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Risk of primary liver cancer in acute hepatic porphyria patients: A matched cohort study of 1244 individuals

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Abstract. Lissing M, Vassiliou D, Floderus Y, Harper P, Bottai M, Kotopouli M, et al. Risk of primary liver cancer in acute hepatic porphyria patients: a matched cohort study of 1244 individuals. *J Intern Med*. 2022;**291:**824–836.

Background. The acute hepatic porphyrias (AHP) are associated with a risk of primary liver cancer (PLC), but risk estimates are unclear, and what AHP characteristics that predict PLC risk are unknown. In this register-based, matched cohort study, we assessed the PLC risk in relation to biochemical and clinical porphyria severity, genotype, age, and sex.

Methods. All patients in the Swedish porphyria register with acute intermittent porphyria (AIP), variegate porphyria (VP), or hereditary coproporphyria (HCP) during 1987–2015 were included. This AHP cohort was compared with age-, sex-, and county-matched reference individuals from the general population. National register-based hospital admissions for AHP were used to indicate the clinical severity. For AIP, the most common AHP type, patients were stratified by genotype and urinary porphobilinogen (U-PBG). Incident PLC data were collected from national health registers.

Results. We identified 1244 individuals with AHP (1063 [85%] AIP). During a median follow-up of 19.5 years, we identified 108 incident PLC cases, including 83 AHP patients (6.7%) and 25 of 12,333 reference individuals (0.2%). The adjusted hazard ratio for AHP-PLC was 38.0 (95% confidence interval: 24.3–59.3). Previously elevated U-PBG and hospitalizations for porphyria, but not AIP genotype or sex, were associated with increased PLC risk. Patients aged >50 years with previously elevated U-PBG (n = 157) had an annual PLC incidence of 1.8%.

Conclusion. This study confirmed a high PLC risk and identified a strong association with clinical and biochemical AIP activity. Regular PLC surveillance is motivated in patients older than 50 years with a history of active AIP.

Keywords: acute intermittent porphyria, hepatocellular carcinoma, inherited disease, rare disease, surveillance

Abbreviations: AHP, acute hepatic porphyria; AIP, acute intermittent porphyria; ALAS 1, aminolevulinic acid synthase 1; CC, cholangiocarcinoma; CPOX, coproporphyrinogen oxidase; HCC, hepatocellular carcinoma; HCP, hereditary coproporphyria; HMBS, hydroxymethylbilane synthase; ICD, international classification of disease; IR, incidence rate; NPR, National Patient Register; PBG, porphobilinogen; PIN, personal identity number; PLC, primary liver cancer; PPOX, protoporphyrinogen oxidase; ULN, upper limit of normal; U-PBG, urinary porphobilinogen; VP, variegate porphyria

Introduction

The porphyrias are a group of rare metabolic diseases caused by altered functions in the different steps of the haem synthesis pathway [1]. Acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), and variegate porphyria (VP) share biochemical and clinical features. However, these conditions are caused by different partial enzyme deficiencies which result from heterozygous pathogenic variants in genes corresponding to different enzymes in the haem synthesis pathway: hydroxymethylbilane synthase (HMBS) in AIP, coproporphyrinogen oxidase (CPOX) in HCP, or protoporphyrinogen oxidase (PPOX) in VP. Together, these conditions are commonly known as acute hepatic porphyrias (AHP). There is also another extremely rare homozygous form of AHP, ALAD-deficient porphyria, which was not considered in the present study.

Triggers, such as drugs, fasting, or hormonal changes, induce expression of the first and rate-limiting enzyme in the pathway, hepatic aminolevulinic acid synthase 1 (ALAS1), and cause downstream accumulation of delta-aminolevulinic acid (ALA) and porphobilinogen (PBG), which are potential neurotoxic porphyrin precursors associated with acute porphyria attacks. Severe abdominal pain, hypertension, gastrointestinal and neurological symptoms, and mental status changes are common features of these attacks. Symptomatic and biochemically active disease is particularly common in women of reproductive age.

The prevalence of AIP mutation carriers is estimated to be ca. 1/1600 in Caucasians, with a higher prevalence in Sweden due to a founder effect [2,3]. Clinical penetrance is low: the disease presents in approximately 1% of the gene carrier population, but in up to 50% in families in which AIP segregates [4].

Several studies have indicated a significant risk of primary liver cancer (PLC) in AIP patients [5–8], with annual incidence rates (IRs) of 0.16%–0.35% [9,10] and 0.6%–0.8% in patients aged 50 years or above [3,10,11]. Hazard ratios (HRs) vary considerably, from 36 to 108, due to differences in the demographics of the AHP cohorts and in PLC rates in reference populations [9,10]. Published data on PLC in VP and HCP are limited to sporadic cases [12–14]. Results from previous studies have led to varying recommendations for radiological surveillance, with several uncertainties remaining [11,15].

Most patients with AHP-related PLC, mainly hepatocellular carcinoma (HCC), but also cholangiocarcinoma (CC), and mixed forms of PLC do not exhibit common risk factors, such as viral hepatitis, alcohol overconsumption, liver cirrhosis, or male sex. In contrast, female sex is overrepresented and liver cirrhosis is rare among reported cases of AHP-related PLC [16]. Data on disease-specific predictors are scarce, and there is a need for identification of risk factors. It has been proposed that the high incidence of AIP-related PLC in Sweden might be related to a common *HMBS* founder mutation, which affects approximately two-thirds of the patients with AIP in Sweden [11,17].

