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Clinical and biochemical characteristics and genotype–phenotype correlation in Finnish variegate porphyria patients

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Variegate porphyria (VP) is an inherited metabolic disease resulting from the partial deficiency of protoporphyrinogen oxidase, the penultimate enzyme in the heme biosynthetic pathway. We have evaluated the clinical and biochemical outcome of 103 Finnish VP patients diagnosed between 1966 and 2001. Fifty-two per cent of patients had experienced clinical symptoms: 40% had photosensitivity, 27% acute attacks and 14% both manifestations. The proportion of patients with acute attacks has decreased dramatically from 38 to 14% in patients diagnosed before and after 1980, whereas the prevalence of skin symptoms had decreased only subtly from 45 to 34%. We have studied the correlation between *PPOX* genotype and clinical outcome of 90 patients with the three most common Finnish mutations I12T, R152C and 338G→C. The patients with the I12T mutation experienced no photosensitivity and acute attacks were rare (8%). Therefore, the occurrence of photosensitivity was lower in the I12T group compared to the R152C group ($P=0.001$), whereas no significant differences between the R152C and 338G→C groups could be observed. Biochemical abnormalities were significantly milder suggesting a milder form of the disease in patients with the I12T mutation. In all VP patients, normal excretion of protoporphyrin in faeces in adulthood predicted freedom from both skin symptoms and acute attacks. The most valuable test predicting an increased risk of symptoms was urinary coproporphyrin, but only a substantially increased excretion exceeding 1000 nmol/day was associated with an increased risk of both skin symptoms and acute attacks. All patients with an excretion of more than 1000 nmol/day experienced either skin symptoms, acute attacks, or both.

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Introduction

Variegate porphyria (VP (MIM 176200)) is an inherited metabolic disease that results from the partial deficiency of protoporphyrinogen oxidase (PPOX, (E.C.1.3.3.4)), the penultimate enzyme in heme biosynthesis.¹ PPOX catalyses the six-electron oxidation of protoporphyrinogen IX to the planar, fully conjugated macrocycle protoporphyrin IX in

the inner membrane of the mitochondrion and requires oxygen for its activity.² The PPOX activity is decreased to approximately half of the normal level in heterozygous VP patients.³ VP is inherited as an autosomal dominant trait displaying incomplete penetrance.⁴

The biochemical abnormalities found in VP patients include overproduction and increased excretion of porphyrins and porphyrin precursors. Faecal excretions of copro- and protoporphyrins are usually elevated together with urinary excretions of uro- and coproporphyrins. Plasma fluorescence spectrum shows an emission maximum at 626 nm, which is specific for VP.^{5–8} The sensitivity of these

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tests in symptom-free individuals is, however, less than 80%. Urinary porphobilinogen (PBG) and delta-aminolevulinic acid (ALA) are elevated during an acute attack and remain mildly elevated in remission in about 50% of patients.⁹

Clinical manifestations of VP include photosensitivity and acute neurovisceral attacks resembling other acute porphyrias. Photosensitivity manifests as skin fragility and blistering in sun-exposed areas. Excess porphyrins in plasma and/or skin interact with light energy inducing a photo-toxic reaction and tissue damage.¹⁰ Symptoms of autonomic neuropathy include abdominal pain, vomiting, constipation, hypertension, and tachycardia.^{11,12} Peripheral neuropathies usually manifest as pain in the extremities or in the back and weakness that may progress to paresis.¹³ In the past, 17–38% of patients experienced acute attacks requiring hospitalisation,^{11,14,15} but milder symptoms of porphyria are more common occurring in 30–40% of patients.¹² Acute attacks are often induced by precipitating factors such as drugs, alcohol, infection, fasting, or the menstrual cycle. The clinical onset of the disease usually occurs after puberty but probably more than 50% of the carriers of the affected gene remain symptom-free throughout their lives.¹¹

The human PPOX-cDNA has been cloned from the human placental cDNA library¹⁶ and the PPOX gene mapped to chromosome 1q23.^{17,18} The gene is 5.5 kb in size including a 660 bp promoter region, and the coding region (1.5 kb) is spread over 13 exons.¹⁹ To date 111 mutations have been reported in the PPOX gene worldwide and no mutational hot spots have been identified.^{20–42} Thirty-eight (34%) of the mutations are small insertions or deletions, 44 (40%) are missense mutations, 17 (15%) change invariant nucleotides at splice sites, 1 (1%) is a gross deletion, and 11 (10%) produce stop codons.

