*CYP1A2*1F* and *GSTM1* Alleles Are Associated with Susceptibility to Porphyria Cutanea Tarda

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Porphyria cutanea tarda (PCT) is a cutaneous porphyria with sporadic (type 1) and familial (type 2) subtypes, both resulting from decreased hepatic uroporphyrinogen decarboxylase (UROD) activity. Environmental and genetic factors are involved in the development of PCT, and genetic variants in the *cytochrome P450* (*CYP*) genes, *CYP1A1* and *CYP1A2*, have been implicated. We investigated the association between PCT and variants in *CYP1A1*, *CYP1A2* and *CYP2E1*, and the *glutathione-S-transferase* (*GST*) genes, *GSTM1* and *GSTT1*. PCT diagnosis was based on urinary or plasma porphyrin profiles. Patients were classified as type 1 or 2 PCT based on *UROD* mutation analysis. The *CYP1A2*1F* promoter A allele frequency was significantly higher ($P < 0.022$) and the A/A genotype frequency marginally higher in PCT patients overall ($P < 0.057$), with the A/A genotype significantly more common in type 1 PCT ($P < 0.043$). The presence of the wild-type *GSTM1* allele also was associated significantly with PCT ($P < 0.019$). Neither *hemochromatosis* (*HFE*) mutations, tobacco smoking, hepatitis C and HIV infection, ethanol consumption, nor estrogen use were associated with these allelic variants. Age at onset was significantly lower in type 2 PCT patients ($P < 0.001$), as observed previously. Thus, positive associations between PCT and the *CYP1A2*1F* promoter A allele and A/A genotype and the wild-type *GSTM1* allele indicates that these functional hepatic biotransformation enzymes are risk factors for the development of this disease.

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INTRODUCTION

Porphyria cutanea tarda (PCT) is the most prevalent of the human porphyrias and results from the reduced activity (~20% of normal) of uroporphyrinogen decarboxylase (UROD) in the liver (1). The disease is classified as type 1 or type 2 based on the absence or presence of a *UROD* mutation, respectively. Most patients do not have a *UROD* mutation and have sporadic or type 1 PCT, while patients who are heterozygous for a *UROD* mutation, encoding an enzyme with no or reduced activity, have familial or type 2 PCT. However, heterozygosity for a *UROD* mutation is not sufficient to cause PCT manifestations, but is a predisposing factor. In both types 1 and 2 PCT, excess iron and multiple susceptibility factors contribute to the development of hepatic UROD deficiency. Recognized susceptibility factors include alcohol, smoking, estrogens, hepatitis C, HIV and mutations in the *hemochromatosis* (*HFE*) gene (2,3). Outbreaks of PCT have occurred following exposure to halogenated polycyclic hydrocarbons such as hexachlorobenzene and tetrachlorodibenzo-p-dioxin (4–6). These and related chemicals can cause hepatic UROD deficiency and uroporphyria in rodents, but it is not clear whether such exposures play a role in isolated cases of human PCT (7).

Although hepatic UROD enzymatic activity is markedly reduced in PCT, the enzyme protein levels remain at their genetically determined levels (normal in type 1 and half-normal in type 2) (8). Therefore, it has been suggested that inhibition of UROD activity is the primary factor causing disease manifestations. An inhibitor of UROD activity has been found in cytosolic extracts from livers of PCT patients and has been identified in a mouse model as a uroporphomethene (9). In mice, cytochrome P450 1a2 (Cyp1a2) plays a major role in the development of uroporphyria and may be involved in the partial oxidation of a heme pathway intermediate (most likely uroporphyrinogen or hydroxymethylbilane) to form the uroporphomethene inhibitor

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of UROD activity (9–11). Unlike Cyp1a2-proficient mice, knockout mice deficient in Cyp1a2 do not develop uroporphyria following exposure to halogenated polyaromatic hydrocarbons (12), and both murine and human CYP1A2 can support the development of uroporphyria in mouse hepatoma cells (13).

