Acute intermittent porphyria, women and sex hormones. Screening for hepatocellular carcinoma in porphyria

Eva Innala
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Abstract

Background: Porphyrias are inherited disorders with impaired heme biosynthesis. Acute intermittent porphyria (AIP) is the most common porphyria in Sweden. AIP attacks may be life-threatening. Female sex hormones are regarded as important precipitating factors. Hepatocellular carcinoma (HCC) is a severe complication in the older AIP population. The aim of the thesis was to describe the clinical expression of AIP in women, experience of hormonal contraception and hormonal replacement therapies (HRT) and of pregnancies. Secondly, we evaluated gonadotropin-releasing hormone (GnRH) agonist treatment for prevention of menstrual-cycle-related AIP attacks. Thirdly, we evaluated whether an altered sex-steroid metabolism was present in AIP women compared with controls. Finally, we evaluated the benefit of screening for HCC in AIP in a 15-year follow-up study.

Methods and results: In a retrospective population-based study in northern Sweden, 166 female AIP gene carriers ≥18 years of age participated. Manifest AIP (MAIP) was reported in 55%; 82% had severe attacks and 39% had menstrual-cycle-related attacks. Hormonal contraceptives were used by 94, and 12 reported that this precipitated AIP attacks. HRT and local vaginal treatments in menopause did not precipitate AIP attacks. Only 10% reported impairment of AIP symptoms during pregnancy.

In the retrospective follow-up study of GnRH-agonist treatment, 11 of 14 women improved during treatment. Porphyria attacks were triggered in two women after estradiol add-back and in 5 of 9 women after progesterone add-back.

In the sex-steroid metabolism study, levels of s-progesterone, estradiol, allopregnanolone and pregnanolone during the menstrual cycle in 32 AIP gene carriers were compared with 20 healthy controls. Progesterone metabolism in the AIP group differed from controls. In the AIP group levels of allopregnanolone, but not pregnanolone, were significantly lower.

In the prospective HCC screening study AIP gene carriers aged >55 years were included. On average 62 subjects participated during 15 years. HCC was diagnosed in 22 of 180 eligible AIP gene carriers in the region (male:female, 12:10, 73% MAIP). The annual incidence of HCC was 0.8%. The risk of HCC was 64-fold higher than in the general population over 50 years of age in this region, and even higher for AIP women (93-fold). Increased 3- and 5-year survival was seen in the regularly screened AIP group. Liver lab tests were not useful in HCC screening.

Conclusion: The clinical expression of AIP in women is pronounced and menstrual-cycle-related attacks are common. Hormonal contraceptives can induce AIP attacks and caution is recommended. GnRH-agonist treatment can ameliorate menstrual-cycle-related attacks of porphyria. Dose findings for GnRH-agonists and add-back regimes, especially for progesterone, are intricate. Progesterone metabolism in the AIP group differs from that in healthy controls. HCC screening in AIP gene carriers >50 years of age enables early diagnosis and a possibility for curative treatments. Annual HCC screening with liver imaging is recommended in AIP gene carriers >50 years of age.
Sammanfattning på svenska

**Bakgrund:** Porfyrier är ärftliga sjukdomar med enzymdefekter i hemsyntesen. Akut intermittent porfyri (AIP) är den vanligaste porfyrin i Sverige. AIP attacker kan vara livshotande. Kvinnliga könshormoner är av betydelse för manifestation av sjukdomen. Levercancer är en allvarlig komplikation för äldre anlagsbärare av AIP.

Syftet med den första studien i avhandlingen var att beskriva manifestationer av AIP hos kvinnor, erfarenheter av hormonella preventivmedel, hormonell substitutionsbehandling (HRT) i klimakteriet och av graviditeter. I studie två, följde vi upp behandlingar med GnRH-agonister (gonadotropinfriättande hormon) för förebyggande av menstruationscykelrelaterade porfyriattacker. I studie tre, undersökte vi om det fanns en förändrad könshormon metabolism vid AIP jämfört med kontroller. Till sist, i en uppföljningsstudie som pågick i 15 år, undersökte vi nyttan av levercancer screening vid AIP.

**Metoder och resultat:** I en retrospektiv populationsbaserad studie i norra Sverige, deltog 166 kvinnliga AIP anlagsbärare ≥18 år, av dessa hade 55% manifest AIP (MAIP), 82% hade svåra attacker och 39% menstruationscykelrelaterade attacker. Hormonella preventivmedel hade använts av 94 och 12 rapporterade att detta utlöst AIP attacker. I klimakteriet hade HRT eller lokal vaginal östrogen behandling inte utlöst AIP attacker. Endast 10% rapporterade försämring av AIP symptom under graviditet.

I den retrospektiva uppföljnings-studien av GnRH-agonist behandling, hade 11 av 14 kvinnor förbättrats av behandlingen. Porfyriattacker hade utlösts av estradiol add-back för två kvinnor och av progesteron add-back för 5 av 9 kvinnor.


I den prospektiva screeningstudien för levercancer inkluderades AIP anlagsbärare >55 år. Uppföljningstiden var 15 år och i medeltal deltog 62 personer. Levercancer diagnostiserades hos 22 av 180 tillgängliga AIP anlagsbärare i regionen (man:kvinn, 12:10, 73% MAIP). Årlig incidens för levercancer var 0.8%. Risken att drabbas av levercancer var 64 gånger högre än för befolkningen i övrigt >50 års ålder i samma region och ännu högre för kvinnorna (93 gånger ökad risk). Förbättrad 3- och 5-års överlevnad sågs i den regelbundet screenade AIP gruppen. Blodprover för leverfunktion kan inte användas vid levercancer screening.

## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>alpha-fetoprotein</td>
</tr>
<tr>
<td>AIP</td>
<td>acute intermittent porphyria</td>
</tr>
<tr>
<td>ALA</td>
<td>5-aminolevulinic acid</td>
</tr>
<tr>
<td>ALAD</td>
<td>5-aminolevulinic acid dehydratase</td>
</tr>
<tr>
<td>ALAS</td>
<td>5-aminolevulinic acid synthase</td>
</tr>
<tr>
<td>CL</td>
<td>corpus luteum</td>
</tr>
<tr>
<td>FSH</td>
<td>follicle-stimulating hormone</td>
</tr>
<tr>
<td>GnRH</td>
<td>gonadotropin-releasing hormone</td>
</tr>
<tr>
<td>HBV</td>
<td>hepatitis B virus</td>
</tr>
<tr>
<td>HCC</td>
<td>hepatocellular carcinoma</td>
</tr>
<tr>
<td>HCV</td>
<td>hepatitis C virus</td>
</tr>
<tr>
<td>HMB</td>
<td>hydroxymethylbilane</td>
</tr>
<tr>
<td>HMBS</td>
<td>hydroxymethylbilane synthase (porphobilinogen deaminase)</td>
</tr>
<tr>
<td>HRT</td>
<td>hormone replacement therapy</td>
</tr>
<tr>
<td>LAIP</td>
<td>latent acute intermittent porphyria</td>
</tr>
<tr>
<td>LH</td>
<td>luteinizing hormone</td>
</tr>
<tr>
<td>MAIP</td>
<td>manifest acute intermittent porphyria</td>
</tr>
<tr>
<td>MPA</td>
<td>medroxyprogesterone acetate</td>
</tr>
<tr>
<td>NETA</td>
<td>norethisterone acetate</td>
</tr>
<tr>
<td>PBG</td>
<td>porphobilinogen</td>
</tr>
<tr>
<td>PBGD</td>
<td>porphobilinogen deaminase</td>
</tr>
<tr>
<td>PCT</td>
<td>porphyria cutanea tarda</td>
</tr>
<tr>
<td>SRD5A1-3</td>
<td>5α-reductase type 1-3</td>
</tr>
<tr>
<td>SRD5B1</td>
<td>5β-reductase type 1</td>
</tr>
<tr>
<td>VP</td>
<td>variegate porphyria</td>
</tr>
</tbody>
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Original papers


III. Innala E, Bixo M, Bäckström T, Sundström-Poromaa I, Andersson C. Women with acute intermittent porphyria have a defect in 5α-steroid production during the menstrual cycle. Manuscript

IV. Innala E, Andersson C. Screening for hepatocellular carcinoma in acute intermittent porphyria – a 15 year follow-up in northern Sweden. Submitted

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Introduction

1 Porphyria

1.1 Background

Porphyrias are a group of inherited metabolic diseases with enzyme deficiencies in one of the eight steps in the heme biosynthesis pathway (1). The porphyrias are classified as erythroid or hepatic according to the principal tissue where the enzymatic defect is expressed. The porphyrias are also classified as acute inducible or cutaneous, depending on the major clinical symptoms. The acute porphyrias are: acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP) and ALA-dehydratase deficiency porphyria (ADP).

1.2 Heme

Heme is synthesized in all living cells, with the major formation in the red bone marrow and the liver. Heme is crucial for biological functions for all aerobic cells. It is a prosthetic group in many important proteins and enzymes, especially in the formation of the cytochrome P450 (1). The first enzyme in the heme biosynthesis pathway is the rate-limiting enzyme 5-aminolevulinic acid synthase (ALAS). This enzyme is induced when demands for heme are increased in the cell metabolism. The mitochondrial enzyme ALAS catalyses the formation of the heme precursor 5-aminolevulinic acid (ALA). In the cytoplasm, the second enzyme aminolevulinic acid dehydrase (ALAD) condenses two molecules of ALA to porphobilinogen (PBG). The third enzyme in this biosynthesis is a cytoplasmatic enzyme, the porphobilinogen deaminase (PBGD). This enzyme catalyses the condensation of four molecules of PBG to the tetrapyrrrole hydroxymethylbilane (HMB). The enzymatic steps in the heme biosynthesis and the different porphyrias are shown in Figure 1.
Figure 1. Heme biosynthetic pathway, the different enzymes and porphyrias. Each enzyme is associated with a specific porphyria. The first and the last three of the enzymes are located in mitochondria and the intermediate enzymes in cytosol. The figure is modified from Anderson and Sassa et al. 2001 (1).

### 1.3 Genetics

AIP is caused by various mutations in the PBGD gene. It is an autosomal dominantly inherited porphyria with incomplete penetrance. The majority of individuals with inherited deficiency of PBGD never develop porphyrpic symptoms (1, 2). PBGD is the third of eight enzymes in the heme biosynthesis.
In AIP, the PBGD gene is mutated, resulting in approximately 50% reduction in enzyme capacity. The enzyme activity of the unaffected PBGD allele is mostly enough for normal demands of heme production (3).

The first identified mutation associated with AIP was reported in 1989 (4). The PBGD genes are encoded at chromosome 11 gene map locus 11q23.3 for AIP mutations (5, 6) and consist of 15 exons (7). The enzymatic deficiency in the PBGD gene is the same in latent and manifest AIP (1).