The definition of AHP, and thereby the potentially associated PLC risk, is not clear. Patients with a typical AHP-related gene variant, family history, elevated porphyrin precursor levels, and symptoms of acute attacks have well-defined disease. On the other hand, some individuals have a disease-causing genotype and elevated porphyrin precursor levels, but never develop acute attacks. Most AHP gene carriers do not develop acute attacks or have elevated porphyrin precursors [2]. The prevalence of porphyria-related gene variants is much higher in the general population than that reflected by the number of known patients with AHP [2,18]. Whether all individuals with mutations and enzyme deficiency, or only those with symptomatic disease and elevated porphyrin precursors, are at risk for PLC is unclear. It is also unclear if the risk is different in AIP, VP, and HCP, and whether sex or genotype influences this risk.

To address these issues, we performed a nationwide register-based matched cohort study to assess the risk of PLC in AHP patients in relation to demographic characteristics, AHP type, AIP genotype, biochemical activity (defined by urinary PGB [U-PBG]), and porphyria-related hospital admissions.

Patients and methods

Data sources

Patients with AHP were identified from the Swedish Porphyria Register, which includes all patients with a confirmed porphyria diagnosis in Sweden since 1976 [19]. The Swedish Total Population Register, administered by Statistics Sweden,

contains demographic information on all Swedish residents, including their sex, year of birth, death, migration status, and place of residence [20].

Four national health registers administered by the National Board of Health and Welfare provided information about diseases and outcomes: (1) the Swedish National Patient Register (NPR) has collected healthcare-related data for all hospitalizations nationwide since 1987. (2) The outpatient NPR contains information about specialized outpatient care since 2001. Primary care visits are not included in the register. (3) The cancer register contains data on all malignancies reported from autopsies and morphological or other laboratory examinations since 1958. (4) The Cause of Death Register contains data on causes of death, how these were assessed, and relevant underlying diseases, since 1961.

Healthcare in Sweden is tax-funded, with activity-based compensation, which ensures virtually complete registration of the population. Register-based research is facilitated by the 12-digit Swedish personal identity number (PIN), which has been maintained by the Swedish Tax Agency since 1947 for all individuals who reside in Sweden [21]. In this study, the PIN was used to link individuals' data in the registers.

Study population

We identified all patients with AIP, VP, and HCP, aged 18 years or older within the study period (1987–2015), in the Swedish Porphyria Register. The AHP diagnoses were verified by a confirmed pathogenic mutation in *HMBS*, *PPOX*, or *CPOX*, by being an obligate carrier of a pathogenic mutation in these genes, or by having biochemical findings consistent with AHP and having family members carrying a known pathogenic mutation.

A reference population was created using the total population register. For each patient with AHP, up to 10 reference individuals matched by sex, birth year, and county of residence were randomly identified. Dates of death or migration were collected for the entire study population. The time of study inclusion was defined as the AHP diagnosis date (or, for the reference population, the corresponding matched date), age 18 years, or 1 January 1987. No individual in the study population had a study baseline before the age of 18 years or 1 January 1987. For instance, a patient with a recorded AIP diagnosis at age 15 would have a study baseline at age 18 years, or, if this was before 1987, on 1 January 1987. The end of the study was either the date of incident PLC, death, migration, or 31 December 2015.

Data collection

Patients with AIP were grouped according to *HMBS* variants. Patients with the most common variant in Sweden, *HMBS*: c.593G>A, were assigned to one group, and those with any other variant to the second group. Patients with AIP were also categorised based on biochemical activity, as defined by U-PBG levels. Both PBG and ALA accumulate during an acute attack. Urinary concentrations of PBG and ALA were measured during routine monitoring and in diagnosis of acute porphyria attacks by ion-exchange chromatography, using the Bio-Rad Laboratories, Inc (Hercules, CA, USA) PBG/ALA test. The concentrations of U-PBG and urinary ALA are reported in millimoles excreted per mole of creatinine, that is, normalized to the creatinine concentration of the specimen [22,23]. The inter-assay variation for the ion exchange chromatography method was 3.7% for U-PBG [24]. The analyses were performed using a validated method at the Porphyria Centre Sweden, an accredited laboratory conforming to internationally recognized standards.

We grouped the patients based on their highest recorded U-PBG in the porphyria register. Only the highest recorded value was used, regardless of the number of samples collected from each individual patient over time. The dates of the individual U-PBG samples were not included in the dataset. Clinical practice regarding U-PBG testing in patients with known AIP, both symptomatic and asymptomatic, varies significantly over time and between hospitals. Some centres have relied mainly on clinical symptoms in diagnosing acute attacks and have not routinely assessed U-PBG in asymptomatic patients. Other centres assessed U-PBG both during attacks and regularly in asymptomatic patients. The highest recorded U-PBG value, dated at any time in the patient's history, was used to define each patient's U-PBG group status: U-PBG-negative, patients with U-PBG levels that never exceeded the upper limit of normal (ULN; 1.6 mmol/mol creatinine); U-PBG-positive; patients with U-PBG > 1.6, mmol/mol creatinine, further divided into moderate (1.6–6.4 mmol/mol creatinine [i.e., 4 × ULN]) and high

(>6.4 mmol/mol creatinine). Patients with no documented U-PBG values in the porphyria register formed a fourth group, the U-PBG-unknown. The cut-off at 4 × ULN was based on previous Swedish studies [24,25].

In order to include a clinical dimension of disease activity other than U-PBG, we also identified hospitalizations for porphyria, after 1986, from the NPR, by recording diagnoses of E802 (international classification of disease [ICD]-10) or 2771 (ICD-9) as the main reason for hospitalization, prior to any diagnosis of PLC. Hospitalizations were assessed to indicate symptomatic porphyria, and thereby possibly patients with more severe AHP disease, but also to identify patients with clinically active porphyria who had no registered U-PBG samples. The time of hospitalization could be at any time during the patient's history. The time at risk was calculated from the study baseline.

