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Hepatocellular carcinoma in acute hepatic porphyrias: a Damocles Sword

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Abstract

Porphyrias are inherited diseases with low penetrance affecting the heme biosynthesis pathway. Acute intermittent porphyria (AIP), variegate porphyria (VP) and hereditary coproporphyria (HCP) together constitute the acute hepatic porphyrias (AHP). These diseases have been identified as risk factors for primary liver cancers (PLC), mainly hepatocellular carcinoma (HCC: range 87-100%) but also cholangiocarcinoma, alone or combination with HCC.

In AHP, HCC annual incidence rates range from 0.16 to 0.35% according to the populations studied. Annual incidence rates are higher in Swedish and Norwegian patients, due to a founder effect. It increases above age 50. The pathophysiology could include both direct toxic effects of heme precursors, particularly δ -aminolevulinic acid (ALA), compound heterozygosity for genes implied in heme biosynthesis pathway or the loss of oxidative stress homeostasis due to a relative lack of heme.

The high HCC incidence justifies radiological surveillance in AHP patients above age 50. Efforts are made to find new biological non-invasive markers. In this respect, we describe here the first report of PIVKA-II clinical utility in the follow-up of an AIP patient that develop an HCC.

In this manuscript we reviewed the epidemiology, the physiopathology, and the screening strategy of HCC in AHP.

Introduction

Porphyrias comprise a group of eight metabolic disorders of the heme biosynthesis pathway. Porphyrias are classified as hepatic or erythropoietic, based on the organ system in which heme precursors (δ -aminolevulinic acid -ALA-, porphobilinogen -PBG- and porphyrins) are overproduced. Clinically, porphyrias are characterized either by acute neurovisceral symptoms, skin lesions or both.

Acute intermittent porphyria (AIP), variegate porphyria (VP) and hereditary coproporphyria (HCP) are the most prevalent types of acute hepatic porphyrias (AHP) [1]. AHP are inherited autosomal diseases with incomplete penetrance. They are caused by a partial deficiency in enzymes of the heme biosynthesis: hydroxymethylbilane synthase (HMBS) in AIP (MIM: #176000), coproporphyrinogen oxidase (CPO) in HCP (MIM: #121300), and protoporphyrinogen oxidase (PPO) in VP (MIM: #176200). The dysfunctions of the heme biosynthesis pathway, lead to an accumulation of porphyrins or their precursors, mainly ALA and PBG that precipitate acute attacks.

Intermittent attacks that include acute abdominal pain, hypertension, tachycardia, various neuropsychiatric symptoms and in VP and HCP also cutaneous lesions clinically characterized these diseases. The biochemical hallmark of acute porphyric attacks is a marked up-regulation of hepatic ALA synthase-1 (ALAS1).

More than 90% of carriers of pathogenic mutations are symptomless throughout life [2].

According to the European Porphyria Network (EPNET), the incidence of new attacks decreased in Europe during the past decades, with a resulting reduced mortality and morbidity [3]. The prevalence of these diseases are however difficult to assess, because environmental factors play a significant role in their expression [4-5].

Some studies have investigated whether AHP were more frequently observed in women. Whereas three studies have evidenced a higher frequency of symptoms in women [6-8], one did not [9], as summarized in the meta-analysis of Baravelli and colleagues [8].

This sex-effect could be indirect and related to the trigger effect of the menstrual cycle, but also to the higher frequency of chronic treatments (including noticeably combined hormonal contraceptives) in women [10].

Diagnosis is currently based on elevated concentrations of ALA and PBG (pathognomonic of the AHP) and, sometimes, of porphyrins in urine, stools or plasma. The type of porphyria is determined by enzyme measurement followed by genetic analysis and the characterization of the corresponding mutations.

Apart from the acute crisis, AHP can manifest in a more recurrent manner. In AIP, the most frequent of AHP, most patients experience one to several attacks during their lifetime, but about 5% of patients in Europe [5] suffer from recurrent acute attacks with no identified precipitating factors and may, in rare cases, result in a need for liver transplantation [11]. In the last century, the prognosis for acute porphyria with neurological complications was dismal, with an associated mortality of 35% [12], but the prevalence of severe neurological complications has markedly decreased since 1990 with the introduction of human hemin in the treatment of acute attacks.