In Finland to date, 143 VP patients, belonging to 21 families,^{11,12,41} have been biochemically and clinically well characterised. According to our data, the prevalence of VP is approximately 1.9:100 000 in the Finnish population of 5 000 000. Six of the Finnish VP mutations are family-specific and found so far only in Finland, whereas the major

mutation (R152C), which was identified in 11 (52%) of the 21 Finnish VP families, has also been reported in France and in the USA.^{29,31} As previously reported,^{40,41} six of the mutations have been expressed in prokaryotic and eukaryotic cell systems. The PPOX activities of the mutated polypeptides were markedly reduced ($\leq 5\%$, Table 1) confirming that the mutations are responsible for the disease.

In this study, we have evaluated the clinical and biochemical outcome of 103 Finnish VP patients with mutations R152C, I12T, 338G→C, 78insC, IVS2-2a→c, 470A→C and 1203A→C. We have investigated the correlation between PPOX genotype and phenotype for the three most common Finnish mutations R152C, I12T and 338G→C. We have studied, (1) whether the occurrence of acute attacks or skin photosensitivity correlates with the mutation type, (2) whether the patients' biochemical characteristics differ depending on the mutation type, and (3) whether the occurrence of symptoms can be predicted by mutation type and/or biochemical tests in remission.

Subjects and methods

Patients and biochemical analyses

Since 1966, we have conducted a systematic follow-up of all Finnish patients known to have VP and informed them of the precipitating factors. For 14 of 21 VP families, ancestors could be traced back to the 18th or 19th centuries using church registers.¹¹ Of the 143 VP patients diagnosed to date, 31 were deceased before 1966 and did not participate in the follow-up. Seven of them had died of an acute attack and an additional four had experienced acute attacks.¹² In addition, four subjects under 14 years of age and one homozygous patient were excluded. Four subjects could not be traced for this study.

The diagnosis of VP was based either on mutation analysis ($n=60$), characteristic clinical symptoms with elevated faecal protoporphyrin excretion ($n=68$),⁴³ typical plasma fluorescence emission spectrum ($n=14$),⁴⁴ low lymphocyte PPOX activity in each family ($n=25$)³ and/or pedigree analysis ($n=6$). The mean PPOX activity measured from the

Table 1 VP patients with PPOX defects by mutation type

					Residual activity (%) ^b			
		Mutation	Exon	Outcome	E. coli	COS-1	Families	Patients
1	R152C	454C→T	Exon 5	R152C	5%	5%	11	67
2	I12T ^a	35T→C	Exon 2	I12T	3%	1%	2	12
3	338G→C	338G→C	Exon 4	Deletion of exon 4	5%	0	2	11
4	Other	78insC	Exon 2	Frameshift	0	0	1	5
		IVS2-2 a→c	IVS 2	34 bp retention of intron 2	0	0	1	2
		470A→C	Exon 5	Deletion of exon 5 and 19 bp retention of intron 5	1%	0	1	1
		1203A→C	Exon 11	L401F	N.D.	N.D.	1	2
		unknown					2	3
Total							21	103

^aThe mutation I12T co-segregated with the polymorphism P256T (767C→G) in both families. ^bStudies described in Kauppinen *et al.* and von und zu Fraunberg *et al.*^{40,41} N.D., not done.

patients' lymphocytes was 2.8 ± 1.0 SD nmol/h/mg protein (range 1.1–6.2, normal 3.9–6.0, $n=28$).

PBG and ALA were measured using ALA/PBG Column test (Bio-Rad, CA, USA) based on Mauzerall and Granick.⁴⁵ Until 1988, urinary excretions of uro- and coproporphyrin and faecal excretions of copro- and protoporphyrin were performed according to Rimington⁴⁶ and Holti *et al.*⁴⁷ Since 1988 all measurements were performed using high-pressure liquid chromatography (HPLC).^{43,48} The mean urinary copro- and uroporphyrin values and faecal copro- and protoporphyrin values of patients did not differ systematically when the method of measurement was changed. All biochemical measurements were performed in adolescence or adulthood (14 to 83 years). Porphyrins and porphyrin precursors were measured during remission of acute symptoms but in the presence, or absence, of skin disease.