In Sweden, the Nordic AIP mutation W198X is the most common mutation (8, 9). It is regarded as a severe mutation, and about 20–50% of these gene carriers experience clinical symptoms (10). A base substitution G to A in exon 10 of the PBGD gene is identified in the mutation W198X. The mutation W198X is due to a founder effect traced back to the 17th century, in a family originating from Arjeplog in Lapland, Sweden (11, 12).

In 2002 about 275 mutations of the PBGD gene were described (5) and 41 of these mutations were found in Sweden (9). Most of the mutations are family-specific. The majority of mutations are nucleotide substitutions, but deletions, insertions and splicing defects are also reported (13). De-novo mutations of the PBGD gene are sporadic (14).

1.4 Diagnosis

DNA analyses are the golden standard to identify gene carriers of AIP (15). AIP diagnosis is based on DNA tests, genealogical data, medical history, clinical symptoms (if applicable), measurement of erythrocyte PBGD activity and of U-ALA and U-PBG levels.

The PBGD enzyme deficiency decreases the ability for heme production in AIP patients. With increased demand for heme, ALAS is induced, resulting in an accumulation of the heme-precursors ALA and PBG (1).

Urinary PBG is the best biochemical test for AIP during attacks but it is unspecific and must be combined with DNA tests for the AIP diagnosis (16). In symptom-free intervals, manifest porphyria (MAIP) patients often have increased levels of urine porphyrins ALA and especially PBG. In a Swedish study of AIP patients 72% in the MAIP group and 38% in the latent porphyria (LAIP) group had elevated U-PBG levels (17). During acute AIP attacks, U-PBG usually increases up to 20–50-fold above normal reference levels (18). In LAIP increased amounts of particularly U-PBG but also U-ALA can be seen, (17, 19). In healthy relatives of AIP patients, levels of U-PBG are found to be significantly higher than in the general population (16).

Analysis of the PBGD enzyme activity was more often used before DNA tests were available. One of the limitations of this analysis is that it does not identify AIP gene carriers if the enzyme defect is not expressed in erythrocytes (3). Another problem may be the risk of both false positive and negative diagnosis due to limitations of the test. Furthermore, the test results could be influenced by concomitant disorders, for example uraemia, chronic polyarthritis, haemolytic disorders, malignancies and liver diseases.
1.5 Prevalence

AIP is the most common form of porphyria. The prevalence of AIP in the general population varies from 0.5–10/100 000. AIP occurs in all races, but is somewhat more common in northern Europe, especially in Sweden, Great Britain and Ireland (1).

In Sweden, the prevalence is 1/10 000 and in the north of Sweden 1/1000, see Figure 2 (9). In Finland, AIP and VP have a prevalence of 3.4/100 000. In the USA, the frequency of AIP gene carriers is about 5/100 000 (1). The prevalence of VP gene carriers in Sweden is 1/100 000 (9).

Around 1000 AIP gene carriers have been diagnosed in Sweden, of whom half are living in the four northernmost counties.
Figure 2. Number of AIP gene carriers and prevalence per 100,000 inhabitants in Sweden in 2002. Different AIP mutations are listed. To the right in the figure, distributions in various parts of the country are shown in the circles. The figure is reprinted with kind permission from Ylva Floderus, Porphyria Centre Sweden, and the copyright holder.
1.6 Clinical symptoms
AIP attacks are characterized by neuro-psychiatric symptoms and may be life-threatening. The acute attacks originate from engagement of the autonomous, peripheral and central nervous system. Frequent symptoms and signs are abdominal pain, constipation, nausea, vomiting, peripheral nerve paresis and paresthesias, back ache and limb pain. Bulbar paralysis and respiratory paresis may occur. Furthermore, various psychiatric symptoms, electrolyte abnormalities (i.e. low levels of sodium and magnesium), hypertension, tachycardia, and red urine can be seen (17). Skin lesions never develop in AIP (1).

1.7 Precipitating factors
Attacks are triggered by different metabolic, environmental and hormonal factors. Various drugs (especially drugs metabolized by the cytochrome P450 enzymes in the liver), psychological stress, fasting, menstruation, alcohol, infections, surgery, smoking and work environment are well-known precipitating factors (1, 2, 17, 19-22). Female sex hormones, especially progesterone and its metabolites, are regarded as important precipitating factors (1, 23, 24). Precipitating factors are thought to act in an additive fashion but sometimes no obvious cause of symptoms is found.

1.8 Latent and manifest porphyria
Most of the gene carriers never develop porphyria symptoms, e.g. latent porphyria (LAIP) but 10–50% have experienced clinical AIP manifestations (1, 17, 25, 26). The AIP is considered manifest (MAIP) if the gene carrier has suffered porphyria symptoms, if levels of the porphyria precursors ALA and PBG are above normal reference values during attacks, and if there was no other obvious reason for these symptoms (17). Less than 10% of acute porphyria patients develop recurrent attacks, and most patients have experienced one or a few attacks and then recover (27). In a Swedish report, a high prevalence of MAIP and recurrent attacks was seen, which may be explained by a predominance of the more severe AIP mutation W198X in this study group (17).

The manifestations of an acute porphyria attack seen in VP are similar to those seen in AIP, but attacks in VP are generally milder and recurrent attacks are less common (2).
2 Women and porphyria

2.1 Women

Women are more affected than men by acute porphyrias (1, 17, 27). Symptoms of AIP in both sexes are rarely seen before puberty (1, 28), but as sexual maturation develops, signs and symptoms of AIP may occur. AIP symptoms are more common and severe in women than in men, especially in reproductive ages. In women, symptoms appear in earlier ages than in men, in women between 20 and 29 years of age and in men about 10 years later (17, 21). The ratio female: male of MAIP is about 2:1 (10, 17, 29). Menstrual-cycle-related AIP attacks are reported in 10–50% of women with AIP (22, 24, 30-32). Porphyria attacks related to the menstrual cycle can frequently arise during the luteal phase (22, 24, 30, 32-34). The frequency of attacks usually declines after menopause, but in some women attacks appear for the first time in the climacteric (1, 17).

