Clinical long-term consequences of acute hepatic porphyria and porphyria cutanea tarda

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Thesis for the degree of Philosophiae Doctor (PhD)
at the University of Bergen

Date of defense: 10.12.2019

Year: 2019

Title: Clinical long-term consequences of acute hepatic porphyria and porphyria cutanea tarda

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Print: Skipnes Kommunikasjon / University of Bergen

Scientific environment

The current dissertation has been carried out at the Norwegian Porphyria Centre (NAPOS), at Haukeland University Hospital, and the Department for Global Health and Primary Health Care at the Faculty of Medicine and Dentistry, University of Bergen. The candidate is affiliated with the Genetic Epidemiology Research Group at the University of Bergen, and the National Research School in Population Based Epidemiology (EPINOR), which partly provided a grant for a research stay abroad to the Department of Statistics, Data Science and Epidemiology at Swinburne University of Technology, Melbourne, Australia.

The present project was funded by a research grant from the Western Norway Regional Health Authority. The cost of acquiring and linking data from several national registries and databases was funded by a grant awarded by the Norwegian National Advisory Unit on Rare Disorders (NKSD), Oslo University Hospital, and NAPOS.

The main supervisor of the project was Dr Mette Christophersen Tollånes (MD, PhD), Medical Consultant and specialist in biological chemistry at the Norwegian Organization for Quality Improvement of Laboratory Examinations (NOKLUS). The candidate was co-supervised by Professor Sverre Sandberg (MD, PhD), Director of NAPOS and NOKLUS and specialist in biological chemistry, and Dr Aasne Karine Aarsand (MD, PhD), Vice-Director of NAPOS and Medical Consultant and specialist in biological chemistry.

Acknowledgements

There have been many individuals who have contributed to this body of work and have assisted me throughout my PhD. First and foremost I would like to thank my three brilliant supervisors, who have been there from the start to the end; Mette Christophersen Tollånes, Sverre Sandberg and Aasne Karine Aarsand. Mette, you conceived much of the original idea of this project, welcomed me to the research group at the department and have contributed significantly to my PhD since. You have given me outstanding guidance in medical research and epidemiology and made me into a better researcher. I don't think a PhD candidate could ask for a more diligent and compassionate supervisor – thank you. Sverre, thank you for your pragmatic guidance through the PhD and your unique insights into the porphyrias. Despite thinking about my findings for weeks and reading widely, you rarely failed to surprise me with a better explanation. Aasne, thank you for both your sharp wit, diligence, sharing your expertise in the porphyrias (spelt here using the plural form) and guidance. Despite your busy schedule you always had your door open to discuss my project and offer solution-oriented advice, following some good banter and lots of laughing of course. You have also always made me feel welcome at NAPOS, both before and during my PhD.

This project would not have been possible without the assistance from all of my colleagues at NAPOS, and I am indebted to them all. I would especially like to thank Ellen Gerd Valberg, Jorild Haugen Villanger and Egil Stole for their assistance, perspectives, attention to detail and witty sense of humour.

Participating in the fortnightly research group meeting in Genetic Epidemiology has broadened my knowledge in the area. I want to thank the previous leader of the research group, Tone Bjørge for her assistance throughout my PhD, kindness and for welcoming me. I would also like to thank the current leader, Rolv Terje Lie, for his interesting insights.

I was fortunate during my PhD to assist in the development of a seminar and a manuscript about the importance of creativity and deep and slow thinking in research

and epidemiology. I have enjoyed my time working on this endeavour with the ever-enthusiastic and clever Rolv Skjærven, Allen Wilcox and Marianna Cortese.

I would also like to express my gratitude to my fellow PhD-candidates and Post Doc fellows at the department, past and present, who have made the process of completing this PhD so much more enjoyable. Namely; Marianna Cortese, Berit Skretting Solberg, Ingeborg Forthun, Hilde Kristin Refvik Riise, Tone Nygaard Flø1o, Maria Winther Gunnes, Marianne Strøm and Julia Romanowska.

I want to thank my friends and family. I am indebted to my father, Mark, and sister, Kristie, who, although they are on the other side of the world, have supported me through this process and in every decision I make in my life. I am indebted to my late and devoted mother and would not be the person I am today if it was not for her. Special thoughts go to my mother-in-law Frances and brother-in-law Adam for their support. To my friends, Andrew and Richard, thank you for your friendship in the previous years and keeping me focused on the other most important thing in life: cycling. Last, but not least, I am indebted to the love of my life, Hildegunn, for her continual support in all my endeavours and being there when it matters, and my children Sofie and Mikal.

"A man should look for what is, and not for what he thinks should be."

Albert Einstein

Abbreviations

AIP	Acute intermittent porphyria
AHP	Acute hepatic porphyria
ALA	5-aminolaevulinic acid
CC	Cholangiocellular carcinoma
CI	Confidence interval
CKD	Chronic kidney disease
CEP	Congenital erythropoietic porphyria
CPOX	Coproporphyinogen oxidase
DAG	Directed acyclical diagram
EPP	Erythropoietic protoporphyria
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCC	hepatocellular carcinoma
HCP	Hereditary coproporphyria
HIV	Human immunodeficiency virus
aHR	Adjusted hazard ratio
HR	Hazard ratio
aIRR	Adjusted incident rate ratio
IRR	Incident rate ratio
NAPOS	Norwegian Porphyria Centre
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PCT	Porphyria cutanea tarda
PLC	Primary liver cancer
PPOX	Protoporphyrinogen oxidase
RCT	Randomised controlled trial
SES	Socioeconomic status
aSHR	Subdistribution hazard ratios
SHR	Subdistribution hazard ratios

URL	Upper reference limit
UROD	Uroporphyrinogen decarboxylase
VP	Variegate porphyria
XLEPP	X-linked erythropoietic protoporphyria
ZIBR	Zero-inflated negative binomial regression

Abstract

Background:
The porphyrias comprise of several rare metabolic disorders in which a crucial enzymatic step in the biosynthesis of haem is affected, mostly due to a genetic defect. The current project focused on two major disease groups of the porphyrias; acute hepatic porphyria (AHP) and porphyria cutanea tarda (PCT).

AHP presents clinically as neurovisceral acute attacks, usually in adulthood, usually requiring inpatient care. Only a small proportion of AHP gene mutation carriers develop symptoms and repeat attacks are common in a minimal, mostly female subtype. It has been proposed that the precursors of haem, which are characteristically overproduced in symptomatic AHP, and to a lesser extent genetically predisposed gene carriers, may be carcinogenic. Indeed AHP is associated with an increased risk of hepatocellular carcinoma (HCC), although the magnitude of this risk remains unclear and it is uncertain if AHP increases risk of other malignancies. The acute attacks and/or chronic symptoms of AHP may also affect daily living and put patients at risk of sick leave absences and disability pension. In addition to HCC, AHP is associated with other long-term complications, such as kidney failure and hypertension, which may lead to premature death.

PCT presents clinically in the form of photosensitivity, blistering, crusts and fragile skin, as a result of abnormal quantities of porphyrins in the skin. Liver damage and iron overload are common in PCT. PCT is also strongly associated with the hepatitis C virus (HCV) infection, abuse of alcohol, hemochromatosis and the use of oestrogens. Consequently, PCT may be associated with premature mortality. PCT may also be a risk factor for HCC and other cancers, but the evidence is unclear.

Aims:
The current project aimed to investigate the long-term consequences of AHP and PCT. Specifically, we aimed to investigate the risk of malignancies, with a particular interest in the risk of HCC. We also aimed to investigate the risk of premature death, both overall and disease-specific mortality. Finally, we investigated morbidity in

persons with AHP and if there was an increased risk of long-term sick leave and/or disability pension compared to the general population.

Methods:

We conducted three nationwide, registry-based cohort studies. Several compulsory data sources were record linked to the Norwegian Porphyria Registry, originally in 2012 and again in 2018. All Norwegian adult residents comprising of over 5 million persons comprised the reference populations. *Study I* investigated cancer risk in AHP from 2000 to 2011. *Study II* investigated cancer and mortality risk in persons with PCT from 2000 to 2016 and *study III* investigated long-term sick leave, disability leave and mortality in persons with AHP from 1992 to 2017, 1992 to 2016 and 1996 to 2017, respectively. The absolute risk was assessed by calculating annual incidence, and we conducted survival analysis using several regression techniques to compare risk between persons with AHP/PCT and the reference population, adjusting for age, sex and educational attainment. We also calculated risk stratified by subtypes of AHP and PCT, namely between persons with symptomatic disease, at some point in time, and asymptomatic AHP gene carriers and between persons with sporadic and familial PCT. Sex differences in *study I* was investigated by a meta-analysis of several published cohort studies. Lastly, given that HCC and PCT share similar risk factors, which would confound our results, we also compared persons with PCT to persons with a history of alcohol abuse in *study II*.

Results:

We found evidence of a 108-fold (95% confidence interval (CI): 56, 207) and a 20-fold (95% CI: 8.8, 44.0) increased risk of HCC in persons with AHP and PCT, respectively. The risk was higher for women than men with AHP according to the findings of the meta-analysis in *study I*. The risk remained, although to a much smaller extent when comparing the risk of HCC in persons with PCT to persons with a history of alcohol abuse/dependence in *study II*. We also found evidence that AHP may be associated with a small increased risk of kidney and endometrial cancers and PCT associated with an increased risk of gallbladder and biliary tract cancer. A 1.5-fold increased overall risk of premature death was observed in individuals with PCT

in *study II*, whereas a sensitivity analysis suggested that there was no increased risk of premature death in persons with AHP in *study III*, despite an increased risk of mortality due to HCC. Lastly, in *study III*, persons with AHP had a 1.5-fold increased risk of long-term sick leave (95% CI 1.3, 1.7) and a 1.9-fold increased risk of disability pension (95% CI 1.5, 2.4). The risk was even greater in persons with symptomatic AHP, but not elevated for asymptomatic AHP gene carriers.

Conclusions:

Persons with PCT and AHP are at substantially increased risk of HCC compared to the general population. Although lifestyle factors likely contribute to these observations in persons with PCT, something specific about PCT itself may contribute to the pathophysiology of HCC. For persons with AHP, who do not generally differ from the general population concerning HCC risk factors, our study supports previous findings that PLC is a serious life-threatening long-term consequence of AHP, and supports the idea that persons 50 years or older from this group would benefit from selective surveillance. Morbidity due to AHP also appears to result in more long-term sick leave absences from work and disability pension in persons with symptomatic AHP. Early diagnosis, counselling about precipitating factors and routine follow-up of symptomatic AHP gene carriers is, therefore, recommended.

List of Publications

I. Baravelli, C.M., Sandberg , S., Aarsand, A.K., Nilsen, R.M., Tollånes, M.C., (2017). 'Acute hepatic porphyria and cancer risk: a nationwide cohort study', Journal of Internal Medicine, Vol. 282, no. 3, pp. 229-240.

II. Baravelli, C.M., Sandberg , S., Aarsand, A.K., Tollånes, M.C., (2019). 'Porphyria cutanea tarda increases risk of hepatocellular carcinoma and premature death: a nationwide cohort study', Orphanet Journal of Rare Diseases, Vol. 282, no. 1, pp. 229-240.

III. Baravelli, C.M., Aarsand, A.K., Sandberg, S., Tollånes, M.C., (2019). 'Sick leave, disability and mortality in acute hepatic porphyria: A population based cohort study'. Submitted.

Contents

1. Background

The porphyrias consist of several rare mainly hereditary metabolic disorders. The laboratory diagnosis and specialised treatment of the porphyrias have received a great deal of attention over the past three decades. This has led to marked improvements in health, especially concerning acute attacks of acute hepatic porphyria (AHP). However, research has recently begun to focus on the natural history and long-term consequences of the porphyrias, both concerning morbidity and mortality, which have been traditionally less in focus.

The background of this thesis will introduce the porphyrias, including the underlying biochemical mechanisms and clinical presentations, and discuss known long-term consequences, particularly hepatocellular carcinoma (HCC) and access to long-term sick leave and the disability pension as well as life expectancy.

1.1 Porphyrias: disorder overview and classification

The porphyrias comprise of several rare, mostly hereditary metabolic diseases. Each type is caused by a specific deficiency of an enzyme involved in the eight steps of haem bio-synthesis (Figure 1) (1). This altered activity of an enzyme can lead to the accumulation of the haem precursors 5-aminolaevulinic acid (ALA), porphobilinogen (PBG), and/or porphyrins in individuals with porphyria, which can have diverse acute and chronic clinical effects (2). Porphyrins are an essential building block of haem, which is vital for oxygen transportation and metabolism in all human cells (3). Haem is in particular abundance in the erythropoietic cells, mostly for the production of haemoglobin (4), and the liver parenchymal cells, for the metabolism of exogenous compounds, such as drugs and chemicals (5). Symptoms of the disorders can present as acute attacks of abdominal pain and neurovisceral symptoms (acute intermittent porphyria (AIP)), cutaneous symptoms (porphyria cutanea tarda (PCT), congenital erythropoietic porphyria (CEP), erythropoietic protoporphyria (EPP) and X-linked erythropoietic protoporphyria (XLEPP)) or both (hereditary coproporphyria (HCP) and variegate porphyria (VP)).

Diagnosis in symptomatic patients is based on the demonstration of increased haem precursors in urine, blood and/or faeces. Porphyria occurs in both sexes and all ethnic groups. AHP refers to four acute forms of porphyria where the enzyme deficiency becomes rate-limiting in the liver: AIP, VP, HCP, and ALAD deficiency. However, given the extreme rarity of ALAD deficiency, the term AHP usually refers to AIP, VP, and HCP, which will be the case hereafter. The current project focussed on the long-term consequences of AHP and PCT only.

Figure 1. The haem biosynthesis pathway. Reprinted from "Porphyrias," by Puy, H., Gouya, L. & Debach, J.C. 2010, Lancet, Vol. 375 (9718), 924-937.
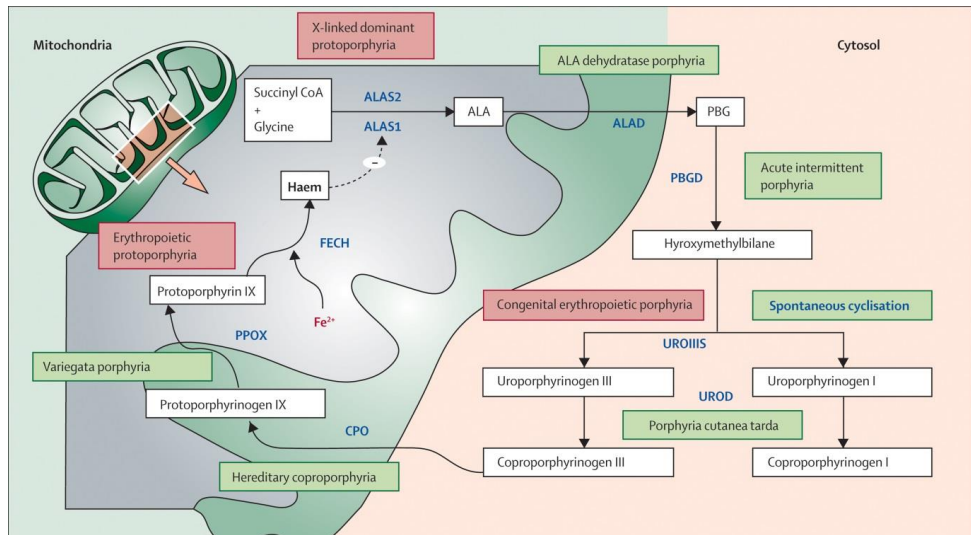
Green boxes=hepatic porphyrias. Red boxes=erythropoietic porphyrias. ALA=5-aminolaevulinic acid. PBG=porphobilinogen. I, III, or IX=type isomers. ALAS=ALA synthase. ALAD=ALA-dehydratase. PBGD=porphobilinogen deaminase. UROIIIS=uroporphyrinogen III synthase. UROD=uroporphyrinogen decarboxylase. CPO=coproporphyrinogen oxidase. PPOX=protoporphyrinogen oxidase. FECH=ferrochelatase. Fe2+=ferrous iron.

1.1.1 Acute hepatic porphyria (AHP)

Epidemiology and pathogenesis

AHP is inherited in an autosomal dominant fashion, meaning that it is 50 per cent likely that an affected parent will pass on the variant to their child and there is roughly a similar sex ratio of affected gene carriers. Although traditionally AHP was

thought to be a monogenic disorder, caused by a single gene, new evidence suggests that AIP at least follows an oligogenic inheritance pattern, and additional genetic and environmental triggers are required for their expression (6). For the current thesis, 'asymptomatic' AHP gene carriers refers to individuals who never have had symptoms of AHP but carry an AHP gene mutation, while 'symptomatic' AHP are individuals who have experienced at least one episode due to an acute attack of AHP. Clinical penetrance is very low, and although it was previously estimated that 10 per cent of mutation carriers develop symptoms (1), a more recent genetic study estimates clinical penetrance to be as low as one per cent for AIP (7). AIP is the most common form of AHP. The incidence of individuals with symptomatic AHP across Europe over three years was estimated at 0.13 per one million for AIP and 0.07 per million for VP (8). The incidence was relatively similar across countries except for Sweden, in which the incidence rate was four times greater due to a founder effect in the Northern regions by the mutation *W198X* (9). Likely, Norway may also have a slightly higher incidence due to this founder effect (10), although this was not demonstrated by Elder *et al*, which may be because of the small study period of only three years. HCP is rarer still. Although a founder effect also accounts for a higher incidence of VP in South Africa (11). At the time of writing the current thesis, over 400 disease-associated sequence variants have been recognised in AIP. However, disease severity is highly variable between individuals, and there is no convincing evidence that a particular mutation is associated with disease severity (12).

Known endogenous and exogenous triggers that may induce clinical penetrance include barbiturates and other porphyrinogenic drugs, alcohol (13), fasting (14), psychological and physical stress (15), infection and menstruation (1, 16), with drug exposure a frequent trigger of an acute attack in VP and hormonal factors more important in AIP (17). These factors induce hepatic delta-aminolevulinic acid synthase 1 (ALAS1), the rate-limiting enzyme in the production of haem in the liver, either directly or indirectly by increasing the demand for haem in the liver (1). Several theories have been proposed regarding the pathophysiology of neuropathic symptoms in AHP, although the exact mechanism remains unknown. Currently, the

hypothesis with the most persuasive evidence is that circulating levels of ALA and/or PBG, which are produced in the liver, are responsible for the neuropathic symptoms of AHP (18). ALA is structurally similar to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and can interact with GABA receptors (19). However, the correlation between ALA and PBG and acute attacks is variable, and asymptomatic AHP gene carriers may have high urinary excretion of ALA and PBG without ever having had symptoms of an acute attack (20). Clinical expression of AHP is uncommon before puberty and after menopause and more common in females between the ages of 30 to 40 years of age (1, 21-23). Most patients have one to a few attacks over their lifetime, whereas, a small sub-set (10%) of mostly women develop recurrent attacks of four or more a year (1, 21).

Clinical presentation

The neurovisceral attacks caused by AHP are mostly characterised by severe abdominal pain that lasts longer than 24 hours, but rarely longer than two weeks (15). Pain in the extremities and muscle weakness is also common. Peripheral neuropathy, motor weakness, electrolyte disturbances, hypertension, tachycardia, and seizures can occur (17, 24-26). Neuropathy can sometimes lead to paralysis of the respiratory muscles and very rarely death (1). Although the three disorders of AHP share similar acute clinical presentation, AIP tends to be more clinically severe than VP at least (17). Hypertension appears to be present in people presenting with AIP (27).

Long-term complications of AHP

Long-term complications of AHP include diseases such as chronic kidney disease (CKD), hypertension (15, 27, 28) and HCC (8, 29-37).

CKD appears increased in over 50 per cent of individuals with symptomatic AIP and over 60 per cent of these individuals have hypertension (28). Symptomatic AIP appears to predict CKD independent of hypertension, even if the latter is a known risk factor of CKD (28). This is supported by the finding that ALA and PBG promote tubular and arteriolar injury of the kidney (28).

HCC risk appears to be dramatically increased in persons with AHP. However, the discrepancies between studies' reported risk estimates of HCC in AHP is large (29, 32-34, 37). The vast majority of detected cases have presented with HCC, although cases of cholangiocarcinoma (CC) have also been reported (34). Therefore, *study I* investigated the risk of primary liver cancer (PLC), rather than just HCC. Predominantly AIP has been linked to PLC, and the evidence for VP has been less. Two cases of HCC in HCP have been reported (34, 38). Typical PLC risk factors, such as alcohol abuse or chronic hepatitis, are not generally reported in persons with AHP and PLC, and only about 26 per cent have presented with liver cirrhosis (39), compared to 80 to 90 per cent of persons with HCC in the general population (40). Likewise, the majority of cases with AHP and PLC are women compared to two-thirds of men in the general population. Further support for an association between AHP and PLC is the finding of an increased urinary ALA and PBG in persons with PLC compared to individuals with AHP and no PLC (39). Although the pathophysiology underlying the development of PLC in AHP is not well understood, a dominant theory proposes that ALA may be carcinogenic (41). Except for the rare case report, there is no evidence that people with an AHP diagnosis are at an increased risk of any other type of cancer (31, 42, 43), despite the accumulation of porphyrins and associated precursors in other areas of the body than the liver, such as the kidneys.

It has been found that individuals with symptomatic AHP, and especially recurrent acute attacks patients, report low health-related quality of life (44-46), chronic symptoms, such as chronic pain and fatigue, between attacks (47-49), and have high rates of unemployment and access of long-term sick leave or the disability pension, especially in persons having recurrent attacks (15, 47, 50). However, it is difficult to determine from these studies if the risk for such life events was comparatively increased to the general population or confounded by age, sex or socio-economic factors. Symptoms outside of an acute attack, which patients ascribe to their AHP, tend to be diffuse, such as chronic pain and fatigue (49). However, these same factors affect about 30 per cent of the adult Norwegian population, especially women, and is

also the most common cause for disability benefit and long-term sick leave as well as years lived with disability (51).

AHP has been associated with an increased risk of premature mortality. In a cohort study of individuals who had been hospitalized for their AIP in Sweden and Denmark, Linet reported a 1.9-fold increased risk of premature death (43). Specific medical diagnoses that contributed to premature death included cancer and ischemic heart disease (43). However, the findings were inconsistent between Sweden and Denmark, and selecting AIP cases based on hospital administration may have biased the findings, despite attempts by the investigators to minimise this. Other studies report despite a high prevalence of hypertension, no increased risk of mortality due to cardiovascular disease (27). However, a significant risk was observed for death due to renal impairment (27).

1.1.2 Porphyria cutanea tarda (PCT)

Pathogenesis, epidemiology and clinical presentation and management
PCT is a non-acute cutaneous hepatic porphyria and the most common form of porphyria worldwide and across Europe (8, 52). The prevalence in Norway is estimated at 1 in 10,000 (10). PCT results from a defect in the fifth enzyme in the synthesis of haem, and specifically a defect of the hepatic enzyme uroporphyrinogen decarboxylase (UROD) (1). The impaired UROD activity causes accumulation of uroporphyrinogens and heptacarboxylated porphyrinogens in the liver, and the corresponding water-soluble porphyrins act as photosensitisers in the skin, giving symptoms in the form of bullae, fragile skin, hypertrichosis and hyperpigmentation, mostly in the sun-exposed areas of the hands and face (53). Symptom debut typically occurs in middle age and has an approximately equal sex ratio.

PCT occurs both as an acquired (sporadic PCT) and an autosomal dominant hereditary form (familial PCT), in which mutations in the *UROD* gene can be identified (54). An acquired toxic type has also been reported, but will not be discussed further in the current thesis (55). Although UROD activity is reduced by up to 50% in familial PCT, exogenous factors are required for overt disease, and clinical

penetrance is low (56). The two types of PCT are clinically indistinguishable, although familial PCT tends to occur at an earlier age. Susceptibility factors for both include excess iron, HCV and hepatitis B virus (HBV), excessive alcohol intake, smoking, human immunodeficiency virus (HIV) and oestrogens (53, 54, 57-61). However, given the already reduced UROD activity, persons with familial PCT have a lower tolerance to exogenous factors and, therefore, such factors tend to be less strongly correlated with this form of PCT (54, 59, 62). In most populations, familial PCT occurs in 20 to 25 per cent of affected individuals (59, 63-65). However, in Norway, the proportion is much larger and estimated closer to 50% (54). This is partially explained by a founder mutation originating in the north-western part of Southern Norway (66). Another explanation may be that Norway has been a low endemic area of HCV infection, a major trigger of sporadic PCT (67), compared to other European countries (64). However, to our knowledge, reliable estimates of HCV infection across Norway are not currently available.

PCT is strongly associated with mild to moderate chronic iron overload, especially in the liver (68). PCT is also associated with diabetes mellitus (69). Treatment includes the removal of precipitating factors, reduction of iron overload by repeated venesection or low dose chloroquine treatment to reduce excretion of uroporphyrins in the liver (70, 71). Such therapies result in prolonged remission in most patients, although relapses occur in some individuals (72, 73).