Biochemical data were not obtained from 20 subjects (four symptomatic and 16 asymptomatic), of whom 14 were only screened with mutation analysis. Information about acute attacks was obtained from hospital records and personal interviews for all patients who had had acute attacks requiring hospitalisation between 1929 and 1966. Since 1966 the criteria for an acute attack were the acute nature of symptoms, urinary excretion of PBG at least five times above the upper limit of normal, and severe abdominal or other pain associated with one or more additional typical porphyric symptoms.^{11,12} The patients were examined clinically or interviewed to evaluate skin fragility in sun-exposed areas.

For mutation analysis (Table 1) DNA was isolated from blood leukocytes, amplified using polymerase chain reaction (PCR) -technique and either analysed using restriction digestion, whenever a specific enzyme was available, or by direct sequencing. The analyses were repeated at least twice for each sample in the presence of negative and positive controls.⁸ Informed consent was obtained for all DNA testing, and the study protocol was approved by the Ethical Committee of the Department of Medicine, University Central Hospital of Helsinki.

Statistical methods

Fischer's exact test was used for the comparison of categorical variables. Continuous variables were analysed using

Mann–Whitney *U*-test, when two groups were compared, or Kruskal–Wallis one-way ANOVA, when more than two groups were compared simultaneously. Logistic regression with maximum likelihood estimation as optimisation criteria was employed to evaluate the association between dichotomous outcome variables (eg occurrence of skin symptoms or acute symptoms) and covariates (eg mutation group and biochemical tests). Statistical calculations were performed with SPSS version 10.04 and NCSS 2000.

Results

The study group consisted of 103 VP patients (36 male, 67 female, age 14–79 years), of whom 27 had experienced acute attacks, 41 photosensitivity and 14 both symptoms. Forty-nine were symptom-free throughout the follow-up period from 1966 to 2001. Twelve patients died during the follow-up. Fifty-two per cent of the patients experienced clinical manifestations before or during the follow-up period (Table 2). The overall frequency for skin symptoms was 40%, and for acute attacks 27%, respectively. The proportion of patients with acute symptoms had decreased dramatically from 38 to 14% among individuals diagnosed before and after 1980, whereas the prevalence of skin symptoms had decreased only subtly from 45 to 34% (Table 3). The decrease in acute symptoms was even more prominent among male patients, since none of the 17 patients diagnosed after 1980 had experienced acute attacks, whereas six of the 20 patients diagnosed before 1980 were symptomatic ($P=0.02$). The median age of the patients at the onset of acute symptoms was 30 years (range 17–55 years) and for skin symptoms 26 years (range 14–56 years), respectively. After the age of 40, only two patients experienced their first acute attacks and only one patient experienced her first skin symptoms.

Correlation between clinical symptoms and mutation type

The age of the patients at the time of diagnosis and gender distribution differed subtly among mutation groups (median age 30, 47 and 41 years and percentage of female patients 57, 75 and 73% for R152C, I12T and 338G→C, respectively). Of note is that none of the patients with I12T substitution manifested photosensitivity, and only

Table 2 Clinical manifestations in VP patients by mutation type

Symptom	R152C	I12T	Mutation 338G→C	Other	Total
Photosensitivity and acute attacks	10 (15%)	0 (0%)	2 (18%)	2 (15%)	14 (14%)
Photosensitivity only	23 (34%)	0 (0%)	1 (9%)	3 (23%)	27 (26%)
Acute attacks only	7 (10%)	1 ^a (8%)	2 (18%)	3 (23%)	13 (13%)
No symptoms	27 (40%)	11 (92%)	6 (55%)	5 (38%)	49 (48%)
Total number of patients	67	12	11	13	103

^aThe patient had experienced acute attacks requiring hospital admission in 1958 and 1959, which were triggered by sulphonamides and barbiturates, but was symptom-free thereafter.

Table 3 Clinical manifestations in VP patients by gender and year of diagnosis

Symptom	Male		Female		Total	
	Before 1980	1980–2001	Before 1980	1980–2001	Before 1980	1980–2001
Photosensitivity and acute attacks	3 (15%)	0	7 (21%)	4 (12%)	10 (19%)	4 (8%)
Photosensitivity only	6 (30%)	4 (24%)	8 (24%)	9 (27%)	14 (26%)	13 (26%)
Acute attacks only	3 (15%)	0	7 (21%)	3 (9%)	10 (19%)	3 (6%)
No symptoms	8 (40%)	13 (76%)	11 (33%)	17 (52%)	19 (36%)	30 (60%)
Total number of patients	20	17	33	33	53	50

one of 12 patients had experienced two acute attacks in her youth. The family became aware of VP mainly due to a homozygous patient with a severe phenotype.⁴⁰ The occurrence of photosensitivity was significantly lower in the I12T group compared to the R152C group ($P=0.001$), whereas no significant differences between the R152C and 338G→C groups could be observed.