Menstrual-cycle-related porphyria attacks in variegate porphyria (VP) are not as frequent as in AIP, and in one report no menstrual-cycle-related VP attacks were noted at all (34, 35).

2.2 Pregnancy

Pregnancy is usually well tolerated in AIP (1, 21, 22). However, in reports about 50 years ago maternal mortality was high, especially in primo gravida. In these women, diagnoses were often unknown until the appearance of severe illness in early pregnancy: porphyria diagnosis was confirmed after delay and maltreatment (36).

The placenta produces large amounts of plasma proteins and steroids that may alter the maternal immune response (37). Most placental hormones are secreted into maternal circulation (38). During pregnancy, maternal sex-hormone levels are largely elevated, serum-progesterone levels increase about 5-fold above non-pregnant levels, estrone and estradiol levels increase 100-fold, and estriol 1000-fold. Sex-steroid levels increase during pregnancy, chiefly in the second and especially the third trimester (39). Estrogen levels begin to increase at gestational week 6–10. The increased levels of progesterone are seen from about the 10th week of gestation and levels are five times higher at the end of the pregnancy.

The corpus luteum (CL) produces progesterone until about 10 weeks of pregnancy. During weeks 7–10 the progesterone production is shared between the CL and placenta and from the 10th week of pregnancy the placenta is the major site for progesterone production. Progesterone is essential for maintenance of pregnancy (38). The placenta produces about 250 mg of progesterone per day at term.

During pregnancy active progesterone metabolites increase significantly, deoxycorticosterone 1200 times at term and the progesterone metabolite, allopregnanolone about 10 times (39). Serum-pregnanolone levels during pregnancy increase about 20 times (40). In the postpartum period hormonal levels rapidly fall to the non-pregnant endocrine state (39).
Experience of pregnancy, delivery and puerperium in AIP and VP were evaluated in a Finnish study. In this study 92% of the pregnancies were without porphyria symptoms (22). In porphyria the most vulnerable periods during pregnancy are the first trimester and the puerperium (41, 42). Hyperemesis gravidarum in early gestation with starvation, for example, might increase risks of attacks.

It is not advisable to discourage pregnancy in AIP. Worsening of porphyria symptoms during pregnancy might be due to harmful drugs, inadequate nutrition, or both (26).

3 Sex hormones

3.1 The menstrual cycle

During the fertile age, the ovary is the main source of the sex steroids estradiol and progesterone. The menstrual cycle is divided into the follicular phase, the ovulation and the luteal phase, see Figure 3 for schematic information. (The correction factor for estradiol levels from pg/mL to pmol/L is ~3.7. The correction factor for progesterone levels from ng/mL to nmol/L is ~3.2.)

![Figure 3](image)

Figure 3. Hormone concentrations in peripheral blood during the menstrual cycle. Picture reprinted with kind permission from the copy right holder (39).
After ovulation, the follicular phase changes to the luteal phase, and the formation of a novel endocrine gland, the corpus luteum (CL), has started. The CL produces estradiol and progesterone. In the normal menstrual cycle, the luteal phase is consistently close to 14 days. A serum-progesterone concentration less than 9.8 nmol/L (3.1 ng/mL) is consistent with follicular phase levels and levels above this as luteal phase levels (43).

Criteria for how to define a normal menstrual cycle length have been discussed in different studies (44). The interval between two menstrual cycles is usually 24–35 days (39). In puberty and in menopausal ages menstrual cycles are often irregular. The length of the menstrual cycle in days at different ages is presented by Vollman (45), see Figure 4.

![Figure 4](image.png)

**Figure 4.** Length in days of menstrual cycle at different ages. Picture reprinted with kind permission from the copyright holder (39).

The menopause is the time when menstruations cease. This mostly occurs at 50–52 years of age. Estradiol levels in the years before menopause remain normal and sometimes elevated until about 6–12 months before the last menstruation when they begin to decline (46). During the climacteric transition, ovarian activity decreases, ovulation ceases and menstruation ends (39). The circulating levels of estradiol after menopause are low, approximately less than 80 pmol/L, see Figure 3 for comparison. Subsequently, estrogen production in postmenopausal women is mainly due to peripheral conversion of androstendione, mainly in fat tissue. The conversion to estrogen correlates to weight and increases with higher body weight (39, 47). Overall, levels of circulating serum-estrone, a less potent estrogen, are higher than estradiol levels in postmenopausal women (48).
3.2 GnRH
The hypothalamic gonadotropin-releasing hormone (GnRH) is functionally inactive in childhood, but starts a pulsatile release when the girl is about eight years of age. The pituitary is stimulated by GnRH pulses and secretes both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). A normal menstrual cycle requires episodic, short bursts of GnRH into the portal system, resulting in a rapid rise in pituitary LH secretion. The FSH response to GnRH is slower and more prolonged. GnRH is a small peptide hormone, with a half-life of 2–4 minutes (38, 39). GnRH also has a direct inhibitory effect in vitro on steroidogenesis in the ovary (49).