As life expectancy improves for patients with AHP, clinicians now need to focus on the potential for long-term complications of AHP. Primary liver cancers (PLC) and particularly hepatocellular carcinoma (HCC), chronic kidney disease (CKD), and hypertension are the more frequent of those complications [13].

Hepatocellular carcinoma (HCC)

HCC is a malignant tumor that usually develops from hepatocellular dysplastic nodules in a liver that is injured by chronic hepatitis and fibrosis, rarely in a healthy liver. HCC accounts for more than 80% of PLC. PLC is the sixth leading cause of cancer morbidity and the second leading cause of cancer-related death in the world, with an estimated 746,000 deaths in 2012 (9.1% of deaths from cancer) [14]. Trends in HCC mortality rates have increased over recent decades in most countries, with variation between countries, and between men and women [15].

The crude incidence rate has been estimated at 0.001-0.010 % (USA, EU) to 0.1 % (Asia, Sub Saharan countries). The ratio of men to women is nearly from 2:1 to 4:1 [16].

Many chronic liver diseases that generate a chronic liver inflammation are risk factors for HCC. Up to 90% of the patients with HCC exhibit pre-existing cirrhosis. Many acquired or hereditary diseases that promote liver cirrhosis may thus cause HCC: viral hepatitis, alcoholic liver disease, nonalcoholic-steatohepatitis, primary biliary cirrhosis, autoimmune hepatitis, primary sclerosing cholangitis, Wilson's disease, inherited deficiency of alpha-1-antitrypsin, some porphyria and especially AIP [17].

The HCC diagnostic criteria are based on the EASL Barcelona Conference in 2000 and an update in 2012 which was published in collaboration with the European Organization for Research and Treatment of Cancer (EORTC) [18]. The treatment is either curative or palliative [19].

The overall 1-year survival rate is 47% if no potentially curative treatment is given [20]. This poor prognosis is partly related to a late diagnosis established in an advanced stage in more than two third of patients, when therapies are less profitable [21].

HCC is usually suspected upon discovery of liver nodular focal lesion on ultrasound of the liver, or due to symptoms in case of an advanced tumor. Multiple phase computed

tomography (CT) or magnetic resonance imaging (MRI) are the reference methods for diagnosis.

Blood alpha-fetoprotein (AFP) measurement has been used for a while for non-invasive diagnosis of HCC, with various cut-offs, but is no longer recommended anymore in this indication. It lacks sensitivity for early diagnosis. Normal concentrations are observed in 10 to 30% of HCC patients. The diagnostic sensitivity of AFP for early-stage HCC (defined as a single lesion <3 cm in diameter or no more than three lesions with each <3 cm and without portal vein thrombosis or extrahepatic metastasis) is less than 50% [22].

Prothrombin or factor II is a vitamin K-dependent coagulation protein synthesized only by the liver, and its synthesis in the presence of vitamin K involves γ -carboxylation of the glutamyl residues necessary for its maturation [23]. In the absence of vitamin K or the presence of anti-vitamin K anticoagulants, a biologically inactive form called Protein Induced by Vitamin K absence or Antagonist II (PIVKA-II), otherwise called Des-gamma-carboxy-prothrombin (DCP) is detected. We and other previously evidenced the good performances of blood PIVKA-II in the diagnosis of early and very early carcinoma [24-26] and the association of this biomarker, currently used in Asia, with the microvascular invasion. This marker could provide a promising additive non-invasive tool for the surveillance of patients at risk.

Earlier non-invasive detection and better treatments are thus unmet needs to improve outcome.

PLC in AHP

AHP are rare diseases. As a consequence, only limited numbers of small, mostly retrospective, studies are currently available (Table 1).

The liver plays a significant role in the pathophysiology of these porphyrias. Besides, hepatic manifestations are frequent in AHP. Elevation of transaminases is observed, although not always present [27-29].

Two types of PLC have been described associated with AHP: HCC and cholangiocarcinoma (CC). HCC is mostly predominant in patients with AHP, with the percentage ranging among studies from 87% to 100% of PLC [6-9].

The most documented association of PLC in AHP is in AIP. In the 1980s, two retrospective studies and one case-control study from Scandinavian teams evidenced for the first time an association between PLC and AHP [30-31]. In the survey of Andersson et al., 11 AHP patients with PLC were found in elderly AIP group (12% of all patients), more in women than in men (63% and 37%, respectively). Liver cirrhosis was identified in five patients (45%). The crude annual incidence rate in AHP was estimated at 0.6 % (600/100,000), whereas the crude annual incidence rate of total PLC in the Umea region of Sweden was 0.0035% (3.5/100,000).

