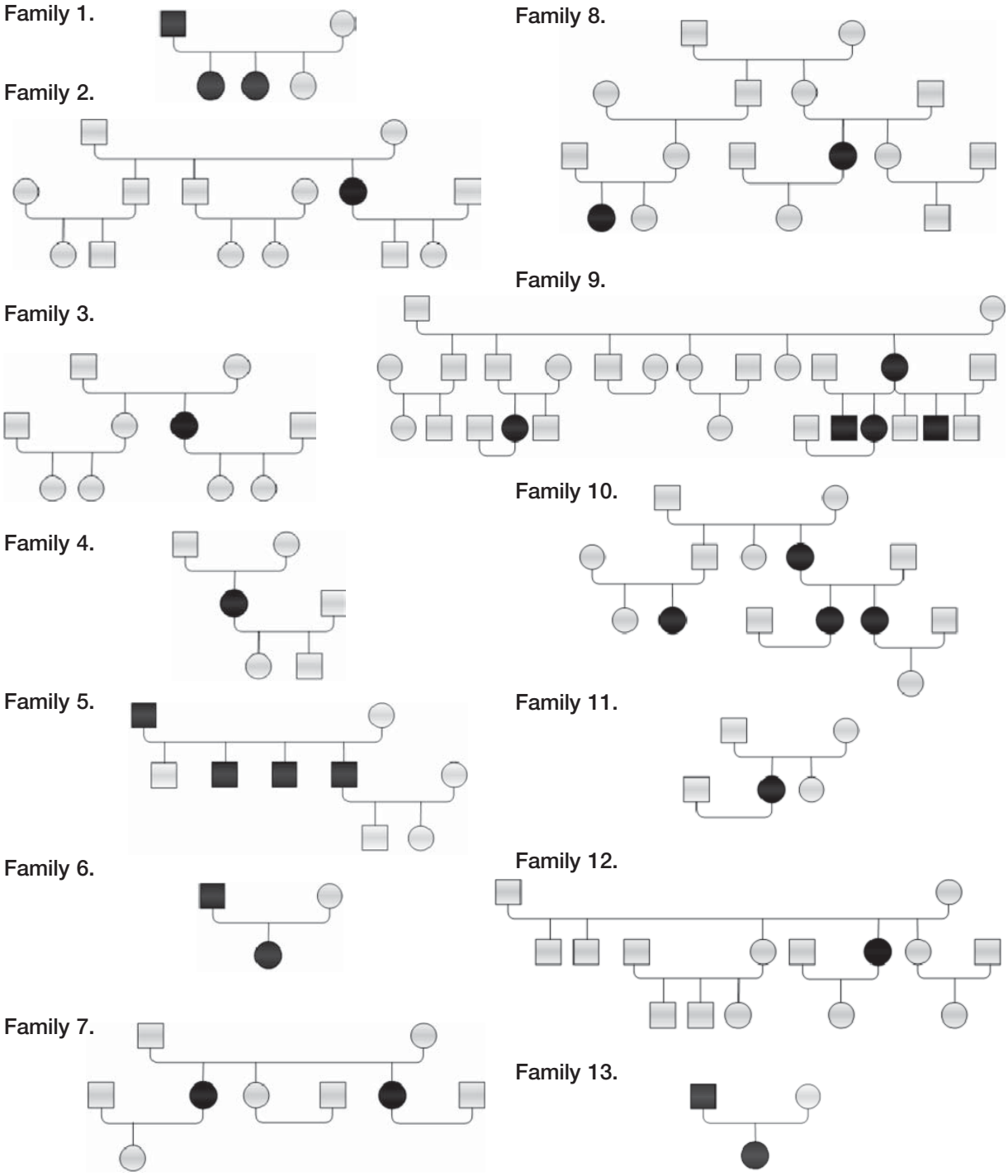

Figure 2. Family backgrounds of Slovenian EPP families (black shading indicates EPP patient).

forms of porphyria, photosensitivity, lead poisoning, iron deficiency, chronic anemia, or hemolytic disorders, was selected from dermatological patients hospitalized at the Department of Dermatology at the Maribor Teaching Hospital and quantitative assays of PP in the peripheral RBCs was performed.