Previous reports have indicated that the *CYP1A2*1F* promoter variant (C > A in intron 1 at nucleotide position 734) increases the amount of this enzyme, thereby increasing its catalytic capacity (14–16). This promoter variant was associated with PCT in two European studies, but not in a third (17–19). Variants in *CYP1A1* have been associated with differences in metabolism of polycyclic aromatic hydrocarbons and steroid hormones (20–24). The *CYP1A1*4* gene variant was associated with PCT in one study, but only with type 2 disease (25).

Common deletion variants in two other important hepatic detoxifying enzymes encoded by the *glutathione-S-transferase* (*GST*) genes, *GSTM1* and *GSTT1*, which result in truncated, nonfunctional enzymes (26), have not been studied previously in PCT. GSTM1 is particularly important in the deactivation of reactive intermediates of polycyclic aromatic hydrocarbons, and several lines of evidence suggest that GSTT1 and/or GSTM1 play a role in the deactivation of reactive oxygen species that are likely to be involved in inflammation (27).

We hypothesized that variants in biotransformation genes that increase cytochrome P450 enzyme activities, or increase oxidative stress through altered GST activity, would constitute susceptibility factors in PCT. Patients with well documented PCT and matched, community-based controls were studied for eight variants in *cytochrome P450* genes including: *CYP1A1*2A*, **2C* and **4*; *CYP1A2*1E*/**1K*; **1K*; **1F*; rs2069522; *CYP2E1*5* and for *GSTM1* and *GSTT1* deletion variants (denoted as *GSTM1 null* and *GSTT1 null*, respectively), which are known or suspected to have functional effects on their respective enzymatic activities (16,28–33). As noted, the *CYP1A2*1F* and *CYP1A1*4* variants have been associated previously with PCT (17,18,25). Associations of these variants with known susceptibility factors, including use of alcohol, tobacco or estrogen, infections with hepatitis C virus or HIV, and *UROD* and *HFE* mutations as well as sex and age of onset, were studied.

METHODS

Study Subjects

The study used a data and sample repository from consented PCT patients and controls and was approved by the UTMB Institutional Review Board. PCT was documented by substantial increases in urinary or plasma total porphyrins with a predominance of the highly carboxylated porphyrins, especially uroporphyrin (octacarboxyl porphyrin) and heptacarboxyl porphyrin. These biochemical measurements were done in the CLIA-approved Porphyria Laboratory and Center at UTMB by previously reported methods (34). *UROD* mutation analysis was performed at the Porphyria Center in the Department of Genetics and Genomic Sciences at The Mount Sinai School of Medicine in New York City, New York.

Of the 73 patients studied, 71 presented initially with typical blistering lesions on sun-exposed skin, most commonly on the dorsa of the hands, forearms and face; 2 patients, both with *UROD* mutations, presented with noncutaneous symptoms and were found to have marked porphyrin elevations with patterns typical of PCT. These findings were consistent with type 2 PCT that was clearly evident biochemically but without cutaneous manifestations and therefore subclinical, which is reported to be common among mutation carriers in type 2 PCT family members (35). *UROD* mutations were identified in 16 (23%) of the 70 patients examined. Patients with *UROD* mutations (type 2) had a significantly lower age at disease onset (Mann–Whitney $U = 148$, $P < 0.001$; $t = 4.728$, df = 66, $P < 0.0001$). Mean and median age at disease onset was 32.7 and 34 years, respectively, in type 2 patients, and 44.3 and 44 years in type 1 patients. Of the 54 type 1 patients, 61% were male, 91% Caucasian, 6% Hispanic, 2% African-American and 1% "Other"; of the type 2 patients, 44% were male , 75% Caucasian, 25% Hispanic and 0% African-American; these differences were not statistically significant. Molecular diagnosis according to *UROD* mutational status was unavailable for three patients (Caucasian, two males, one female), who were included in analyses comparing PCT to normals, but not in comparisons of types 1 and 2 disease, although normal erythrocyte UROD activity suggests they were type 1. Two patients in the type 1 group were siblings without *UROD* mutations, and might have been designated type 3 (7), but were designated as type 1 in this study. Rare type 3 cases, like type 1, have normal erythrocyte UROD activity; a molecular basis for distinguishing types 1 and 3 is not established. All the other PCT patients were unrelated. Healthy community-based controls were matched with the study subjects by age (within 5 years) and self-reported ethnicity (Caucasian/non-Hispanic White, Hispanic, African-American or Other).