Long-term complications
PCT is associated with HCC (43, 74-76). HCV, HBV and excessive alcohol intake constitute the main risk factors for HCC in the general population (77, 78). Histopathological examinations of liver biopsy samples show liver abnormalities, including liver cirrhosis, in some patients with PCT. If such hepatic injury is caused by porphyrins or their associated precipitants, iron overload, hepatitis or hepatotoxins is unclear (79). It has been hypothesised that HCC risk is greater in PCT cirrhosis than other types of cirrhosis and that HCC risk may be greatest in persons with a long treatment delay (80). This has been partially supported by animal studies demonstrating the induction of liver tumours in mice with induced experimental PCT

(81); and a finding from a case-control study that patients with PCT had a 5-fold increased risk of HCC compared to matched control patients with chronic liver disease (76). However, the small and highly selective sample of this latter study makes drawing strong conclusions difficult. Others have suggested that with improvements in the diagnosis and consequently reduced treatment delays in persons with PCT, hepatic injury from PCT would be reduced, and excessive risk of HCC may no longer be apparent (82). Whether PCT increases the risk for HCC above the risk caused by common HCC risk factors and PCT susceptibility factors are still controversial. It also remains unclear if the risk for HCC warrants selective HCC surveillance, as recommended for individuals with AHP (83). Other than HCC, the risk for other malignancies is less clear. A single cohort study suggests that patients with PCT may have an increased risk of lung cancer and suggest that porphyrins may increase susceptibility to tobacco-related cancers (43). Case studies suggest that PCT may also be precipitated by leukaemia (84-86), and therefore, reverse causality is an important factor in the design of a study investigating PCT and cancer. Persons with PCT may also have an increased risk of premature death (43), although the finding is not consistent between studies and populations (59).

2. Aims

The overall aim of the current project was to investigate the long-term consequences of AHP and PCT. Specific research aims included:

- to investigate the absolute risk of malignancies in persons with AHP and if this risk was increased compared to the general population, with a specific interest in PLC (*study I*)
- to investigate the absolute risk of malignancies in persons with PCT and if this risk was increased compared to the general population, with a specific interest in the risk of PLC, as well the increased risk of premature death and to compare differences of these risks between persons with familial and sporadic PCT (*study II*)
- to investigate if persons with AHP were at increased risk of long-term sick leave, disability pension and premature death compared to the general population and if there were any differences in risk between asymptomatic and symptomatic gene carriers (*study III*).

3. Methods

Table 1. Overview of materials and methods

Paper	Study I-AHP and cancer	Study II-PCT, cancer and mortality	Study III-AHP, long-term sick leave, disability pension and mortality
Main aim	To examine the risk of malignancies in persons with AHP compared to the general population, with a specific interest in primary liver cancer (PLC)	To examine the risk of malignancies and of premature death in persons with PCT compared to the general population, with a specific interest in hepatocellular carcinoma	To examine if persons with AHP were at increased risk of long-term sick leave, disability pension and premature death compared to the general population
Study design	A population-based nationwide cohort study	A population-based nationwide cohort study	A population-based nationwide cohort study
Study population	All Norwegian residents aged 18 years or older from 01-2000 to 12-2011	All Norwegian residents aged 18 years or older from 01-2000 to 12-2016	All Norwegian residents aged 18 years or older from 01-1992 to 12-2017 (long-term sick leave), 01-1992 to 12-2016 (disability leave) and 01-1996 to 12-2017 (premature mortality)
Observation period	From the study start to the date of emigration, death of first primary cancer or study end, or whichever occurred first	From the study start or the date of the respective person's 18th birthday to the date of death, first primary cancer or study end, or whichever occurred first	From the study start or the date of the respective person's 18th birthday to the date of death event of interest or study end, or whichever occurred first
Exposure	AHP	PCT	AHP
Reference/ unexposed	General population	General population; persons with a history of alcohol abuse/dependence	General population; matched cohort (10 controls to each case)
Main outcomes	Primary liver cancer (PLC). ICD codes: C22 (ICD-10) and 155 (ICD-7)	Hepatocellular carcinoma (HCC). 155.0 (ICD-10) and C22.0 (ICD-7), and premature death (all-cause and disease specific - mortality)	Long-term sick leave, disability pension and premature death (all-cause and disease specific - mortality)
Statistical analysis	Survival analysis using Cox proportional hazards regression models (time scale=time on the study). Meta-analysis to explore sex differences in risk of PLC	Survival analysis using Cox proportional regression models (time scale=age on the study). Competing risks regression to assess risk compared to persons with a history of chronic alcohol abuse/dependence	Survival analysis using Cox proportional hazard regression models for the primary outcomes (time scale=age on the study). Differences in diagnostic reasons assessed by Poisson regression. Annual events for long-term sick leave episodes and total days, determined by zero-inflated negative binomial regression
Adjustments	Year of birth, sex, highest attained education	Year of birth, sex, highest attained education	Year of birth, sex, highest attained education
Stratifications/ subtypes	Male and female; symptomatic and asymptomatic AHP	Familial PCT, sporadic PCT, unclassified PCT	Hospitalised, non- hospitalised, asymptomatic, unclassified AHP
Sensitivity analyses	Reduced cases with outcome and AHP by 1. Assessed effect of non-consent	E-value	Assessed impact of non-consent

3.1 Data sources

3.1.1 The National Registry

The National Registry contains demographic information of all Norwegian residents since 1960 and is administered by the Norwegian Tax Administration (87). Specifically, the registry contains information regarding gender, date of birth, place of birth, date of emigration and date of death.

3.1.2 The Norwegian Porphyria Centre

The Norwegian Porphyria Centre (NAPOS) is located at Haukeland University Hospital, Bergen, Norway and was established in December 1999. All individuals with symptomatic disease and asymptomatic AHP and overt PCT are invited to participate in the national Norwegian Porphyria Registry. The registry was established in 2002 and obtained status as a national medical quality registry in 2012. Data collection is based on participant informed consent, and the data is derived from patient-reported questionnaires supplemented with biochemical and genetic laboratory results. The questionnaires are disease-specific (i.e., AIP questionnaire specifically for AIP patients, VP questionnaires specifically for VP patients, HCP questionnaire specifically for HCP patients, PCT questionnaires specifically for PCT patients) and include a rich array of data elements concerning diagnosis, provoking factors, symptoms, treatment, medication, lifestyle habits, daily life activities and comorbidities. Laboratory data of porphyrin and porphyrin precursor analyses are included when samples are sent for routine analysis as well as by biobanking. In 2018 there was a 71 per cent response rate to the registry (PCT=71%, AIP=69%, VP=69%, HCP=71%), which means that 71 per cent of all known porphyria patients and porphyria gene mutation carriers in Norway participate by completing the questionnaire at the time of diagnosis and every second to fourth year thereafter (88). In addition to the registry, NAPOS maintains an administrative database of all known persons with a porphyria diagnosis, updated periodically with life status.

3.1.3 The Cancer Registry of Norway

Since 1951 all physicians, hospitals and pathology libraries across Norway were instructed by law to notify all new neoplasms to the mandatory national Norwegian Cancer Registry (89). Cancer information comes from several independent sources, thus securing a high grade of accuracy and completeness (89). Up until 1992, diagnoses were based on a modified version of the 7th revision of the International Classification of Diseases (ICD-7). The International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3) was used since 1993 for coding the site (topography) and the histology (morphology) of neoplasms. Since 1986, non-solid tumours have been coded according to a separate coding system. All new primary cancer diagnoses for each individual are recorded, meaning one person can have up to several primary cancer diagnoses.

3.1.4 Norwegian Cause of Death Registry

The Norwegian Cause of Death Registry, maintained by the Norwegian Institute of Public Health since 2014, records all deaths that occur in Norway and deaths of citizens who die abroad. The digitalised registry maintains records since 1951. The registry has a coverage greater than 98% (90). Diagnostic codes are prepared in accordance to the ICD, with the 10th revision implemented in Norway in 1996 and includes both the underlying cause of death (i.e., the disease or injury which initiated the death) and contributing causes of death (i.e., other significant factors related to the cause of death but not related to the disease or condition causing it).

3.1.5 Statistics Norway

Statistics Norway (SSB) administers the National Education Database, which maintains individual-based education statistics for all residents of Norway from primary to tertiary level since 1970 (91). Information about students aboard was included in 1986 (91). This information is available for 90% of the population, with missing data mostly comprising of persons who immigrated to Norway.

3.1.6 Norwegian Labour and Welfare Administration

The Norwegian Labour and Welfare Administration has maintained records regarding disbursements of different benefits, including long-term sick leave benefit, medical and occupational - rehabilitation and disability pension, since 1992 (92). The database includes data on the start and end date of each benefit for all Norwegian residents and, for long-term sick leave, the total number of days it was accessed. Diagnostic codes for physician-certified long-term sick leave episodes, medical rehabilitation and disability leave, included the second revision of the International Classification of Primary Care (ICPC-2), ICD-9 and 10.

3.1.7 Record linkage

Precise record linkage between the data sources was performed in 2012 and again in 2018 by Statistics Norway (SSB). *Study I* was based on record linked data from 2012, whereas *study II* and *study III* were based on data that was record linked in 2018. All personal identification numbers were replaced by unique study numbers, producing a de-identified research database for further analyses.

3.2 Study design and study population

We conducted a population based, nationwide, cohort study using registry data. Data regarding the exposure (AHP/PCT diagnosis) were collected before the outcomes (e.g., cancer diagnosis/cause of death). The study sample comprised of all Norwegian adult residents alive during the study periods. The study period varied between the three studies and outcomes according to the availability of data.

3.2.1 Study I (AHP and cancer)

Study I included 251 adults with a confirmed AHP diagnosis (AIP, n=222; VP, n =21; and HCP, n=8) and 4,398,546 adults from the general population (reference population). The study period was from January 2000 to December 2011. The primary endpoint was a primary first cancer diagnosis and, therefore, people with a cancer diagnosis registered in the Cancer Registry of Norway (not including non-melanoma skin cancers) prior to 2000 were excluded.

3.2.2 Study II (PCT, cancer and mortality)

Study II included 612 adults with a confirmed overt PCT diagnosis from January 2000 to December 2016. To avoid issues of reverse causality, 23 persons who had a cancer diagnosis proceeding PCT symptoms were excluded from analyses where cancer was the primary outcome.

3.2.3 Study III (AHP, mobidity and mortality)

Study III comprised of 319 persons with a confirmed diagnosis of AHP (AIP=281; VP=30; HCP=8). The study period was from January 1992 to December 2016 for investigating the risk of disability pension; January 1992 to December 2017 for investigating the risk of long-term sick leave and January 1996 to December 2017 for investigating the risk of premature death.

3.3 Exposures, outcomes and confounders

3.3.1 Exposures (AHP/PCT diagnosis)

NAPOS is tasked with the responsibility of diagnosing the porphyrias across Norway and, therefore, has an overview of almost all Norwegian porphyria patients (10). When the centre was established in 1999, all laboratories diagnosing the porphyrias were contacted and requested to send information about all patients with a porphyria diagnosis (10). Porphyria diagnoses are established in accordance with diagnostic algorithms by Badminton *et al* (93). Biochemical testing of porphyrins and their precursors was conducted by the Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital and DNA sequencing of the *UROD,* porphobilinogen deaminase (*PBGD),* coproporphyinogen oxidase (*CPOX*) and protoporphyrinogen oxidase (*PPOX*) genes by the Centre for Medical Genetics and Molecular Medicine at the same hospital. NAPOS offers predictive testing to all AHP patients' family members. However, predictive testing is voluntary and in addition, Norwegian law requires the patients themselves to inform their relatives. Thus, not all family members at risk undergo predictive genetic testing. Additional to the Norwegian Porphyria Registry, NAPOS maintains a record of all porphyria diagnoses. In 2017, a request of signed consent was mail posted to persons with a

confirmed porphyria diagnosis and not included in the registry to link their porphyria diagnosis and laboratory data to other national registries. Persons who were deceased with a confirmed porphyria diagnosis and not included in the registry were included in all studies, as permitted by the Regional Ethics Committee. In total, the participation rate of persons with a known AHP diagnosis, both symptomatic and asymptomatic, or PCT diagnosis was 73% for *study I*, 78% for *study II* and 77% for *study III*.

It is common to classify persons with an AHP diagnosis either as having symptomatic disease (referred to as 'manifest' in *study I*) or being an asymptomatic predictively tested gene mutation carrier (referred to as 'predisposed cases' in *study I*). Broadly speaking, the former refers to persons who have had at least one acute attack throughout their life and/or skin symptoms in the case of VP/HCP, whereas the latter refers to people who have been found to be genetically predisposed to AHP porphyria but remain symptom-free. However, to date, there remains no consensus on the definition of an acute attack. For *study I* we classified persons as symptomatic if they had reported having had porphyria related symptoms, in the form of acute attacks, and/or skin lesions if VP or HCP in the Norwegian Porphyria Registry. Patients reporting acute attacks experienced abdominal pain as their main symptom (92%). Other frequently reported symptoms were nausea, obstipation, muscular pain, muscular weakness, palpitations, red-brown coloured urine, fatigue and psychiatric symptoms. For *study III* we further classified individuals with symptomatic AHP as either 'hospitalised AHP' if they reported having been hospitalised at least once due to an acute attack or non-hospitalised AHP' if they reported having had symptoms of porphyria but never having been hospitalised specifically for acute attack. Eighty-one per cent of the persons with hospitalised AHP had a urinary PBG concentration four times greater than the upper reference limit (URL) recorded outside of an acute attack, compared to 66 per cent of persons with non-hospitalised AHP, and 26 per cent of asymptomatic AHP gene carriers. Of the individuals who had not responded to any questionnaires and, therefore, could not be classified as symptomatic or

asymptomatic, 37 per cent had a urinary PBG concentration four times greater than the URL.

Persons with PCT were classified as having familial (inherited) or sporadic PCT based on sequencing of the *UROD* gene. Participants for which DNA sequencing had not been performed were registered as unclassified PCT.

3.3.2 Outcomes

Study I (AHP and cancer)

The primary outcome of *Study I* was primary liver cancer (PLC) defined with the following ICD codes: C22 (ICD-10) and 155 (ICD-7). The most prevalent histological form of PLC (90% of cases) is HCC (ICD-10: C22.0; ICD-7: 155.0). Intrahepatic cholangiocellular carcinoma (CC) (ICD-10: C22.1; ICD-7: 155.1) is less common; however, cases of AHP and CC have been reported (34). Secondary outcomes included other cancer diagnoses in which 3 or more persons with an AHP diagnosis from our study sample were affected. Therefore, these diagnoses were exploratory, rather than determined *a priori*, and the findings from these secondary outcomes considered hypothesis-generating for future studies. The secondary outcomes for *study I* included: kidney cancer, including the renal pelvis=C64-65 (ICD-10), 180 (ICD-7); endometrial cancer=C54 (ICD-10), 172 (ICD-7); breast cancer=C50 (ICD-10), 170 (ICD-7); and prostate cancer=C61 (ICD-10), 177 (ICD-7). We classified all non-PLC malignancies using the ICD codes C00-96 (ICD-10), excluding PLC codes and non-melanoma skin cancer codes.

Study II (PCT, cancer and mortality)

The primary outcomes for *study II* (PCT, cancer and mortality) included HCC (ICD-7: 155.0; ICD-10: C22.0), and overall risk of premature death. Secondary outcomes included the following cancer diagnoses of *a priori* interest: all sites (ICD-7: 140-207; ICD-10: C00-96, D45-47), lung (ICD-7: 162; ICD-10: C33-34); and leukaemia (ICD-7: 207; ICD-10: C91-95, D45-47). We also investigated the following secondary outcomes related to a premature death and of *a prior* interest: malignant neoplasms (ICD-10: C00-96), diabetes mellitus (ICD-10: E10-14), cerebrovascular

diseases (ICD-10: I60-I69), chronic obstructive pulmonary disease (ICD-10: J43-44), and diseases of the liver (ICD-10: K70-77, B15-19, E83.1). Similar to *study I,* we explored cancer and mortality diagnoses in which three or more persons with AHP were affected. These diagnoses included: colon/rectum (ICD-7: 153-154; ICD-10: C18-C21), gallbladder and biliary tract (ICD-7: 156; ICD-10:C23-24), pancreas (ICD-7: 157; ICD-10; C25), lung (ICD-7: 162; ICD-10: C33-34); non-melanoma skin (ICD-7: 191; ICD-10: C44), breast (ICD-7: 170; ICD-10: C50), prostate (ICD-7: 177; ICD-10: C61), and all-second primary cancers.

Study III (AHP, mobidity and mortality)

The primary outcomes for *study III* included long-term sick leave, disability pension and overall risk of premature death. The first 16 days of a sick-leave absence is compensated by the employer and paid for by the Labour and Welfare Administration (NAV). Therefore, long-term sick leave is defined in *study III* as any sick leave absence of 17 days or more as it is not possible to acquire data regarding sick leave episodes of a shorter duration. Sick leave can be granted for a maximum of one year. In Norway, to qualify for disability pension, a person has to be aged 18 years or older and have a permanently reduced earning capacity by at least 50% due to illness or injury. Disability pension is seen as a last resort, and a person must first be on sick leave for one year before they qualify for work or medical rehabilitation, and if they still are unable to return to work, can be granted disability pension. Therefore, it is rare that an individual will return to full-time work following admission to disability pension.

Specific diagnoses for long-term sick leave coded using the ICPC-2 included: general and unspecified (A01-A99), weakness/tiredness general (A04), abdominal pain (D01-D02, D06), high blood pressure/hypertensive disorder (K85, K86, K87), ischemic heart disease (K76), muscle/joint – pain/symptoms (L18, L19, L20), neurological (N01-N99), psychological (P01-P99), acute stress reaction (P02), feeling depressed (P02), depressive disorder (P03), endocrine/metabolism dis. other (T99) and urology (U01-U99). Diagnoses for disability pension were coded using the ICD-10, and included: neoplasms (C00-96, D45-47), disorders of porphyrin and

bilirubin metabolism (E80), mental and behavioural disorders (F00-F99), epilepsy (G40), diseases of the circulatory system (I00-I99), hypertension (I10-I15), ischemic heart disease (I20-I25), diseases of the musculoskeletal system and connective tissue (M00-M99) and renal failure (N17-N19). The following underlying causes of death were investigated using the ICD-10: malignant neoplasms (C00-96, D45-47), HCC (C22.0), renal carcinoma (C64), type I diabetes (E10), hypertension (I10-I11), ischemic heart disease (I20-I25), and renal failure (N17-N19).

3.3.3 Potential confounders

Sex and age

Incidence and risk factors for many cancers are strongly influenced by sex and age. In the general population, HCC has a strong male predominance, with males estimated to have a four-fold increased risk of PLC (94). PLC risk also increases with advancing age (95). Although asymptomatic AHP gene carriers have an equal sex distribution, there is a female predominance among persons with symptomatic AHP. Despite there is roughly an equal sex distribution for persons with both familial and sporadic PCT in Norway (54), triggering factors do vary between the sexes. In relation to long-term sick leave and disability leave, female sex and older age status are strongly correlated factors (96). Therefore, given the association between age and sex with both the exposures AHP and PCT, as well as our outcomes, study results that fail to account for these factors are likely to be confounded and invalid. Previous studies investigating the association between PLC and AHP have found very different sex profiles compared to the general population, while the risk is also highest in old age. Therefore, sex and age are important covariates that need to be adjusted for in the survival models to control for confounding bias (Figure 2).
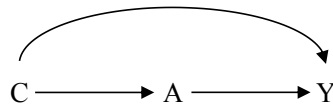
Figure 2. Controlling for measured covariates C (age, sex) reduces confounding of the relationship between the exposure A (e.g., AHP) and the outcome Y (e.g., PLC) (97).

Socio-economic status (SES), liver diseases and chronic alcohol abuse

Socioeconomic status (SES) refers to a person's social standing. It has been found to negatively correlate with cancer outcome (98), premature death (99), long-term sick leave and disability pension (96). These effects hold constant in Norway, despite a policy of universal access to health care for over 70 years (100). In relation to long-term sick leave and disability pension, the strong correlation with lower socio-economic status is mostly explained by health behaviours, such as diet, alcohol, smoking, exercise and differences in working conditions (96). In relation to premature death, studies in Norway have found that persons with tertiary education live five to six years longer and have better health than those with lower education (99). A recent study further found substantial and increasing disparities in life-expectancy by household income in Norway (101). The relationship between SES and cancer incidence is more complicated. SES is positively correlated with the incidence of prostate and breast cancers (102, 103), and a negatively correlated with lung, colorectal and PLC (98, 104-106). Some studies have indicated that this negative association may reflect variances in exposures to lifestyles or carcinogens that determine cancer risk (103).

While socioeconomic status is viewed as a multidimensional latent variable, encompassing education, income level and occupation, education may be the best measure for health related socioeconomic status of the three constructs. It is generally available for both sexes and excludes few members of the population. It encompasses much of the same information as occupation and household income but also reflects individual differences in terms of access to information. Including all three measures can cause multicollinearity problems (i.e., correlation coefficient of above 0.8 or 0.9) with regression models. Factor analytical techniques, which are

often considered for creating a single score from multiple variables, is not appropriate for formative models. Therefore, the highest educational level achieved was used alone as the best estimation of SES.

While socioeconomic status may not directly affect PLC incidence, it acts as a proxy for major unmeasured risk factors, such as chronic alcohol intake and HCV and HBV, which are more prevalent in persons with greater SES deprivation (104). PLC and PCT, especially sporadic PCT, share some of the same risk factors, such as liver disease and chronic alcohol use. However, reliable statistics of these factors do not exist at the population level. Therefore, SES was included as a proxy for these factors for *Study II* (Figure 3).

U ⟶ C ⟶ A ⟶ Y
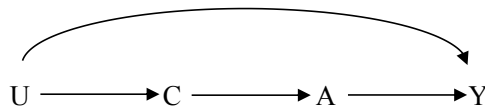
Figure 3. Controlling for measured covariate C (SES), even in the presence of unmeasured variables U (e.g., liver disease, health behaviour), eliminates, or more likely reduces, confounding of the relationship between exposure A (e.g., AHP) and the outcome Y (e.g., PLC, long-term sick leave), even though C itself is not a common cause of A and Y (97).

Although symptomatic AHP may be triggered by alcohol and drugs like barbiturates, there is no indication that this patient group differ in relation to PLC risk factors compared to the general population. Furthermore, unlike persons with PCT, liver disease due to lifestyle factors is uncommon. Some cases of liver cirrhosis have been reported in persons with AHP and PLC (34). However, it is unclear if this association is due to porphyrins/precursors, old age or other factors (34). We included SES as a proxy of potential "backdoor" confounders in the analysis of *study I*, in accordance with the principals of confounder selection outlined by VanderWeele (97). However, we expected there to be a small overall effect for this adjustment. In regards to social benefits, sickness benefits and disability pension increase with decreasing SES (107) and, therefore, the inclusion of education was included as a potential confounder for *study III,* again in accordance to the principals of confounder selection outlined by VanderWeele (97) .

As stated above, a challenge of *study II* was to adjust for spurious effects of liver disease and chronic alcohol abuse in the causal pathway between persons with PCT and HCC, given this data was not available for the entire population. Therefore, as well as including SES as a proxy in adjusted regression analyses of the entire population, we conducted a sub-group analysis, investigating the risk of HCC in persons with PCT compared to a subset of persons with a diagnosis of chronic alcohol abuse/dependence. This control group was derived from medical registrations in the social benefits registries. Chronic alcohol abuse/dependence was defined by the following codes: ICPC-2: P15; ICD-9: 303, 305.0; ICD-10: F10 specifically, from long-term sick leave, medical and vocational rehabilitation and disability pension registries. The group is by no means a complete list of individuals with this diagnosis across Norway.

3.4 Statistical methods

3.4.1 Analyses of primary endpoints

Stata/SE Version 14 and 15 for Windows was used for all statistical analyses (StataCorp Stata Statistical, Software, College Station, TX, USA).

The incidence rate is a measure of the number of new occurrences of a disease over a given time period or age divided by the corresponding person-years at risk among members of the source population (108). Person-years at risk is the summation of all persons within a study by the potential time at risk of the outcome of interest of the study (109). Incidence rate was used across all studies to indicate absolute risk both in the exposed and the general population.

In its simplest form, the risk ratio refers to the ratio of the incidence of an outcome in the exposed compared to the unexposed or reference population and provides the strength of association between the exposure/risk factor and the outcome. Although there are some important differences between them, there are a number of specific measures of risk estimates. In the current project, risk ratios were estimated by hazard ratios (HRs), subdistribution hazard ratios (SHRs) and incident rate ratios

(IRRs), dependent on the aim of the analysis and statistical procedure used. A risk ratio of one indicates that the exposure is not related to the outcome; a risk ratio greater than one indicates that an increase in exposure is associated with increased risk of the outcome, and a risk ratio less than one indicates the outcome is decreased by the exposure.

To compare the time-to-event from exposure to the main outcomes between the exposed and general population, HRs with 95% confidence intervals (CIs) were estimated using Cox proportional hazards regression models (110). The porphyria diagnosis AHP/PCT (no/yes) or AHP/PCT subtype (e.g., symptomatic AHP) was the exposure and cancer, cause of death of interest, or social security benefit (no/yes) the outcome. Time on the study was used as the time scale for *study I*, whereas age on the study was used as the time scale for *study II* and *study III*. Entry time was from the time (*study I*) or age (*study II, study III*) of the start of the study. Additionally, for *study II* and *study III*, persons aged 18 years after the study start were included at the time of their 18th birthday (i.e., left truncated). The exit time was the time (*study I*) or age (*study II, study III*) of the event (e.g., cancer) or censoring (emigration (*study I* only), death due to other factors, or end of study follow-up, whichever occurred first. For *study III*, we additionally censored for the time a person entered disability pension when assessing long-term sick leave. Units of time were measured in years for *study I* and *study II* (due to the lack of availability of month of cancer diagnosis for privacy reasons) and months for *study III*.