In 1998, a prospective cohort study assessed the crude annual incidence rate of PLC in 650 French AHP followed for seven years [32]. PLC was described in 1% of the patients, found mainly in AIP subjects but also in VP and HCP, both in symptomatic and latent carriers [6] [33-34]. Most patients did not have any other risk factors for HCC. There was no evidence of fibrosis, inflammatory-cell infiltrate, necrosis nor of iron overload. Cirrhosis was observed in only 28% of the patients. The low frequency of cirrhosis in AHP patients with HCC has also been pointed out in two further studies [7] [9], with a frequency comprised between 24 and 31%.

Baravelli et al. [8] published the most extensive study concerning PLC in porphyria. In this study, based on Norwegian cancer registry which includes more than 4 millions inhabitants, they established that whereas PLC represents 0.6% of all cancers in the Norway population,

PLC represent 31% of cancers reported in the 251 patients identified as suffering from AHP (as they self-report having had porphyria-related symptoms, in the form of acute attacks and/or skin lesions).

CC is a rare tumor of the biliary duct- Sardh et al. described four patients (17% among patients with PLC) with CC in a cohort of 179 AHP patients above the age of 50 years, either alone or combined with HCC [35]. However, the authors were unable to determine whether CC was related to AHP or incidental.

PLC in PV

Evidence linking HCC to VP is insufficient and is related to a small number of published case reports and some individuals identified in cohort studies [36-37]. The cohort studies gave frequencies ranging from 1.3% to 5.4% [38]. Interestingly, no case has been reported in the South African population where the disease is more frequent due to a founder effect. Whether this reflects differences in health data organizational arrangements or differences in HCC incidence is unclear.

Pathophysiology

The pathophysiology of HCC is highly complex and partially elucidated. Several risk factors may lead to the development of "chronic liver disease" (viral hepatitis, alcoholic hepatitis, nonalcoholic steatohepatitis noticeably) which results, following repeated attacks, to the formation of a scar tissue that replaces the damaged liver cells: liver fibrosis and ultimately cirrhosis. The most characteristic lesion of hepatic carcinogenesis is an underlying chronic liver injury, which leads to repeated cycles of cell death, inflammation and compensatory cell proliferation (regeneration of hepatocytes), in the context of oxidative stress, providing a mutagenic environment leading to the development of HCC. Hepatic carcinogenesis is a

multi-stage process, with chronic hepatitis and cirrhosis being the preneoplastic stages [39]. Some genetic alterations have been described, involving the activation of oncogenes or inactivation of tumor suppressor genes, noticeably by epigenetic mechanisms [40], and recently, through non-coding RNAs [41]. Several groups of genes including telomere maintenance, cell cycle gene, Wnt/ β -catenin, have been identified as pathways implied in the liver carcinogenesis [42].

In AHP, some peculiar mechanisms could participate in the physiopathology of the disease. The combination of somatic and germline mutations of genes implicated in the heme biosynthesis could also favor the development of HCC [33].

The hypotheses evoked in the appearance of HCC on the healthy liver are noticeably a direct carcinogenic effect of ALA, a loss of antioxidant effects due to heme deficiency [43] and a direct or indirect effect of mutations [8].

A paradigmatic model of the toxic effect of ALA in AHP is found in hereditary tyrosinemia, which is associated with early HCC due to an accumulation of ALA [44]. Indeed, ALA has been evidenced to exert some toxic effects on hepatocellular cells [45-46]. A few studies have described increased ALA and PBG in patients with HCC compared with other patients [5][47].

As a consequence, one question is to know whether HCC is more frequent in symptomatic (with acute crisis) or latent AHP (with no acute crisis). Baravelli et al. [8] reported that eight of the nine Norwegian patients with self-reported AHP manifest disease. In a Europe-wide study, Elder et al. described that HCC was mainly observed in symptomatic patients [5]. In other studies 0-43% of PLC have been reported in latent AHP-patients, as summarized in Table 2. However, the results are not sufficient for robust conclusions to be made.