Correlation between the biochemical characteristics of patients and mutation type

Figure 1 shows the biochemical data of the individuals with the three most common Finnish mutations. Overall, the urinary PBG and ALA were elevated in 73 and 52% of individuals with VP (data not shown). Urinary copro- and uroporphyrin were elevated in 59 and 72% and faecal coproporphyrin and protoporphyrin in 56 and 84% of the subjects, respectively. No significant difference existed between the excretions of the male and female patients.

The urinary excretions of uro- and coproporphyrins were significantly lower in the I12T group compared to the R152C group ($P=0.001$ for coproporphyrin and $P=0.01$ for uroporphyrin) and 338G→C group ($P=0.01$ for coproporphyrin). In addition, the excretions of PBG and ALA were at the lowest in the I12T group, although the differences between the groups were not significant (data not shown). The urinary excretions of uro- and coproporphyrins in the R152C and 338G→C groups were comparable. The faecal excretions of coproporphyrins and protoporphyrins were significantly lower in the I12T group compared to the R152C group ($P=0.001$ for coproporphyrin and $P=0.0002$ for protoporphyrin). Faecal excretion of protoporphyrin was also significantly lower in the I12T group than in the 338G→C group ($P=0.01$). The plasma fluorescence spectrum was normal in three patients tested in the I12T group, whereas in the R152C group it was positive in eight of nine patients and in the 338G→C group in two of three patients tested.

Correlation between biochemical characteristics and patients' symptoms

Table 4 shows the correlation between biochemical characteristics and patients' symptoms. The patients were defined in remission, if they had not experienced acute symptoms in 3 months, but may have manifested skin symptoms such

as increased fragility of sun-exposed skin. The excretions of the patients, who are known to have experienced acute symptoms and/or skin symptoms, were compared to asymptomatic patients, who have never been clinically active. Urinary and faecal excretions of porphyrins were significantly higher in patients with prior symptoms, even during remission. This finding applied to all mutation groups. Figure 2D demonstrates that the faecal protoporphyrin test was 92% accurate in predicting those individuals who will remain symptom-free with respect to acute attacks. One patient with previous cyclical acute attacks, showed normal faecal protoporphyrin excretion in her post-menopausal phase. For skin symptoms, the negative predictive value was 92%, respectively. A 64-year-old patient, who had experienced mild skin fragility in his youth, had normal porphyrin excretion in faeces and urine, but a positive plasma fluorescence spectrum for VP. In two patients, a single measurement conducted before 18 years of age showed normal excretion even though the patients had skin symptoms and increased faecal protoporphyrin excretion later in adulthood. The negative predictive values of other biochemical tests varied from 61 to 77% for skin symptoms and 70 to 82% for acute attacks, when the normal value limits shown in Table 4 were used. In addition, urinary coproporphyrin excretion of more than 1000 nmol/day was associated with an increased occurrence of both skin symptoms and acute attacks with a positive predictive value of 100% (Figure 2A).

Prediction of clinical symptoms

To predict the risk of clinical symptoms in previously asymptomatic patients, we constructed a logistic regression model⁴⁹ where the occurrence of clinical symptoms was explained by the mutation type and/or biochemical tests. Because all patients with normal faecal protoporphyrin excretion were symptom-free at present, we included in the analysis only those individuals who had elevated excretion of faecal protoporphyrin. Table 5 shows that a moderate increase in urinary excretion of coproporphyrins in remission did not substantially increase the likelihood of symptoms, whereas a higher excretion (>1000 nmol, corresponding to the 80% percentage point of patients studied, normal value <236 nmol) was related to a significantly increased likelihood of skin symptoms, which was further

Figure 1 Urinary and faecal porphyrin excretions of VP patients with the three most common mutations ($n=69$). The individual values were calculated as the mean of 1 to 7 single measurements in adulthood. Dashed line denotes the normal value limit and dash-dotted line shows the boundary between moderately and substantially elevated excretion.

increased by a markedly elevated excretion of protoporphyrin in the faeces (> 644 nmol, normal value < 130 nmol).