The Cox model was stratified by birth cohorts to adjust for cohort effects, roughly equating to the time of a generation of 20 years. The multivariate models adjusted for the covariates sex, age (as a continuous covariate in *study I* and as a continuous time-scale in *study II* and *study III*), and educational attainment. The proportionality assumption of the Cox models was assessed by inspecting Kaplan-Meier curves and the log(-log(survival)) versus log(time) graphs for fixed covariates, including time-dependent covariates in the model for all covariates, and tests of the non-zero slope. In the rare circumstance in which a covariate violated the assumption of proportionality of the hazards, the covariate was consequently entered as time-

dependent in an extended model. Obtained level of education was categorized into: no schooling, compulsory education (year 1-10), upper secondary education (11-13, or 14 years if including post-secondary non-tertiary education), tertiary education (14+ years), and unspecified/missing.

Given a combination of a rare exposure (i.e., AHP/PCT) and many outcomes of interest (e.g., PLC) of the current project, we were mostly underpowered to investigate interactions. Therefore, where interaction analysis was not possible, we stratified on subtypes in which we thought the hazard ratio might differ. Specifically, we conducted separate Cox regression models for sex and persons aged 50 years or older in *study I*. We also stratified by sex in a meta-analysis in *study I* (details below). *Study III*, in which outcomes such as long-term sick leave and disability pension were common, we investigated interactions between the exposure and educational attainment (tertiary or upper secondary vs less education) and sex, and none were found.

3.4.2 Analyses of secondary endpoints

In *study II*, we compared the risk of cancer and causes of death in persons with PCT to persons with a history of chronic alcohol abuse/dependence, who have a high mortality risk by a competing risks regression survival analysis. Death, due to other causes, was the competing risk in the analysis. Persons with both PCT and a history of chronic alcohol abuse/dependence (n=17) were excluded from this analysis.

In *study III*, we conducted a Poisson regression to investigate differences in the diagnostic reasons for long-term sick leave and the disability pension between persons with AHP and the general population. All analyses were conducted using robust standard errors to estimate the IRRs and CIs, and were offset for months on the study (defined as month and year of exit minus month and year of entry).

To assess total annual episodes and days of long-term sick leave in persons with AHP and sub-types, we estimated IRRs and CIs using a zero-inflated negative binomial regression (ZIBR) with robust standard errors. The total number of days or events was divided by each participant's number of years on the study to obtain an

annual rate for each person. For computational efficiency in predicting the IRR and to adjust more precisely, we frequency-matched ten randomly selected controls to every AHP case, on sex, age at study start and educational attainment for this analysis. Frequency matching, especially with so many controls, will result in approximately the same results, but with some loss of power (111).

3.4.3 Sensitivity analyses

For *study I* in which both the exposure and the outcome were infrequent, we conducted a sensitivity analysis to investigate the effect on the HR by subtracting one case with AHP and the outcome of interest. So, for example, we subtracted the total number of cases with AHP and PLC by one and re-ran the analysis to investigate the overall effect. We also investigated the impact of including all known persons with AHP in the denominator while specifying no new cases of PLC and AHP, to investigate the maximum potential for selection bias by non-consenters in a crude analysis. Overall, these changes had a negligible effect on the estimates and interpretations of the study findings.

For *study II* we calculated the evidence value (E-value) for each adjusted HR. The E-value is the minimum size of a risk ratio of an unmeasured confounder that is required to explain away the association between the exposure and the outcome. The E-value formula is: *hazard ratio (HR) + sqrt[HR x (HR-1)]* (97).

For *study III*, we investigated the impact of non-consent bias specifically for the outcome of premature death by including all known persons with an AHP diagnosis in the denominator in a crude analysis.

3.4.4 Literature review and meta-analysis

For *study I*, we conducted a random-effects meta-analysis of the PLC risk in men and women. In October 2016, a systematic literature search for relevant studies was performed using the PubMed database with the following search terms: "Porphyrias, Hepatic" [MeSH Major Topic]) AND "Liver Neoplasms"[MeSH Major Topic]. This resulted in 46 hits. Following quality assessment and rejection of case series and basic research studies, seven studies investigating specifically AHP were identified.

An additional four studies of interest were further identified by scanning the reference lists of these publications. Of the 11 published studies examining AHP and PLC, there were six studies which reported separate risk ratios for men and women (29-34). The meta-analysis included these estimates and ours. Risk ratios were stratified by sex and then weighted using the inverse-variance method to calculate separately pooled risk ratios for females and males.

3.5 Ethical considerations/ approval

All data were de-identified of personal identification numbers before delivery to the investigators. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by approval by the Regional Committees for Medical and Health Research Ethics, Norway (reference number: 2012/753).

4. Summary of main results

4.1 Acute hepatic porphyria and cancer risk (*study I*)

From 2000 to 2012 and across 251 persons with AHP, we found 9 cases of PLC
(AIP=8, VP=1), constituting a burden of 30% of all neoplasms reported for AHP
participants. The annual incidence of PLC was 0.35% in all adults and 0.63% in
persons aged 50 years or older, compared to 0.003% in the general population. In the
adjusted analysis, this constituted a 108-fold increased risk of PLC compared to the
general population (95% CI: 56, 207). In an exploratory analysis, we found a 7-fold
(95% CI: 2.4, 23.1) and 6-fold (95% CI: 2.0, 19.3) increased risk of kidney and
endometrial cancers, respectively. In total, we found no evidence of an increased risk
of other malignancies or non-PLC malignancies in total compared to the general
population.

In a sensitivity analysis, we found that hypothetically decreasing the number of cases
with AHP and PLC by one over the study period would reduce the hazard ratio to 96
(95% CI: 56, 205). If we included all known persons with an AHP diagnosis who did
not consent to the Norwegian Porphyria Registry, hypothetically specifying that no
new cases of PLC were found, the annual incidence would decrease to 0.27% and
hazard ratio to 86 (95% CI: 45-166).

In our study, 67% of persons with PLC and AHP were female compared to 37% in
the general population. When we stratified the Cox regression by sex, females had a
168-fold increased risk (95% CI: 75-376) and males a 70-fold increased risk (95%
CI: 22-217). In a meta-analysis of our and previous studies, the pooled risk ratio
estimate was 131 for women (95% CI: 92-186) and 51 for men (95% CI: 37-71),
suggesting that the relative risk of PLC in persons with AHP is different between the
sexes (Figure 4). When comparing persons within the AHP cohort, we found that
eight out of the nine cases with PLC had symptomatic AHP, compared to 59% of
cases without PLC (p=0.09). The risk in persons with only symptomatic AHP was
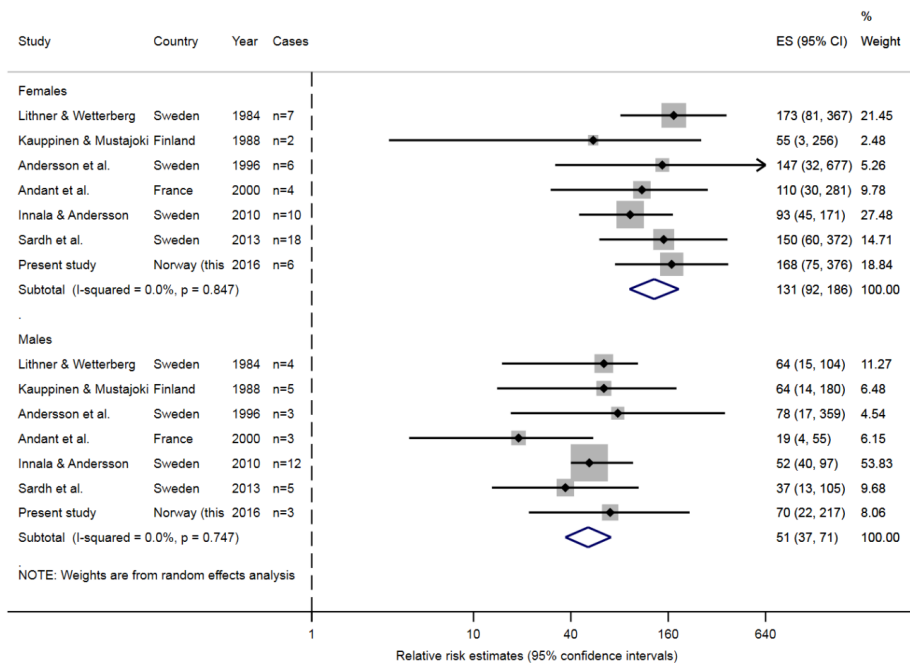160 compared to the general population (95% CI: 80-321).

Figure 4. Forest plot showing an inverse-weighted random-effect meta-analysis of the risk of primary liver cancer in persons with acute hepatic porphyria. Results are stratified by sex. The area of each square is proportional to the study's weight in the meta-analysis and the diamonds represent the measure of the risk ratio estimate for each sex; 95% confidence intervals for these estimates are shown (horizontal lines). ES, effect size. The X-scale is logarithmic.

4.2 Porphyria cutanea tarda and cancer/mortality risk (*study II*)

We found 6 cases of HCC and 3 cases of gallbladder or biliary tract cancer in 589 persons with PCT from 2000 to 2016. This constituted a 19.7-fold (95% CI: 8.8, 44.0) increased risk of HCC and a 6.8-fold (95% CI: 2.2, 21.0) increased risk of gallbladder and biliary tract cancer (Figure 5). The excess risk was reduced when compared to persons with a history of chronic alcohol abuse/dependence (HCC, SHR=3.1, 95% CI: 1.2, 7.7; Gallbladder and biliary tract cancer, SHR=4.0, 95% CI: 1.1, 14.4). We also observed a 1.6-fold increased risk for lung cancer compared to

the general population, although the lower bound confidence interval was slightly below one (Figure 5). There was no indication of an increased risk of other malignancies, or all malignancies, compared to the general population or persons with a history of alcohol abuse/dependence.
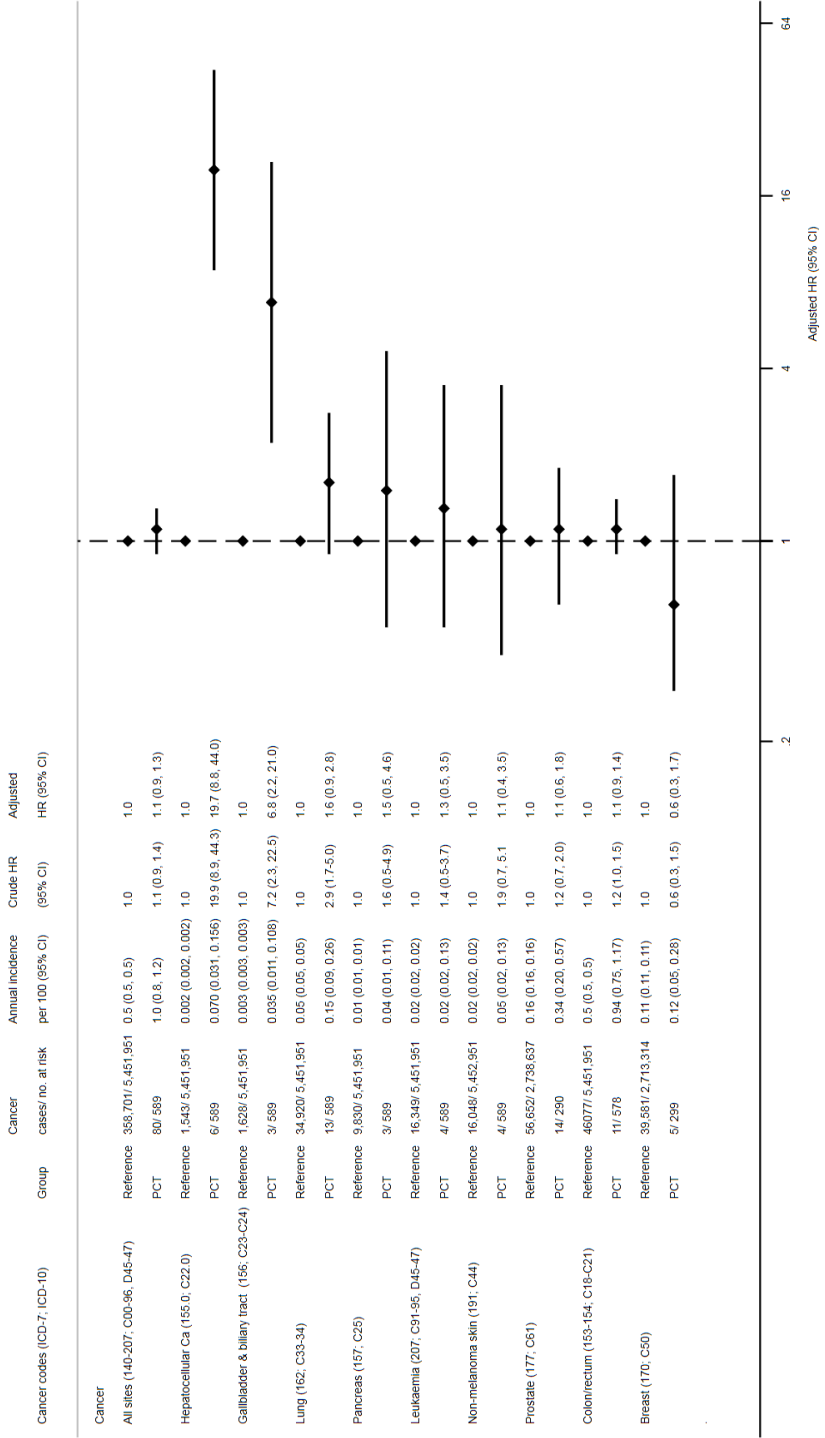
Cancer codes (ICD-7, ICD-10)	Group	Cancer cases/ no. at risk	Annual incidence per 100 (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
Cancer					
All sites (140-207; C00-96, D45-47)	Reference	358,701/5,451,951	0.5 (0.5, 0.5)	1.0	1.0
	PCT	80/589	1.0 (0.8, 1.2)	1.1 (0.9, 1.4)	1.1 (0.9, 1.3)
Hepatocellular Ca (155.0; C22.0)	Reference	1,543/5,451,951	0.002 (0.002, 0.002)	1.0	1.0
	PCT	6/589	0.070 (0.031, 0.156)	19.9 (8.9, 44.3)	19.7 (8.8, 44.0)
Gallbladder & biliary tract (156; C23-C24)	Reference	1,628/5,451,951	0.003 (0.003, 0.003)	1.0	1.0
	PCT	3/589	0.035 (0.011, 0.108)	7.2 (2.3, 22.5)	6.8 (2.2, 21.0)
Lung (162; C33-34)	Reference	34,920/5,451,951	0.05 (0.05, 0.05)	1.0	1.0
	PCT	13/589	0.15 (0.09, 0.26)	2.9 (1.7-5.0)	1.6 (0.9, 2.8)
Pancreas (157; C25)	Reference	9,830/5,451,951	0.01 (0.01, 0.01)	1.0	1.0
	PCT	3/589	0.04 (0.01, 0.11)	1.6 (0.5-4.9)	1.5 (0.5, 4.6)
Leukaemia (207; C91-95, D45-47)	Reference	16,349/5,451,951	0.02 (0.02, 0.02)	1.0	1.0
	PCT	4/589	0.02 (0.02, 0.13)	1.4 (0.5-3.7)	1.3 (0.5, 3.5)
Non-melanoma skin (191; C44)	Reference	16,048/5,452,951	0.02 (0.02, 0.02)	1.0	1.0
	PCT	4/589	0.05 (0.02, 0.13)	1.9 (0.7, 5.1)	1.1 (0.4, 3.5)
Prostate (177; C61)	Reference	56,652/2,738,637	0.16 (0.16, 0.16)	1.0	1.0
	PCT	14/290	0.34 (0.20, 0.57)	1.2 (0.7, 2.0)	1.1 (0.6, 1.8)
Colon/rectum (153-154; C18-C21)	Reference	46077/5,451,951	0.5 (0.5, 0.5)	1.0	1.0
	PCT	11/578	0.94 (0.75, 1.17)	1.2 (1.0, 1.5)	1.1 (0.9, 1.4)
Breast (170; C50)	Reference	39,581/2,713,314	0.11 (0.11, 0.11)	1.0	1.0
	PCT	5/299	0.12 (0.05, 0.28)	0.6 (0.3, 1.5)	0.6 (0.3, 1.7)

Figure 5. Hazard ratios and annual incidence rates for neoplasms in persons with porphyria cutanea tarda

Persons with sporadic PCT (aHR=1.4, 95% CI: 1.1, 1.8) and unclassified PCT (aHR=2.5, 95% CI: 1.9, 3.1) were at an increased risk of premature death compared to the general population. There was no increased risk observed for individuals with familial PCT (aHR=0.8, 95% CI: 0.1–1.1). Mean age at death was 71.8 years for persons with sporadic PCT (95% CI: 68.9–74.7), 72.1 years for persons with familial PCT (95% CI: 67.0–77.1) and 73.3 years (95% CI: 70.5–76.1) for persons with unclassified PCT, compared to 78.5 years (95% CI: 78.4–78.5) in the general population. Specifically, persons with PCT had a 1.4-fold (95% CI: 1.0, 1.9) increased risk of death by malignant neoplasms (all-sites), a 5.5-fold excess risk of death by liver diseases (95% CI: 2.5, 12.2), and a 9.9-fold excess risk of death by alcohol or drug overdose (95% CI: 4.7, 20.8). We found no evidence of an increased risk of premature death due to an underlying diagnosis of diabetes mellitus, ischemic heart disease, cerebrovascular diseases, chronic obstructive pulmonary disease or renal failure.

4.3 Acute hepatic porphyria and long-term sick leave, disability leave and risk of premature death (*study III*)

Persons with AHP were more likely to access long-term sick leave (aHR=1.5, 95% CI: 1.3, 1.7) at least once over their lifetime, and to access disability pension (aHR=1.9, 95% CI: 1.5, 2.4). They were, on average, 5 to 6 years younger at the time of first long-term sick leave episode and disability pension compared to the general population. A dose-response for both long-term sick leave and disability pension was suggested by the different AHP subtypes, with the highest risk detected in persons with hospitalised AHP, less so in persons with non-hospitalised AHP and unclassified AHP, while no excess risk was detected in asymptomatic AHP gene carriers (Figure 6). Compared to the general population, we observed no increased risk for premature death in individuals with AHP, or the subtypes.

Social benefit type/ Group	Cases/ No. at risk	Median age at event (IQR)	Annual incidence per 100 (95% CI)	Crude hazard ratios (95% CI)	Adjusted hazard ratios (95% CI)
Long-term sick leave					
Reference	2,597,165/5,021,163	33 (26,47)	4.9(4.9,4.9)	1.0	1.0
AHP (total)	205/293	28 (23,34)	9.5(8.3,10.9)	1.8 (1.6, 2.1)	1.5 (1.3,1.7)
Hospitalised	33/42	24 (22,29)	13.8(9.8,19.3)	2.7 (1.9, 3.8)	2.1 (1.5,3.0)
Non-hospitalised	86/104	27 (23,33)	11.8(9.5,14.6)	2.0 (1.6-2.5)	1.6 (1.3,2.0)
Asymptomatic	52/101	33 (27,44)	5.7(4.4,7.5)	1.2 (0.9, 1.6)	1.0 (0.8,1.4)
Unclassified	34/46	22 (21,31)	10.4(7.5,14.6)	1.9 (1.4, 2.7)	1.6 (1.2,2.3)
Disability leave					
Reference	618,053/4,952,682	67 (60,67)	0.7(0.7,0.7)	1.0	1.0
AHP (total)	82/290	61 (47,67)	1.8(1.4,2.2)	2.1 (1.7, 2.6)	1.9 (1.5,2.4)
Hospitalised	19/41	46 (38,58)	3.0(1.9,4.7)	4.6 (2.9, 7.2)	4.0 (2.6,6.3)
Non-hospitalised	34/104	61 (51,67)	1.8(1.3,2.6)	1.9 (1.4, 2.7)	1.8 (1.3,2.5)
Asymptomatic	16/100	62 (54,67)	1.1(0.6,1.7)	1.4 (0.9, 2.3)	1.4 (0.8,2.2)
Unclassified	13/45	61 (39,67)	1.9(1.1,3.4)	2.1 (1.2, 3.6)	1.6 (0.9,2.8)

Figure 6. Annual incidence rates, median age and hazard ratios for first long-term sick leave and disability pension in persons with acute hepatic porphyria

Note: HR=Hazard ratio; CI=Confidence intervals; IQR= interquartile range (25th, 75th percentiles)

Compared to matched controls, persons with symptomatic AHP, hospitalised and non-hospitalised, had on average 1.6 (95% CI: 1.1, 2.2) and 1.4 (95% CI: 1.2, 1.6) times more total days of long-term sick leave per year, respectively. This equated to a difference of 4.5 (0.4, 8.6) and 3.0 (95% CI: 1.1, 4.8) additional days of annual long-term sick leave, respectively. There was no difference found between matched controls and asymptomatic gene carriers or persons with unclassified AHP. Additionally, compared to matched controls, persons with non-hospitalised AHP were expected to have 1.3 (95% CI: 1.0, 1.7) times more episodes of long-term sick leave per year. No differences were detected, however, between matched controls and persons with hospitalised AHP (IRR=1.1, 95% CI: 0.8, 1.5), the unclassified group (IRR=0.9, 95% CI: 0.7, 1.1) or the asymptomatic group (IRR=0.9, 0.7, 1.2), in relation to annual episodes.

The main diagnostic reason for long-term sick leave in patients with porphyria was 'endocrine/metabolism/nutritional disorder' (n=52 patients), a category that AHP diagnoses fall under. Following this 'psychological symptoms/disorders' were common, both in AHP and the general population. However, compared to the general population, individuals with an AHP diagnosis had an increased risk of a long-term sick leave episode due to ischemic heart disease (aIRR=3.0, 95%CI: 1.4, 6.6), endocrine/metabolism/nutritional disorder (aIRR=46.2, 95% CI: 36.2, 59.0), urological symptom/disorder (aIRR=2.4, 95% CI: 1.4, 4.3) and high blood pressure (aIRR=2.1, 95% CI: 1.2, 3.7).

The main diagnostic reason for accessing disability pension was a diagnosis of AHP (ICD-10: E80.2), n=16. Of these 16 cases, six were classified as hospitalised AHP, nine non-hospitalised AHP and one was unclassified. Other diagnostic reasons for accessing disability pension included mental and behavioural disorders, n=10; diseases of the circulatory system, n=10; and diseases of the musculoskeletal system and connective tissue, n=10. There was, however, no evidence that risk of disability due to these specific diagnostic groups was elevated among persons with AHP compared to the general population, except for diseases of the circulatory system (aIRR=3.8, 95% CI: 2.0, 7.1).

The most prominent cause of mortality was HCC (ICD-10: C22) (n=6, adjusted mortality rate ratio (aMRR)=84.4, 95% CI: 37.8, 188.2). Other causes of death of interest, but with a count less than four, included: renal carcinoma (C64): n=2; porphyria (E80.2), n=2; and renal failure (N17-19), n=2. There was one additional count of HCC, three counts of hypertensive disorder (I10, I11) and five other cases of renal failure (N17-19) listed as contributing, but not the leading underlying cause of death.

5.   Discussion

5.1 Summary of main findings

We found evidence of a substantial risk of PLC in persons with AHP and
considerable risk of HCC in persons with PCT, compared to the general population.
The finding is significant given the very poor prognosis of PLC (112). However,
although the hazard ratios were large, the absolute risk estimates were small,
especially for PCT. For example, the annual incidence of PLC in individuals with
AHP was 0.35%, meaning that for every 285 persons with AHP screened annually,
we would expect to find a single case of PLC. On the other hand, we would need to
test annually over 1,429 persons with PCT to find an individual instance of HCC,
even though in 2017, only 780 persons were registered with a PCT diagnosis in
Norway.

We also found evidence that persons with AHP had an increased risk of kidney and
endometrial cancers and persons with PCT gallbladder and biliary cancer. However,
given the findings were exploratory, novel and the total number of positive cases,
small, the results are seen as hypothesis-generating rather than confirmatory, and
further research is required. We found evidence of an increased risk of premature
death in persons with sporadic PCT due to lifestyle diseases, such as liver diseases
and alcohol or drug overdose. On the other hand, we found no evidence of an
increased risk of early death in persons with AHP. Persons with symptomatic AHP
were more likely to access long-term sick leave and disability pension than the
general population due to their porphyria, and this appeared pronounced in persons
with more severe disease.

5.2 Methodological considerations

5.2.1 Study design

Double-blinded randomised control trials (RCTs) are seen as the gold standard in
epidemiology (113), given that by randomly allocating participants to receive the

exposure or treatment, potential confounders are also randomly distributed between the experimental groups. If well designed with a sufficient sample size this results in two groups that differ based on only the allocated exposure, whereas potential confounders, including confounding factors not even considered by researchers, will be roughly equally distributed between the groups by chance (114). Therefore, researchers can be more confident that a strong association between the exposure and outcome is indicative of a causal relationship. RCTs do, however, suffer from some inherent critical limitations. For example, given the expense of RCTs, they are very rarely population-based and generally highly selective of study participants (113). Most importantly, randomly allocating persons to receive an exposure, such as porphyria, in epidemiological studies is usually not ethical or even possible.