Analysis of Genetic Variants

To study the *CYP1A1*, *CYP1A2*, *CYP2E1*, *GSTM1* and *GSTT1* genetic variants, genomic DNA was isolated from 10 mL of whole blood collected in EDTA using the Gentra Puregene Blood Kit according to the manufacturer's procedure (QIAGEN; Valencia, CA, USA). Approximately 1–10 ng of genomic DNA was used for PCR amplification for each gene. Genotypes for *GSTT1* and *GSTM1* were determined by a multiplex PCR assay that detects the homozygous truncating deletion (0 copies) versus 1 or 2 copies of each GST gene (36). Genotypes for eight variants in the three *cytochrome P450* genes were determined by TaqMan assays (Applied Biosystems Incorporated, ABI; Foster City, CA, USA). Custom assays were designed and synthesized by ABI for the three variants in the *CYP1A1* gene. All PCR amplifications were performed in duplicate, and along with appropriate negative and positive controls for each al-

lele, when possible. Fluorescent PCR amplifications were run in 10 μl total volumes according to the manufacturer's instructions for the TaqMan Genotyping Master Mix or the TaqMan Universal PCR Master Mix (ABI). A DNA Engine (formerly MJ Research) equipped with a Chromo4 Real-Time PCR Detection System (Bio-Rad Laboratories; Hercules, CA, USA) was used for amplification and fluorescent detection. All reactions were carried out in tube strips fitted with optical caps (Bio-Rad Laboratories).

Statistical Analysis

The frequencies of the variants for each gene were evaluated for Hardy-Weinberg Equilibrium (HWE) using the Linkage Disequilibrium Analyzer program (version 1.0) (37). The chi-square test was used in addition to a Monte-Carlo permutation based exact test. The criterion for significance was $P < 0.05$. Allele frequencies failing to meet expected HWE in both statistical tests were excluded from further analyses.

The Fisher exact test or the likelihood ratio chi-square test was used to detect differences in allele and genotype frequencies and to compute odds ratios (OR) and 95% CL representing the relative risk for the disease. Additional analyses included testing the allelic effects on PCT using simple genetic models. Each variant that might be significantly associated with PCT was evaluated as a recessive allele having an effect in the homozygous state, a dominant allele having an effect in the homozygous or heterozygous state, or an additive allele having an increasing effect from the heterozygous to the homozygous state.

Within PCT cases, we tested for associations between genotype frequencies and factors established as influencing susceptibility. Genetic factors examined included presence or absence of *UROD* mutations, and *HFE* mutations—that is, whether heterozygous for C282Y or H63D or having genotypes C282Y/C282Y, C282Y/H63D or H63D/H63D. Acquired factors studied included hepatitis C, HIV infection, alcohol consumption, smoking and estrogen use. Associations of these factors with demographic variables such as sex, ethnicity and age at disease onset were examined using nonparametric tests (Mann–Whitney and Kruskal–Wallis) and parametric tests (Student *t* test, one-way analysis of variance [ANOVA]). The criterion for significance was $P < 0.05$. SPSS 16.0 for Windows was used for statistical analyses.

RESULTS

A total of 73 cases and 72 matched controls were enrolled in the study. Table 1 summarizes the genotype and allele frequencies for the seven *CYP* and two *GST* variants for both groups. An additional *CYP1A1* gene variant rs1048943 deviated significantly from the expected HWE frequencies ($\chi^2 = 13.5$, $P < 0.001$; permutation test, $P < 0.005$) and was excluded. Allele or genotype frequencies for the *CYP1A2*1F* allele and the presence of at least one intact *GSTM1* allele were associated statistically with PCT (see below). For the three other *CYP1A2* variants, the 3 *CYP1A1* variants, the *CYP2E1* variant and the *GSTT1* variants, no associations with PCT, or differences between types 1 and 2 PCT, were found. None of the *CYP* or *GST* variants we examined correlated with previously established susceptibility factors, namely *HFE* mutations, hepatitis C or HIV infection, estrogen or alcohol use or smoking, although sample sizes became too small in these analyses to definitely exclude such associations.