3.3 Progestins and progesterone
The only indication for the use of progestins or progesterone in hormonal replacement therapies is the prevention of estrogen-induced endometrial hyperplasia. Apart from naturally occurring progesterone, there are different types of synthetic progestins that can be used for this purpose. The activity and potency of progestins and progesterone are mostly evaluated by means of parameters associated with endometrial effects (50). If the endometrium is ≤5 mm thick the risk of endometrial pathology is small (51). Equipotent doses of progestins and progesterone due to endometrial effects are described in Table 1. Different progesterone and progestin regimes are seen in HRT therapies and in oral contraceptives. Progestin is administered either cyclically or in continuous regimes. Some progestins are pro-drugs and are metabolized in the liver into active compounds (52). The metabolic effect on different tissues and organs varies. Different progestins and progesterone differ in efficacy, activity, and potency. The effectiveness depends on the galenic preparation, how the hormone is administered, and how high the doses are. Addition of progestins can cause negative mood symptoms and physical symptoms in some women (53).

Table 1. Equipotent doses of different progestins and natural progesterone regarding the effect on the endometrium.

<table>
<thead>
<tr>
<th>Progestins, progesterone</th>
<th>dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medroxyprogesterone acetate</td>
<td>10 mg</td>
</tr>
<tr>
<td>Norethisterone acetate</td>
<td>0.7–1 mg</td>
</tr>
<tr>
<td>Levonorgestrel</td>
<td>0.075 mg</td>
</tr>
<tr>
<td>Micronized progesterone</td>
<td>200–300 mg</td>
</tr>
</tbody>
</table>
3.4 Progesterone metabolites

Steroids are synthesized from cholesterol. Progesterone levels rise in the luteal phase, see Figure 3. The metabolic reduction along the 5α or 5β pathway is similar for all natural steroids having a keto group at the 3-position and a double bond between carbon atoms 4 and 5 in the steroid molecule (38, 50). The stereo chemical structural feature of steroid α-metabolites is flat and the β-metabolites are angulated (54).

Progesterone is metabolized along the 5α pathway to allopregnanolone (3α-hydroxy-5α-pregnan-20-one) or by the 5β-reductive pathway to pregnanolone (3α-hydroxy-5β-pregnan-20-one), see Figures 5 and 6. In fertile healthy women during an ovulatory menstrual cycle, allopregnanolone and pregnanolone levels are correlated to progesterone levels. Steroid levels increase in the luteal phase, see Table 2. In the luteal phase allopregnanolone levels are two to three times higher than pregnanolone. Furthermore, serum-allopregnanolone levels are significantly higher in the mid luteal phase compared to early and late luteal phase. Levels of s-pregnanolone are unaltered across the luteal phase (55).

Table 2. Serum concentrations of progesterone, allopregnanolone and pregnanolone during the follicular and luteal phases in women in the fertile and the postmenopausal period. Concentrations are given as mean ±SEM.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Follicular phase</th>
<th>Luteal phase</th>
<th>Postmenopausal period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone (nmol/L)</td>
<td>5.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.7 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Allopregnanolone (nmol/L)</td>
<td>0.5 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnanolone (nmol/L)</td>
<td>0.6 ± 0.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.1 ± 0.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.7 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The data are cited from the following references: <sup>a</sup>(56), <sup>b</sup>(57), <sup>c</sup>(58), <sup>d</sup>(59).
Figure 5. Steroid biosynthesis.
3.5 5α- and 5β-reductases

Two studies by Kappas et al. in 1972 investigated the 5α- and 5β-reductive capacity in AIP patients. In the first study, they showed that in 15 AIP patients and 12 controls receiving injected 14C-labelled testosterone and dehydroisoandrosterone, steroids were preferably metabolized along the β-reductive pathway (60).

In the second study, decreased levels of 5-α reduced 14C-androstendione and testosterone metabolites were found in the AIP group compared to controls (61). The research group discussed whether this reduced 5-α reductive capacity in AIP is of both endocrine and genetic origin, and that steroid metabolism is shunted from the α-reductive pathway to the β-reductive way. In addition their conclusion was that the 5β-steroid metabolites are potent inducers of ALAS.

This phenomena was further analysed and evaluated by Bradlow (62) in 1973. In a follow-up study, markedly reduced metabolism along the 5α-pathway for injected 14C-labelled testosterone was seen in both sexes in 7 MAIP patients and 3 patients with porphyria cutanea tarda (PCT). The reduction of the enzymatic deficiency ranged from 34–70%, compared to normal subjects when metabolism of a given tracer dose of 11β-hydroxyandrostenedione was investigated. There was no increased production of the 5β-metabolites. LAIP patients were not studied in this report. Bradlow et al. discussed whether the 5α-reductase deficiency in AIP was acquired or had partial genetic determinants. Work by Anderson et al. in 1979 found indications of a deficient hepatic 5α-reductive steroid metabolism in MAIP but not in LAIP when using radio-labelled hormone.
tracers for testosterone and 11β-hydroxyandrostenedione. A control group was also included (63).

One the other hand, in a study by Marks et al. in 1979, equal potency for 5α- and 5β-steroids in inducing ALAS was seen in chick embryo liver cells (64). That study could not confirm the theory that the β-steroids were more porphyrin-inducing than α-steroids.

All 5α- and 5β-reductases are coded on distinct genes. There are, to our knowledge, three types of 5α-reductase: type 1 (SRD5A1), type 2 (SRD5A2), and type 3 (SRD5A3) (65-68), and there is one type of 5β-reductase: type 1 (SRD5B1) (69), see Figure 7.

**Figure 7.** Progesterone conversion to allopregnanolone and pregnanolone with the different enzymatic steps and gene map locus for each enzyme. SRD5A1 (5α-reductase type 1), SRD5A2 (5α-reductase type 2), SRD5A3 (5α-reductase type 3), SRD5B1 (5β-reductase type 1), 5α-DHP (5α-dihydro progesterone), 5β-DHP (5β-dihydro progesterone), 3α-HSD (3α-hydroxysteroid dehydrogenase), 5α-THP (3α-hydroxy-5α-pregn-20-one), 5β-THP (3α-hydroxy-5β-pregn-20-one).