In observational research, participants are not randomly allocated to each group, but rather have the exposure of interest or do not. Therefore, observational research does not present the same ethical issues inherent to RCT design (115, 116). Observational studies, like the current project, can also be population-based and less selective than RCTs, meaning there is better generalisability of the results (115). Such studies are, however, prone to systematic differences between the groups, and such variations may be confounding factors. Researchers can control for potential measured confounders by several experimental or statistical methods, such as matching or regression. Residual confounding cannot, however, be ruled out in such studies. Therefore, traditionally, researchers have been cautious when drawing causative inferences from observational studies.

There are three main types of observational studies, including cohort studies, case-control studies and cross-sectional studies. Of the three, cohort studies are considered to provide the best evidence, yielding better validity than case-control studies, and given that the exposure is measured before the outcome of interest, generally eliminate issues of reverse causality, which is an inherent issue in cross-sectional studies (108, 117). The current project used a registry-based cohort design (118). Although the cohort is historical and the study aims developed following data collection, the exposure was measured before the outcome at the study start or

follow-up, and independent of the study aims. This type of cohort design is advantages because data collection is complete at the time of the studies conception and study findings are, therefore, expedited. A limitation of this design compared to cohort studies which collect data prospectively is that often information on potential confounding is not available, at the population level at least. Nordic countries have developed population-based registries, such as the Cancer Registry of Norway, over decades and enable linkage at the level of the individual by the unique personal identifier. The Norwegian personal identification number is an 11 digit personal Id used nationally and assigned to every Norwegian resident at the time of birth or immigration by the National Population Register (87). The Id is used ubiquitously in every facet of Norwegian society from health appointments and employment to the access of social benefits, and across all data sources used in the current project. In health and national registries, the number enables data linkage, dramatically reducing the risk of duplication, even in the case of twins.

5.2.2 Causal inferences

Epidemiological studies are typically concerned by the causal relationship between an exposure and the outcome. However, spurious effects, or bias, from common causes are likely in observational studies. The definition of a confounder has been disputed in the causal inference literature (119). However, a useful description of the term confounder is a factor associated with the outcome conditional on the exposure and not causally related between the exposure and outcome pathway (120). According to this definition, a confounder is a factor that leads to an outcome, entirely or in part, and this effect is mistakenly attributing to the exposure. Conditioning on a confounder, either by restriction, stratification or statistical adjustment, is considered to remove the bias by comparing groups within strata of covariates (114).

A standard method to explore graphically and theoretically, the presence of common causes and, therefore, potential confounders, is to draw a directed acyclical diagram (DAG) (121-123). DAGs are directed, in that the relationship between two variables is depicted by arrows, representing causal relationships, and the relationship between

two variables cannot be unidirectional (i.e., they cannot cause each other). DAGs are acyclical, which means that there is no feedback loop where a variable causes itself. DAGs can have either directed/open paths, in which all arrows point in the same direction, reflecting a causal relationship; backdoor paths, in which two variables share the same cause, reflecting confounding (Figure 9), or closed pathways, in which two variables have the same effect, also known as collider bias (Figure 10) (120). According to VanderWeele, in situations where complete knowledge of a causal diagram is lacking, researchers should control for each covariate that is a cause of the exposure, outcome, or both; excluding any variables known to be an instrumental variable; and include as a covariate, any proxy for unmeasured variables known to be a cause of both the exposure and the outcome (97).
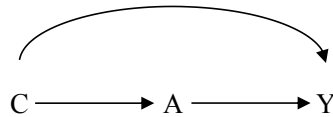
Figure 9. DAG illustrating confounding (C) from the exposure (A) to outcome (Y)
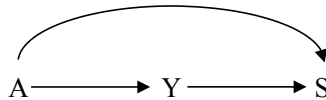
Figure 10. DAG illustrating collider bias (S), in which A (exposure) and Y (outcome) have a common effect

5.2.3 Choice of statistical methods

Cox proportional hazards regression

Cox proportional hazards regression models are used extensively throughout all studies presented in the current thesis. The hazard ratio, produced by the Cox model, evaluates the ratio of the hazard rate between the exposed and unexposed at each unit of time, rather than the ratio of the cumulative incidence. In this way, the hazard of

the outcome can vary over time between groups and can be interpreted as the instantaneous risk of developing the event at that time, assuming that a person remains at risk of the outcome at that time (109). Cox regression assesses time-to-event while accounting for person-time, potential confounders as covariates and censoring and, therefore, is typically the preferred method for assessing cohort studies. Relative risk ratios or odds ratios, on the other hand, produced by Poisson or logistic – regression, respectively, evaluates the cumulative risk or odds of an event and cannot account for censoring. The traditional Cox model assumes, however, that the HR between the exposed and unexposed remains constant over time (i.e., proportional hazard assumption). However, time-varying covariates can be included in a so-called extended Cox model by including an interaction term of the covariate by time.

Competing risks regression

In *study II*, we compared the risk of cancer and causes of death in persons with PCT to persons with a history of chronic alcohol abuse/dependence. Given the latter group are more likely to die young before they are at risk of cancer, we conducted a competing risks regression survival analysis calculating the adjusted subdistribution hazard ratio (aSHR). A competing risk is considered an event that hinders the observation of the outcome of interest or modifies the chance that this outcome will occur (26). This was the case for persons with a history of alcohol abuse/dependence, who were at a higher risk of early death due to their condition before they could develop cancer or die from other causes. When calculating the SHR, those having a competing event are maintained in the risk set and only censored when the event of interest occurs. Consequently, over time, a higher proportion of the risk set becomes full of individuals who have had a competing event before that time. Therefore, whereas the hazard function (Cox proportional regression), is the incidence of a particular outcome of interest in individuals who are currently event free, the subdistribution hazard function (competing risks regression) is the incidence of a specific outcome of interest in individuals who have not yet experienced an outcome

of that type (124). It is noteworthy, however, that we found very similar outcomes when conducting a Cox proportional regression analysis.

Zero-inflated negative binomial regression

To assess annual events for total long-term sick leave episodes and total days in persons with AHP and subtypes (*Study III*), we estimated incident rate ratios (IRRs) and CIs using zero-inflated negative binomial regression (ZIBR) with robust standard errors. A binomial model was chosen because the outcomes of interest were count variables and, therefore, it is expected the response will follow a Poisson or binomial distribution, rather than a normal distribution. A binomial model was chosen over a Poisson model, because of overdispersion of our data. Negative binomial regression accounts for overdispersion of the mean by estimating a dispersion parameter, whereas Poisson regression holds constant the dispersion parameter equal to the mean, which may bias the standard errors (125). The model was zero-inflated given that most individuals never experience long-term sick leave and, therefore, there was an excess number of zero events. To account for differences in follow-up time, we offset the analysis by time-on-study. We also averaged the total number of sick days by years on study to estimate the annual rate. Lastly, we used frequency-matched controls, rather than the whole population, given persons of a similar age had a similar time of exposure.

5.2.4 Precision

Precision refers to how close estimates from different samples, or sampling distribution, drawn from the same population are to each other (126). It is dependent of the natural variation from the population they were drawn, sample size, the magnitude of the effect of the outcome under investigation and the level of random error an investigator is willing to accept in the final estimate. The latter is nearly always, and arbitrarily, set to five per cent, meaning that we reject the null hypothesis five per cent of the time when, in fact correct. In statistics, this relates to the Type I error rate and null hypothesis significance testing (NHST). However, NHST has several limitations (127), and we rarely depended on such methods in assessing the importance or weaknesses of our research findings. As the sample size of a

population increases in a study, the higher chance the sample will accurately represent the population under investigation. Consequently, as sample size increases, variation, usually measured by the standard error, will decrease as random error decreases. The standard error refers to the dispersion of the sample estimate around the population estimate. From the standard error, we can calculate the 95% CI, which can be interpreted as the probability, or our confidence, that our estimate will fall between the upper and lower bound probability distribution 95 per cent of the time (128).

Investigating diseases with low prevalence, such as the porphyrias, is plagued by small sample sizes. Small sample sizes lead to more substantial standard errors and wide CIs, which in turn decreases statistical power. Statistical power relates to the probability of rejecting the null hypothesis when the null hypothesis is false and is the inverse of Type II error (129). However, we were able to mostly overcome this issue when investigating our primary outcomes at least, by conducting a population based cohort study. We included most persons with a known AHP/PCT diagnosis across Norway in the exposed groups and the entire population as our reference group, meaning that the sampling distribution and population distribution were virtually identical. Therefore, the confidence interval of the incidence of the reference population is minimal, indicating high precision of this estimate within this group. Importantly, this enables us to investigate not only the associations between AHP/PCT and shared outcomes, such as all-cause mortality or long-term sick leave, but also rare outcomes, such as PLC.

Although we were sufficiently powered to investigate our primary outcomes, we lacked sufficient sample sizes to investigate some interactions of interest (see sub-section 'Interactions' below for further detail). Additionally, some of our research findings may have been due to small sample bias and chance alone. For example in *study I* and *study II* we found an association between AHP/PCT and kidney cancer, endometrial cancer and bile duct and biliary tract cancer, respectively, even though only three cases of each cancer were detected during the study periods. We approach these research findings with caution, given several other factors other than just the

small number of cases. This included the exploratory nature of investigating such results (e.g., we were not interested in investigating endometrial cancer *a priori* when specifying our research aims) and the novelty of such findings (i.e., not reported in previous studies).

It is important to note also the difference between risk ratios and absolute risk. Three cases among the exposed over a long study period among 251 or 589 do not indicate a high absolute risk, even if the proportion was more substantial than that observed in the reference population. Small number bias could also be used to criticise our findings of an increased risk of PLC in persons with AHP (n=9 of 256) and PCT (n=6 of 589). In *study I* we conducted a sensitivity analysis and decreased cases with the outcome of interest by one, hence from 9 to 8, and re-ran the analysis to observe the effect on the risk ratios. This decreased our aHR from 108 to 96 (95% CI: 48, 192). Therefore, given the large effect size (i.e., aHR=108), the *a priori* nature of our investigation and support from previous findings, we do not believe that small sample biases our result regarding the association between AHP and PLC. If we were to apply the same scenario to *study II,* the aHR would decrease from 19.6 to 16.5 (95% CI: 6.8, 39.7), and again, we can draw a similar conclusion. In *study III* we were mostly interested in prevalent outcomes, and therefore, small sample bias mainly was not an issue. We conducted, however, a sensitivity analysis to investigate the effect of non-consent bias for mortality using a crude analysis in *study III* (see sub-section 'Selection bias' below for further detail).

Another benefit of using a cohort design for the current project was the very long study period over many years/decades, ranging from 12 years (*study I*) to 24 years (*study III*). Therefore, we were able to observe a higher number of incident cases of rare outcomes, improving each studies' precision.

5.2.5 Selection bias

Selection bias occurs when the individuals included in the study are not representative of the target population (108). In DAG terminology, selection bias is

considered to cause collider bias, given that the probability of being selected is affected by the exposure and outcome (130).

For the current project, the entire target population served as the reference group, ensuring the generalisability of the research findings. However, not all known persons with a confirmed diagnosis of AHP or PCT were included in the project. All participants of the Norwegian Porphyria Registry were included, as well as persons who were deceased by the study end. For *studies II and III*, we additionally sent out letters to all non-consenters of the Norwegian Porphyria Registry requesting their participation. The consent rate in *study I* was 73 per cent, and increased to 78 per cent for *study II* and 77 per cent for *study III*.

Persons who did not participate may differ to those who did concerning the severity of their AHP/PCT or the outcome of interest. Individuals with symptomatic AHP may, for example, be more motivated to participate than an asymptomatic AHP gene carrier who have never had clinical symptoms. Evidence of this was semi-supported by the finding in *Study I* that a higher proportion of females (77%) than males (62%) participated, and as discussed previously, generally, more females present with symptomatic AHP than males. This could consequently bias the assessment of an association between AHP and the outcome of interest (131). It is difficult to predict if such a bias would lead to an under or over-estimation of the risk ratios. One could speculate that in the case of AHP, if the association between AHP and PLC was due to AHP *per se*, then including more persons with severe AHP would result in an overestimation. Additionally, all persons who were deceased by the end of the study were included in the analysis. Given the poor prognosis of PLC, it was less likely, although not impossible, that we, therefore, missed incident cases of PLC in non-consenters. Due to this concern, we conducted a sensitivity analysis in *study I*, in which we reduced the hazard ratio of the main finding by the calculated per cent difference between the relative risk of the original study's findings and, hypothetically, including all non-participants in the study and specifying no new cases of PLC. The two relative risk estimates were calculated in a simple two-by-two table, and do not adjust for covariates or account for time effects, such as person-

years or censoring, so is quite crude. The total impact of such covariates in *study I* was, however, minimal, as persons with AHP generally did not differ by such factors relative to the general population. Based on this estimate, we would observe an 18% reduction in the hazard ratio (sensitivity analysis, HR=86) from the original analysis.

If we were to apply the same sensitivity analysis to our estimated aHR of PCT and HCC in *study II*, we would again expect an 18% reduction in the estimated HR, from 19.7 to 16.2. In *study III*, again applying the above crude calculation would result in a 19% and 18% reduction of the aHR for long-term sick leave and disability pension, respectively. This would hypothetically reduce the HR from 1.5 to 1.2 for long-term sick leave and 1.9 to 1.5 for disability pension. This sensitivity analysis is hypothetical, and we do not know if any of the non-participants developed PLC in *study I or study II*. HCC, the most common type of PLC, has a poor three-year survival rate of between 10 to 50 per cent in non-operable HCC (132). Therefore, it may be argued that given the poor prognosis of non-operable HCC it is more likely than not that such individuals would be captured in our study within a small number of years as our sample did include all persons with AHP/PCT who were deceased by the study end. However, five-year survival may be greater than 70 per cent in selectively screened surgical HCC patients (133). Therefore, it is not certain all individuals with both PLC and HCC were captured. Regarding *study III*, given the frequent occurrence of the two outcomes, this scenario of no new cases among non-participants is improbable. However, the sensitivity analysis demonstrates that even if no new cases were observed, the potential selection bias caused by non-participation would have little effect on the interpretation of the risk ratios.

Given the non-interventional nature of the study and that only pre-existing data would be investigated, we were granted permission by the Regional Ethical Committee to include all persons who were deceased by the end of the study period, irrespective of whether or not they had consented to participate in the Norwegian Porphyria Registry. Although this increased our sample and the study power, this was a particular source for selection bias when investigating mortality as an outcome in *study II* and *study III*.

In *study III,* we conducted a sensitivity analysis to reassess our risk ratio estimates of premature death and HCC, using the same method used above. The finding decreased the MRR from 1.3 to 0.7 for early death, suggesting that selection bias accounted for the observed increased risk in the initial analysis. This was further supported by the sub-group analysis, in which no evidence of an increased risk was observed in the three subtypes based on responses from the Norwegian Porphyria Registry (i.e., hospitalised AHP, non-hospitalised AHP and asymptomatic AHP gene carriers). Whereas, an increased risk was observed for the subtype formed of persons who were not participants of the Norwegian Porphyria Registry (i.e., unclassified). This is because this group comprised of a disproportionate number of deceased persons, whereas, the proportions of deceased persons in the other groups was not affected by this selection bias.

NAPOS offers predictive testing to all AHP patients' family members. However, predictive testing is voluntary, and Norwegian law requires the patients themselves to inform their relatives, which reduces the total number of genetically predisposed cases diagnosed and included in our project. Forty per cent and 39 per cent of individuals classified in *study I* and *study III*, respectively, were classified as asymptomatic. However, as discussed previously, a recent genetic study on AIP estimated clinical penetrance to be as low as one per cent (7). Therefore, the number of persons with asymptomatic AHP is underrepresented in *study I* and *study III*. It is difficult to say how this affects estimates for asymptomatic AHP gene carriers and how, if at all, persons unknown to NAPOS differ from those who were genetically tested. However, we have no reason to believe it is an important systematic bias.

5.2.6 Information bias

Information bias, also sometimes referred to as misclassification, occurs when there is a measurement error of the exposure, outcome or covariates and is a threat to the validity of research findings (126). Information bias can either be non-differential or differential. Non-differential bias arises when the measurement error in the exposure is unrelated to measurement error in the outcome (126), and generally, but not always, the bias affects estimates of risk ratios (e.g., HR) towards the null hypothesis

(134). Differential bias, on the other hand, refers to a measurement error which impacts groups disproportionately and can result in an under or overestimation of the risk ratios in either direction (135).

Misclassification of the exposure

All persons included in our project were diagnosed with either an AHP or PCT diagnosis by biochemical testing and/or DNA analysis, depending on the clinical presentation. Diagnostic tests were carried out by NAPOS, a European specialist centre, in collaboration with the Section of Porphyrin Analysis at the Department of Medical Biochemistry and Pharmacology, and the Centre for Medical Genetics and Molecular Medicine, Haukeland University Hospital. The procedure has been summarised elsewhere (136). However, the diagnostic procedure demonstrates good sensitivity and specificity, and definitive diagnosis in the case of DNA analysis for healthy at risk relatives. DNA analysis is further applied to differentiate sporadic and familial PCT (54). However, there is a small subset of cases where no mutation is found but where a genetic factor is still suspected, commonly referred to as type III PCT, which allows the possibility of some miss-classification.

Individuals with AHP are classified as 'symptomatic' (referred to as 'manifest' in *study I*) or 'asymptomatic' (referred to as 'predisposed cases' in *study I*). Although different criteria may be applied, generally it is agreed that symptomatic AHP status is given to AHP gene mutation carriers who have had at least one hospitalised acute attack, characterised by severe abdominal pain in the absence of significant abdominal tenderness and the absence of any other cause for the symptoms (17), accompanied by an increased concentration of PBG and ALA in urine or blood (15, 137). An acute attack usually persists for days to weeks (1). However, the clinical presentation of AHP may be non-specific, and symptoms could also reflect other illnesses (138). In remission from an acute attack, persons with AIP may still have increased ALA and PBG concentrations (15), whereas in persons with VP and HCP, concentrations usually normalise (1). In AIP the likelihood of having an acute attack is correlated with increasing levels of urinary PBG (137). However, asymptomatic AIP gene carriers may also excrete PBG concentrations greater than the URL (137).

Therefore, the values themselves have low levels of sensitivity and specificity when used to classify a person with AIP as symptomatic and asymptomatic, although this can be improved with multiple samplings.

In *study I*, participants of the Norwegian Porphyria Registry self-reported by questionnaire if they had porphyria-related symptoms, in the form of acute attacks and/or skin lesions. Given the unspecific presentation of an acute attack, it is likely there is some miss-classification in *study I* of symptomatic patients. It is unlikely a patient would say they have never had an acute attack when they have, given the severity of an attack. It is, however, conceivable some patients ascribe various non-AHP related symptoms to their predisposition for AHP, resulting in misclassification into the wrong subgroup (139, 140). We have also not used the demonstration of increased urinary ALA and PBG concentration as a requirement for the definition of a symptomatic person.

In *study III* we classified persons with AHP into 4 subtypes: 1) 'Hospitalised AHP' - persons who reported having been hospitalised at least once due to an acute attack '; 2) 'Non-hospitalised AHP' - persons who reported having had symptoms of porphyria but never having been hospitalised specifically for an acute attack; 3) 'Asymptomatic AHP gene carriers' - persons who reported never having had symptoms of porphyria over the study period; and 4) 'Unclassified' - persons with a confirmed AHP diagnosis but who had not participated in the Norwegian Porphyria Registry and, therefore, had not answered clinically relevant questions. Again, we were dependent on self-reported clinical data to define these subtypes, which may have resulted in some level of miss-classification. However, biochemical data was available for all persons. Eighty-one per cent of the persons reporting having been hospitalised for an acute attack had at one point in time a urinary PBG value four times the laboratory URL, compared to 65 per cent of persons not hospitalised, 25 per cent of asymptomatic AHP gene carriers and 37 per cent unclassified.

Misclassification of the outcome

Cancer diagnoses in *study I* and *study II* were derived from the Cancer Registry of Norway. The registry is a compulsory national registry with high levels of validity and completeness (89). Cancer information comes from several independent sources, thus securing a high grade of accuracy and completeness. Clinical notifications, pathological notifications and death certificates are the most used sources of reporting. In a validation study looking at registry data from 2001 to 2005, completeness was found to be very high at 99 per cent for overall cancer sites, and PLC and 94 per cent of cancer cases were morphologically verified, although with some variations according to cancer site (PLC=85%) (89).

Cause of death was derived from the Norwegian Cause of Death Registry, which has high levels of completeness (90). We explicitly used the underlying cause of death when investigating the primary outcomes. One identified problem in the Cause of the Death Registry of Norway is the use of unspecific and non-meaningful 'garbage codes' in place of the underlying cause of death in some instances (10-20% of registrations in 2005) (141). However, in a more recent quality assessment of the Norwegian Cause of Death Registry, the registry was ranked in the highest group compared to other countries (142). Where diagnosis-specific causes of death, rather than all-cause mortality, was investigated, this may result in fewer identified incident cases. We, however, have no reason to believe any misclassification would be dependent on porphyria status. Thus, any such bias would likely be non-differential.

Long-term sick leave and disability pension were derived from the Norwegian Labour and Welfare Administration. The data are primarily used for administrative purposes and, therefore, have not been validated. However, the information is ubiquitous, available for the entire population and include both persons consulted as inpatients and outpatients. Furthermore, benefits/payouts are based on the database. Any misclassification for accessing long-term sick leave or disability pension due to incorrect registration would be non-differential in *study III*. However, it is possible physicians may be more likely to code an AHP diagnosis as the reason for a visit or disability pension rather than using a more general code, such as 'chronic fatigue' or

'chronic pain'. Therefore, although we found no evidence that individuals with AHP had a higher incidence of such health complaints, this may be inaccurate.

5.2.7 Confounding

Confounding in AHP

Symptomatic AHP is extremely rare before puberty and more prevalent in females (21, 23). Therefore, age and sex were adjusted for by regression. Additionally, we adjusted for education as a proxy for lifestyle factors, such as heavy alcohol consumption, given the strong relationship to cancer in general, PLC specifically and early death. However, persons with AHP are counselled by NAPOS regarding lifestyle factors, such as alcohol avoidance, to reduce the risk of an acute attack (140). Further, as discussed previously, alcohol abuse and hepatitis are generally not observed in persons with AHP and PLC, and only a small proportion present with liver cirrhosis (39, 82). Overall, we found little evidence of confounding due to these three covariates. For example, in *study I* we found only a two per cent difference in hazard ratios from the crude to the adjusted - analysis when investigating the risk of PLC. In *study III*, additional to an adjusted analysis, we frequency-matched each case across the studies outcomes to 10 randomly selected controls from the entire population based on age in years at the study start, sex and educational attainment. The results from the matched analysis were essentially identical when compared to using covariate adjustment by regression. The largest difference was for the unclassified group, which may reflect that the group comprised of a more substantial proportion of the deceased, who were older at the start of the study, on average, than the other AHP subtypes and had lower educational attainment. The matched analysis, in this instance, may have more precisely adjusted for residual confounding than regression (143), possibly due to the small sample size in our exposure group. However, this difference did not affect the interpretation of the study results and generally supported the findings from the adjusted Cox regression analysis.

Confounding in PCT

Unlike AHP, triggering factors in both familial and, to a more considerable degree, sporadic PCT are known risk factors of cancer, HCC and premature death. It is

important to note, however, that not all individuals with sporadic PCT have liver disease or consume alcohol excessively, and susceptibility factors include, for example, exposure to oestrogens in women (59, 144). PCT typically develops later in life, and individuals with sporadic PCT in our study population had a mean age of 53 years and familial PCT 48 years compared to 39 years of the adult reference population at the study start. Age is most likely one of the most critical risk factors for cancer and, obviously, an early death. In *study II* age was adjusted by the time-scale in the Cox regression, rather than time on study, meaning incidence cases in persons with PCT and the reference population was compared at each year of age both in the crude and adjusted analyses. We additionally adjusted for calendar time in these models. Overall, there was a minimal change from 19.9 to 19.7 in the crude to adjusted analysis for HCC. This suggests that sex and educational attainment had little impact on the estimated HR. If we were simply to divide the annual incidence rate of HCC in persons with PCT (0.07%) by the annual incidence rate in persons with HCC from the reference population (0.002%) we would have a crude risk ratio of 35, suggesting that holding the effect of age constant in the model was important to not overestimate the impact of PCT on HCC.

Ideally, we would have liked to have data regarding the diagnosis of liver diseases, such as HBV and HCV, and liver scarring, such as fibrosis and cirrhosis, across Norway, to adjust for these factors in our models. This would, therefore, enable us to more confidently state if high rates of HCC in persons with PCT was related to PCT *per se* or other co-dependent factors. However, to our knowledge, this data is not available. We included educational attainment. However, this was likely an imperfect proxy for the true confounder, as not all persons with a lower obtained education will develop liver disease and vice versa. Therefore, residual confounding by lifestyle factors is likely in *study II*.