Statistical analysis. The descriptive statistics were calculated for acute photosensitivity and erythrocyte PP. Because the data were not normally distributed, a Spearman (rank) correlation test was used for determining correlation between acute photosensitivity and erythrocyte PP. A nonparametric Kruskal-Wallis test was

Table 2. Erythrocyte protoporphyrin values in the control group.

Control	Erythrocyte protoporphyrin (nmol/l)
C1	184
C2	222
C3	318
C4	307
C5	209
C6	311
C7	248
C8	85
C9	184
C10	163
C11	431
C12	203
C13	365
C14	220
C15	0
C16	85
C17	98
C18	169
C19	222
C20	274
C21	382
C22	281
C23	320
C24	483
C25	170
C26	226
C27	156
C28	219
C29	490
C30	87
C31	222
C32	294

performed for assessing the difference in erythrocyte PP among three grades of acute photodermatosis.

Results

Values above 530 nmol/l were recognized as elevated, with the PP values in patients ranging from 550 to 46,286 nmol/l, the mean statistical value being 10,725 nmol/l (± 10,368 nmol/l) and the median value 9,616 nmol/l. Altogether, 32 persons displayed elevated PP values and photosensitivity, and they were therefore considered EPP patients (Table 1). The PP values in the control group ranged from 0 to 490 nmol/l, the mean statistical value being 238 nmol/l (± 114 nmol/l) and the median value 222 nmol/l (Table 2).

A statistically significant correlation between acute photosensitivity and erythrocyte PP values was established using Spearman's rank correlation coefficient ($r_S = 0.849$; $p < 0.01$). Because the coefficient is close to +1, there is a fairly strong positive relationship between the variables. The erythrocyte PP values of patients with mild acute photodermatosis varied between 550 and 18,700 nmol/l, with a median value of 938 nmol/l. Patients with medium acute photodermatosis had minimum erythrocyte PP values of 5,130 nmol/l and maximum values of 12,800, with a median value of 9,913 nmol/l. Patients with severe acute photodermatosis had erythrocyte PP values between 9,640 nmol/l and 46,286 nmol/l, with a median value 21,300 nmol/l (Fig. 1). Using the Kruskal-Wallis test, the observed difference in erythrocyte PP among the three grades of acute photodermatosis is significant ($H = 22.37$; $p < 0.01$). The results show that more acute photodermatosis leads to higher erythrocyte PP.

The patients belonged to 13 families: in 3 the autosomal dominant mode of inheritance was evident and in 5 families incomplete penetrance was observed, whereas in 5 instances there was only one affected member per family. The family backgrounds are presented in Figure 2. The prevalence was estimated at 1.75 per 100,000 inhabitants.

Discussion

With regard to publications, EPP ranked third among the porphyrias, following *cutanea tarda* and *acuta intermittens,* but statistically comparable data are not available. In our study the latent cases represented a problem because they did not display increased PP values, but they still expressed a certain degree of photosensitivity. Chronic cutaneous symptoms were not a reliable criterion because experience shows that the skin of persons exposed to UV irradiation over many years can mimic chronic EPP lesions to a certain extent. On the other hand, patients that avoid UV light (mostly females) may display a clinically almost normal skin surface. Fluorescent microscopy of diluted peripheral blood could help detect latent cases because fluorescent erythrocytes are normally not observed. Nevertheless, a few can be observed in iron deficiency, lead poisoning, certain anemias, other porphyrias, pellagroid, and other conditions, but this is due to zinc-chelated PP, not PP as seen in EPP. An enzymatic test for lymphocyte ferrochelatase activity may also help to detect latent cases. In the symptomatic patient, activity on the order of 30% of normal or lower is to be expected, whereas in latent cases the deficiency generally exhibits ferrochelatase activities above 60 to 70% of normal.