Notably, the allele frequencies for the *CYP1A2*1F* promoter variant were significantly different among PCT cases and controls (Fisher exact test, $P < 0.022$, Figure 1), with the A allele, which is more common in the general population, associated with PCT with an odds ratio (OR) of 1.8 (95% CI, 1.1–3.0). Genotype frequencies for this variant were almost significantly different (likelihood ratio chi-square = 5.742, df = 2, $P < 0.057$, Figure 1). In a genetic model treating the A allele as recessive, the association between PCT and the homozygous A/A genotype in comparison with the C/A and C/C genotypes was significant (Fisher exact test, $P < 0.028$, Figure 2) with a corresponding OR of 2.2 (95% CI, 1.1–4.3).

Among the PCT cases classified as type 1 or 2 based on *UROD* mutation analysis, the *CYP1A2*1F* promoter A allele and A/A genotype were more common in patients with type 1 than in those with type 2 PCT (Fisher exact test, $P < 0.082$; likelihood ratio chi-square = 4.077, df = 1, $P < 0.043$, Figure 3). The frequency of the A allele in type 1 PCT cases was 0.78, while its frequency in Type 2 PCT patients and controls were 0.59 and 0.62, respectively (Table 1). The corresponding frequencies of the A/A genotype in types 1 and 2 PCT patients and matched controls were 0.60, 0.31 and 0.36, respectively (Table 1).

The presence of at least one intact *GSTM1* allele rather than the *GSTM1* deletion allele, was significantly more common in PCT cases compared with controls (Fisher exact test, $P < 0.019$). The OR for this association with PCT was 2.3 (95% CI 1.2–4.5,). There were no differences in *GSTM1* genotype or allele frequencies in types 1 and 2 disease and no association with the *CYP* genotypes examined, including *CYP1A2*F*.

DISCUSSION

PCT is caused by the interaction of genetic and environmental factors which leads to the deficient activity of hepatic UROD, accumulation of highly carboxylated porphyrins and blistering cutaneous photosensitivity (1,2,7). The important roles of iron overload as well as for genes that affect iron absorption and phase I biotransformation have been demonstrated in mouse models of PCT. For example, uroporphyria develops *urod* allele and two *hfe* null alleles, and in wild-type mice treated with iron, the porphyrin precursor δ-aminolevulinic acid, and polychlorinated biphenyls or other halogenated polycyclic aromatic hydrocarbons, which are potent CYP inducers (12,38). In particular, CYP1A2 appears to be essential for the development of uroporphyria in mice (10–13).

This study extends previous findings by examining the relationship between PCT and *CYP1A1* and *CYP1A2* variants in a North American population and by examining, for the first time, the relation-

Table 1. *Cytochrome P450* (*CYP*) and *glutathione-S-Transferase* (*GST*) allele and genotype frequencies in patients with porphyria cutanea tarda (PCT) and matched controls. Gene symbols in addition to traditional allelic designations and reference SNP identification numbers are provided.

	PCT patients							Controls					
					Genotype frequency						Genotype frequency		
CYP gene variant	PCT subtype	N[a]	% Male	% Common allele	C/C[b]	C/R	R/R	N	% Male	% Common allele	C/C	C/R	R/R
*CYP1A1*2A* (m1, rs4646903)	1 & 2	69	59	83	0.72	0.22	0.06	72	68	82	0.65	0.34	0.01
	1	50	64	84	0.72	0.24	0.04						
	2	16	44	88	0.81	0.12	0.07						
*CYP1A1*2C* (m2, rs1048943)	1 & 2	69	59	94	0.90	0.09	0.01	72	68	90	0.85	0.11	0.04
	1	50	64	95	0.90	0.1	0						
	2	16	44	100	1.00	0	0						
*CYP1A1*4* (m4, rs1799814)	1 & 2	71	59	99	0.97	0.03	0	70	53	96	0.91	0.09	0
	1	53	62	98	0.96	0.04	0						
	2	16	44	100	1.00	0	0						
CYP1A2 (rs2069522)	1 & 2	54	56	98	0.96	0.04	0	36	67	100	1	0	0
	1	41	61	98	0.95	0.05	0						
	2	10	30	100	1.00	0	0						
*CYP1A2*1E* and **1K* (rs2069526)	1 & 2	55	55	97	0.95	0.05	0	41	56	99	0.98	0.02	0
	1	41	61	96	0.93	0.07	0						
	2	11	27	100	1.00	0	0						
*CYP1A2*1K* (rs12720461)	1 & 2	48	56	96	0.92	0.08	0	44	61	99	0.98	0.02	0
	1	35	63	94	0.89	0.11	0						
	2	10	30	100	1.00	0	0						
*CYP1A2*1F* (rs762551)	1 & 2	69	59	75	0.55	0.39	0.06	72	68	62	0.36	0.51	0.13
	1	50	64	78	0.60	0.36	0.04						
	2	16	44	59	0.31	0.56	0.13						
*CYP2E1*5* (rs3813867)	1 & 2	72	58	87	0.78	0.18	0.04	64	53	84	0.73	0.22	0.05
	1	53	62	89	0.81	0.15	0.04						
	2	16	44	81	0.69	0.25	0.06						