Residual confounding was modelled by calculating the evidence value (E-value) for each HR. This is defined as the minimum risk ratio an unmeasured confounder requires to explain away the outcome (97). The sensitivity analysis suggested that an unmeasured confounder would need to have a substantial hazard ratio of at 39, at a

minimum, to remove the effect of an increased HCC risk. Given that alcohol abuse/dependence and HBV are substantial risk factors for both PCT and HCC, it is possible that unmeasured confounding may explain away any direct relationship between PCT and HCC.

We further compared the risk of HCC and other factors of interest to persons with a history of chronic alcohol abuse/dependence. This control group consisted of 30,468 persons with a diagnosis of chronic alcohol abuse/dependence, identified through the Norwegian Labour and Welfare Administration registrations of social security benefits using ICPC-2, ICD-9 and ICD-10 codes. In this analysis, we found a 3-fold increased risk of HCC in persons with PCT, when compared to persons with a history of chronic alcohol abuse/dependence. We also found that although HCC risk was higher, persons with PCT had an 80% reduced risk of dying from non-malignant liver diseases than this reference population. This is interesting as alcohol abuse/dependence, and liver disease typically leads to cirrhosis and eventual HCC. The remaining high risk, although lower when compared to the reference population, suggests that alcohol was an essential confounder in the analysis, but may not thoroughly explain the association between PCT and HCC.

5.2.8 Interactions

Interaction occurs when the relationship between the exposure and outcome depends on a third interacting factor. Interactions can be tested by including a product term in a regression model. However, this procedure requires substantial statistical power and large sample sizes across categories. Given the small sample of the exposed and some outcomes, such as PLC, we were generally underpowered to investigate all interactions of interest. Another approach is to stratify/separate the regression analysis of the exposure and outcome by the third factor, with non-overlapping CIs of the risk estimates indicating interaction.

In *study I,* consistent with previous studies, we found that although the risk of PLC in the general population was higher in men than women (37%), most cases with AHP and PLC were female (67%). This finding is generally supported by previous studies

(29, 31, 34, 35, 32, 33, 34, 35, 36). However, although the risk difference between men and women was substantial in *study I*, our study, as well as previous studies, was underpowered to conduct an interaction analysis. Therefore, we conducted a random-effects meta-analysis of the risk of PLC in men and women from six studies reporting separate risk ratios for men and women were identified, as well as our own (*study I*). Overall, we found that there was no overlap between the relative risk estimates between the studies, suggesting the relative risk of AHP and PLC was dependent on sex, with the risk being higher in women than men.

In *study II* no interactions of PCT by educational attainment (tertiary or upper secondary vs less education) and by sex were found, although again there was limited power for interaction analyses. In *study III*, tests of interactions between AHP and sex were also investigated, although there was no evidence that the risk of accessing disability pension in AHP was dependent on sex (p-value for interaction=.919).

5.2.9 External validity

As discussed previously (see 'study design' section), observational population-based studies have excellent external validity compared to other methods, such as RCTs or case-control studies. In our project, the study population was essentially the same as the target population (i.e., Norwegian population), and, therefore, the findings are valid to this population. However, caution is required when comparing our results to other populations, due to some critical differences concerning the outcomes of interest.

In relation to PLC, Norway has one of the lowest incidences of PLC across Europe. The age-adjusted rate per 100,000 was 3.0 in men and 1.4 in women in 2011, compared to 12.5 in men and 2.9 in women in France for the same year (112). The differences are quite stark compared to Spain, France and Italy, especially in men, where incidence is high (112). The differences are less compared to the Netherlands, Sweden, Denmark, Finland and Germany (112). Outside of Europe, the incidence of PLC is also very heterogeneous because of the variable prevalence of risk factors,

and are highest in Eastern and South-Eastern Asia and lowest in South-Central Asia
and Western Asia (Figure 7) (145).
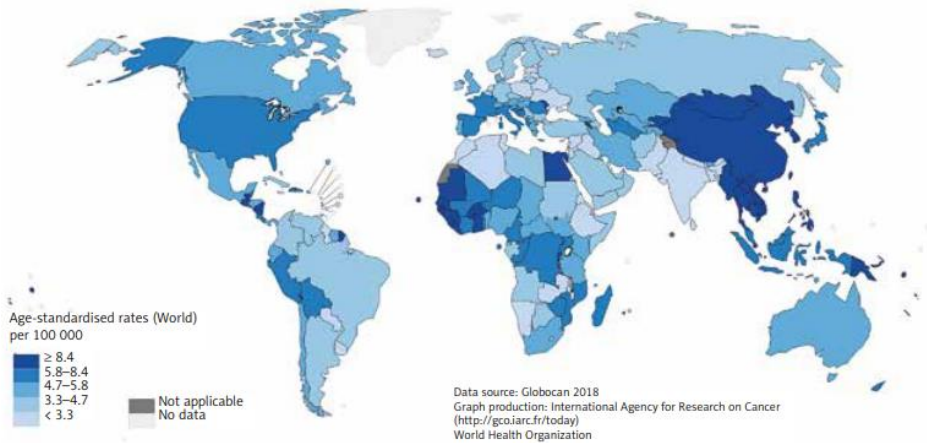
*Figure 6. Estimated age-standardised rates of incident cases of primary
liver cancer for both sexes, worldwide in 2018. Reprinted from "Update in
global trends and aetiology of hepatocellular carcinoma," by Prashanth, R,
et al. 2018, Contemporary oncology (Poznan, Poland), Vol. 22 (3), 141-
150.*

The prevalence of more common risk factors for PLC varies greatly between
countries, which explains such national differences. Overall, such baseline
differences in rates of PLC will affect risk ratio estimates, and we should be cautious
when comparing such relative statistics between countries. Instead, we should
compare absolute rates, such as annual incidence rates, although, again, caution is
required given such values typically have not adjusted for potential confounders. One
potential solution may be to calculate the population attributable fraction (PAF) for
AHP and to compare this between countries. The PAF combines the prevalence of a
risk factor in a particular population with the adjusted risk ratio and indicates the
proportion of incident cases in a population attributable to a specific risk factor like
AHP.

Concerning long-term sick leave and disability pension (*study III*), given
administrative, political and cultural differences between countries, direct
comparisons are difficult. Even between Norway, Sweden, Denmark and Finland,

although patterns of access between demographic groups are similar (highest among women and older employees), rates of uptake are different (96). Generally speaking, Norway has a generous welfare system in which an employee's job security is legally protected, and illness or disability does not constitute grounds for dismissal (43). An individual who is unable to work due to illness following one year of sick leave and vocational rehabilitation can be entitled to disability pension. Therefore, it follows that acute illness and chronic disability due to AHP and its late effects will manifest in access to long-term sick leave and disability pension in Norway. Where these protections do not exist, illness and disability due to AHP may result in unemployment, as reported elsewhere (50). However, although it may be difficult to generalise our findings from *study III* to populations outside of Norway specific to uptake of long-term sick leave and disability pension, the results are universally relevant. The effect of which is a loss of income and working years lost, and reflect an association between symptomatic AHP and chronic morbidity, regardless of how that manifests.

5.3 Interpretation and contribution of the findings

5.3.1 Porphyrins, porphyria and cancer

In 1993 Batlle argued that accumulation of porphyrins, which occurs across disorders of porphyria, initiates carcinogenesis, and that cells with abnormal haem metabolism will increase cancer risk (146). Therefore, her model predicts that people with porphyria should be at an increased risk of cancer. Given that in the hepatic porphyrias, such as AHP and PCT, porphyrins and their associated precursors accumulate mostly in the liver, a higher incidence of liver cancer in these disorders follows. However, given these porphyrins and their precursors circulate and accumulate in other areas of the body, such as the spleen and kidneys (147, 148), it does not exclude observations of increased risk in other cancers as well.

The aetiology of PLC cancer in AHP

The exact underlying mechanisms that lead to tumour growth in the liver of persons with AHP is currently unknown, and several theories have been proposed (149-151).

One dominant hypothesis suggests that the accumulation of ALA may act via a pro-oxidant and genotoxic effect (149). In Vitro, ALA has been found to damage plasmid and isolated DNA by reactive oxygen species (149). ALA has also been found to promote in isolated DNA the formation of radical-induced base degradation products (149). If this was true, then given that acute attacks are likely due to elevated levels of ALA (1, 152), then we may predict more persons with symptomatic AHP than asymptomatic gene carriers with normal concentrations of ALA and PBG to develop PLC. We found only one person with asymptomatic AHP and PLC of nine in total (*study I*), and this trend is a general finding across other studies (39). We also were able to demonstrate in a meta-analysis that females with AHP were more likely to develop PLC than males. Females are six times more likely than men to develop symptomatic AHP and, therefore, sex my act as a proxy in this instance for symptomatic disease. We would further predict that PLC is more likely in AIP than VP or HCP, given the chronic elevation of ALA is observed in some patients during remission in the former but not the two latter diseases. This is, however, difficult to investigate as VP and especially HCP are so rare in most countries, excluding South Africa. We found one case of VP with PLC out of 22 persons with VP. Therefore, if a small incidence of PLC in VP and HCP is due to differences in exposure to ALA or low incidence of VP and HCP, or a combination, is currently difficult to determine. Furthermore, PBG, uroporphyrin III, coproporphyrinogen III and protoporphyrinogen IX, which accumulate in the liver depending on type of AHP, may independently contribute to the tumour development (151).

The aetiology of kidney cancer in AHP

After the liver and bone marrow, the kidneys are third regarding the accumulation of ALA and amounts of haem synthesised (147). It is, therefore, possible that the same underlying mechanism was responsible for the observation of increased risks of PLC and kidney cancer in our study (*study I*). However, another possible explanation may be due to renal impairment. Kidney cancer is more common in patients with kidney failure, a known AIP comorbidity (28). However, the cause of this association between renal impairment and kidney cancer is not well understood (153).

The aetiology of HCC in PCT

Unlike AHP, there is no accumulation of ALA and associated neurotoxicity in PCT. It has been suggested that under chronic conditions of exposure, such as when there is a long diagnostic delay, the accumulation of uroporphyrins in individuals with PCT may result in liver injury and the development of a liver tumour (74, 151). Rodent models further suggest the potential synergistic role of iron overload and genetic predispositions in this process (151). However, the confounding role of hepatotoxic factors, such as alcohol, which are implicated in the pathogenesis of PCT and HCC, makes it difficult to determine if PCT independently gives rise to HCC. Our findings provide some support for the hypothesis that PCT may by parallel, or interaction with, hepatotoxic factors, contribute to the formation of a liver tumour. In *study II,* we observed an increase in the risk after adjusting for educational attainment and comparing persons with PCT to persons with a history of chronic alcohol abuse/dependence. Of the six persons in our cohort with PCT and HCC, two had familial PCT, which is less associated with other types of liver disease or excessive alcohol intake than sporadic PCT (54). We also found a trend towards higher concentrations of total porphyrins and uroporphyrins in PCT patients with HCC compared to those without. However, the evidence is not clear, and the small number of persons with PCT and HCC, as well as the variability in the findings, limit our ability to draw any firm conclusions. It has been further suggested that with improved recognition and treatment of PCT, there will be a decrease in the incidence of HCC (82). We also found an increased risk of gallbladder and biliary tract cancer in persons with PCT. The finding is interesting, given that it is located beneath the liver and connected by the common hepatic duct. Although small numbers of observed cases tested over multiple cancers mean this could reflect a chance finding, the finding has been recently replicated in a Danish cohort (154).

5.3.2 Long-term sick leave and disability pension in AHP

In *study III,* we found a very high proportion of persons with hospitalised and non-hospitalised symptomatic AHP accessed long-term sick leave at least once (82%) and disability pension (36%). The HRs were 1.5 and 1.9, respectively. However, given

that long-term sick leave and disability pension are common in the general population, it is not possible to observe a much higher risk ratio (i.e., ceiling effect). The median age when accessing disability pension was 46 years in persons with hospitalised AHP, which was substantially younger than the general population (21 years) and other AHP subtypes. In a population-based study in Northern Sweden, a mean age of 45 years was reported in symptomatic patients accessing long-term sick leave or disability leave (15). We also found that persons who had been hospitalised at least once for an acute attack had 60 per cent more long-term sick leave days per year than the reference population. These findings suggest that severity in AHP may dramatically increase the number of working years lost due to disability.

The most common symptoms associated with accessing long-term sick leave in *study III* included weakness/tiredness (6%), abdominal pain (2%), muscle/joint pain (3%), acute stress reaction (8%) and feeling depressed/depressive disorder (14%). However, such complaints were also common in the reference population, and we found no evidence that persons with AHP had increased risk of long-term sick leave due to these reasons. However, such medical complaints may have been coded with a porphyria diagnostic code instead, which was the most prevalent cause of long-term sick leave or for accessing disability pension.

5.3.3 Causes of death in AHP

We found an 84-fold excess risk of mortality due to HCC in persons with AHP compared to the general population. No other types of PLC were reported in the Cause of Death Registry. Although HCC has a very poor prognosis and low survival times, not all persons with AHP and HCC reported in *study I* were deceased in *study III*, suggesting the tumour was detected early and curatively treated in two persons.

Another general finding regarding mortality and AHP is an excess risk of death due to renal impairment. Andersson and Lithner found that renal impairment was the cause in 9% of AIP deaths between 1978 and 1990 in Northern Sweden (27). In our study, renal failure was cited as the underlying cause in 4%, and as a contributing cause in 9% of deaths in individuals with AHP. Therefore, our results support

previous findings and suggest that renal disease remains a common cause of death in persons with AHP.

Initially, in *study III,* we found a 30 per cent increased risk of premature death. However, the finding was isolated to the AHP unclassified subtype, who were overrepresented by persons included in our study due to their deceased status and, therefore, had not completed clinical questionnaires. As discussed previously, we conducted a sensitivity analysis which contradicted our initial finding. This stands in contrast to a study by Linet and colleagues (21), who reported a 90 per cent increased risk of premature death in AIP patients relative to respective Swedish and Danish populations, and specifically due to cancer and ischemic heart disease (excluding AIP as a cause of death) (43). Despite an increased risk of death due to HCC in persons with AHP in *study III*, the risk was not substantial enough to suggest persons with AHP were at an overall increased risk of early death.

5.3.4 Causes of death in PCT

In *study II*, we found that persons with PCT were at a seven-fold increased risk of dying from liver diseases compared to the general population. The specific diagnoses were alcoholic cirrhosis of the liver, hepatic alcohol failure, primary biliary cirrhosis, hepatic failure, and two cases of chronic HCV. Liver disease is associated with UROD inhibition and a known trigger of PCT (58, 62, 155, 156). It is, therefore, unsurprising to find an increased risk of mortality due to liver diseases in our PCT cohort. It is interesting though that of the six persons with PCT and HCC, none had liver disease listed as the underlying or contributing cause of their death. Although, one of the three persons with cancer of the gallbladder and biliary tract had cirrhosis of the liver listed as a contributing cause of their death. In addition, seven persons had died from alcohol poisoning or substance overdose, which constituted an 11-fold increased risk compared to the general population. Given the association between drug and alcohol abuse and liver disease, such as chronic HCV, and between liver disease and PCT, again, this finding is not surprising. Two of the six persons with liver disease and one of the seven who died due to alcohol poisoning or overdose due to substance abuse were female. This reflects a general finding that a larger

proportion of males than females with PCT have liver disease related to poor lifestyle, whereas many females develop PCT due to contraceptive use and not necessarily due to lifestyle factors (59). The median age of the persons who died due to alcohol poisoning or substance overdose was 53 years, compared to 63 years in persons who died from liver diseases and 73 years in persons with PCT, but who died due to other causes. Therefore, alcohol poisoning and substance overdose were the most significant causes of years of life lost.

In a study published after the publication of *study II*, Danish researchers reported a 20 per cent increased risk of premature death in all individuals registered with a PCT diagnosis from 1989 to 2012 compared to age and gender-matched controls (154). The researchers controlled for alcohol-related diseases, hepatitis, hemochromatosis, HIV, diabetes, acute mesenteric ischemia acute myocardial infarction, stroke, cancer, chronic obstructive pulmonary disease, and cirrhosis. Cause-specific diagnoses included non-malignant gastrointestinal diseases (HR=5.3), cancers of the gut (HR=2.1), liver/gallbladder (HR=11.24), and lungs (HR=2.17). Overall, the study supports our finding of an increased risk of premature death and due to the specific causes of cancers of the lung, liver and gallbladder. The differences in the magnitude of the HRs and cause-specific mortality may be explained by either national differences in baseline risk, these investigators including additional constraints for potential confounding, and/or these investigators not accounting for differences in all-cause mortality between individuals with familial and sporadic PCT.

Lung cancer is associated with a poor prognosis (157). We found a 1.6 increased risk of lung cancer in PCT patients compared to the general population, although the finding was non-significant. Smoking was also the second most prevalent cancer, accounting for 16 per cent of all cancers. Christiansen *et al* reported a 2.2 increased risk of mortality in PCT patients compared to matched controls (154). Similarly, Linet *et al* reported a 2.9-fold increased risk of lung cancer compared to the general population in Swedish and Danish cohorts (43). Linet *et al* speculated that smoking alone could not explain the increased risk of lung cancer and that PCT may enhance susceptibility to cancer of the lung given evidence that smoking can lead to earlier

onset of cutaneous symptoms in patients with sporadic disease (53). In our study, of the 6 persons who completed questionnaires, all were smokers, and given the strong association between lifestyle factors and PCT and the small size of the relative risk, it is difficult to rule out that this confounded the results. We also found no evidence of a difference in the diagnostic delay of PCT patients with a diagnosis of lung cancer to those without. Still the similar finding between the three studies supports the idea that cancer of the lung is prevalent among persons with PCT and an important contributing factor to premature mortality.

5.4 HCC surveillance in persons with AHP and PCT

When clinical symptoms of HCC present, typically the tumour is far advanced, and there are few therapeutic options. The European Association for the Study of the Liver (EASL) and European Organization for Research and Treatment of Cancer (EORTC) clinical practice guidelines for the management of HCC recommend biannual hepatic ultrasound screening by experienced personnel for individuals at high risk of HCC (127). This includes persons aged 50 years or older (127, 158).

Additionally, according to expert opinion, an annual incidence cut-off of 1.5% would justify HCC surveillance in chronic hepatitis patients with cirrhosis, and 0.2% would justify surveillance in non-cirrhotic patients (159, 160). This is because the detection of HCC by ultrasound in the cirrhotic liver is more complicated and decreases the sensitivity and specificity of the diagnostic method (127). Although the porphyrias has not been considered in the above recommendations, two Swedish studies investigating surveillance in adults over the age of 50 years with AHP suggest that screening may lead to earlier tumour detection and reduced mortality rates in both individuals with and without symptomatic disease (33, 34). There is no such evidence, however, for persons with PCT. In *study I* we found an annual incidence of 0.6 per cent in persons with AHP aged 50 years or older. Therefore, if we were to apply the cut-off for non-cirrhotic HBV patients, those with AHP, and particularly with symptomatic AHP, and 50 years or older should be targeted for screening as a high-risk group. In regards to PCT, we observed an annual incidence of HCC of 0.07

per cent, with an upper bound 95 per cent CI of 0.17 per cent. Additionally, unlike in AHP, confounding by concurrent cirrhosis cannot be ruled out in patients with PCT. Therefore, although surveillance is recommended for persons with AHP aged 50 years or older, and especially with symptomatic disease, it cannot be currently recommended based on a PCT diagnosis alone. However it has been suggested that individuals with PCT should likely be tested for HCV/HBV infection and have a liver biopsy - with those with concurrent disease or cirrhosis included in a standard surveillance programme (75). Another recent recommendation is that individuals with AHP should additionally be vaccinated against viral HCV/HBV (161).

6. Conclusions

The current thesis contributes with valuable knowledge to the long-term consequences of AHP and PCT. Specifically, we found that compared to the general population, persons with AHP, and especially symptomatic AHP, had an extremely high risk of PLC. We also showed in a meta-analysis that the risk was higher in women than in men. Our findings of moderately increased risks of endometrial and kidney cancers, however, need to be validated in future studies and may reflect chance findings. We also showed that compared to the general population, persons with PCT had a high risk of HCC and premature death due to lifestyle factors, such as alcohol abuse. We found moderate excess risks of gallbladder and biliary tract cancers, which again may be chance findings, and of premature death in persons with sporadic PCT. Given the risk of HCC was increased even when compared to persons with a history of alcohol abuse/dependence, PCT *per se* may contribute in part, or by interaction with lifestyle factors, to the development of HCC. Lastly, we found that individuals with symptomatic AHP were at increased risk of accessing long-term sick leave and disability pension due to their porphyria. We found no evidence of overall increased risk of premature death in persons with symptomatic or asymptomatic AHP, despite an increased risk of dying from HCC.

7. Future perspectives

The very high risk of PLC in AHP has been confirmed, and although the underlying mechanisms are not entirely understood, there is good epidemiological evidence that the cause is due to something inherent to AHP, such as the accumulation of ALA in the liver. However, it remains unclear if risk of PLC is increased in asymptomatic AHP gene carriers and in VP and HCP. The costs and benefits of PLC surveillance programs for persons aged 50 years or older and with symptomatic AHP vs asymptomatic AHP gene carriers should be further explored. It would also be interesting to investigate if we can better identify high risk subtypes, such as asymptomatic high excretors of ALA and PBG, or recurrent attack patients. It is also of important scientific inquiry to investigate if prophylactic treatments to lower ALA and PBG concentrations and frequency of recurrent attacks reduce risk of PLC in these patients (1, 162, 163).

The reason for the association between PCT and PLC is more complicated and remains controversial than the association between AHP and PLC. However, it is possible that with improvements to PCT diagnosis in the previous decade that observations of PLC may decrease over time due to reductions in diagnostic delay. Theoretically, these questions could be better investigated in a future study, by conducting a prospectively designed cohort study in which PLC risk factors, such as alcohol use, and HCV/HBV – status are recorded and adjusted for across groups. Ideally, this would be conducted across countries to improve the generalisability of research findings and increase the number of exposed cases with the outcome of interest. However, such studies are expensive, take years and require international collaboration.

One example of an international collaborative effort investigating such questions is the European Porphyria Registry (164), which was established in 2012. However, the registry is not epidemiological, and although high-quality data regarding acute attacks, comorbidities and specifically PLC are collected, patients are included based on convenience. Another possible solution would be to combine Nordic registries,

especially between Norway, Finland and Sweden who all have excellent lists of persons with a porphyria diagnosis - and cancer population-based registries. This would improve small sample bias, although data regarding risk factors would still be incomplete.

We found an increased risk of long-term sick leave and disability pension in persons mainly due to an AHP diagnosis. However, as previously discussed, there is presently no diagnostic test that may determine which symptoms may be caused by AHP, and it is uncertain under what conditions physicians use the code. For example, does the doctor use the code if a patient complains of chronic pain outside of an acute attack? Therefore, another interesting line of investigation for future studies could be detailed clinical reasons in persons with AHP who access long-term sick leave and disability pension. Ideally, the data would be collected via questionnaires to patients and physicians as well as subtracted from medical notes. Given we found some indication of a dose-effect in our analyses, it would be vital to collect physician-reported data regarding if the patients have previously had a confirmed acute attack, and if the patient was treated for recurrent acute attacks of four or more attacks within a year. The Norwegian Porphyria Registry has in recent years started to collect this type of data from general practitioners.

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Studies I to III

Study I

Acute hepatic porphyria and cancer risk: a nationwide cohort study

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Abstract. Baravelli CM, Sandberg S, Aarsand AK, Nilsen RM, Tollånes MC (Haukeland University Hospital, University of Bergen, Haraldsplass Deaconess Hospital; Western Norway University of Applied Sciences, Norwegian Institute of Public Health, Bergen, Norway). Acute hepatic porphyria and cancer risk: a nationwide cohort study. *J Intern Med* 2017 https://doi.org/10.1111/joim.12646

Background. Acute hepatic porphyria (AHP) is considered to be a risk factor for primary liver cancer (PLC), but varying risk estimates have been published.

Objectives. Our aim was to investigate the risk of PLC and other cancers in persons with AHP using a nationwide cohort design. Given that greater numbers of women than men tend to have manifest and more severe AHP, a further aim was to investigate sex differences in this risk.

Methods. The study sample consisted of all Norwegian residents aged 18 years or older during the period 2000–2011. Persons with AHP ($n = 251$) were identified through the Norwegian Porphyria Centre, and patients with a cancer diagnosis were

identified by linkage to the Cancer Registry of Norway.

Results. For persons with AHP, the annual incidence rate of PLC was 0.35%. PLC risk was substantially higher for individuals with an AHP diagnosis compared to the reference population [adjusted hazard ratio (aHR) 108, 95% confidence interval (CI) 56–207]. In a meta-analysis of published studies on PLC and AHP, including ours, women had a higher risk than men. In addition, our results suggested that persons with AHP may have increased risks of kidney (aHR 7.4, 95% CI 2.4–23.1) and endometrial cancers (aHR 6.2, 95% CI 2.0–19.3).

Conclusions. Our findings confirmed a substantially higher risk of PLC associated with AHP compared to the general population. In a meta-analysis, the risk was shown to be greater for women than men. The novel findings of a moderate to substantial association between AHP and kidney and endometrial cancers should be investigated further.