The national health registers identified incident cases of PLC, defined as C220–C229 (ICD-10) or 1550–1552 (ICD-9). The incidence of HCC has historically been underestimated in the Swedish cancer register, which relies on mandatory reporting of histopathological samples, while HCC diagnosis in at-risk individuals is often based on radiology [26]. We also used the cause of death and inpatient and outpatient registers to identify incident PLC and applied a simple validation algorithm to increase the precision of case identification. A PLC diagnosis was considered confirmed if (1) a diagnosis of PLC appeared in the cancer register, (2) a diagnosis of PLC appeared in the other registers at least twice, and (3) a diagnosis of PLC in the patient registers was not followed by another cancer diagnosis within 2 months. If several registrations of PLC occurred in the registers, the time of PLC was defined by the date of the first recorded PLC diagnosis.

HCC, the most common PLC, is frequently preceded by chronic liver disease and liver cirrhosis. To assess the possible impact of liver disease on the outcome, we used the NPR and the specialized outpatient care register (from 2001) to identify any main or contributing diagnosis of liver disease prior to the PLC occurrence. Liver disease was defined as ICD codes 571–5733 (ICD9) and K700–K779 (ICD10). Data on viral hepatitis (B150–B199 [ICD 10] or 0700–0709 [ICD 9]) were not included in the dataset. A sensitivity analysis was performed in which patients with any liver disease were censored at the time of diagnosis of liver disease or were excluded if liver disease was diagnosed before baseline.

Statistical analysis

Continuous variables were summarized as medians and interquartile ranges (IQRs), and categorical variables as counts and percentages. The hazard of developing PLC, as an estimated relative risk, was computed using univariable and multivariable Cox proportional hazards models. To account for intragroup correlation, given the matched nature of the data, the cluster sandwich estimator for standard errors was used. The multivariable Cox models were adjusted for age and sex, where applicable. To evaluate effect modification by sex, an interaction term between patient status (patients with AHP/reference population) and sex was introduced. Subgroup analyses, based on a priori decisions for patients aged >50 years, sex, hospitalization, and for patients with AIP, U-PBG, and *HMBS* genotypes, were performed using Cox regression models. The results are presented as HRs with 95% confidence intervals (CIs). For each categorical variable used as exposure in the Cox models, the IR per 1000 person-years was calculated and reported. Kaplan–Meier curves depicting cumulative incidences were plotted to describe PLC incidence by sex, hospitalization, U-PBG, and genotype as categorical groups. Survival analysis for patients who developed PLC was performed using the Kaplan–Meier method and the log-rank test for equality of survivor functions.

STATA Statistical Software Release 15 (StataCorp 2017, StataCorp LLC, College Station, TX, USA) was used to perform all statistical analyses. All p-values were two-sided, and a p-value < 0.05 was considered statistically significant.

This study was approved by the Regional Ethical Review Board in Stockholm (Dnr: 2017/117–31). Individual informed consent was waived due to the observational nature of the study.

Results

The study cohort included 1244 patients with AHP and 12,333 matched reference individuals from the general population (Table 1). The median age at inclusion was 36 years (IQR 19–52) and 53% were women. Within the AHP population, 1063 (85%) had AIP, 125 (10%) had VP, and 56 (5%) had HCP. During the study period, 266 (21%) patients were

Table 1. *Characteristics of the study population*

	AHP	Reference population
Total study population	1244	12,333
Sex, female, *n* (%)	654 (53)	6500 (53)
Age at study entry, median (IQR)	36 (19–53)	36 (19–52)
Birthyear, median (IQR)	1957 (1939–1974)	1957 (1940–1974)
AHP type		
Acute intermittent porphyria, *n* (%)	1063 (85)	–
Variegate porphyria, *n* (%)	125 (10)	–
Hereditary coproporphyria, *n* (%)	56 (5)	–
Hospitalized for AHP 1987–2015, *n* (%)	266 (21)	–
U-PBG, highest value, AIP only:		
– No data, *n* (%)	224 (21)	–
– Negative, never elevated, *n* (%)	345 (32)	–
– Positive, moderate 1.6–6.4 mmol/mol creatinine, *n* (%)	140 (13)	–
– Positive, high >6.4 mmol/mol creatinine, *n* (%)	354 (33)	–
HMBS gene variant, AIP only:		
– c.593G>A, *n* (%)	693 (65)	–
– All other variants[a], *n* (%)	370 (35)	–

Abbreviations: AHP, acute hepatic porphyria; HMBS, hydroxymethylbilane synthase; U-PBG, urinary porphobilinogen.
[a]Listed in detail in Table 5.

Table 2. *Characteristics of the acute intermittent porphyria (AIP) subgroup by urinary porphobilinogen (U-PBG)*

	All U-PBG subgroups	U-PBG-negative	U-PBG-positive (moderate)	U-PBG- positive (high)	U-PBG-unknown
Total, *n* (%)	1063	345 (32)	140 (13)	354 (33)	224 (21)
Sex, female, *n* (%)	536 (50)	112 (32)	83 (59)	260 (73)	81 (36)
Age, median (IQR)	36 (18–52)	23 (18–41)	47 (32.5–60.5)	40 (25–51)	35 (18–60)
Birthyear, median (IQR)	1957 (1939–1974)	1967 (1951–1982)	1942 (1932–1960)	1949.5 (1938–1967)	1960 (1933–1981.5)
Hospitalized for AIP, *n* (%)	241 (23)	29 (8)	31 (22)	157 (44)	24 (11)
Not hospitalized for AIP, *n* (%)	822 (77)	316 (92)	109 (78)	197 (56)	200 (89)
HMBS c.593G>A variant, *n* (%)	693 (65)	225 (65)	84 (60)	219 (62)	165 (74)
HMBS all other variants, *n* (%)	370 (35)	120 (35)	56 (40)	135 (38)	59 (26)

Abbreviations: AIP, acute intermittent porphyria; HMBS, hydroxymethylbilane synthase; U-PBG, urinary porphobilinogen.

hospitalized due to porphyria. Elevated U-PBG levels (U-PBG-positive) were recorded in 494 (46%) of the patients with AIP, while 345 (32%) had U-PBG levels below the ULN (U-PBG-negative). Data on U-PBG were missing in 224 (21%) patients with AIP. The U-PBG subgroups are presented in Table 2. Almost two-thirds of the patients with AIP ($n = 693$, 65%) had the founder gene variant *HMBS*: c.593G>A. The time at risk was 243,470 years in total: 21,544 years for patients with AHP and 221,926 years for the reference population.