Figure 1 summarizes the different factors that have been evoked in the physiopathology of PLC in AHP. Interestingly, in AIP, the p.W198X mutation has been suggested to be more prone to be associated with HCC [5].

Chronic treatment administrated in AIP, heme arginate, could participate in the physiopathology of the disease in recurrent patients by an imbalance between the heme-oxygenase 1 and heme [48]. However, to date in France, no PLC has been recorded in the 48 recurrent patients followed in the French reference center for Porphyria (LG, HP, JCD: personal communication).

Figure 2 summarizes the central pathways that could contribute to the PLC development in AIP.

Screening: risk versus benefit

Due to the rarity of the disease, the screening and follow-up of patients and their relatives have to take into account the balance of benefits and harms. Any form of surveillance is potentially harmful, for example, by causing anxiety to otherwise asymptomatic individuals and by generating some false positive screening tests.

Some questions still have to be solved regarding the long-term follow up of patients and relatives. First, does the follow-up improve the survival? Is the strategy cost-effective, which exam has to be chosen, and with which frequency?

Regarding the first interrogation, two main studies are available. Innala et al. showed that the surveillance significantly improved the 3 and 5-year survival ($p=0.005$ and $p=0.038$, respectively) [9]. Conversely, Sardh et al. evidenced a non-significant survival benefit in patients under surveillance [7]. However, the mean tumor diameter at diagnosis was 4.3 cm in patients with surveillance, compared to a 10.3 cm mean diameter in patients with no monitoring in Sardh et al. study. To date, the 5-year survival rate of HCC remains low in

advanced forms. Indeed, this apparent conflicting image could be related to the overall poor performance of abdominal ultrasonography in the diagnosis of very early and early HCC, that minimize the impact of surveillance onto survival [49].

A pragmatic approach to surveillance was however suggested in Sweden: annual abdominal ultrasound monitoring is recommended for patients over 50 years of age [9]. Alpha-fetoprotein does not seem relevant for monitoring AHP patients [9][50].

In other European countries, ultrasound is suggested at least annually [3]. The establishment of an International consensus by the two main porphyria dedicated societies (American Porphyria Consortium, APC & EPNET) is needed.

Within the frame of the French reference center for Porphyria (CRM Porphyries), we began to evaluate in some patients that gave their informed consent the potential clinical utility of PIVKA-II in the follow-up of patients with AHP, and report the first description of potential PIVKA-II clinical utility (Table 3). This marker could be a promising biological alternative to AFP. Its use is recommended by the *Asian Pacific Association for the Study of the Liver* [51], and its performances are recognized through some meta-analysis [52], although further investigations are needed in the setting of AHP and other metabolic inherited diseases.

Other non-invasive biomarkers have been proposed as early biomarkers of HCC in addition to abdominal ultrasonography. It includes noticeably some circulating miRNA, long-non coding RNA, or biochemical markers as Glypican-3, Osteopontin or fucosylated variants of AFP [53]. None of them are presently recommended by any clinical society, but further investigations in AHP could be undertaken.

The benefits of early diagnosis through systematic surveillance remain to be demonstrated in populations with a lower background prevalence of acute porphyria and incidence rates of PLC/HCC.

Conclusions

AIP is associated with an increased risk for PLC (mainly HCC) in European countries, whereas the risk in VP and HCP remains unclear.

Cost-benefit modelings are not currently available but a 0.2% annual incidence would warrant surveillance like in a non-cirrhotic patient with HBV. Indeed,ultrasound surveillance using CT in AIP over 50 years (0.6% incidence) is recommended in Europe, probably biannually as for other liver disease with an increased HCC risk (EORTC). PIVKA-II, which is already recommended in some countries, could provide an alternative non-invasive biomarker for monitoring at-risk patients although results from on-going international studies are anticipated. Clinicians should consider the diagnosis of PLC in any AHP patient with unexplained abnormal liver function tests, but also with symptoms and signs suggestive of underlying liver disease or malignancy. This consideration should be extended to patients with manifestations of acute porphyria (skin lesions or acute attacks) after the age of 50 years, particularly for the first time or after a period of latency. As a treatment option of PLC in AIP patients, liver transplantation should also be considered as it will even cure AIP disease.

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Figure and tables legends

Table 1: Previous studies of the literature describing PLC in AHP patients in Europe.

n: number of patients suffering from PLC associated with AHP

This table includes patients with HCC, CC or both.