Keywords: acute hepatic porphyria, acute intermittent porphyria, cancer, hepatocellular carcinoma, hereditary coproporphyria, variegate porphyria.

Introduction

The porphyrias are a group of several rare metabolic disorders of the haem biosynthesis pathway. Acute hepatic porphyria (AHP) usually refers to three types of autosomal dominant porphyrias in which the liver is considered the primary site of haem pathway enzyme deficiency: acute intermittent porphyria, variegate porphyria and hereditary coproporphyria. AHP is characterized by the accumulation of porphyrins and/or the precursors delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) in the biosynthesis of haem. The liver is

the main location of this build-up because of its inherent central role in haem production and regulation. Clinical penetrance is low, and clinical and biochemical studies suggest that the majority of individuals who inherit an AHP mutation never develop porphyria-related symptoms [1, 2]. Manifest disease typically does not present before adulthood, is more common in women [1] and is characterized by acute neurovisceral attacks and/or skin lesions in variegate porphyria and hereditary coproporphyria. Acute intermittent porphyria has an estimated prevalence in Norway of one in 14 000 [3]. In European countries, the prevalence

of variegate porphyria and hereditary copropor-phyria is estimated at one in 30 000 and less than one in 50 000, respectively [4]. Long-term compli-cations of AHP include recurrent severe acute attacks, kidney failure, hypertension [5] and pri-mary liver cancer (PLC) [6].

A strong association between AHP and PLC was first described by Swedish investigators in 1984 [7], and has since been reported in other case–control and cohort studies although, interestingly, no focused studies outside Europe have been reported [6, 8–12]. There is disagreement regarding incidence and risk estimates between studies con-ducted in Sweden compared to other European countries, with Swedish estimates being higher. Liver cirrhosis, which is found in over 80% of patients with PLC in the general population [13], is typically not observed in AHP patients with PLC [6, 8, 12]. Although leading theories regarding the pathogenesis linking AHP and PLC suggest that AHP could increase the risk of cancers in general [14–16], currently there is not, to our knowledge, any published evidence that persons with AHP are at an increased risk of any other cancers. Using a nationwide historical cohort study design, we aimed to investigate (i) the incidence and risk of PLC and other malignant neoplasms in persons with AHP, and (ii) whether there is a difference in this risk between men and women.

Materials and methods

Data sources

The Norwegian Porphyria Registry was established in 2002 by the Norwegian Porphyria Centre (NAPOS) and acquired status as a national medical quality registry in 2012 [17]. The registry is based on patient consent and records porphyria diagno-sis and clinical information [18], derived from patient-reported questionnaires supplemented with biochemical laboratory results.

The Cancer Registry of Norway was established in 1953 as a compulsory registry of all new neo-plasms and has been found to have a high degree of validity, accuracy and completeness [19]. Our study included information about cancer diagnosis and date of diagnosis.

The Norwegian Population Registry became a com-pulsory registry in 1946 and records demographic information for all Norwegian residents. The reg-istry supplied information about sex, life status,

year of birth, emigration status, year of emigration and year of death.

The Norwegian Standard Classification of Educa-tion (NUS2000) was revised by Statistics Norway in 2000. Formally recognized education is categorized as: no education and preschool education (0 years); primary education (1–10 years); interme-diate education [11–13 years (or 14 years if includ-ing postsecondary nontertiary education)]; tertiary education (≥14 years); and unspecified. We used a person's highest level of formal education by year of emigration, death or end of study as a marker of socio-economic status. This information was avail-able for over 90% of the study population.

Record linkage of the Norwegian Porphyria Regis-try, Cancer Registry of Norway, Norwegian Popula-tion Registry and Norwegian Standard Classification of Education database was carried out in 2012 by Statistics Norway using each person's unique national identification number.

Study population and case definitions

The study sample comprised all Norwegian adult residents, born before 1994 and registered in the Norwegian Population Registry from 2000 (the year NAPOS was established) to 2011. As the outcome of interest was a primary cancer diagnosis, we excluded individuals who, prior to the year 2000, had a cancer diagnosis registered in the Cancer Registry of Norway (not including nonmelanoma skin cancers).

NAPOS keeps a record of both patients with man-ifest porphyria disease and predictively tested cases without reported porphyria-related symp-toms, hereafter referred to as genetically predis-posed cases [3]. Porphyria diagnosis is derived from biochemical and/or DNA analyses conducted at the Laboratory for Clinical Biochemistry and the Center for Medical Genetics and Molecular Medi-cine at Haukeland University Hospital. Participa-tion in the Norwegian Porphyria Registry is voluntary, and the study population included 73% of known manifest and genetically predis-posed AHP cases (consent rate: 62% for men versus 77% for women). Persons with AHP are categorized in the Norwegian Porphyria Registry as having manifest disease if they report having had por-phyria-related symptoms, in the form of acute attacks and/or skin lesions. Patients reporting acute attacks experienced abdominal pain as their

main symptom (92%). Other frequently reported symptoms were nausea, obstipation, muscular pain, muscular weakness, palpitations, red/brown coloured urine, fatigue and psychiatric symptoms such as feelings of anxiety, depression, confusion and irritability. NAPOS offers predictive testing to all AHP patients' family members. However, predictive testing is voluntary, and Norwegian law requires the patients themselves to inform their relatives, which reduces the total number of genetically predisposed cases diagnosed and, therefore, included in our study.

We classified cancer diagnoses using the following International Classification of Diseases, 10th revision (ICD-10) and seventh revision (ICD-7) codes: PLC, C22 (ICD-10), 155 (ICD-7); hepatocellular carcinoma (HCC), C22.0 (ICD-10), 155.0 (ICD-7); kidney cancer, including the renal pelvis, C64-65 (ICD-10), 180 (ICD-7); endometrial cancer, C54 (ICD-10), 172 (ICD-7); breast cancer, C50 (ICD-10), 170 (ICD-7); and prostate cancer, C61 (ICD-10), 177 (ICD-7). We classified all non-PLC malignancies using the codes C00–96 (ICD-10), excluding codes for PLC and nonmelanoma skin cancer.

Our study population consisted of 251 adults with AHP (acute intermittent porphyria, $n = 222$; variegate porphyria, $n = 21$; and hereditary coproporphyria, $n = 8$). The reference population comprised 4 398 546 adults.

Statistical analysis

STATA/SE version 14 for Windows was used for all statistical analyses (StataCorp Stata Statistical Software: Release 14, College Station, TX, USA). All *P*-values were two-sided, and values <0.05 were considered statistically significant. Comparisons between groups were performed using the Wilcoxon–Mann–Whitney, chi-squared or Fisher's exact probability tests, where appropriate. The occurrence of PLC alone, all non-PLC malignancies and diagnosis-specific malignancies with more than two cases were reported as annual incidence rates (in person-years). We calculated person-years at risk by adding the total number of years for each cohort member from January 2000 to the time of cancer diagnosis, emigration, death or the end of the study in January 2011, whichever occurred first. Annual incidence rates in person-years were then calculated by dividing the number of new cases during the whole study period (2000–2011) by person-years at risk.

Cox proportional hazard regression models were used to calculate the hazard ratio as an approximation of relative risk. The AHP diagnosis (no/yes) was the exposure variable, and the cancer of interest (no/yes) was the outcome variable. Persons who died from causes other than cancer, emigrated or were free of the outcome of interest by the end of the study period in 2011 were censored. The potential confounders sex, age and education were included in the adjusted model. Age was added as a time-dependent (continuous) variable. Based on visual inspection of the log–log plots we confirmed the assumption of proportionality of the hazard function. The models were further stratified by sex, and subgroup analyses were conducted in participants aged 50 years or older and in those with self-reported manifest disease.

Sensitivity analyses were conducted to assess the robustness of the results. The model was used to determine the effects of reducing the number of cases with AHP and the outcome by one, and of including all persons who refused participation in the denominator.

Consistent with previous studies, we reported sex-specific risk estimates of PLC in AHP. Although the risk difference between men and women was substantial, our study as well as previous studies was underpowered to detect a statistically significant interaction between sex and AHP. Therefore, we conducted a random-effects meta-analysis of the risk of PLC in men and women. In October 2016, a systematic literature search for relevant studies was performed using the PubMed database with the following search terms: 'Porphyrias, Hepatic' (MeSH Major Topic) AND 'Liver Neoplasms' (MeSH Major Topic). This resulted in 46 studies. Following quality assessment and rejection of case series and basic research studies, seven studies investigating specifically an acute form of hepatic porphyria were identified. An additional four studies of interest were further identified by scanning the reference lists of these publications. Of these 11 published studies [4, 6–12, 16, 20, 21], six reported separate risk ratios for men and women [6–10, 12]. The meta-analysis included these estimates and ours, and there is unlikely to be overlap or double counting of patients from this study and others. Risk ratios were stratified by sex and then weighted using the inverse-variance method to calculate separate-pooled risk ratios for women and men.

Ethical approval

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Regional Committees for Medical and Health Research Ethics, Norway (reference number: 2012/753).

Results

Demographic and clinical characteristics and a list of all cancer diagnoses identified in persons with AHP from the Cancer Registry of Norway are shown in Table 1. Nine participants with AHP were registered with PLC (acute intermittent porphyria, $n = 8$; variegate porphyria, $n = 1$), compared to 1478 cases in the reference population (Table 1). PLC constituted a burden of 31% of all cancers reported for the AHP cohort and 0.6% in the reference population.

Six of the nine PLC cases in the AHP population were female (67%), compared to 545 of the 1478 PLC cases (37%) in the reference population (*P*-value for

Table 1 *Demographic and clinical characteristics of persons with acute hepatic porphyria compared with the reference population (2000–2011, ≥18 years of age)*

	AHP (n = 251)		Reference (n = 4 398 546)	
	n	%	n	(%)
Sex, female	151	60.2	2 191 730	49.8
Age, years (median and range)[a]	53	19–96	48	19–104
Manifest porphyria[b]	147	60.5	NA	NA
Level of education				
Not specified	0	0.0	246 013	5.6
No or preschool education only	0	0.0	15 713	0.4
Compulsory education (1–10 years)	66	26.3	1 116 662	25.4
Intermediate education (11–13 years)	107	42.6	1 786 905	40.6
Tertiary education (≥14 years)	78	31.1	1 233 253	28.0
Emigrated	2	0.8	168 818	3.8
Died	29	11.6	431 605	9.8
Cancer diagnoses[c]				
All sites (C00–96, excluding C44)	29	11.6	253 732	5.8
Site specific[d]				
Liver, primary (C22)	9	3.6	1478	0.03
Prostate (C61)	4	4.0	41 336	1.9
Kidney (C64–65)	3	1.2	6868	0.2
Endometrium (C54)	3	2.0	7000	0.3
Breast (C50)	3	2.0	30 718	1.4
Colon (C18)	1	0.4	7311	0.2
Peritoneum (C48)	1	0.4	132	0.01
Lung (C34)	1	0.4	24 701	0.6
Cervix uteri (C53)	1	0.7	3400	0.2
Bladder (C67)	1	0.4	11 463	0.3
Other endocrine glands (C75)	1	0.4	123	<0.01
Non-Hodgkin lymphoma (C82–85)	1	0.4	2021	0.5

AHP, acute hepatic porphyria; NA, not applicable.
[a]Age in years at the end of study, time of death or emigration; [b]manifest porphyria status was missing for eight persons; [c]cancer diagnoses were classified using the International Classification of Diseases, 10th revision codes; [d]site-specific comparisons exclude other sex where appropriate.

difference = 0.07). Median age at the time of PLC diagnosis was 71 years in both groups. All cancer cases in the AHP group were 50 years or older at the time of PLC diagnosis, compared to 91% in the reference population (P = 0.42). Amongst persons within the AHP cohort with PLC, we found that 89% had manifest disease (n = 8); by comparison, 59% of persons with AHP but without PLC had manifest disease (n = 139; P = 0.09).

The annual incidence of PLC was 0.35% in individuals with AHP compared to 0.003% in the reference population (Table 2). When restricted to those aged 50 years or older, the annual incidence of PLC increased to 0.63%, compared to 0.006% in the reference population. The annual incidence of PLC was 0.52% when restricted to those with self-reported manifest porphyria disease only, and 0.86% when further restricted to individuals with manifest AHP disease and aged 50 years or older. Only one of the nine patients with PLC and AHP did not report manifest AHP. There was generally only a small difference between the crude and adjusted relative risk estimates, suggesting a

Table 2 *Annual incidence rates and relative risk estimates for primary liver cancer in persons with acute hepatic porphyria (2000–2011)*

Sample	PLC cases/no. at risk	Annual incidence of PLC (person-years per 100)	Relative risk estimates (hazard ratios) of PLC diagnosis (95% CI)	
			Crude	Adjusted[a]
≥18 years				
Total				
Reference	1478/4 398 546	45 528 370 (0.003)	1	1
AHP	9/251	2610 (0.35)	106 (55–205)	108 (56–207)
Females				
Reference	545/2 191 730	22 693 793 (0.002)	1	1
AHP	6/151	1587 (0.38)	158 (71–352)	168 (75–376)
Males				
Reference	933/2 206 816	22 834 577 (0.004)	1	1
AHP	3/100	1024 (0.29)	72 (23–223)	70 (22–217)
Manifest AHP				
Reference	1478/4 398 546	45 528 370 (0.003)	1	1
AHP	8/147	1542 (0.52)	160 (80–320)	160 (80–321)
≥50 years				
Total				
Reference	1341/2 081 781	20 669 976 (0.006)	1	1
AHP	9/144	1434 (0.63)	97 (50–187)	114 (59–219)
Females				
Reference	503/1 069 248	10 640 720 (0.005)	1	1
AHP	6/86	872 (0.69)	146 (65–326)	167 (75–375)
Males				
Reference	838/1 012 533	10 029 256 (0.008)	1	1
AHP	3/58	562 (0.47)	64 (21–199)	70 (22–217)
Manifest AHP				
Reference	1341/2 081 781	20 669 976 (0.006)	1	1
AHP	8/91	926 (0.86)	133 (66–267)	154 (76–309)

PLC, primary liver cancer; AHP, acute hepatic porphyria; CI, confidence interval.
[a]Adjusted for sex (where applicable), education and age. Participants who died, emigrated or were free of PLC by the end of the study were censored.

small degree of confounding in relation to sex, age and socio-economic status (Tables 2 and 3). The relative risk estimated from the adjusted Cox regression model suggested that the risk of PLC was 108-fold higher in individuals with AHP compared with the reference population (Table 2 and Fig. 1). Women had a 168-fold increased relative risk of PLC, whilst men had a 70-fold increased relative risk. When analyses were restricted to persons aged 50 years or more, those with AHP had a 114-fold increased relative risk of PLC (167-fold and 70-fold increased risk in women and men, respectively). Individuals with self-reported manifest disease had a 160-fold increased relative risk of PLC. When this analysis was further restricted to participants aged 50 years or older, those with manifest disease had a 154-fold increased relative risk of PLC (Table 2). Finally, when the outcome was restricted to a diagnosis of HCC alone ($n = 946$), persons with AHP had a 170-fold increased relative risk [95% confidence interval (CI) 88–328].

The meta-analysis showed that when pooled across studies, there was no overlap of the confidence bands, suggesting that women had a higher relative risk compared with men [adjusted hazard ratio 130 (95% CI 91–184) and 51 (95% CI 37–71) in women and men, respectively] (Fig. 2).

Table 3 *Annual incidence rates and relative risk estimates for nonprimary liver cancer malignancies in persons with acute hepatic porphyria (2000–2011)*

Sample	Cancer cases/no. at risk	Annual incidence of PLC (person-years per 100)	Relative risk estimates (hazard ratios) of cancer diagnosis (95% CI)	
			Crude	Adjusted[a]
All non-PLC sites[b]				
Total				
Reference	252 254/4 398 546	44 796 383 (0.56)	1.0	1.0
AHP	20/251	2557 (0.78)	1.4 (0.9–2.2)	1.3 (0.9–2.1)
Females				
Reference	120 278/2 191 730	22 324 127 (0.54)	1.0	1.0
AHP	14/151	1546 (0.91)	1.7 (1.0–2.9)	1.7 (1.0–2.8)
Males				
Reference	131 976/2 206 816	22 472 256 (0.59)	1.0	1.0
AHP	6/100	1011 (0.59)	1.0 (0.5–2.2)	0.9 (0.4–2.0)
Site specific				
Kidney (C64–65)[c]				
Reference	6868/4 398 546	45 510 844 (0.02)	1.0	1.0
AHP	3/251	2629 (0.11)	7.6 (2.4–23.5)	7.4 (2.4–23.1)
Endometrium (C54)				
Reference	7003/2 191 730	22 667 406 (0.03)	1.0	1.0
AHP	3/151	1587 (0.19)	6.1 (2.0–19.0)	6.2 (2.0–19.3)
Breast (C50)				
Reference	30 718/2 191 730	22 566 055 (0.14)	1.0	1.0
AHP	3/151	1585 (0.19)	1.4 (0.4–4.3)	1.4 (0.4–4.2)
Prostate (C61)				
Reference	41 336/2 206 816	22 694 030 (0.18)	1.0	1.0
AHP	4/100	1016 (0.39)	2.1 (0.8–5.8)	2.0 (0.8–5.4)

AHP, acute hepatic porphyria; CI, confidence interval.
[a]Adjusted for sex (where applicable), education and age. Participants who died, emigrated or were free of PLC by the end of the study were censored; [b]includes all sites [International Classification of Diseases, 10th revision (ICD-10) codes: C00–96] except PLC and nonmelanoma skin cancers (ICD-10: C44); [c]classified using ICD-10 codes.
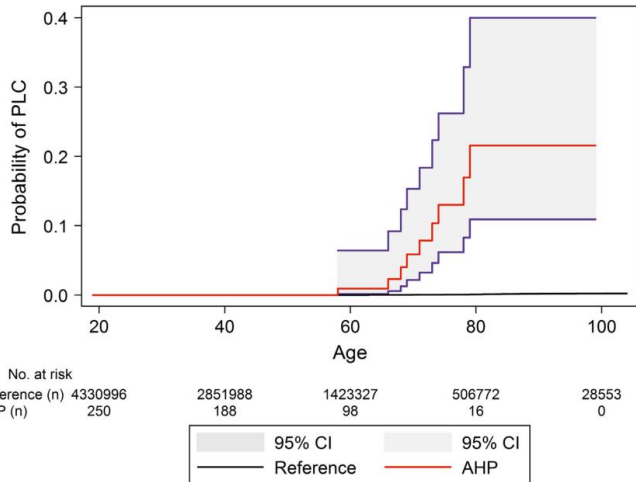
Fig. 1 *Kaplan–Meier plot showing risk of primary liver cancer with age for persons with acute hepatic porphyria compared to the reference population.*

Twenty cases of non-PLC malignancies were registered for the AHP cohort (Table 3). There were no overall differences observed in risk between persons with AHP and the reference population. The majority of cases with a non-PLC diagnosis were female (n = 14, 70%), whereas women comprised just under half of the non-PLC cases (48%) in the general population (P = 0.07).

Four types of diagnosis-specific non-PLC malignancies with more than two cases were identified as follows: kidney (n = 3), endometrial (n = 3), breast (n = 3) and prostate cancers (n = 4). The exposed were at a sevenfold increased risk of kidney cancer compared with the reference population and a sixfold increased risk of endometrial cancer (Table 3). There were no statistically significant differences regarding risk or incidence for either breast or prostate cancer (Table 3). The sensitivity analysis had a negligible effect on the estimates and interpretations of the study (Table 4).

Discussion

Main findings

In adults with AHP, we found an annual incidence of PLC of 0.35%, with a 108-fold increased risk of PLC over their lifetime compared to the reference population. PLC was the most prevalent cancer, constituting 30% of the total cancer burden for this patient group, compared to 0.6% in the reference population. The findings of a meta-analysis, including previously published data on PLC in AHP and our study, further suggested that PLC risk is greater in women than in men for this patient group. Finally, we found that persons with AHP had an additional sevenfold and sixfold increased relative risk of kidney and endometrial cancers, respectively.

Comparison with previous studies

The estimates of relative risk of PLC we report are greater than those from any previous study, where relative risks have varied from 31 in France [8] to 86 in Sweden [12]. Norway has one of the lowest incidences of PLC in Europe and the lowest in Scandinavia, at 3.5 per 100 000 inhabitants in 2011 compared with 12.9 in France, 7.5 in Finland and 6.6 in Sweden [13]. Therefore, national differences in baseline PLC incidence may explain much of this variation, even after accounting for changes in PLC incidence over time [22]. Additionally, whereas we investigated the risk of all types of PLC, many previous studies have investigated the risk of HCC alone. We were motivated by a recent study in which Sardh and colleagues found four cases of cholangiocarcinoma in patients with AHP [12]. When restricting our analysis to HCC cases alone (i.e. ICD-10 code C22.0), our relative risk estimate increased to 170. However, this is probably an overestimation as data from the Norwegian Cancer Registry indicate that some HCC diagnoses
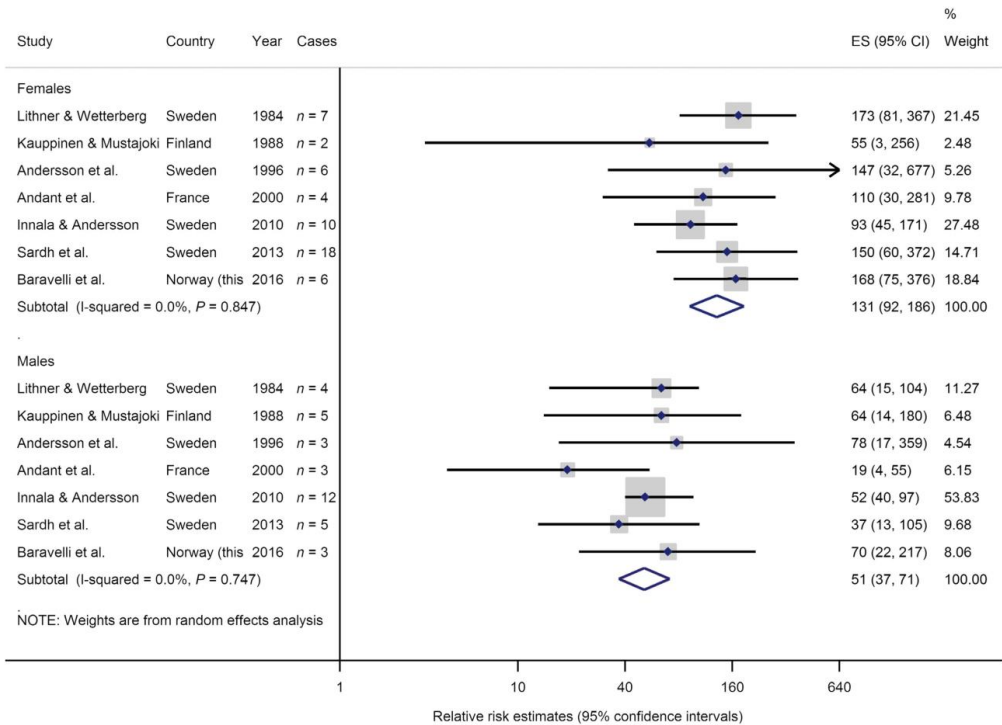
Fig. 2 *Forest plot showing an inverse-weighted random-effect meta-analysis of the risk of primary liver cancer in persons with acute hepatic porphyria. Results are stratified by sex. The area of each square is proportional to the study's weight in the meta-analysis and the diamonds represent the measure of effect for each sex; 95% confidence intervals for these estimates are shown (horizontal lines). ES, effect size.*

in the reference population have been coded as unspecified (i.e. C22.9). Overall, both the characteristics of the reference population and the definition of the outcome need to be considered when comparing relative risk estimates across different populations.

Annual incidence rates (a measure of absolute risk) of PLC in persons with AHP have also varied between studies and, unlike relative risk estimates, this cannot be explained by national differences in PLC incidence in the reference population. We found annual incidences of PLC of 0.35% for individuals of all ages and 0.63% for adults aged 50 years or older. In a multinational study by the European Porphyria Network, the annual incidence of PLC across all ages of AHP

patients in Sweden was 0.33%, which was eight-fold greater than in the five other participating European countries reporting cases [4]. However, a previous prospective cohort study conducted in France over a 7-year period found an annual incidence rate of 0.16% [8]. In a study from Northern Sweden, Innala and Andersson reported an annual incidence of 0.8% in adults aged 55 years or older with acute intermittent porphyria [6]. Our results confirm findings from Sweden, and also support a higher incidence in those aged 50 years or older.

Innala and Andersson speculated that higher incidence rates reported from Swedish studies could, in part, be due to properties of the acute intermittent porphyria-specific W198X mutation of the

HMBS gene [6], which is more common in northern parts of Sweden and, albeit to a lesser extent, in Norway than elsewhere. However, this was not supported by another Swedish study, which demonstrated correspondingly high incidence rate ratios of PLC in a porphyria population with predominantly other disease-specific mutations [12]. In Norway, 22% of persons with AHP have the W198X mutation, most of whom reside in Northern Norway [23]. AHP mutation data were not available in our study, but the nine PLC cases were not from Northern Norway, and one case had variegate porphyria. Both Sweden and Norway have small populations, a relatively high prevalence of acute intermittent porphyria and national diagnostic porphyria centres, which have a good coverage of at least most manifest cases of AHP [3, 4]. The generally higher incidence rates reported from our study as well from Swedish studies may thus be related to a better coverage of AHP cases within the respective study populations, rather than mutation-specific properties.