* It is not established that SRD5A3 is involved in progesterone metabolism in normal tissue.

** 3α-HSD type 1-4 are all located on the same gene map locus.
Two isozymes of 5α-reductase in humans were detected by Russell et al. in 1993; the 5α-reductase type 1 (SRD5A1) and 5α-reductase type 2 (SRD5A2) (67). The homology between the two isozymes of 5α-reductases is weak. The two 5α-reductases have different enzyme kinetic parameters, tissue expression and chromosomal localization. The tissue expression is not completely known and expression varies between target tissues and period of life. The SRD5A1 occurs in liver, skin and in the ovary, for example in the corpus luteum (70, 71). SRD5A2 is expressed mainly in the human prostate and androgen-dependent organs and in the liver, skin and pituitary (70, 72, 73). Hanning et al. in 1996 investigated the expression of the two 5α-reductases in human ovarian follicles, stroma and corpus luteum (CL). They concluded that the ovary apparently only expresses the 5α-reductase type 1 and that the expression was higher in the CL than in the surrounding ovarian tissue (70).

In a study with normally menstruating healthy women Ottander et al. in 2005 concluded that the corpus luteum of the human ovary express both 5α-reductase type 1 and 5β-reductase (55). In addition, the 5β-reductase is expressed in the human liver (74).

3.6 Estrogen

Estrogen therapy alleviates hypoestrogenic symptoms, and stabilizes and prevents the occurrence of osteoporosis (39). Unopposed estrogen treatment (daily use of estrogen without the addition of progestins) is a risk factor for endometrial hyperplasia and carcinoma (39, 52, 75, 76). Adding a progestin to estrogen significantly reduces the risk of endometrial hyperplasia (77). The risk of endometrial cancer in women treated with unopposed estrogen increases with prolonged treatment duration. Less than a year of solely estrogen use increases the relative risk to 1.4, and the risk estimate for more than 10 years of use was 9.5 (76). Estrogens vary in dose equivalency and metabolic effect on different tissues and organs. Ethinyl estradiol in dosages of <0.010 mg/day and 17β-estradiol in dosages of 0.5–1.0 mg/day are regarded as low-dose therapies (52). A schematic classification of different estrogens regarding the source and clinical use are shown in Table 3.

<table>
<thead>
<tr>
<th>Estrogens</th>
<th>Source/clinical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethinyl estradiol</td>
<td>Synthetic/combined oral contraceptives</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Endogenous/vaginal preparations, HRT</td>
</tr>
<tr>
<td>Estrone</td>
<td>Endogenous/none</td>
</tr>
<tr>
<td>Estriol</td>
<td>Endogenous/vaginal preparations</td>
</tr>
</tbody>
</table>

Table 3. Different estrogens, source and clinical use.
3.7 **GnRH-receptor agonists**

GnRH-agonists are used in treatments of different sex-hormone-dependent conditions, such as endometriosis, premenstrual dysphoric disorder (PMDD), uterine leiomyomas, for treatment of hormone-dependent tumours (39) and in the prevention of menstrual-cycle-related porphyria attacks when symptoms are confined to the luteal phase of the menstrual cycle (26, 33).

By changing the amino acid composition, the half-life of many synthetic GnRH-agonists is prolonged. The GnRH-agonists can be administered by either intranasal absorption or subcutaneous/intramuscular injections/implants (39). GnRH-agonist treatment inhibits gonadotropins, ovulation, and decreases serum sex steroids (49). This inhibition is reversible when treatment is interrupted. To maintain effective drug concentrations, nasal sprays are administered 2–6 times per day, subcutaneous/intramuscular depot injections are administered monthly or every second/third month. After 1–3 weeks, the pituitary is desensitized (GnRH receptor down-regulation), gonadotropin secretion is suppressed, and a hypogonadotropic state is established. During the first 1–3 weeks of GnRH-agonist treatment an initial agonistic effect, with elevated levels of FSH, and LH (the flare-up effect) is seen. To minimize the flare-up effect it is preferable to start down-regulation of the pituitary in the mid luteal phase, but down-regulation can also be initiated on the first or second day of the menstrual bleeding (39). After 2–3 weeks of down-regulation, menopausal levels of s-estradiol and s-progesterone are reached and menopausal symptoms may occur.

3.8 **Steroid add-back**

GnRH-agonist treatment induces a pharmacological hypogonadotropic state. Several adverse effects are seen during this treatment, for example hot flushes, mood disturbances, insomnia, decreased libido and vaginal dryness. These adverse effects can make GnRH-agonist treatment intolerable for some women. In addition, the risk of osteoporosis increases during long-term treatment with GnRH-agonists (26).

Add-back with estrogens in low doses can ameliorate menopausal symptoms and also protect from osteoporosis. Estrogen deficiency is a well known cause of bone loss (78). Early menopause (menopause before the age of 45 years), is statistically associated with the presence of fractures during lifetime, after age 50 and after menopause. Especially at older age, early menopause is an important predictor of fractures (79).

The sole purpose of progesterone/progestin add-back is endometrial protection in women with an intact uterus.
4 Hormonal contraceptives

The estrogen component in oral contraceptives up to now mostly is synthetic ethinyl estradiol. Oral contraceptives containing 50 µg ethinyl estradiol or more are known as a “first-generation oral contraceptives”. Oral contraceptives containing less than 50 µg ethinyl estradiol are known as low-dose oral contraceptives (39), e.g. “second-generation contraceptives”.