During follow-up, 108 individuals developed PLC: 25 (0.2%) in the reference population and 83 (6.7%)

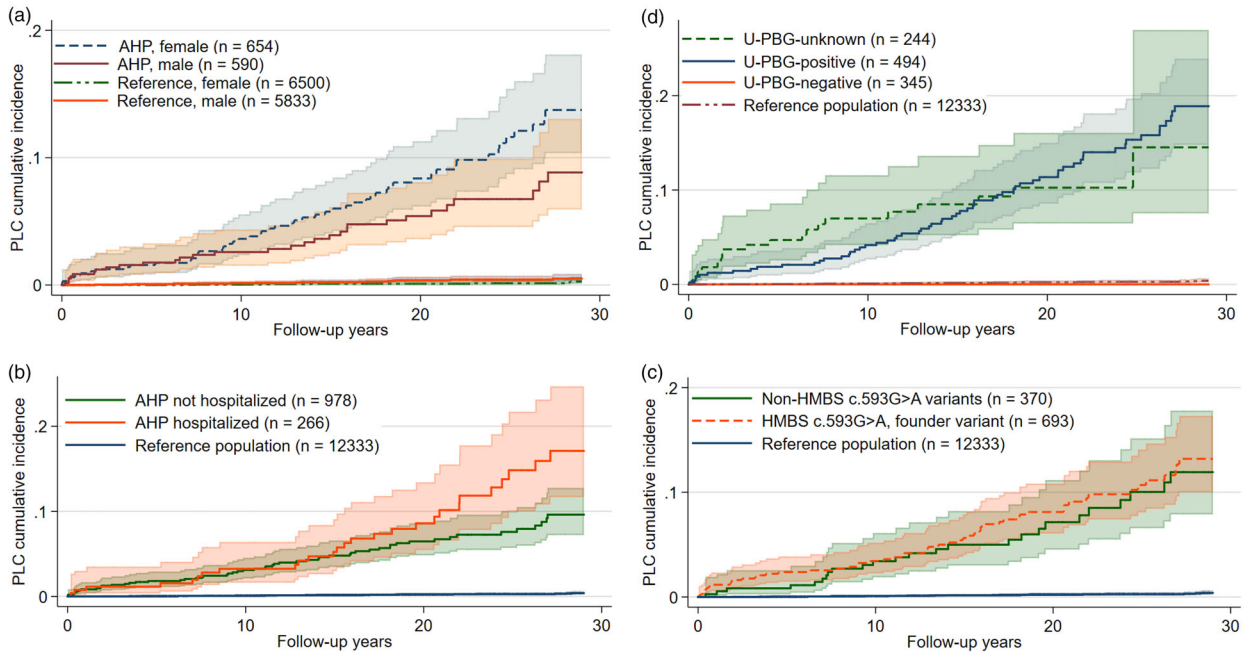

Fig. 1 *Kaplan–Meier plots depicting cumulative incidence of primary liver cancer with 95% confidence interval (CI) for the period 1987–2015. (a) Patients with acute hepatic porphyria (AHP) versus the reference population by sex. (b) Patients with AHP hospitalized during 1987–2015 for AHP (main diagnosis) versus those not hospitalized and the reference population. (c) Patients with acute intermittent porphyria (AIP) by urinary porphobilinogen (U-PBG) group (biochemical activity): U-PBG-negative, below upper limit of normal (ULN); U-PBG-positive, above ULN; U-PBG-unknown, no data on U-PBG; and the reference population. (d) Patients with AIP by HMBS variant: c.593G>A (founder gene variant) versus all other HMBS variants and the reference population.*

among the patients with AHP. The IRs were 0.11 and 3.85/1000 person-years, respectively, and the adjusted hazard ratio (aHR) was 38.0 (95% CI = 24.3–59.3). In the reference population, men had a higher PLC incidence than women (IR = 0.17 vs. 0.07/1000 person-years), while in the AHP cohort, males had a lower incidence (IR = 2.99 vs. 4.60/1000 person-years) (Fig. 1a, Table 3).

AHP-type

Among the 83 patients with AHP who developed PLC, 81 (98%) had AIP, one had VP, and one had HCP. The PLC IR in the AIP subgroup was 4.30/1000 person-years and aHR 44.5 (95% CI = 28.3–70.0). In VP and HCP patients, the PLC IRs were 0.57 and 1.04 and the aHRs were 4.8 (95% CI = 0.6–36.3, $p = 0.127$) and 7.0 (95% CI = 0.9–57.8, $p = 0.07$).