We found a nonsignificant trend towards female dominance in the risk of PLC, whereas two-thirds of PLC cases were men in the reference population, an observation that is mirrored in other populations across the world [24]. This female dominance is consistent with previous research regarding AHP and PLC [6–9, 12]. However, given the rarity of AHP, our study and previous studies have been underpowered to establish statistically significant sex differences for PLC in AHP. We, therefore, conducted a meta-analysis to pool previous estimates with our findings. The results support a significant female dominance with relative risk for women, on average across studies, 2.5 times higher than for men.

Eight of the nine PLC patients in our AHP population self-reported manifest disease, which is in line with the majority of previous findings [6, 8, 9, 12]. Insufficient reporting in previous studies meant that we were unable to assess this difference by meta-analysis. It could also have been informative to investigate whether those who developed PLC had elevated or higher levels of ALA and PBG than other AHP patients, which has been reported in previous studies [8, 12]. However, we lacked data to investigate this question.

In our study, we further found that persons with AHP had a sevenfold and sixfold increased risk of kidney and endometrial cancers, respectively.

There have been previous reports of single cases of kidney cancer in AHP patients [9, 11]. In a recent cross-sectional study in a cohort of 49 German AHP participants, one case with endometrial cancer was found [16]. To our knowledge, no other study has reported cases of kidney and/or endometrial cancer, and therefore, caution is required when generalizing from these findings.

Strengths and limitations

The nationwide cohort study design is an important strength of the current study, in addition to the reasonable coverage of the exposure and extensive coverage of the outcomes. All Norwegian adult residents at risk of a first primary malignancy during the years 2000–2011 were included. Since the year 2000, NAPOS has kept a record of all diagnosed cases of porphyria in Norway and thus has good coverage of known manifest AHP cases across the country. In relation to the outcome variables, data derived from the Cancer Registry of Norway are considered reliable, accurate and complete [19]. The cohort method is not selective, and consequently there is only a small degree of sampling bias.

There was a participation rate of 73% in our study, with the consent rate higher for women (77%) than for men (62%). Given that manifest disease is more common in women than men, it may be that nonconsenters differed in relation to their AHP. Additionally, 60% of the participants in our study self-reported manifest AHP, which is higher than expected for a low penetrance disease like AHP. It is probable that those with manifest disease are more motivated to participate in the Norwegian Porphyria Registry than genetically disposed persons. Hypothetically, if all nonconsenters participated ($n = 93$) and no additional cases of PLC were observed, then in the current study we would have observed at most a 0.09% reduction in the estimated annual incidence. Therefore, nonconsent bias had at most a moderate impact on the estimation of the exposure.

Previous studies estimated that manifest disease occurs in about 10–40% of individuals with AHP. It has been suggested that penetrance may be increased in patients with the W198X mutation of the HMBS gene [6]. In our study, 61% of participants self-reported manifest disease. This categorization was not validated, which is a limitation of the study. Furthermore, healthy at-risk relatives are offered predictive DNA testing, but this is voluntary, and

Table 4 *Sensitivity analysis comparing the original analysis with alternative hypothetical scenarios*

Sample	Original analysis[a] IR, adj RR (95% CI)	One less case[b] IR, adj RR (95% CI)	Non-consenters included, no additional persons with PLC[c] IR, adj RR (95% CI)
Liver, primary (C22)[d]			
Reference	0.003, 1	0.003, 1	0.003, 1
AHP	0.35, 108.0 (56–205)	0.31, 96.0 (48–192)	0.27, 86.0 (45–166)
Kidney (C64–65)[d]			
Reference	0.02, 1	0.02, 1	0.02, 1
AHP	0.11, 7.4 (2.4–23.1)	0.08, 5.0 (1.2–19.8)	0.09, 6.0 (1.9–18.6)
Endometrium (C54)[d]			
Reference	0.03, 1	0.03, 1	0.03, 1
AHP	0.19, 6.2 (2.0–19.3)	0.13, 4.2 (1.0–16.6)	0.15, 5.6 (1.8–17.2)

AHP, acute hepatic porphyria; IR, annual person-years incidence rate (per 100); adj, adjusted analysis (variables: sex, age and education); RR, relative risk; CI, confidence interval. Hazard ratios produced by Cox regression models were used to produce relative risk estimates.
[a]As reported in Tables 1 and 2; [b]hypothetical scenario if one less person with AHP and the outcome of interest was found (PLC, *n* = 8; all non-PLC cases, *n* = 19; kidney and endometrial cancers, *n* = 3); [c]hypothetical scenario if all persons with the outcome of interest (e.g., PLC) who did not consent to the porphyria registry (*n* = 65) were included in the analysis (i.e. coded as exposed) and no cases of the outcome were detected in these persons; [d]cancer diagnoses were classified using the International Classification of Diseases, 10th revision codes.

therefore, coverage of genetically predisposed persons is not complete and underrepresented in the study cohort. This selection bias is a common issue in rare disease research in which the disorder has a low penetrance, and is a common limitation across most previous nationwide or population-based studies that have investigated AHP. Even where active case finding has been undertaken, families without an AHP diagnosis may not be included, also adding to the likely underrepresentation of genetically predisposed persons. This underrepresentation of genetically predisposed persons may be reflected in our and previous studies by an overrepresentation of women [6, 7, 11, 12], given that mutations in the *HMBS* gene related to AHP are inherited with an equal sex ratio. Therefore, the findings of the current study are limited when generalizing to all persons with AHP and specifically those who are genetically predisposed.

Alcohol consumption can precipitate acute attacks [1] and is a known risk factor for PLC [25]. If alcohol consumption was unevenly distributed between persons with AHP and the general population, this could have confounded the results. However, there is no evidence that alcohol consumption varies between individuals with AHP and the general population. As an improvement compared to previous studies, we were able to control for education

as a proxy for socio-economic status, and indirectly lifestyle factors. This did not affect the results. Therefore, we do not believe that differences in alcohol intake or other lifestyle factors could be the cause of our results, although residual confounding cannot be excluded.

Finally, the total number exposed was small, which is a common issue in studies of rare diseases. We were able to increase statistical power by conducting a meta-analysis to investigate the effect of sex differences in the PLC outcome; however, the same data were not available for differences between those with and without manifest disease or for other malignancies. The very small numbers of cases with kidney and endometrial cancers also mean that the results could reflect chance findings rather than valid statistical associations.

Interpretation of the study findings

Currently, the aetiology of PLC in persons with AHP is unknown, but two main hypotheses have been proposed. First, ALA, which generally accumulates in patients with manifest acute intermittent porphyria, may act via a pro-oxidant and genotoxic effect [14]; secondly, dysfunctional haem synthesis, which has been linked to carcinogenesis and tumour development, may have an important role

[26]. In a meta-analysis, we showed a sex difference in relative risk of PLC in persons with AHP. In general, AHP is more common in women than men [1], and this may explain the observed sex difference in PLC risk, for example, mediated by ALA accumulation in women with manifest disease. Furthermore, Onuki and colleagues have found that ALA can cause DNA damage in the kidney, liver and spleen *in vitro* [14]. Liver and kidney cancers may, therefore, share the same carcinogenesis in individuals with AHP. A link between AHP and kidney cancer through renal impairment, possibly secondary to hypertension, is another possible explanation. Kidney cancer is more common in patients with kidney failure, although the cause of this association is not well understood [27]. Renal impairment is a known cause of morbidity in those with acute intermittent porphyria [28].

Recommendations

Detection of early-stage PLC is possible. Two Swedish studies investigating surveillance in adults over the age of 50 years with AHP suggest that screening may lead to earlier tumour detection and reduced mortality rates in both individuals with and without manifest disease [6, 12]. For those at high risk of HCC, the European Organisation for Research and Treatment of Cancer recommends biannual abdominal ultrasound screening by experienced personnel [29]. Ultrasound provides adequate, although not perfect, sensitivity and specificity [30]. To prevent high false-positive rates (i.e. the proportion of cases with a positive test result but without PLC), it is recommended that surveillance programmes target groups of individuals who are at high risk. Although to date cost–benefit modelling data are not available, according to expert opinion an annual incidence cut-off of 0.2% would warrant surveillance in noncirrhotic patients with hepatitis B virus (HBV) [31, 32]. In individuals aged 50 years or older, we found an annual incidence of 0.6%; if applying the cut-off for noncirrhotic HBV patients, those with AHP, and particularly with manifest disease, should be targeted for screening as a high-risk group. However, studies to investigate the cost–benefit of such programmes should be conducted.

Conclusion

We showed in a nationwide cohort study in Norway that there is a high relative risk of PLC in persons with AHP. Furthermore, we showed that the risk was higher in women than in men. We also found a moderate increase in the risk of endometrial and kidney cancers in individuals with AHP compared to the general population, which needs to be validated in future studies.

Conflict of interest statement

The authors declare no competing financial, professional or personal conflict of interests relevant to the manuscript.

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Study II

RESEARCH   **Open Access**

Porphyria cutanea tarda increases risk of hepatocellular carcinoma and premature death: a nationwide cohort study

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Abstract

Background: Porphyria cutanea tarda (PCT) is a skin disorder originating from a deficit of the liver enzyme uroporphyrinogen decarboxylase. PCT may be a risk factor for hepatocellular carcinoma (HCC) and other cancers, but the evidence is unclear. We aimed to investigate cancer and premature mortality risk in persons with PCT.

Methods: The cohort study consisted of all Norwegian residents from 18 years between 2000 and 2016 ($n = 5.4$ million). 612 persons with PCT, and all cancer diagnoses and causes of death were identified through record linkage between national registries. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were adjusted for age, sex, education and calendar years. We additionally compared persons with PCT to persons with a history of chronic alcohol abuse ($n = 30,468$).

Results: Persons with PCT were more likely to be diagnosed with HCC [adjusted HR (aHR) = 19.7, CI = 8.8–44.0) and gallbladder and biliary tract cancer (aHR = 6.8, CI = 2.2–21.0) than the reference population. A moderate increased risk for HCC (aHR = 3.1, CI = 1.2–7.7) and gallbladder and biliary tract cancer (aHR = 4.0, CI = 1.1–14.4) remained when compared to persons with a history of chronic alcohol abuse. Additionally, compared to the reference population, persons with PCT had an increased risk of premature death (aHR = 1.5, CI = 1.2–1.7), due to the following causes of death: malignant neoplasms (aHR = 1.4, CI = 1.0–1.9), diseases of the liver (HR = 5.5, CI = 2.5–12.2), and drug and alcohol overdose (HR = 9.9, CI = 4.7–20.8).

Conclusions: Persons with PCT had an increased risk of HCC and cancer of the gallbladder and biliary tract, as well as premature death. Although most of our findings can likely be explained by common lifestyle risk factors, something inherent in PCT may contribute to the development of HCC.

Keywords: Porphyria cutanea tarda, Neoplasms, Liver neoplasms, Hepatocellular carcinoma, Gallbladder and bile duct neoplasms, Cause of death

Background

Porphyria cutanea tarda (PCT) results from a defect of hepatic enzyme uroporphyrinogen decarboxylase (UROD). The impaired UROD activity in the liver causes accumulation of uro and heptacarboxylated porphyrins, which act as photosensitizer in the skin and give symptoms in the form of bullae, fragile skin, hypertrichosis and hyperpigmentation [1].

PCT is the most prevalent form of porphyria worldwide, with symptom debut typically in middle age and an approximate equal sex ratio. In Norway, the prevalence is estimated at 1 in 10,000 [2]. PCT occurs in both an acquired (sporadic PCT) and a hereditary form (familial PCT) [3], which are clinically indistinguishable. Susceptibility factors for both include excess iron, hepatitis C and B infection, excessive alcohol intake, smoking, human immunodeficiency virus (HIV) and oestrogens [3–8], although susceptibility factors appear less strongly correlated with familial PCT [3, 6]. PCT is associated with hepatocellular carcinoma (HCC) [9–12], and persons with PCT may also have increased risk of premature death

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[12]. The main risk factors for HCC in the general population include Hepatitis C and B and an excessive alcohol intake [13, 14]. Whether PCT increases the risk for HCC over and above the risk caused by associated susceptibility factors is controversial. It also remains unclear whether the risk for HCC warrants HCC surveillance, as indicated for other types of hepatic porphyrias [15].

We aimed to investigate risks of cancer and premature mortality in persons with PCT compared to the general population, and to examine whether any increased risk was likely caused by PCT associated susceptibility factors or, in part, PCT itself.

Methods

Data sources

Data collection was derived from five nationwide Norwegian population based sources: The Norwegian Porphyria Registry [16], The Cancer Registry of Norway [17], The Cause of Death Registry of Norway [18], Statistics Norway, and the Norwegian Labour and Welfare Administration. Using a unique national 11-digit personal identification number, record linkage was performed in 2018.

Persons in Norway who are diagnosed with porphyria are invited to participate in the national Norwegian Porphyria Registry, administered by the Norwegian Porphyria Centre, established in 2000 [16]. Data is derived from patient-reported questionnaires supplemented with biochemical and genetic laboratory results. Data included in this report were updated one or two years after the first questionnaire, and thereafter every four years.

The mandatory national Norwegian Cancer Registry, records all new neoplasms since 1953. Diagnoses are based on a modified version of the 7th revision of the International Classification of Diseases (ICD-7), and completeness is greater than 98% [17]. The Cause of Death Registry of Norway has a coverage greater than 98% [18]. Diagnostic codes are based on ICD-9 and ICD-10.

Statistics Norway has maintained the Population Database, with demographic data for the entire population, since 1876, and The Norwegian Standard Classification of Education, with information about educational attainment, since 1970.

Statistics Norway and Norwegian Labour and Welfare Administration have maintained records regarding access to social security benefits: sick leave benefit, the disability pension, and medical and occupational - rehabilitation benefit since 1992. Diagnostic codes include the second revision of the International Classification of Primary Care (ICPC-2), ICD-9 and ICD-10.

Study population

All Norwegian residents, registered in the Norwegian Population Registry and 18 years or older from 2000 to 2016 were included in our cohort, and the reference population comprised of 5,451,951 adults. Of the 790

persons with a known overt diagnosis of PCT in Norway, registered at the Norwegian Porphyria Centre, alive and aged 18 years or older between January 2000 and December 2016, 612 (78%) agreed to participate (Fig. 1). Where a cancer diagnosis was the outcome, we excluded 23 persons who had a cancer diagnosis proceeding PCT symptoms (Fig. 1).

PCT diagnosis was based on biochemical testing performed at the Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital [3], applying diagnostic algorithms as described by Badminton and colleagues [19]. Urinary porphyrins were expressed in nmol/mmol creatinine. Sequencing of the *UROD* gene was performed at the Center for Medical Genetics and Molecular Medicine at Haukeland University Hospital to determine if the patient had familial or sporadic PCT. Participants where DNA analysis had not been performed, were registered as unclassified PCT.

For our sub-group analyses, we identified 30,468 persons with a diagnosis of chronic alcohol abuse/
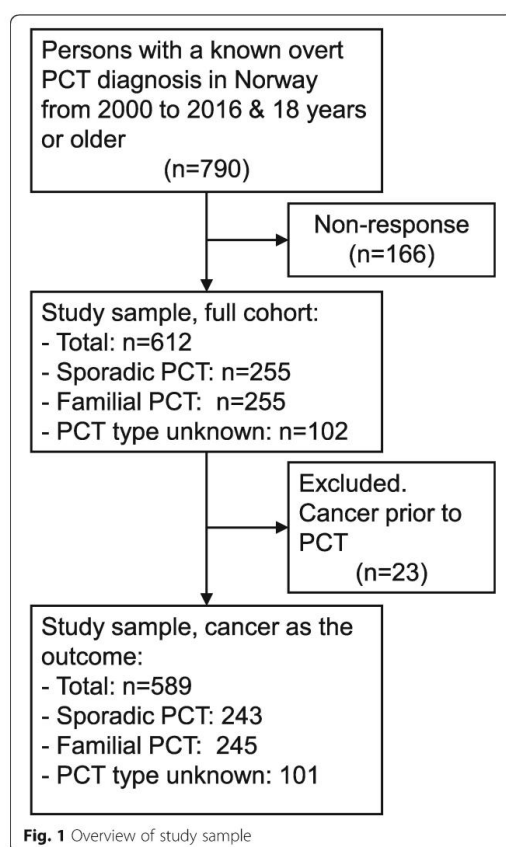
Fig. 1 Overview of study sample

Persons with a known overt PCT diagnosis in Norway from 2000 to 2016 & 18 years or older
(n=790)

Non-response
(n=166)

Study sample, full cohort:
- Total: n=612
- Sporadic PCT: n=255
- Familial PCT: n=255
- PCT type unknown: n=102

Excluded. Cancer prior to PCT
(n=23)

Study sample, cancer as the outcome:
- Total: n=589
- Sporadic PCT: 243
- Familial PCT: 245
- PCT type unknown: 101

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dependence through the Norwegian Labour and Welfare Administration registrations of social security benefits.

Disease classification

Primary cancer diagnoses and underlying causes of death of theoretical interest, or with a count of three or greater, were investigated. This resulted in the following primary cancers: all sites (ICD-7: 140–207; ICD-10: C00–96, D45–47), colon/rectum (ICD-7: 153–154; ICD-10: C18-C21), HCC (ICD-7: 155.0; C22.0), gallbladder and biliary tract (ICD-7: 156; ICD-10:C23–24), pancreas (ICD-7: 157; ICD-10: C25), lung (ICD-7: 162; C33–34); non-melanoma skin (ICD-7: 191; ICD-10: C44), breast (ICD-7: 170; ICD-10: C50), prostate (ICD-7: 177; ICD-10: C61), and leukemia (ICD-9: 207; ICD-10: C91–95, D45–47). We included any new cases of primary cancer diagnosis from January 2000. We also assessed second primary cancers specifically. Similarly, the following underlying causes of death were investigated: malignant neoplasms (ICD-10: C00–96, D45–47), diabetes mellitus (ICD-10: E10–14), ischemic heart disease (ICD-10: I20–25), cerebrovascular diseases (ICD-10: I60-I69), chronic obstructive pulmonary disease (ICD-10: J43-J44), diseases of the liver (ICD-10: K70–77, B15–19, E83.1), renal failure (ICD-10: N17–19), and alcohol or drug overdose (ICD-10: X40–49). Chronic alcohol abuse/ dependence was defined by the following codes: ICPC-2: P15; ICD-9: 303, 305.0; ICD-10: F10.

Statistical analysis

Stata/SE Version 14 for Windows was used for all statistical analyses (StataCorp Stata Statistical, Software: Release 14, College Station, TX, USA). Annual incidence was calculated by dividing the number of new cases by the total person-years of all participants. The entry time was age at baseline or 18 if younger than 18 years in 2000 (i.e., left truncated). The exit time was age at event (cancer/mortality) or censoring (death due to other factors, or end of study follow-up in 2016, whichever occurred first). To assess the relationship between PCT and cancer/mortality, a Cox regression was used to estimate the hazard ratios (HRs) with 95% confidence intervals (CIs), in which PCT diagnosis (no/yes) was the exposure and the cancer or cause of death of interest (no/yes) the outcome. Where numbers permitted, we additionally investigated PCT type (0 = reference, 1 = sporadic PCT, 2 = familial PCT, 3 = PCT type unclassified PCT) as the exposure. Age was used as the time scale, stratifying the model by birth cohorts (< 1937, 1937–1960, 1961–1983, > 1983), to adjust for calendar effects. The multivariate models were adjusted for sex, age (as the time-scale), and educational attainment. The proportionality assumption was assessed and covariates entered as time-dependent when the assumption was

violated. Obtained level of education was categorized into: no schooling, compulsory education (year 1–10), upper secondary education (11–13, or 14 years if including post-secondary non-tertiary education), tertiary education (14+ years), and unspecified/missing. Interactions of exposure by educational obtainment (tertiary or upper secondary vs less education) and by sex were explored, and none were found (although limited power for interaction analyses). Differences in age at time of death were investigated by independent samples t-tests.

For each adjusted hazard ratio (aHR), we calculated the corresponding evidence value (E-value). The E-value is defined as the minimum risk ratio an unmeasured confounder requires to explain away the outcome and is calculated by the formula: *E-value = hazard ratio (HR) + sqrt[HR x (HR-1)]* [20].

We compared cancer and causes of death risk in persons with PCT to persons with a history of chronic alcohol abuse/dependence, who have a high mortality risk. This was achieved by conducting a competing risks regression survival analysis calculating the adjusted subdistribution hazard ratio (aSHR). Persons with both PCT and a history of chronic alcohol abuse/dependence (n = 17) were excluded.

Ethical approval

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Regional Committees for Medical and Health Research Ethics, Norway (reference number: 2012/753).

Results

At entry into the study, persons with familial PCT, sporadic PCT and unclassified PCT were, on average, 9, 14 and 21 years older than the reference population, respectively. Additionally, persons with sporadic and unclassified PCT types, generally had less education and were more likely to smoke daily, and have type II diabetes than persons with familial PCT. However, there was no apparent difference regarding self-reported alcohol use (Table 1).

Persons with PCT had, on average, a 20-fold increased risk of HCC and an 7-fold increased risk of gallbladder and biliary tract cancer, compared to the general population. There was no evidence of an increased risk of all first primary cancers in persons with PCT, but we observed a 2-fold increased risk of all second primary cancer diagnoses. When assessed by PCT type (sporadic, familial, unclassified PCT), the risk was greatest in persons with unclassified PCT type (Table 2). The individual characteristics of persons with PCT and cancers are displayed in Additional file 1: Table S1.

The mean age at death for persons with PCT was 72.2 years (95% CI: 70.3–74.1) compared to 78.5 years (95%

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Table 1 Baseline characteristics of participants with sporadic (s-PCT), familial (f-PCT) and unclassified PCT compared to the reference population (2000 to 2016, 18 years or older)

Characteristics	s-PCT (n = 255)		f-PCT (n = 255)		Unclassified PCT (n = 102)		Reference (n = 5452,010)	
	n	Valid %	N	Valid %	n	Valid %	n	Valid %
Sex – male	125	49.0	122	47.9	46	45.1	2713,666	50.2
Age in years at study start (mean, SD)	52.8	(10.9)	47.5	(13.7)	60.1	(13.9)	38.9	(22.2)
Highest level of education obtained								
Not specified	4	1.6	2	0.8	0	0	649,456	11.9
No schooling, primary/middle education (year 1–10)	90	35.3	55	21.6	46	45.1	1334,399	24.8
Upper secondary education (11–12, 13 or 14 years)	124	48.6	123	48.2	43	42.2	1994,025	36.6
Tertiary education (14+ years)	37	14.5	75	29.4	13	12.8	1474,130	27.0
Lifestyle factors								
Alcohol use (yes)	163	93.6	173	93.5	35	87.5		
Alcohol – amount of standard drinks per week (mean, SD)	1.7	(1.3)	1.6	(1.3)	1.5	(1.3)		
Tobacco: cigarettes (yes)	83	32.6	50	19.6	30	61.2		
Never smoked / have quit	65	37.8	127	69.4	22	53.63		
Occasional smokers/ daily smokers	107	62.2	56	30.6	19	46.3		
Amount of cigarettes consumed per day (mean, SD)	2.8	(6.2)	1.2	(3.8)	1.7	(4.6)		
Body mass index (BMI)								
Underweight (BMI Under 18.5)	8	4.8	5	2.8	2	5.1		
Normal (BMI between 18.5 and 25)	67	40.4	56	30.9	13	33.3		
Overweight (BMI between 25 and 30)	68	41.0	90	49.7	21	53.8		
Obese (BMI of 30 or greater)	23	13.9	30	16.6	3	7.7		
Other diseases								
Liver disease	24	11.0	12	5.2	6	5.2		
Haemochromatosis	11	5.1	12	5.2	2	4.1		
Type II diabetes	11	5.1	3	1.3	2	7.4		
Biochemical characteristics								
Total porphyrins (mean, 95% CI)	880.5	803.6–957.5	941.6	835.7–1047.5	989.2	660.58–1317.81		
Uroporphyrin (mean, 95% CI)	608.5	553.5–663.4	706.1	625.03–787.1	768.45	504.01–1032.89		

Abbreviations: *PCT* porphyria cutanea tarda

Lifestyle factors are based on self-reported questionnaires sent to the Norwegian Porphyria Registry. 500 of 612 responded to the questionnaire. Biochemical characteristics are based on the highest ever recorded value (generally at time of diagnosis). Urinary porphyrins were expressed in nmol/mmol creatinine (upper reference limit < 30).

CI: 78.4–78.5) in the general population (mean difference = 6.3, 95% CI: 4.4–8.2, *p* < .001). There was no detectable difference in the mean age at death between persons with sporadic PCT (mean = 71.8; 95% CI: 68.9–74.7), familial PCT (mean = 72.1; 95% CI: 67.0–77.1) and unclassified PCT type (mean = 73.3; 95% CI: 70.5–76.1).

Compared to the general population, persons with PCT had, on average, a 50% increased risk of premature death, 40% increased risk of death by malignant neoplasms (all-sites), a 6-fold excess risk of death by liver diseases, and a 10-fold excess risk of death by alcohol or drug overdose. The risk of premature death and death by malignancies was highest in persons with unclassified PCT (Table 3).