GST gene	PCT subtype	N	% Male	Patients with a full-length allele (%)	N	% Male	Controls with a full-length allele (%)
GSTM1	1 + 2	71	58	62	72	68	42
	1	52	62	63			
	2	16	44	63			
GSTT1	1 + 2	71	58	83	72	68	78
	1	52	62	81			
	2	16	44	88			

[a]For each variant examined, 2–3 patients not examined for *UROD* mutations were included in comparisons of PCT versus controls but not in comparisons of types 1 and 2.
[b]C = common allele; *R* = rare allele.

ship between this disease and *CYP2E1* and *GSTM1* and *GSTT1* deletion variants. Matched, healthy, community-based controls were used as the comparison population. The results confirm an association of PCT with the *CYP1A2*1F* promoter variant, specifically the "A" allele, as previously reported in Danish and Spanish patients, although not in French patients (17-19). Table 2 compares the *CYP1A2*1F* genotype and allele frequencies in patients and controls in this and the previous three studies. Frequencies of the A allele in controls ranged from 62% to 68% in these four studies, and from 67% to 83% in PCT patients. Furthermore, we found that inheritance of at least one full length, and presumably functional, *GSTM1* allele was significantly more common in PCT cases than in controls, suggesting that this enzyme plays a role in the susceptibility to PCT.

We also examined interactions between these biotransformation variants and other susceptibility factors and demographic variables in patients with PCT. Presence of a *UROD* mutation was associated significantly with an earlier age of disease onset in PCT cases, as described previously (7). The *HFE* mutations and other *CYP* and

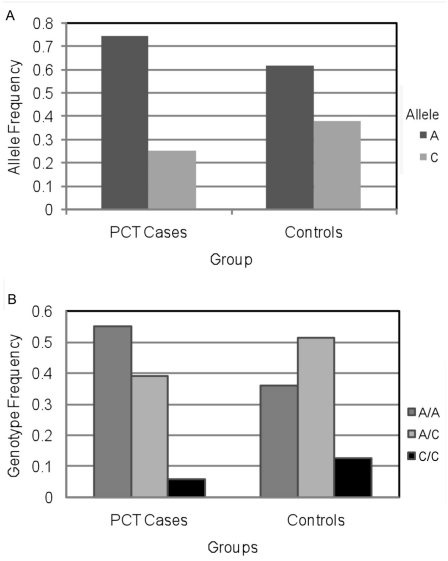

Figure 1. *CYP1A2*1F* promoter A allele and genotype frequencies in PCT cases and matched controls. Allele frequency (Panel A) was significantly higher in PCT patients than in controls (Fisher exact test, $P < 0.022$). The higher A/A genotype frequency in PCT patients (Panel B) was marginally significant (likelihood ratio chi-square, $P < 0.057$).

GST alleles were not associated with age at onset of disease, even when type 1 and 2 cases were considered separately.

In this study, *CYP1A1*2A*, **2C* and **4* variants were not associated with PCT, and were not different between types 1 and 2 PCT. Gardlo *et al*. in Germany, also found no association of PCT with the *CYP1A1*2A* allele (25), but in contrast to our findings, reported an association between the **4* allele and PCT, which was statistically significant in type 2 cases (25). These *CYP1A2*4* (previously known as m4) genotype and allele frequencies are compared in Table 3, showing comparable frequencies in controls but higher frequencies in PCT in the previous study. These divergent findings might be due to geographic differences among the various underlying susceptibility factors leading to PCT expression.