Clinical disease activity

Hospitalized patients ($n = 266$) had a PLC IR of 5.37 compared to 3.39/1000 person-years in those not hospitalized ($n = 978$) (Fig. 1b). The crude HR for hospitalized versus not hospitalized groups was 1.6 (95% CI = 1.0–2.5, $p = 0.05$) and the aHR in the age- and sex-adjusted model was 1.9 (95% CI = 1.2–3.2, $p = 0.01$). The lack of data on hospital admissions before 1987 might explain similar PLC incidences in the hospitalized and non-hospitalized groups during the first 15 years of the study (Fig. 1b). Subgroups based on hospitalizations and biochemical activity overlapped. If tested, all patients hospitalized with acute porphyria attacks also had elevated porphyrin precursors (U-PBG-positive).

Biochemical disease activity

Patients with AIP were stratified based on the highest recorded U-PBG values (Tables 1 and 2). We found that 62 U-PBG-positive patients developed PLC, IR = 6.47/1000 person-years (95% CI = 5.05–8.30), while no patients in the U-PBG-negative group ($n = 345$) developed PLC (Fig. 1c). The PLC incidence in the group with unknown

Table 3. *Primary liver cancer incidence, crude and adjusted hazard ratios for acute hepatic porphyria, and matched reference population*

	Total (n)	PLC (n)	IR/1000 person-years (95% CI)	HR crude (95% CI)	aHR (95% CI)	*p*
Reference population	12,333	25	0.11 (0.08–0.17)	1		
AHP-cohort	1244	83	3.85 (3.11–4.78)	34.3 (22.3–52.8)	38.0 (24.3–59.3)	<0.005
Sex						
Reference females	6500	8	0.07 (0.03–0.13)	1		
AHP females	654	53	4.60 (3.51–6.02)	70.1 (35.5–138.5)	83.3 (42.0–165.4)*	<0.005
Reference males	5833	17	0.17 (0.10–0.27)	1		
AHP males	590	30	2.99 (2.09–4.28)	17.8 (9.9–31.9)	17.8 (9.7–32.6)*	<0.005
AHP type						
Reference individuals	12,333	25	0.11 (0.08–0.17)	1		
AIP	1063	81	4.30 (3.46–5.35)	38.2 (24.8–58.8)	44.5 (28.3–70.0)	<0.005
VP	125	1	0.57 (0.08–4.06)	5.2 (0.7–39.1)	4.8 (0.6–36.3)	0.127
HCP	56	1	1.04 (0.15–7.38)	9.3 (1.2–69.8)	7.0 (0.9–57.8)	0.069
Symptomatic disease						
AHP, hospitalized	266	27	5.37 (3.68– 7.83)	47.4 (27.7–81.2)	65.3 (37.9–112.6)	<0.005
AHP, never hospitalized	978	56	3.39 (2.61–4.41)	30.3 (19.3–47.5)	31.5 (19.6–50.6)	<0.005
Biochemical activity (AIP only)						
U-PB- negative	345	0	0.00	≈0	≈0	<0.005
U-PBG-positive (moderate)	140	15	5.58 (3.37–9.26)	48.7 (25.3–93.5)	37.8 (19.6–73.1)	<0.005
U-PBG-positive (high)	354	47	6.82 (5.12–9.08)	59.6 (37.4–94.8)	77.1 (47.2–126.1)	<0.005
U-PBG-positive (all)	494	62	6.47 (5.05–8.30)	56.5 (35.9–88.9)	61.3 (38.6–97.5)	<0.005
U-PBG No data/unknown	224	19	6.14 (3.92–9.63)	58.1 (32.7–103.3)	50.5 (27.0–94.3)	<0.005
HMBS variant (AIP only)						
c.593G>A	693	56	4.52 (3.47–5.87)	40.1 (25.5–63.0)	47.1 (29.0–76.6)	<0.005
Other variants	370	25	3.89 (2.63–5.75)	34.6 (20.0–59.8)	39.9 (23.0–69.4)	<0.005
Subgroup: age >50 years at inclusion						
Reference individuals	3411	23	0.41 (0.27–0.62)	1		
AIP	290	60	14.47 (11.23–18.63)	35.4 (22.3–56.1)	36.0 (22.3–58.0)	<0.005
AIP, hospitalized	62	18	20.62 (12.99–32.73)	52.1 (28.4–95.6)	61.0 (32.7–113.9)	<0.005
AIP, U-PBG-positive	157	44	17.87 (13.30–24.01)	43.6 (26.8–70.9)	47.9 (29.0–79.0)	<0.005
AIP, U-PBG-positive and/or hospitalized	175	45	16.95 (12.65–22.70)	41.5 (25.5–67.5)	45.9 (27.8–75.8	<0.005

Note: Hazard ratios are adjusted (aHR) for age and sex (except aHRs marked * which are adjusted for age).
Abbreviations: AHP, acute hepatic porphyria; aHR, adjusted hazard ratio; AIP, acute intermittent porphyria; HCP, hepatic coproporphyria; HMBS, hydroxymethylbilane synthase; IR, incidence rate; U-PBG, urinary porphobilinogen; VP, variegate porphyria.

U-PBG was similar to that in the U-PBG-positive group. The aHR for the U-PBG-positive group versus the reference population was 61.3 (95% CI = 38.6–97.5). We observed a higher PLC incidence among the U-PBG-positive patients with high (>6.4 mmol/mol creatinine) U-PBG levels, as compared to those with moderate (1.6–6.4 mmol/mol creatinine) U-PBG levels, aHR = 2.1 (95% CI = 1.1–3.9, $p = 0.02$) (Table 4). The tendency toward a lower PLC risk in males than in females with AHP (crude HR = 0.7 [95% CI = 0.4–1.0], $p = 0.06$) was related to U-PBG levels. The HR for males versus females in the U-PBG-positive subgroup was 1.0 (95% CI = 0.6–1.7, $p = 0.94$).