There are different progestins in different oral contraceptives.

Natural progesterone cannot be used in oral contraceptives because of high first-pass effect (extensive metabolism in the gastrointestinal tract and the liver, and high and individually variable concentrations of circulating metabolites). Data on the potency of various progestins are tissue-specific and cannot be generalized; moreover data of various clinical trials differ greatly (50). Prolonged progestin efficacy is needed in oral contraceptive treatments. Progestins in oral contraceptives are usually from the 19-nor testosterone family, for example, levonorgestrel (LNG), norgestrel (with its active isomer levonorgestrel), lynestrenol, norethisterone acetate (NETA), norgestimate and desogestrel. All these progestins have some androgenic properties (39). Progestins from the 17α-hydroxyprogesterone family are, for example, medroxyprogesterone acetate (MPA) and cyproterone acetate. These are more closely related to the natural progesterone than 19-nor steroids, and they also have less androgenicity.

All these progestins are regarded as porphyrinogenic (80).

5 Sex steroids in acute porphyria

5.1 Background

Sex steroids are metabolized by heme-dependent enzymes in the liver. This results in increased demands for production of heme proteins, which can induce porphyria attacks. All estrogens, progesterones and progestins are metabolized in the liver by cytochrome P450 enzymes. Heme is a key constituent in heme proteins, for example in hemoglobin and in the cytochrome P450 enzymes (1). In AIP, progesterone and progestin treatments are regarded as provoking attacks (80). Progesterone and progestins are on the list of unsafe drugs in acute porphyria in the USA (1).

Estrogens have been regarded as harmful in acute porphyria. However, transdermal low doses of estrogens have been used successfully to prevent side effects of GnRH-agonist treatment in women with menstrual-cycle-related attacks (26).

According to some authors hormonal replacement therapies for menopausal symptoms can be permitted (22).
5.2 Hormonal contraceptives

Benefits from treatment with oral contraceptives in preventing menstrual-cycle-related porphyria attacks are reported from studies and case reports (18, 22, 81, 82) but attacks can also be induced by contraceptives (1, 83, 84).

In three case reports benefits were reported in preventing menstrual-cycle-related porphyria attacks. In the first one, the authors found indications that suppressed ovulation by natural causes or by administered exogenous estrogens prevented menstrual-cycle-related AIP attacks in one woman. This raised the question whether both endogenous progesterone and administered exogenous progesterone might provoke AIP attacks (23). In the second case report, three women were successfully treated with combined oral hormonal contraceptives (81). In the third case series, three women were treated with either combined oral hormonal contraceptives or contraceptives containing only progestins with improvement regarding menstrual-cycle-related porphyria attacks (82).

However, recent studies have shown that menstrual-cycle-related porphyria attacks can be prevented by oral contraceptive pills. In a follow-up study of acute porphyria, about one third of women with AIP and VP (n=95) had cyclical symptoms suggestive of porphyria. Sex-hormone preparations were used in 46%. The reason for treatment was menopausal symptoms in 7 women and contraception in the others. Severe acute menstrual-cycle-related attacks were successfully prevented with oral hormonal contraceptives. The treatment with sex steroids provoked symptoms suggestive of porphyria in 14% of the women. In two women (4.5%) an acute attack requiring hospitalization was seen (22). According to the study above, the authors conclude that oral contraceptives could be allowed under supervision in women with acute porphyria but only in the quiescent phases, and also that low-dose hormonal contraceptive pills can be an option for prevention of menstrual-cycle-related porphyria attacks (18).

In contrast to this, several researchers discourage the use of oral combined hormonal contraceptives and also contraceptives with progestins alone, because of the risk of severe porphyria attacks (1, 83, 84). In Sweden, progestins are considered to be a high-risk drug in porphyria. It is recommended that hormonal therapies are prescribed in cooperation with a porphyria specialist. Hormonal contraceptives are considered porphyrinogenic, and are not recommended in Sweden according to the European drug database (80).
6 Prophylaxis and therapies

To prevent AIP attacks early detection of gene carriers in kindred families is essential and should preferably be performed before puberty. AIP gene carriers should have counselling about lifestyle factors, drugs, and environmental factors to avoid attack-precipitating factors.

6.1 Prophylaxis
Counselling of gene carriers has improved the possibility to remain asymptomatic. Drugs are among the most important precipitating factors. Lists of safe and harmful drugs are available at the drug database for acute porphyria (80). Patients are recommended to join patient porphyria associations for information about drugs and lifestyle factors (85). Porphyria prognosis has improved during the last 40 years. Better prevention/information has contributed to this improvement (26).

6.2 Therapy of acute attacks
Removal of precipitating factors and unsafe medications is necessary, as well as restoring nutritional status.

Mild attacks of porphyria can be treated with increased carbohydrate intake. In treatment of attacks glucose infusion is well-documented in mild and moderate attacks, but treatment response varies (26).

Intravenous heme arginate therapy is the most effective treatment in acute porphyria attacks (18, 26, 27, 86, 87). In severe acute attacks heme arginate treatment should be started as soon as possible. After the introduction of heme arginate in therapy in 1971, mortality in the acute phase has become extremely rare.

Acute therapy usually includes β-adrenergic blockers for hypertension and tachycardia, antiemetics for nausea and vomiting, mainly opiates as painkillers and consideration of seizure precautions. Intravenous fluids are used to correct electrolytes, mainly low levels of sodium and magnesium, and to increase caloric intake. Medications known to be safe in acute porphyrias must be chosen. Hospitalization is often necessary during acute attacks.