For an individual with normal faecal protoporphyrin excretion, the probability of developing skin symptoms is virtually zero. For the patients with normal urinary excretion of coproporphyrin and moderately elevated excretion of protoporphyrin in faeces, the estimated frequency of skin

symptoms is 31%, whereas for an individual with a markedly increased excretion of urinary coproporphyrin and faecal protoporphyrin, the probability reaches 80% and the probabilities for other patients remain between these two extremes. A model, which could significantly predict the occurrence of acute attacks for these patients, could not be constructed.

Table 4 Comparison of mean values ($\pm$ SEM) of biochemical tests in VP patients with the three most common Finnish mutations. In parenthesis the number of patients in each group

	Normal value	Photosensitivity and acute attacks	Photosensitivity only	Acute attacks only	No symptoms	P ^a
Urinary coproporphyrin	< 236 nmol/day					
R152C		1543 $\pm$ 413 (7)	718 $\pm$ 203 (17)	687 $\pm$ 351 (6)	396 $\pm$ 74 (14)	0.03*
I12T		–	–	213 (1)	157 $\pm$ 12 (5)	0.13
338G→C		1972 $\pm$ 1400 (2)	1146 (1)	319 $\pm$ 101 (2)	161 (1)	0.23
Urinary uroporphyrin	< 36 nmol/day					
R152C		172 $\pm$ 50 (7)	105 $\pm$ 20 (14)	105 $\pm$ 52 (5)	61 $\pm$ 17 (11)	0.12
I12T		–	–	83 (1)	20 $\pm$ 3 (4)	0.14
338G→C		347 $\pm$ 67 (2)	65 (1)	78 $\pm$ 61 (2)	31 (1)	0.31
Faecal coproporphyrin	< 100 nmol/g (dry faeces)					
R152C		337 $\pm$ 82 (7)	248 $\pm$ 51 (18)	217 $\pm$ 79 (7)	119 $\pm$ 25 (17)	0.02*
I12T		–	–	379 (1)	32 $\pm$ 18 (8)	0.12
338G→C		187 $\pm$ 93 (2)	112 (1)	79 $\pm$ 69 (2)	75 $\pm$ 59 (2)	0.89
Faecal protoporphyrin	< 130 nmol/g (dry faeces)					
R152C		726 $\pm$ 130 (7)	622 $\pm$ 77 (20)	611 $\pm$ 147 (7)	369 $\pm$ 74 (16)	0.04*
I12T		–	–	521 (1)	116 $\pm$ 60 (8)	0.15
338G→C		401 $\pm$ 196 (2)	569 (1)	262 $\pm$ 46 (2)	316 $\pm$ 58 (2)	0.91

^aKruskal-Wallis one-way ANOVA for difference in medians.

Discussion

A systematic follow-up of Finnish VP patients, which was conducted from the early 1960s and based on hospital records from the beginning of the 20th century, provided an excellent opportunity for the genotype–phenotype analysis in VP. Our series includes both patients with symptoms (52%) and phenotypically normal carriers (48%) and thus provides information about the clinical and biochemical outcome among VP patients in general. The proportion of patients with acute attacks (27%) in this series is somewhat lower than in some extensive family studies in which up to 38% of patients had experienced acute attacks,^{14,31} but higher than 4–15% reported recently from a large South African kindred (Hift R, personal communication). The frequency of photosensitivity (40%) is lower than reported previously in South Africa and France (70%),^{14,31} but comparable with that of the recent South African study (39%) (Hift R, personal communication). Extended mutation screening among symptom-free family members and improved counselling explain the difference in these numbers.

The patients with the I12T mutation experienced no photosensitivity and acute attacks were rare. In addition, biochemical abnormalities were milder suggesting a milder form of the disease than in patients with other mutations. However, this could not be predicted by expression studies. The I12T mutation lies in the highly conserved FAD-binding domain in the amino-terminal region of the *PPOX* gene,^{16,50} whereas the R152C mutation causes an amino acid substitution in exon 5 and the 338G→C mutation results in exon 4 deletion and a truncated polypeptide. *In vitro*, the I12T mutation caused a dramatic decrease in the enzyme activity that was comparable to the activities found in the R152C and 338G→C mutations. Post-translational factors and interaction between the mutant and normal

polypeptide may vary and modify the enzyme activity *in vivo* explaining the milder phenotype in patients with the I12T mutation. Interestingly, the homozygous patient with the I12T mutation demonstrated as high as 10% residual activity *in vivo*, which is sufficient to enable the patient to survive.⁴⁰ Alternatively, another familial factor may be present in the subjects with the I12T mutation that influences their phenotype.