Table 4. *Within-group comparisons of primary liver cancer incidences, and crude and adjusted hazard ratios*

	n	PLC (n)	IR /1000 person years (95% CI)	HR crude (95% CI)	aHR (95% CI)	P
AHP, all						
females	654	53	4.60 (3.51–6.02)	ref	ref	
males	590	30	2.99 (2.10–4.28)	0.7 (0.4–1.0)	0.7 (0.5–1.2)*	0.219
AHP never hospitalized	978	56	3.39 (2.61–4.41)	ref	ref	
AHP hospitalized	266	27	5.37 (3.68– 7.83)	1.6 (1.0–2.5)	1.9 (1.2–3.2)	0.008
AIP only						
Non c.593G>A *HMBS* variants	370	25	3.89 (2.63–5.75)	ref	ref	
HMBS: c.593G>A	693	56	4.52 (3.47–5.87)	1.2 (0.7–1.9)	1.3 (0.8–2.0)	0.338
U-PBG-negative	345	0	0.00	ref	ref	
U-PBG-positive	494	62	6.47 (5.05–8.30)	NA	NA	
U-PBG-positive, moderate	140	15	5.58 (3.37–9.26)	ref	ref	
U-PBG-positive, high	354	47	6.82 (5.12–9.08)	1.2 (0.7–2.2)	2.1 (1.1–3.9)	0.019
UPBG-positive, females	343	43	6.44 (4.77–8.68)	ref	ref	
UPBG-positive, males	151	19	6.56 (4.18–10.28)	1.0 (0.6–1.7)	0.8 (0.4–1.4)*	0.392

Note: Hazard ratios are adjusted (aHR) for age and sex (except aHRs marked *, which are adjusted for age only).
Abbreviations: AHP, acute hepatic porphyria; AIP, acute intermittent porphyria; IR, incidence rate; U-PBG, urinary porphobilinogen.

Table 5. *Incidence of primary liver cancer associated with different HMBS variants in the acute intermittent porphyria subgroup*

HMBS variant	*n* (%)	PLC (*n*)	IR /1000 person-years (95% CI)
c.593G>A (founder variant)	693 (65)	56	4.51 (3.47–5.87)
c.517C>T	57 (5)	3	2.92 (0.94–9.06)
c.499C>T	49 (5)	2	2.56 (0.64–10.26)
c.499-1G>A	41 (4)	5	5.76 (2.40–13.84)
c.847_848delTG	27 (3)	0	0.00
c.356C>T	20 (2)	0	0.00
c.87+1G>A	17 (2)	2	7.22 (1.81–28.89)
c.500G>A	19 (2)	0	0.00
Remaining less-frequent variants	138 (13)	13	5.77 (3.36–9.95
Unknown	2 (0.2)	0	0.00
All variants	1063 (100)	81	4.30 (3.46–5.35)

AIP genotype

We found no significant difference in PLC risk between patients with the most common *HMBS* variant (c.593G>A) in Sweden ($n = 693$), and those with other variants (n = 370), aHR 1.3 (95% CI = 0.8–2.0) (Fig. 1d). The number of patients with each of the less-common genotypes was relatively low and no further subgroup analyses were performed (Table 5). Data on AIP disease characteristics by *HMBS* variants are presented in Table S3.

Primary liver cancer

Sixty-six (80%) patients with AHP-PLC and 19 (76%) patients with PLC from the reference population were included in the cancer registry. The remaining cases were identified in the patient registers. Sixteen patients (three with AHP, 13 from the reference group) who had one or more diagnoses of PLC in the patient registers were defined as not having PLC based on the algorithm criteria.

Among the AHP-associated PLCs in this study ($n = 83$), 67 (81%) were HCCs (C220 [ICD-10] or 1550 [ICD-9]), three (4%) were CC (C221 [ICD-10] or 1551 [ICD-9]), and 13 (16%) were classified as unspecified (C229 [ICD-10], 1552 [ICD-9] or codes for both HCC and CC were registered for the same patient). Among patients with PLC from the reference population ($n = 25$), 16 (64%) had HCC, three (12%) had CC, and six (25%) had unspecified PLC. The overall survival after PLC diagnosis was significantly longer in patients with AHP-associated PLC than in those from the reference population with PLC, with 1- and 5-year survival rates of 64% and 30% versus 15% and 0%, respectively ($p < 0.001$).

Age

The median age at PLC diagnosis was 71 years (range 53–89 years) in the AHP cohort and 74 years (range 56–92 years) in the reference population. We observed a non-significant trend in that, among the 47 patients in the high U-PBG-positive subgroup, six (13%) developed PLC before age 60 years as compared to none in the moderate U-PBG-positive subgroup (chi-square test: $p = 0.145$). The difference in mean age between the two U-PBG-positive subgroups was not statistically significant.

No patient in the AHP cohort developed PLC before the age of 50 years, and the lowest recorded age at PLC diagnosis was 53 years. Among the 175 patients with AHP who were aged >50 years and who were U-PBG-positive and/or had been hospitalized for porphyria, we found an IR of 16.9 (95% CI = 12.6–22.7)/1000 person-years or 1.7% per year. The incidence was similar in hospitalized and U-PBG-positive patients over the age of 50 years when analysed separately (Table 3).

Liver disease

Among the 108 individuals who developed PLC, we found a main or contributing diagnosis of liver disease prior to PLC in three (12%) individuals from the reference population. Two AIP-PLC patients (2%) had previously been diagnosed with liver disease, alcoholic cirrhosis, and unspecified hepatitis. Neither patients with PLC with VP nor those with HCP had any registered liver disease. Of note, data on viral hepatitis and liver disease diagnosed prior to 1987 were not available. To assess possible bias from liver diseases in the results, a sensitivity analysis was performed in which subjects with a diagnosis of liver disease were censored (Tables S1 and S2). IRs and HRs were only marginally altered, and no changes in statistically significant results were observed.