Table 2: PLC in AHP patients with either symptomatic or latent porphyria

This table includes patients with HCC, CC or both, as described in the published studies.

Table 3: Description of the clinical history of a patient with AIP that develops HCC

Figure 1: Physiopathology of HCC (and possibly CC) in AHP

The main features implied are inflammatory lesions of the hepatocytes, potentially associated with mutations, either in genes related to the heme biosynthesis, or to the oncogenesis process. Several distinct mechanisms could participate in the carcinogenesis. The direct toxicity of ALA could exert its effect either via oxidant or genotoxicity properties. The loss of antioxidant effects could be due to a relative lack of heme. The energetic mitochondrial failure related to the relative lack of succinyl-CoA, which is produced by the citric acid cycle, has also been suggested. Melatonin modulates the glucose metabolism and decreases the over-induction of the heme biosynthesis in porphyria.

ALAS1: amino-levulinate synthase 1; ROS: reactive oxygen species; HCC: Hepatocellular Carcinoma

Figure 2: Pathways that could participate in the liver carcinogenesis in recurrent AIP patients.

Patients present a higher level of ALA and PBG. The inflammation, exogenous heme, and release of iron, associated with the high level of ALA and PBG, could participate in the hepatocarcinogenesis. From Schmitt et al. [48].

Table 1

Authors	Country	Year	Patients with PLC (n)
Lithner and Wetterberg [54]	Sweden	1984	11
Hardell et al. [31]	Sweden	1984	3
Kauppinen and Mustajoki [29]	Finland	1988	7
Andersson et al. [30]	Sweden	1996	9
Bjersing et al. [55]	Sweden	1996	22
Linnet et al. [56]	Sweden & Denmark	1999	5
Andant et al. [6]	France	2000	7
Schneider-Yin et al. [57]	Switzerland	2009	4
Innala and Andersson [9]	Sweden	2011	22
Sardh et al. [7]	Sweden	2013	23
Elder et al. [5]	Europe (EPNET)	2014	14
Baravelli et al. [8]	Norway	2017	9

Table 2

Reference	Country	Manifest-AHP patients n (%)	Latent-AHP patients n (%)
Andant et al. (2000) [6]	France	4 (57.1)	3 (42.9)
Innala and Andersson (2011) [9]	Sweden	16 (73)	8 (37)
Sardh et al. (2013) [7]	Sweden	23 (100)	0 (0)
Elder et al. (2013) [5]	Europe (EPNET)	8 (57)	6 (43)
Baravelli et al. (2017) [8]	Norway	8 (89)	1 (11)

Case report

After 50 years-old, annual screening for HCC was systematically proposed to AHP patients and their pre-symptomatic relatives. This screening was based on ultrasonic imaging of the liver, AFP and PIVKA-II measurements in plasma.

A 53-year old female AHP pre-symptomatic patient was included, for the first time, in the follow-up study. The diagnosis of AHP was made in the course of a familial survey by both measuring HMBS activity at a time when the patient had no symptoms and genetic testing. AFP and PIVKA-II assays were performed in the Department of Biochemistry (Beaujon Hospital). AFP was determined by an automated system (Architect I1000, ABBOTT, normal range < 9 µg/L). PIVKA-II level was measured on a LUMIPULSE® G1200 (Fujirebio Europe, Belgium), using a chemiluminescent enzyme immunoassay (PIVKA-II; Fujirebio Europe, Belgium) per the manufacturer's instructions. All samples were performed in duplicates. The patient presented in good clinical condition without fever, weight loss or pain. The AFP level was in usual values (5 µg/L; normal <9) and PIVKA-II level was increased (378 mAU/mL; normal range <42). Ultrasonic imaging of the liver revealed a suspected mass in the left liver lobe. Doppler-ultrasound, CT-scan, and MR-cholangiography were performed. CT-scan detected a 1.8 cm mass in the left liver lobe. There were no morphological or biochemical signs of liver cirrhosis and preoperative laboratory values were normal. The patient, that presents no distant metastasis, underwent hepatic resection: an extended left half-hepatectomy was performed without surgical complications. Results of exhaustive investigation of etiological factors for HCC were negative: alcohol consumption, blood transfusion, hepatotoxic drug intake, aflatoxin, professional exposure to organic solvents, iron overload, smooth muscle, antinuclear and anti-mitochondrial antibodies. Liver and serum samples were screened for hepatitis B and C virus by both immunological and molecular techniques. Microscopic histological analysis of the non-tumor liver was normal: no fibrosis, no inflammatory cell infiltrate, and no necrosis nor iron overload was found. The postoperative course was uneventful. PIVKA-II level returned to normal values within the first month. Histology confirmed a moderately differentiated HCC. After one year of follow-up, no recurrence of HCC has been detected.