Urinary and faecal excretions of porphyrins in remission differed significantly between the mutation groups. Environmental influences may be important and other metabolic gene(s) that are presently not identified may modify the porphyrin excretion in general. Several polymorphisms in the cytochrome P450 enzymes exist⁵¹ and these genes are good candidates in searching genes modifying porphyrin metabolism and VP phenotype. Diminished supply of heme may lead to insufficient function of P450-mediated reactions in VP patients.^{52–54} P450 enzymes may also associate with the excretion of porphyrins, since CYP3A induction attenuated the hepatic accumulation and urinary excretion of uro- and heptacarboxylporphyrins in a rat porphyria cutanea tarda model.⁵⁵

Since only 26% of symptomatic patients suffered both photosensitivity and acute attacks, the majority of the patients with each of these manifestations were in two distinct groups. This is in line with previous studies, where 79 and 77% of symptomatic patients experienced either photosensitivity or acute attacks, but not both.^{14,31} We have shown that the occurrence of acute attacks has decreased markedly during the last two decades, whereas no such tendency has been observed for skin symptoms.^{12,56} The decrease has been more prominent in males, since they are not prone to hormonal factors. This indicates that different pathogenetic mechanisms may

Figure 2 Clinical manifestations and porphyrin excretions of VP patients with seven different and two unknown mutations in remission ($n=80$). The values for individual persons were calculated as a mean of 1 to 7 single measurements in adulthood. Dashed line denotes the normal value limit.

underlie the development of skin symptoms and acute attacks. The latter may be more readily prevented by avoiding precipitating factors.

The occurrence of skin symptoms was related to a more than fourfold increase in urinary copro- and uroporphyrin excretion. In contrast, normal faecal protoporphyrin excretion as well as negative plasma fluorescence predicted freedom from skin symptoms. These findings support the

theory that the severity of chronic skin symptoms is likely to depend on the permanent circulating levels of porphyrins.^{10,56} In the pathogenesis of acute attacks, the induction of ALA-synthase (ALAS), which is the rate-limiting enzyme of the pathway, plays a key role. Moreover, if heme requirements are increased in the liver, ALAS is induced resulting in the accumulation of porphyrins and porphyrin precursors in those patients.⁵⁷

Table 5 Logistic regression model for predicting the occurrence of skin symptoms in patients with increased excretion of protoporphyrin in feces (>130 nmol/g, dry faeces)

	OR ^a	Skin symptoms 95% CI	P-value ^b
Urinary coproporphyrin			0.001
<236 nmol/day (reference)	1		
236–1000 nmol/day	11.8	0.9–164	
>1000 nmol/day	85.6	4.1–1798	
Faecal protoporphyrin			0.093
130–644 nmol/g (reference)	1		
>644 nmol/g	4.7	0.7–34	

^aAdjusted for gender, age at time of diagnosis, year of diagnosis, and mutation type. ^bSignificance of the single variable in the model (Log-likelihood test).

In our series, normal excretion of protoporphyrin in faeces in adulthood predicted freedom from both skin symptoms and acute attacks for patients. In contrast, normal urinary excretion of porphyrins, PBG or ALA, or normal faecal excretion of coproporphyrin did not predict freedom from symptoms for VP patients. The most valuable test predicting an increased risk of symptoms was urinary coproporphyrin, but only a substantially increased excretion exceeding 1000 nmol/day was associated with an increased risk of both skin symptoms and acute attacks and virtually all patients with an excretion of more than 1000 nmol/day experienced either skin symptoms, acute attacks, or both.

Electronic-Database information

Human Gene Mutation Database, <http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html> (for mutations in the *PPOX* gene); Human Genome Mapping Project, <http://www.hgmp.mrc.ac.uk> (for accession numbers for *PPOX* genomic/cDNA sequences: X99450 [human]); Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for VP [MIM 176200]).

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