Discussion and conclusion

This study analysed the relative and absolute PLC risk in AHP patients, and investigated whether this risk is related to age, sex, disease severity, AHP type, or AIP genotype.

Since its first publication in 1984, several studies have reported an association between AHP, mainly AIP, and primary liver cancer [3,5,6,8–11,13,16,27,28]. The majority of these studies emanated from the Nordic countries, Sweden [3,5,8,11,16], Norway [10], and Finland [6]. The number of studies from this region, in combination with a lack of reports on AHP-related PLC from other parts of the world, has led to speculation about whether PLC risk in AHP is an isolated Nordic phenomenon. The high prevalence of AIP and compulsory national patient registers in Nordic countries may be more likely explanations. PLC often occurs decades after porphyria has become clinically active, and the patient is no longer in active follow-up. Furthermore, cohort-based studies performed in France [9], the United States [28], Germany [27], and Switzerland [13] all confirmed significantly increased PLC risks in AHP patients. Risk estimates in previous studies have, however, varied significantly, mainly due to limited sample sizes, and data on risk in subgroups have been scarce. In this, the largest AHP cohort assembled to date, we confirm that AHP is associated with an increased PLC risk compared to the general population. The whole AHP cohort of 1244 individuals had an aHR of 38 when compared with 12,333 matched reference individuals. Our results clearly identify a group with a high risk of PLC: AIP patients >50 years of age with active disease during their lifetime have an annual PLC incidence of 1.7–2.1, comparable to the incidence of 1%–6% in patients with liver cirrhosis caused by alcohol, NAFLD, or viral aetiology [29–31]. Low-risk groups were also identified: younger patients and those with asymptomatic AIP who never had elevated U-PBG. As discussed below, our results are less clear for the remaining AHP patients.

Since both AIP, VP, and HCP can manifest in acute attacks and accumulated ALA and PBG, a similar PLC risk has been assumed. Published data on PLC in VP and HCP are, however, limited to case reports and sporadic cases found in AHP cohorts

[3,6,9,10,13,16]. Although not formally studied, the absence of reports on PLC from the large VP population in South Africa could be an indication that the PLC risk in VP is low. Despite large sample sizes (by VP and HCP standards) and up to 29 years of follow-up, we found only one PLC case in each of the VP and HCP cohorts and could not demonstrate a significant risk increase as compared to the reference population. VP and HCP patients generally have lower levels of PBG and ALA than those with AIP [32] and are considered less prone to severe acute attacks [3,33]. Our study did not include data on the biochemical activities in VP and HCP patients. An increased risk in patients with high biochemical activity cannot be dismissed. The much larger AIP cohort (1063 patients), with a higher number of incident PLCs ($n = 81$) and more comprehensive data, permitted a more detailed analysis of PLC risk in AIP in this study.

Previous studies have shown an association between female sex and PLC risk in AHP [10,16,28]. We indeed found a higher relative risk in AHP females than in the matched reference individuals (female HR = 70.1 versus male HR = 17.8). This difference was highly influenced by the higher PLC risk in the male reference population, reflecting the more than twofold male-to-female PLC risk increase in the general population [34]. The risk difference between female and male patients with AHP disappeared when patients were stratified for U-PBG levels. This suggests that higher AHP disease activity, which is more common in females [32], rather than female sex per se, is associated with increased PLC risk.

The severity of symptoms and biochemical disease activity vary significantly in patients with AIP. While most gene carriers have neither symptoms nor biochemical activity, others have increased U-PBG levels, despite having few or no attacks during their lifetime. Some have several attacks and are frequently hospitalized. Both ALA and PBG reflect the biochemical activity related to the disease. During acute porphyria attacks, U-PBG levels are elevated and often remain elevated for years after the attack [35,36].

Among the 345 patients with AIP who were tested and never had elevated U-PBG, none developed PLC. In contrast, 62 of the 494 patients with elevated U-PBG developed PLC, corresponding to an aHR of 61.3 when compared to the matched reference population. Among U-PBG-positive patients, the aHR for PLC development for those with high U-PBG versus those with moderate U-PBG was 2.1 (95% CI = 1.1–3.9). This suggests that PLC risk increases with higher biochemical activity in AIP patients. The absence of PLC in U-PBG-negative patients suggests a low risk in biochemically inactive AIP. However, this finding should be interpreted with caution because the median age was lower in the U-PBG-negative subgroup (Table 2).

Six of the 47 patients in the high-U-PBG group developed PLC between 53 and 60 years of age, while all in the moderate U-PBG group were above age 60 years at PLC diagnosis. This suggests a correlation between earlier PLC and higher biochemical activity. However, this difference was not statistically significant.

We used hospital admissions for AHP as a surrogate for symptomatic disease. There is a considerable overlap between the hospitalized group and the U-PBG-positive group, since hospitalized patients, if tested, always had elevated U-PBG. Among the 266 patients who had been hospitalized, we found an association with PLC that was similar to that in the U-PBG-positive group (aHR = 65.3 vs. 61.3), as compared to the reference population.

Our study results refute the suggestion that the Swedish *HMBS* founder gene variant explains the high incidence of AIP-related PLC reported in previous studies [11,16]. As shown in Fig. 1d, we found no difference in PLC risk between carriers of the Swedish founder variant and those who carried other variants.

We observed longer overall survival after PLC diagnosis among patients with AHP than in PLC patients from the reference population. AHP-related PLCs are often found in non-cirrhotic livers and are therefore more likely to be amenable to curative treatment options. Another explanation might be better coverage by surveillance, which potentially increases the likelihood of early detection and curative treatments, but also introduces a risk of surveillance bias [11,16].