Compared to persons with a history of chronic alcohol abuse/dependence, persons with PCT were at a 3-fold

increased risk of HCC and 4-fold increased risk of gallbladder and biliary tract cancer, and had an 80% reduced risk of dying from non-malignant liver diseases (Table 4).

Discussion

We found a 20-fold increased risk of HCC and a 7-fold increased risk of gallbladder and biliary tract cancer in persons with PCT compared to the general population. An increased risk remained also when comparing to persons with a history of chronic alcohol abuse/dependence. Further, persons with PCT had an overall 50% increased risk of premature mortality compared to the general population with 6- and 10-fold increased risks of dying from liver disease and drug or alcohol overdose, respectively.

Table 2 Annual incidence rates and hazard ratios for neoplasms in persons with porphyria cutanea tarda (2000–2016, 18 years or older)

Cancer codes (ICD-7; ICD-10)	Cancer cases/no. at risk	Person-years; annual incidence of cancer diagnosis per 100 (95% CI)	Hazard ratios (95% confidence intervals)		E-value
			Crude	Adjusted	
All sites – first primary C (140–207; C00–96, D45–47)					
Reference	358,701/5451951	70,185,262; 0.507 (0.506–0.509)	1.0	1.0	1.0
PCT (total)	80/589	8368; 0.956 (0.768–1.190)	1.1 (0.9–1.4)	1.1 (0.9–1.3)	1.4
Familial PCT	25/245	3728; 0.671 (0.453–0.992)	0.9 (0.6–1.4)	0.9 (0.6–1.3)	1.0
Sporadic PCT	34/243	3547; 0.959 (0.685–1.342)	1.1 (0.8–1.5)	1.0 (0.7–1.4)	1.1
Unclassified PCT	21/101	1094; 1.920 (1.252–2.945)	1.8 (1.1–2.7)	1.6 (1.0–2.5)	2.6
All sites – second primary C (140–207; C00–96, D45–47)					
Reference	57,505/5451951	71,305,121; 0.081 (0.080–0.081)	1.0	1.0	1.0
PCT (total)	26/589	8424; 0.309 (0.210–0.453)	2.4 (1.6–3.5)	2.2 (1.6–3.3)	3.8
Familial PCT	6/245	3749; 0.160 (0.72–0.356)	1.5 (0.7–3.3)	1.4 (0.6–3.1)	2.2
Sporadic PCT	14/243	3557; 0.394 (0.233–0.665)	2.9 (1.7–4.9)	2.8 (1.7–4.7)	5.0
Unclassified PCT	6/101	1119; 0.536 (0.241–1.194)	2.8 (1.2–6.1)	2.5 (1.1–5.6)	4.4
Colon/rectum (153–154; C18-C21)					
Reference	46,077/5451951	71,375,873; 0.503 (0.501–0.504)	1.0	1.0	1.0
PCT	11/578	8564; 0.936 (0.752–1.165)	1.2 (1.0–1.5)	1.1 (0.9–1.4)	1.4
Hepatocellular carcinoma (155.0; C22.0)					
Reference	1543/5451951	71,538,150; 0.002 (0.002–0.002)	1.0	1.0	1.0
PCT (total)	6/589	8567; 0.070 (0.031–0.156)	19.9 (8.9–44.3)	19.7 (8.8–44.0)	38.9
Gallbladder and biliary tract (156; C23-C24)					
Reference	1628/5451951	71,537,477; 0.003 (0.003–0.003)	1.0	1.0	1.0
PCT	3/589	8576; 0.035 (0.011–0.108)	7.2 (2.3–22.5)	6.8 (2.2–21.0)	13.0
Pancreas (157, C25)					
Reference	9830/5451951	71,375,873; 0.014 (0.014–0.014)	1.0	1.0	1.0
PCT	3/589	8546; 0.035 (0.011–0.109)	1.6 (0.5–4.9)	1.5 (0.5–4.6)	2.4
Lung (162, C33–34)					
Reference	34,920/5451951	71,498,290; 0.049 (0.048–0.049)	1.0	1.0	1.0
PCT	13/589	8571; 0.152 (0.088–0.261)	2.9 (1.7–5.0)	1.6 (0.9–2.8)	2.6
Non-melanoma skin (191, C44)					
Reference	16,048/5452951	66,463,540; 0.024 (0.024–0.0024)	1.0	1.0	1.0
PCT	4/589	8533; 0.047 (0.018–0.125)	1.9 (0.7–5.1)	1.1 (0.4–3.5)	1.4
Breast (170; C50)					
Reference	39,581/2713314	35,414,769; 0.112 (0.111–0.113)	1.0	1.0	1.0
PCT	5/299	4337; 0115 (0.048–0.277)	0.6 (0.3–1.5)	0.6 (0.3–1.7)	1.0
Prostate (177; C61)					
Reference	56,652/2738637	35,644,567; 0.158 (0.157–0.159)	1.0	1.0	1.0
PCT	14/290	4170; 0.336 (0.199–0.567)	1.2 (0.7–2.0)	1.1 (0.6–1.8)	1.4
Leukaemia (207; C91–95, D45–47)					
Reference	16,349/5451951	72,014,044; 0.023 (0.022–0.023)	1.0	1.0	1.0
PCT	4/589	8560; 0.023 (0.018–0.125)	1.4 (0.5–3.7)	1.3 (0.5–3.5)	1.1

Crude analysis adjusted for age as the time-scale. Adjusted analysis covariates include age as time-scale, sex (except for gender specific cancers), educational obtainment, and birth cohorts.
Note that 23 persons diagnosed with cancer prior to their first reported PCT symptoms or diagnosis were excluded. Diagnosis based on the International Classification of Diseases, 7th (ICD-7) and 10th revision (ICD-10) codes.

Table 3 Annual mortality rates and hazard ratios for causes of death in persons with porphyria cutanea tarda (2000–2016, 18 years or older)

Sample	Mortality cases/ no. at risk	Person-years; annual mortality rate per 100 (95% CI)	Hazard ratios (95% confidence intervals)		E-value
			Crude	Adjusted	
Premature death					
Reference	748,900/5451,951	71,559,947; 1.047 (1.044–1.049)	1.0	1.0	1.0
PCT (total)	158/612	8921; 1.771 (1.515–2.070)	1.5 (1.3–1.8)	1.5 (1.2–1.7)	2.4
Familial PCT	27/255	3933; 0.686 (0.471–1.001)	0.7 (0.5–1.1)	0.8 (0.1–1.1)	1.0
Sporadic PCT	64/255	3828; 1.672 (1.309–2.136)	1.5 (1.2–1.9)	1.4 (1.1–1.8)	2.2
Unclassified PCT	67/102	1160; 5.776 (4.546–7.339)	2.6 (2.1–3-4)	2.5 (1.9–3.1)	4.4
Malignant neoplasms (C00–96)					
Reference	181,205/5,451,951	71,559,947; 0.254 (0.253–0.255)	1.0	1.0	1.0
PCT (total)	48/612	8921; 0.538 (0.405–0.714)	1.4 (1.1–1.9)	1.4 (1.0–1.9)	2.2
Sporadic PCT	23/255	3828; 0.601 (0.399–0.904)	1.6 (1.0–2.4)	1.5 (1.0–2.2)	2.4
Familial PCT	11/255	3933; 0.280 (0.155–0.505)	0.9 (0.5–1.7)	0.9 (0.5–1.6)	1.0
Unclassified PCT	14/102	1160; 1.207 (0.715–2.038)	2.0 (1.2–3.4)	1.8 (1.1–3.1)	3.3
Diabetes mellitus (E10–14)					
Reference	11,826/5452,010	71,559,947; 0.016 (0.016–0.017)	1.0	1.0	1.0
PCT	3/612	8921; 0.034 (0.011–0.104)	2.0 (0.7–6.3)	1.7 (0.5–5.3)	2.8
Ischemic heart disease (I20–25)					
Reference	5,451,951	71,559,947; 0.135 (0.134–0.136)	1.0	1.0	1.0
PCT	7/612	8921; 0.079 (0.037–0.165)	0.6 (0.3–1.2)	0.6 (0.3–1.2)	1.0
Cerebrovascular diseases (I60-I69)					
Reference	57,639/5,451,951	71,559,947; 0.081 (0.080–0.081)	1.0	1.0	1.0
PCT	8/612	8915; 0.090 (0.045–0.179)	1.2 (0.6–2.4)	1.1 (0.6–2.3)	1.4
Chronic obstructive pulmonary disease (J43–44)					
Reference	31,172/5,451,951	71,559,947; 0.043 (0.043–0.044)	1.0	1.0	1.0
PCT	7/612	8921; 0.078 (0.037–0.165)	1.4 (0.6–2.9)	1.4 (0.6–2.8)	2.2
Diseases of the liver (K70–77, B15–19, E83.1) [a]					
Reference	4661/5,452,010	71,559,947; 0.007 (0.00–0.007)	1.0	1.0	1.0
PCT	6/612	8921; 0.067 (0.030–0.150)	5.9 (2.7–13.2)	5.5 (2.5–12.2)	10.5
Renal failure (N17–19)					
Reference	6686/5,452,010	71,559,947; 0.009 (0.009–0.010)	1.0	1.0	1.0
PCT	2/612	8921; 0.022 (0.006–0.090)	2.9 (0.7–11.8)	2.9 (0.7–11.7)	5.3
Drug and alcohol overdose (X40–49) [b]					
Reference	4961/5452,010	71,559,947; 0.007 (0.007–0.007)	1.0	1.0	1.0
PCT	7/612	8921; 0.078 (0.037–0.165)	12.1 (5.8–25.5)	9.9 (4.7–20.8)	19.3

Disease codes defined using the International Statistical Classification of Diseases 10th (ICD-10) revision

[a]Specific diagnoses of the 6 PCT patients with liver disease were: alcoholic cirrhosis of liver (K70.3), alcohol hepatic failure (K70.4) primary biliary cirrhosis (K74.3), hepatic failure (K72.9), 2 x chronic viral hepatitis C (B18.2). Familial PCT, *n*=0, sporadic PCT, *n*=2, Not tested, *n*=3. None of the six persons with liver disease were diagnosed with HCC or gallbladder and biliary tract cancer.

[b]Specific diagnosis of the 7 persons with PCT and drug and alcohol overdose included: 4 x accidental poisoning by and exposure to narcotics and psychodysleptics [hallucinogens], not elsewhere classified (ICD-10: X42); 1 x accidental poisoning by and exposure to non-opioid analgesics, antipyretics and antirheumatics (ICD-10: X40); 1 x accidental poisoning by and exposure to antiepileptic, sedative-hypnotic, antiparkinsonism and psychotropic drugs, not elsewhere classified (ICD-10: X41); 1 x accidental poisoning by and exposure to alcohol (X45). Familial PCT, *n*=1, sporadic PCT, *n*=3, Not tested, *n*=3

A similar excess risk for HCC has been reported from a combined registry cohort study of 530 PCT inpatients from Sweden and Denmark [12]. Although the relative risk of HCC in our study was high when compared to the general population, the annual incidence rate (absolute risk) could be considered low at 0.07%, considering that HCC risk factors are prominent among persons with PCT. However, Norway has one of the lowest prevalence's of HCC and chronic hepatitis B and C across Europe [21–23]. Therefore, the proportion of

Table 4 Annual incidence/mortality rates and subdistribution hazard ratios for malignancies and causes of death in persons with porphyria cutanea tarda (2000–2016, 18 years or older)

	Incidence cases/no. at risk	Person-years; annual mortality rate per 100 (95% CI)	Subdistribution hazard ratios (95% confidence intervals)	
			Crude SHR (95% CI)	Adjusted SHR (95% CI)
All sites – first primary C (C00–96, D45–47)				
Reference	3549/30468	393,347; 0.902 (0.873–0.932)	1.0	1.0
PCT	78/572	8132; 0.959 (0.768–1.198)	0.7 (0.5–0.8)	0.7 (0.6–0.9)
HCC (155.0; C22.0)				
Reference	80/30,468	402,342; 0.020; 0.020 (0.16–0.025)	1.0	1.0
PCT	5/572	8330; 0.060 (0.025–0.144)	2.2 (0.9–5.5)	3.1 (1.2–7.7)
Gallbladder & biliary tract (156; C23–24)				
Reference	19/30,468	402,403; 0.003 (0.003–0.007)	1.0	1.0
PCT	3/572	8339; 0.036 (0.012–0.112)	4.4 (1.3–15.0)	4.0 (1.1–14.4)
All-cause mortality				
Reference	11,756/30468	402,757; 2.919 (2.867–2.972)	1.0	1.0
PCT	150/595	8684; 1.728 (1.472–2.027)	0.4 (0.3–0.4)	0.4 (0.4–0.5)
Diseases of the liver (K70–77, B15–19, E83.1)				
Reference	735/30468	402,746; 0.182 (0.170–0.196)	1.0	1.0
PCT	4/595	8683; 0.046 (0.017–0.123)	0.2 (0.1–0.6)	0.2 (0.1–0.6)
Malignant neoplasms (C00–96)				
Reference	2323/30,468	402,746; 0.577 (0.554–0.601)	1.0	1.0
PCT	45/595	8683; 0.518 (0.387–0.694)	0.5 (0.4–0.7)	0.6 (0.4–0.8)
Drug and alcohol overdose (X40–49)				
Reference	484/30,468	402,746; 0.134 (0.134–0.157)	1.0	1.0
PCT	5/597	8.683; 0.058 (0.024–0.138)	0.5 (0.2–1.1)	0.5 (0.2–1.2)

Disease codes defined using the International Classification of Primary Care – 2nd Edition (ICPC-2) and the International Statistical Classification of Diseases 7th (ICD-7) and 10th (ICD-10) revisions. Reference group = Chronic alcohol abuse/dependence (ICD-10 = F10, ICD-9 = 303, 305.0, ICPC2 = P15). 17 persons with both PCT and a diagnosis of chronic alcohol abuse or dependence, including one person with HCC, were excluded from the analyses. 23 persons with a cancer diagnosis prior to their PCT diagnosis were excluded from any analysis in which cancer was the outcome
Mean age at death for persons with chronic alcohol abuse = 63.6 (SD: 11.7) vs. PCT = 72.5 (SD = 11.7), proportion of males = 78% vs. PCT = 50%

persons with PCT in Norway precipitated by a common HCC risk factor is likely lower than countries where such risk factors are more frequent (which is supported by the proportionally larger group of familial PCT in Norway). Consequently, it is likely that annual incidence rates of HCC in persons with PCT are higher in non-Scandinavian countries. We found in addition to HCC, a 7-fold increased risk of gallbladder and biliary tract cancer, which, to our knowledge, has only been reported once before in a case-report [24]. We also found 13 persons with PCT and lung cancer, which implied a non-significant excess risk of 60%.

Fracanzani and colleagues found that having PCT resulted in a 5-fold increased risk of HCC in 53 PCT patients compared to age, sex, liver disease severity and hepatitis C infection matched control patients with chronic liver disease [11]. However, the study may have suffered from selection bias as PCT patients were drawn from a single liver unit with a 90% prevalence of hepatitis C infection. In our population based cohort study,

we found a 3-fold increased risk of HCC in persons with PCT, when compared to persons with a history of chronic alcohol abuse/dependence. Typically, alcohol abuse/dependence and liver disease, such as hepatitis C, may lead to cirrhosis and eventual HCC. Interestingly, we found that although HCC risk was greater, persons with PCT had an 80% reduced risk of dying from non-malignant liver diseases, such as viral hepatitis or liver cirrhosis, than this control group. This finding suggests that something specific to PCT may contribute to the development of HCC independent of, or by interaction with, liver disease and an excessive alcohol intake.

In line with Linet and colleagues, who reported a 70% excess risk of premature death for persons with PCT in their study [12], we found a 50% excess risk. When stratified by PCT type, however, this increased risk was only evident in persons with sporadic or unclassified PCT. We also found that having PCT resulted in a 6-fold increased risk of dying from liver diseases compared to the general population. Similarly, Linet and

colleagues found increased odds of dying from cirrhosis of the liver in persons with PCT [12]. Lastly, we found an 10-fold excess risk of mortality from alcohol or drug overdose, which, to our knowledge, has not been previously reported.

Strengths and weaknesses

Major strengths of our study are the population-based cohort design, the use of valid information from compulsory national registries, and a long period of follow-up. Persons with PCT were drawn from a nationwide registry with a good participation rate (78%). However, it is possible that participants differed from non-participants and thus selection bias may affect the validity of the results. Given the complex relationship between PCT and cancer, we chose to exclude cancer cases preceding PCT. This design reduces the risk of selection and information bias. We extend previous analyses by adjusting for education (as a proxy of socioeconomic position and lifestyle), comparing outcomes for patients with familial, sporadic and unclassified PCT, and comparing risks of PCT patients with persons with a history of alcohol abuse/dependence. A limitation of the study was the small numbers of cases. We also did not have access to liver biopsies of patients with HCC or gallbladder and biliary tract cancer. Therefore, we are uncertain regarding the proportion of these persons with concurrent fibrosis/cirrhosis.

Several common causes of PCT and cancer exist, such as alcohol use, liver disease and smoking. This information is not available in our study for the general population. Given the relationship between lifestyle factors such as excessive alcohol intake [25] and smoking habits [26] with socio-economic disadvantage and lower educational attainment, we were able to extend previous findings by controlling for educational attainment as a proxy for lifestyle.

Residual confounding by lifestyle factors is likely. We modelled residual confounding by calculating the E-value and found that an unmeasured confounder would need to have a large risk ratio of at least 39 to explain away the findings of increased HCC risk. Given the strong association between alcohol abuse/dependence and hepatitis C and both PCT and HCC, it is conceivable that unmeasured confounding may explain away any direct relationship. We were, however, able to identify persons with a history of chronic alcohol abuse/dependence to further investigate this to partially account for their generally younger age at the time of death (i.e., may have died before they could develop HCC), we conducted a competing risks survival analysis. Results, suggested persons with PCT had a 3-fold excess risk of HCC even when compared to persons with a history of alcohol abuse. The remaining high risk, albeit lower than when comparing to the general population, suggests that alcohol is an important confounder, but may not completely explain the association between PCT and HCC.

Interpretation

It has been proposed that the accumulation of uroporphyrins, of which levels are high in (untreated) PCT, may cause liver injury, resulting in the formation of a hepatic tumor [27]. This may occur in parallel or by interaction with exposure to hepatotoxic factors, such as alcohol. However, if the increased risk of HCC is related in part to PCT itself or is due only to confounding lifestyle factors, is difficult to conclude. Our findings give some support to the former, given that we observed this increase in the risk ratio even after controlling for educational attainment and comparing our cohort to persons with a history of chronic alcohol abuse/dependence. Of the six persons in our cohort with PCT and HCC, two had familial PCT, which is less associated with other types of liver disease or excessive alcohol intake than sporadic PCT [3]. There was a trend towards higher concentrations of total porphyrins and uroporphyrins in PCT patients with HCC compared to those without. However, the small number of cases and variability in the findings limit our ability to draw any firm conclusions. We also observed an increased risk of gallbladder and biliary tract cancer in persons with PCT. However, given we only found three cases and tested over multiple cancers, this could reflect a chance finding.

Additionally, we found an increased risk of premature death overall, death by liver diseases and drug or alcohol overdose in persons with PCT. Liver diseases, such as chronic hepatitis C virus, are known precipitating factor for PCT [10, 28], and are also associated with excessive alcohol intake and injection drug use. We also found that persons with sporadic PCT were generally more likely to die from causes associated with lifestyle factors than persons with familial PCT. Therefore, increased mortality rates in persons with PCT, and specifically sporadic PCT, could be caused by lifestyle factors.

Clinical recommendations

According to the European Association for the Study of the Liver (EASL) and European Organization for Research and Treatment of Cancer (EORTC) clinical practice guidelines for the management of HCC, an annual incidence of 1.5% would warrant surveillance of HCC in cirrhotic patients and 0.2% would warrant surveillance in non-cirrhotic patients [29]. We found an annual incidence of HCC of 0.07% in our PCT patients, with an upper bound 95% CI of 0.17%. Additionally, unlike in acute forms of hepatic porphyria, confounding by concurrent cirrhosis cannot be ruled out in patients with PCT. Therefore, surveillance cannot be currently recommended based on a PCT diagnosis alone.

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Conclusion

We found that compared to the general population, persons with PCT had a high risk of HCC and death from alcohol or drug abuse. In addition, they had moderate excess risks of gallbladder and biliary tract cancers and of premature death in general. Although it is likely that most of our findings can be explained by common lifestyle risk factors, something inherent in PCT may contribute in part, or by interaction with lifestyle factors, to the development of HCC.

Additional file

Additional file 1: Table S1. Individual characteristics of persons with hepatocellular carcinoma and gallbladder and bile duct cancer (DOCX 16 kb)

Availability of data and materials
The data that support the findings of this study are available from Statistics Norway but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Statistics Norway.

Authors' contributions
Conceptualisation: MCT CB SS AKA. Analysed the data: CB. Interpretation of data: CB MCT AKA SS. Wrote the first draft of the manuscript: CB. Contributed to the writing of the manuscript: CB MCT AKA SS. Agree with the manuscript's results and conclusions: CB MCT AKA SS. All authors have read, and confirm that they meet the Journal's criteria for authorship. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Regional Committees for Medical and Health Research Ethics, Norway (reference number: 2012/753).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Table S1. *Individual characteristics of persons with hepatocellular carcinoma and gallbladder and bile duct cancer*

Sex	Diagnosis[†]	Topography; morphology (ICD-03)‡	Porphyria symptoms onset – age (years)	Cancer diagnosis age (years)	Deceased at end of follow-up (Yes/no)	PCT type	Max value - total porphyrins/ uroporphyrins	HFE-status	Hemochromatosis (Yes/ no/ unclassified PCT)	Chronic Liver disease / alcohol abuse or dependence (yes/no)
Male	HCC	C22.0; 8170/3	70	84	Yes	S-PCT	369 / 234	Very low risk	Unknown	Yes[§]
Male	HCC	C22.0; 8170/3	62	74	Yes	F-PCT	2,073 / 1,557	Normal	No	No
Male	HCC	C22.0; 8170/3	64	72	Yes	S-PCT	1,460 / 1,056	Very low risk	No	No
Male	HCC	C22.0; 8170/3	54	66	Yes	S-PCT	2,172 / 1,600	Normal	Unknown	No
Male	HCC	C22.0; 8170/3	45	66	Yes	F-PCT	171 / 118.6	Normal	No	No
Male	HCC	C22.0; 8170/3	45	61	Yes	Unknown	150 / 60	Not tested	Unknown	Yes[¶]
Male	E-CC	C24.1 816039	64	69	Yes	S-PCT	2,395 / 1,619	Very low risk	Unknown	Yes[#]
Male	Ampulla of vater	C24.0 / 814039	50	62	Yes	Unknown	1,280 / 909	Not tested	Yes[‡‡]	No
Female	Biliary tract	C24.9 / 816039	54	63	Yes	S-PCT	986 / 681	Normal	No	No

Note: Disease codes defined using the International Classification of Diseases of Oncology (3rd Edition) (ICD-O3). PCT type: S-PCT=sporadic PCT, F-PCT=familial PCT. Concentration of urinary total porphyrin and uroporphyrin reflect maximum value ever recorded. Urinary porphyrins were expressed in nmol/mmol creatinine (upper reference limit < 30 nmol/mmol). HFE status refers to testing of the HFE gene variant as an indication of risk for hemochromatosis. Very low risk = "heterozygous for C282Y and negative for H63D". 'Hemochromatosis' was self-reported data from the Norwegian Porphyria Registry. 'Liver disease and alcohol chronic abuse or dependence' registered diagnosis in the Norwegian Labour and Welfare Administration social benefit databases or the Norwegian Causes of Death Registry, including underlying and contributory causes of death. Liver disease codes defined using the International Classification of Primary Care – 2nd Edition (ICPC-2): D72 & D97; and the International Statistical Classification of Diseases 9th revision (ICD-9): 570-573 & 070 and 10th revision (ICD-10): B15-B19 & K70-K77. Disease codes defined using the ICPC-2: P15; and the ICD-9: 303, 305.0 and 10th revision (ICD-10): F10.

† HCC=hepatocellular carcinoma; E-CC=extrahepatic cholangiocarcinoma

‡ C22.0=hepatocellular carcinoma; C24.0=extrahepatic bile duct cancer; C24.1=ampulla of vater cancer; C24.9=biliary tract cancer unclassified PCT.

§ Specific ICD-10 code=K75.4 (autoimmune hepatitis)

¶ Specific ICD-9 code=303.0 (acute alcohol intoxication) & 303.9 (other and unspecified alcohol dependence, unspecified)

Specific ICD-10 code=K74.6 (other and unspecified cirrhosis of liver); F10 (mental and behavioural disorders due to use of alcohol)

Errata for
Clinical long-term consequences of acute hepatic porphyria and porphyria cutanea tarda

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Thesis for the degree philosophiae doctor (PhD)

at the University of Bergen

19/11/2019

(date and sign. of candidate)

19.11.2019

(date and sign. of faculty)

Errata

Page 26 Incorrect reference number: reference "[16]" changed to "(92)"

Page 73 Incorrect reference number: references "[6, 12]" changed to "(33, 34)"

Page 87 Incorrect order of references within the reference list: references 165 to 172 were incorrectly numbered in the reference list and were renumbered and reordered.

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