Detection of new patients and latent cases is of great importance today because people are exposed to a number of drugs and substances that may trigger the pathological metabolism of porphyrins, increase pho-

tosensitivity, and provoke hepatobiliary disease. This epidemiological data will be used to create a plan for monitoring EPP patients for early detection of hepatobiliary complications. Finally, our further efforts are directed to performing molecular analysis in order to detect the causal genetic mutations and contribution of a single-nucleotide polymorphism to the genetic predisposition for EPP in Slovenian EPP patients.

REFERENCES

1. Black MM, Gawkrodger DJ, Seymour CA, Weisman K. Metabolic and Nutritional Disorders. In: Champion RH, et al., editors. Textbook of dermatology. 6th ed. Oxford: Blackwell 1998; p. 2597.

2. Kossenow W, Treibs A. Lichtüberampfindlichkeit und Porphyrinämie. Z Kinderheilk 1953;73:82–92.

3. Langhof H, Müller H, Rietschel L. Untersuchungen zur familiären protoporphyrinemischen Lichturticaria. Arch Klin Exp Derm 1961;212:306–18.

4. Magnus IA, Jarret A, Pankard et al. A new porphyria syndrome with solar urticaria due to porphyrinemia. Lancet 1961;II:448–51.

5. Gouya L, Puy H, Lamoril et al. Inheritance in erythropoietic protoporphyria: a common wild-type ferochelatase allele variant with low expression accounts for clinical manifestations. Blood 1999;93:2105–10.

6. Lamoril J, Boulechfar S, de Verneil H et al. Human erythropoietic protoporphyria: Two point mutation in the ferrochelatase gene. Biochem Biophys Res Commun 1991;2:594–9.

7. Whatley SD, Mason NG, Khan M et al. Autosomal recessive EPP in United Kingdom: prevalence and relationship to liver disease. JMG Online. J Med Gen 2004;41:e105.

8. Gouya L, Puy H, Robreaui et al. The penetrance of dominant EPP is modulated by expression of wildtype FeCH Nat Genet 2002;30(1):27–8

9. Haeger Aronsen B, Krook G. EPP. A study of known cases in Sweden. Acta Med Scand 1966;179;Suppl 445.

10. Suurmond D, Van Stevenick J, Went LN. Some clinical and fundamental aspects of EPP. Br J Dermatol 1970;82:323–8.

11. Schmidt H, Snitker BG,Thomsen K, Lintrup J. EPP. A clinical Study on 29 cases in 14 families. Arch Dermatol 1974;110:58–64.

12. Langhof H, Heilmeyer L, Clotten R, Rietschel L. Die erythropoietische Protoporphyrie. Dtsch med Wschr 1964;89:1289-93.

13. Mirzoeva MG. K voprosu o EPP. Vestn Dermatol Venerol. (Moscow), 1969;43:73–5.

14. Peterka ES, Fusaro RM, Runge WJ et al. EPP. Clinical and laboratory features in 7 new cases. JAMA 1963;193:1036–42.

15. Harber LC, Fleischer AS, Baer RL. *Erythropoietic protoporphyria.* JAMA 1964;189:1911-6.

16. Redeker AG, Bronov R, Sterling S. Erythropoietic protoporphyria. S Afr J Lab Clin Med 1963;9:235–8.

17. Reed WB, Wuepper KD, Epstein JH et al. EPP. A clinical and genetic study. JAMA 1970;214:1060–6.

18. De Leo VA, Poh-Fitzpatrick M, Mathews-Roth M et al. EPP. 10 years experience. Am J Med 1976;60:8–22.

19. Kansky A. Eriropoetska protoporfirija. Zdrav Vestn 1969;38:439–42.

20. Kansky A. Erythropoietic protoporphyria. A report of five cases. Int J Dermatol 1972;11:21–5.

21. Kansky A, Berčič M. Erythropoietic protoporphyria in Slovenia, Epidemiologic study. Dermatologica 1981;163:232–8.

22. Rimington C, Sweinsson L. The spectrophotometric determination of UP. Scand J Clin Lab Invest 1950;2: 209–16.

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