This first case report demonstrates the potential utility of the PIVKA-II assay to detect HCC in AHP carriers. Further investigations are needed to evaluate the benefit of this assay in this context.

Figure 1

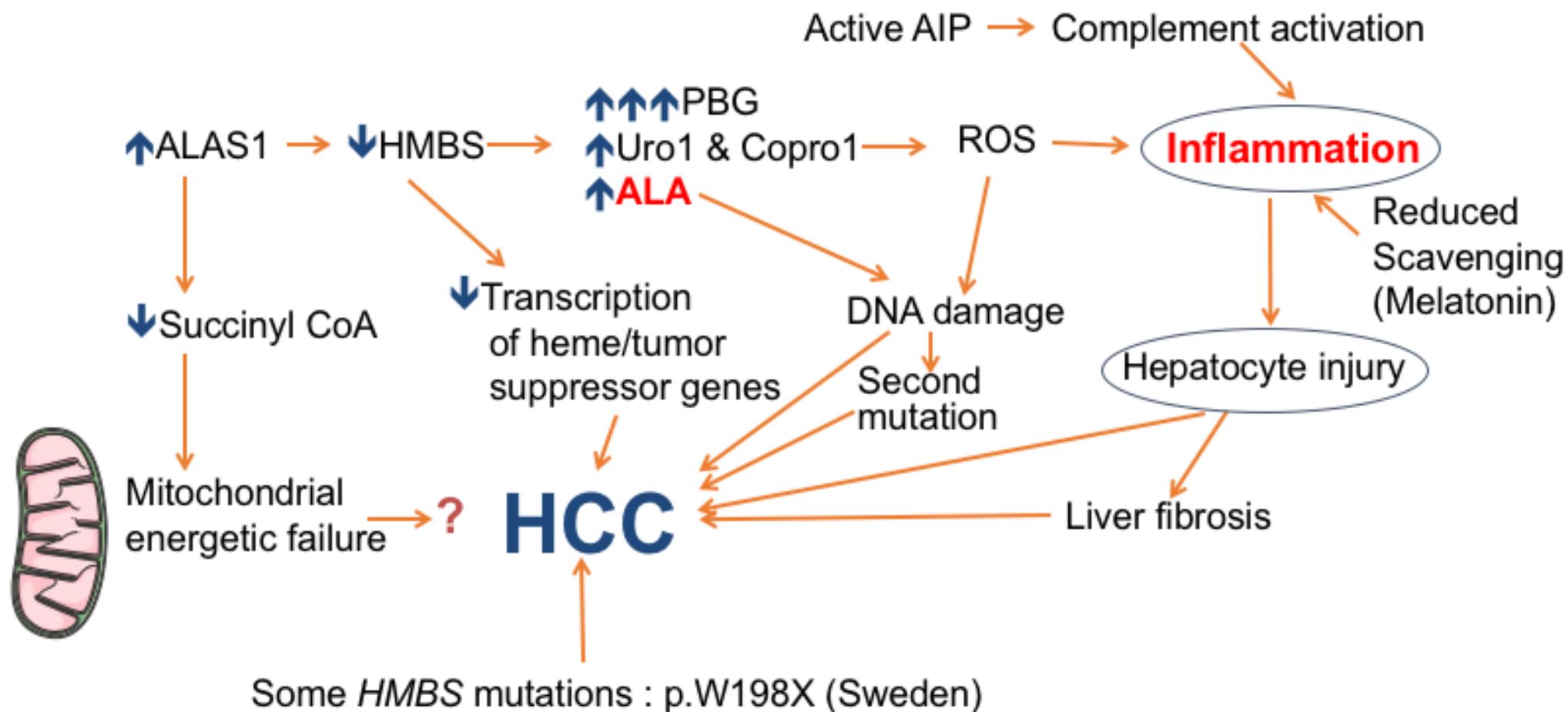


Figure 2