In the general population, patients with HCC are predominantly male and typically have a history of liver cirrhosis, while AHP-PLC patients are mainly women with low rates of underlying liver diseases [10,16]. Several hypotheses have been proposed for the mechanisms involved in the development

of PLC in AHP. Impaired antioxidant levels due to haem deficiency, a second somatic mutation in genes controlling haem biosynthesis, and carcinogenic effects of ALA might contribute to cancer development in non-cirrhotic livers in patients with AHP [37–39]. Several reports have identified ALA as a key mediator [38]. ALA accumulates in hepatocytes during attacks and can cause oxidative stress and DNA damage and is cytotoxic in vitro [40–42]. ALA is also increased in the liver in tyrosinemia type 1, which has been associated with a high risk of early liver cancer [43]. Our finding of a correlation between biochemical disease activity and the risk of PLC in AIP supports the theory that porphyrin precursors are involved in hepatic carcinogenesis. ALA and PBG are both increased in patients with biochemically active AHP. ALA is suggested to be a noxious precursor that contributes to neurotoxicity and carcinogenicity. PBG was used as a marker of biochemical activity in this study because it is considered a more robust analyte. PBG elevation is specific for acute attacks of AHP and is used as the sole diagnostic tool in clinical practice in several countries [44,45]. It is more reliable as a marker of AHP biochemical activity over time, and concentrations of U-PBG (but not urinary ALA) are stable in patients who develop renal insufficiency [46]. No U-PBG-negative patients developed PLC, and patients with increased U-PBG, regardless of genotype and sex, had a significantly increased risk of PLC. In addition, patients with higher U-PBG levels had a higher risk of PLC.

The strengths of this study include the unprecedented study size, the use of a matched population-based reference cohort, the long follow-up period, and the high quality of data from the national porphyria register and national health registers. The linking of data on biochemical activity and genotype for the AIP cohort to outcome was also an advantage. Register-based porphyria research in Sweden is facilitated by comprehensive long-term registers at an individual level and by the high AIP prevalence.

This study had several limitations. Register-based research entails the risk of information bias. Registration practice, clinical practice, and diagnostic criteria for HCC all changed during the study period. However, the Swedish national health registers are reported to have high validity, including in terms of data on liver diseases and HCC [47–50]. Assessment of AIP disease severity has several weaknesses. U-PBG testing has varied among Swedish hospitals and over time. No U-PBG data were available for 244 (21%) patients with AIP. These patients were similar in age to the tested patients but were more often male than female (64% vs. 50%) and were overrepresented in the three northernmost counties in Sweden. The PLC risk in the patients with no data on U-PBG was similar to the risk in the U-PBG-positive group. Misclassification bias might occur with the use of hospitalizations as a surrogate measure of active disease. Patients were not identified as hospitalized if they were admitted before 1987, if porphyria attacks were misdiagnosed, or if porphyria was not recorded as the primary discharge diagnosis. Patients with symptomatic or biochemically active disease may be more likely to undergo radiological examinations, which may result in non-differential surveillance bias. The recording of data on U-PBG and hospitalizations at any time during the study rather than at baseline entailed potential immortal time bias. Elevated U-PBG levels and clinical symptoms are most common in patients aged 20–50 years, while PLC was not observed in any patient before the age of 50 years.

This study has practical implications for the care of patients with AHP. The HCC guidelines advocate biannual ultrasound surveillance in patients with liver cirrhosis who have an annual risk of $\geq$1.5% [51]. An even lower risk in non-cirrhotic patients has been suggested to warrant surveillance based on cost-effectiveness estimates. Some countries have PLC surveillance programs for patients with AHP, but international guidelines are lacking. We found an annual PLC incidence of 1.7%–2.1% in patients with AIP aged over 50 years who had a history of clinically or biochemically active disease. These patients can be considered a high-risk group that may benefit the most from PLC surveillance. It is difficult to define clinically useful criteria to identify low-risk patients who would not benefit from surveillance. AHP patients aged <50 years and asymptomatic AIP gene carriers who have been regularly tested to have normal U-PBG appear to have a low PLC risk. Unfortunately, few asymptomatic patients are regularly tested in clinical practice. These patients lack data to confirm a biochemically inactive disease, and the PLC risk is difficult to assess. Regular testing of porphyrin precursors, also in asymptomatic AIP patients, would improve the assessment of PLC risk and facilitate the decision on whether to include these patients in PLC surveillance. The small number of incident cancers does not permit robust recommendations

for PLC surveillance in VP and HCP patients. Studies on larger HCP and VP cohorts that include data on clinical and biochemical activity might provide better risk estimates.

This study represents the largest and most comprehensive analysis of the risk of PLC in AHP patients to date. The results confirm that their PLC risk is significantly increased as compared to that in the general population. We propose that clinically identified patients with AHP, with exceptions based on individual risk assessment, as discussed above, should be advised to undergo biannual ultrasound surveillance from the age of 50 years.

Conflict of interest

Mattias Lissing has consulted and received grants from Alnylam Pharmaceuticals. Pauline Harper, Eliane Sardh, and Staffan Wahlin received grants from Alnylam Pharmaceuticals.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary table 1. Primary liver cancer incidence and adjusted hazard ratios for acute hepatic porphyria and matched reference population before and after censoring subjects with any liver disease. aHRs are adjusted for age and sex (except aHRs marked * which are adjusted for age).

Supplementary table 2. Within group comparisons of primary liver cancer incidences and adjusted hazard ratios (aHR) before and after censoring subjects with any liver disease. aHRs are adjusted for age and sex (except aHRs marked * are adjusted for age).

Supplementary table 3. Primary liver cancer, U-PBG activity and hospitalizations by HMBS variants in the acute intermittent porphyria subgroup. ■