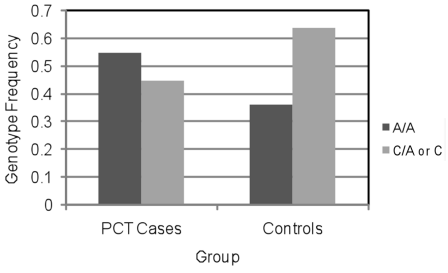

Figure 2. *CYP1A2*1F* genotype frequencies in PCT patients and matched controls under a model where A is recessive. The frequency of the A/A genotype in comparison with the C/A and C/C genotypes was significantly higher in PCT than in controls (Fisher exact test, $P < 0.028$).

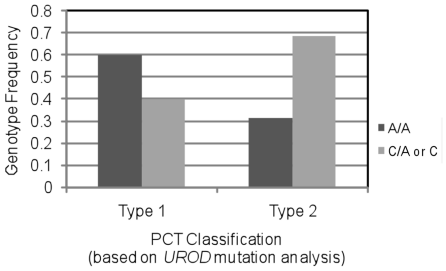

Figure 3. *CYP1A2*1F* genotype frequencies in PCT patients with and without *UROD* mutations (types 1 and 2 respectively). Under a model where A is recessive, the frequency of the A/A genotype in comparison with the C/A and C/C genotypes was significantly higher in type 1 than type 2 cases (Fisher exact test, $P < 0.082$; likelihood ratio chi-square, $P < 0.043$).

The *CYP1A2* alleles rs2069522**1E*/**1K* and **1K*, the *CYP2E1*5* allele, and the *GSTT1* deletion variant were not associated with PCT in this study. The minor allele frequencies of these three *CYP1A2* alleles were quite low, and the sample size may not have been adequate to detect an association, if present. The association of PCT with the *CYP1A2*1F* promoter A allele and the A/A genotype is plausible because the C to A base substitution in the promoter region increases the amount of wild type CYP1A2 enzyme, although the mechanism by which this nucleotide substitution increases the amount of the encoded mRNA and enzyme is not yet defined (16). Because the *CYP1A2*1F* A allele and A/A genotype are frequent in diverse populations, including European Caucasians (frequencies of 0.692 and 0.483, respectively), Yoruba Africans (0.567 and 0.317, respectively), Japanese (0.614 and 0.386, respectively), and Han Chinese (0.667 and 0.422, respectively) (39), they may con-

Table 2. Genotype and allele frequency of the *CYP1A2*1F* (A) allele in patients with PCT and controls in this study and in three previous studies.

			Genotype frequency			Allele frequency	
Study	PCT subtype	n	A/A, %	A/C, %	C/C, %	A, %	C, %
This study (USA)	1 & 2	66	55.1	39.1	5.8	75	25
	1	50	60.0	36.0	4.0	78	22
	2	16	31.0	56.0	13.0	59	41
	Controls	72	36.1	51.4	12.5	62	38
Christiansen *et al.* (Denmark) (17)	1 & 2	53	71.7	22.6	5.7	83	17
	1	40	70.0	27.5	2.5	84	16
	2	13	76.5	7.7	15.4	81	19
	Controls	60	46.7	43.3	10.0	68	32
Dereure *et al.* (France) (19)	1 & 2	49	38.8	53.1	6.1	67	33
	1	45	N/A	N/A	N/A	N/A	N/A
	2	4	N/A	N/A	N/A	N/A	N/A
	Controls	48	39.6	47.9	12.5	64	37
Fontanella *et al.* (Spain) (18)	1 & 2	102	48.0	49.0	2.9	73	28
	1	80	45.0	52.5	2.5	71	29
	2	22	59.1	36.5	4.5	77	23
	Controls	150	40.0	48.0	12.0	64	36

[a]N/A, not available.

Table 3. Genotype and allele frequencies of the *CYP1A1*1* and **4* in patients with PCT and controls in this study and in a previous study in Germany.

			Genotype frequency			Allele frequency	
Study	PCT subtype	n	*1/*1, %	*1/*4, %	*4/*4, %	*1, %	*4, %
This study (USA)	PCT 1 & 2	71	97.2	2.8	0.0	98.6	1.4
	PCT 1	53	96.0	4.0	0.0	98.0	2.0
	PCT 2	16	100.0	0.0	0.0	100.0	0.0
	Controls	70	91.4	8.6	0.0	95.7	4.3
Gardlo *et al.* (Germany) (29)	PCT 1 & 2	46	82.6	17.4	0.0	85.3	14.7
	PCT 1	29	89.7	10.3	0.0	94.8	5.2
	PCT 2	17	70.6	29.4	0.0	85.3	14.7
	Controls	101	92.6	7.4	0.0	100.0	0.0

tribute to PCT susceptibility in many geographic areas.

An important PCT pathogenic role for this enzyme has been suggested by studies in mice, where murine CYP1A2 is essential for the development of uroporphyria. CYP1A2 oxidizes uroporphyrinogen to uroporphyrin *in vitro* (that is, uroporphyrinogen oxidase activity) and, in the presence of iron, may catalyze the generation of the uroporphomethene inhibitor of hepatic UROD (9,10). Murine CYP1A2 is significantly induced by hexachlorobenzene and related chemicals (12). CYP1A2 also may contribute to uroporphyrinogen oxidase activity and uroporphyrin accumulation in humans, based on studies of mouse hepatoma cells in culture (13). Oxidation of uroporphyrinogen in mice and in human PCT may occur enzymatically or indirectly by reactive oxygen species produced by a Fenton-type reaction during the CYP1A2 catalytic cycle, and other CYPs may contribute. Environmental risk factors for PCT, such as tobacco smoke, may contain chemicals that are substrates for CYP1A2 with metabolic products that contribute to the development of PCT (40). Our findings indicate that the A/A genotype may be a more important contributor to PCT susceptibility in patients without a *UROD* mutation who, because they have a higher genetically determined level of UROD protein, may require a greater amount of UROD inhibitor to develop overt PCT.

The association of PCT with the presence of at least one wild-type *GSTM1* allele in this study seems counter-intuitive, given the prominent role of GST enzymes in detoxifying reactive chemical metabolites by glutathione conjugation. However, a number of mechanisms of glutathione-dependent activation of xenobiotics have been described. Some glutathione conjugates are reactive compounds, and others can undergo further chemical modifications to form more reactive products (40–42). Examples include quinone metabolites of benzene that are conjugated to glutathione and then may undergo autoxidation resulting in the formation of toxic compounds (42), and glutathione conjugates of haloalkanes, thiocyanates and nitrosoguanidines that release toxic metabolites (43,44). Whether such glutathione conjugates might be important in PCT, perhaps by increasing oxidative stress or generating reactive glutathionyl compounds that might contribute to the generation of a UROD inhibitor, deserves further study.

No significant two-way interactions between the studied pharmacogenetic variants and other risk factors were observed, including the environmental risk factors of tobacco use, alcohol consumption, estrogen use, chronic infections (hepatitis C, HIV) or *HFE* mutations. However, the small sample size limits the power of analyzing gene-to-environment and gene-to-gene interactions in this study, so further research on a larger patient population is required to adequately investigate such potential relationships. For example, we recently demonstrated associations among behavior-related susceptibility factors (alcohol use, smoking and hepatitis C) in a larger group of PCT patients studied retrospectively (2).

In summary, this study suggests that individuals with the *CYP1A2*1F* A allele and A/A genotype in conjunction with other susceptibility factors have an increased risk of developing PCT. This increased susceptibility seems most pronounced for development of type 1 PCT. Therefore, this gene variant, which enhances CYP1A2 enzymatic activity, increases an individual's susceptibility to PCT. The new finding that a full-length *GSTM1* allele contributes to PCT susceptibility requires additional molecular epidemiological studies in other PCT populations for verification. It is also possible but unlikely that these gene variants do not themselves contribute to the development of this porphyria, but are tightly linked to other genes that contribute to PCT susceptibility. These results provide further evidence that human PCT is a highly heterogeneous disease in which inherited and environmental factors contribute to disease susceptibility and expression.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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