6.3 GnRH-agonist treatment
GnRH-agonist treatment has been considered a safe alternative in preventing menstrual-cycle-related AIP attacks (33).

High-dose GnRH-agonist treatment can prevent menstrual-cycle-related porphyria attacks by suppressing endogenous production of sex hormones (30, 33, 83, 88). The therapy is reversible and when the treatment is interrupted ovulations rapidly return (89).

At start of GnRH-agonist treatment there is an initial but transient hormonal stimulation, a flare-up reaction (39). Increased AIP symptoms after about
two weeks from start of GnRH-agonist treatment are seen in some women, but this deterioration is transient (33, 90).

Treatment with GnRH-agonists in women with frequent luteal-phase-related porphyria attacks is recommended by the American Porphyria Foundation (an expert panel on acute porphyrias) (26). The panel furthermore suggests a low-dose estrogen add-back for preventing menopausal symptoms. Bone density measurements and gynaecological examinations are recommended every 6th month during treatment (26). Low-dose GnRH-agonist therapy in preventing menstrual-cycle-related porphyria attacks may be a treatment option to maintain some endogenous estrogen production (33). If high-dose regimes of GnRH-agonists are given, add-back with low doses of estrogens is advocated (1). Add-back therapy with low-dose estrogen patches is recommended, as the first liver passage is avoided, with reduced risk of inducing porphyria attacks (26). To our knowledge there are only a few reports published of GnRH-agonist treatment combined with estrogen add-back in the treatment of menstrual-cycle-related AIP (91, 92).

### 6.4 Alternative therapies

In severe situations of selected patients, liver transplantation is a possibility to cure AIP. In a 19-year-old woman with severe AIP and frequent, not menstrual-cycle-related attacks, a liver transplantation was successful. Treatment with liver transplantation normalized her urinary ALA and PBG levels within 24 hours and completely eliminated her recurrent neurological attacks. Her quality of life was good 1.5 years after the transplant (93). Up until today, nine liver transplantations in acute porphyria have been reported, among them two patients from Sweden (94).

Gene therapy for the most severely stricken AIP patients may be an option in the future; such therapies are under development (95, 96).

### 7 Associated diseases, late complications

AIP gene carriers are at increased risk of developing long-term complications such as hypertension, renal dysfunction (1, 17, 97, 98) and hepatocellular carcinoma (99-103). Some patients may experience chronic neuropathic pain, pareses, depressions, and also have an increased risk of suicide. In a follow-up study from the United States the mortality rate in MAIP was three times that of the general population (104). However, prognosis has improved since hematin treatment became available.
8 Hepatocellular carcinoma

8.1 Prevalence

Hepatocellular carcinoma (HCC) is the third most common cause of cancer deaths worldwide (105). The highest global incidence of HCC is seen in East Asia and sub-Saharan Africa, with 50–100 cases per 100 000 population. A relatively low incidence of HCC is seen in Scandinavia, Canada and the United States, less than 5 cases per 100 000 population. In Sweden the annual incidence of HCC is 4.3 cases per 100 000 population (male:female 5.8:2.8) (106). HCC is rare before 50 years of age in North American and Western European populations; however, the incidence has increased in the last two decades. Overall, HCC incidence increases with age. HCC primarily affects people from 65 years of age and older. It is also more common among men. The reason for the male preponderance is unclear.

8.2 Risk factors

The single major risk factor for development of HCC is cirrhosis of the liver (105). Liver cirrhosis is present in 80–90% of HCC cases (107). Hepatitis B (HBV) and hepatitis C (HCV) virus account for the majority of liver cirrhosis and HCC worldwide (108). Other global major risk factors for HCC are toxic (alcohol and aflatoxins), metabolic (diabetes, non-alcoholic fatty liver, hemochromatosis) and immune-related (primary biliary cirrhosis, autoimmune hepatitis) (105, 107). In Sweden the prevalence of HBV and HCV infections is low, as is the exposure to aflatoxins.

AIP is a known risk factor for HCC (99-103, 109, 110). The risk of HCC is also increased in patients with VP (22, 111) and in hereditary coproporphyria (102).

8.3 Prognosis

Most HCC patients are diagnosed at advanced tumour stages that preclude radical treatments. Several classification systems are available for tumour staging in HCC. In recent years the Barcelona Clinic Liver Cancer (BCLC) classification has been preferred for clinical management of HCC, including tumour status, liver function (according to Child-Pugh A-D criteria) and the performance status (the general health status) (112, 113). HCC detected after onset of symptoms has a poor prognosis (0–10% 5-year survival). Small tumours can be cured with appreciable frequency, 50% disease-free five-year survival (107). The natural course of early HCC is unknown (107). In recent years early detection and new treatments have improved the outcome of HCC (113).

8.4 Current treatments and prevention

Treatment possibilities of HCC depend on tumour stage, liver function and general health status. Curative treatments, such as liver resection, percutaneous ablations and liver transplantation are options to improve survival. In 10–20% of patients with HCC, surgical resection and radiofrequency ablation (RFA) can be a treatment possibility, or constitute a bridge to liver transplantation (114). Palliative treatment, i.e. transarterial
chemoembolization, does not aim to cure but can increase quality of life (107).

Surveillance programmes in the population at risk are recommended in prevention of HCC, for example in established liver cirrhosis, HBV and HCV carriers, genetic hemochromatosis, primary biliary cirrhosis and autoimmune hepatitis (107, 115). Screening for HCC should be performed by ultrasonography. In areas with high prevalence of HBV infections, an HBV vaccination programme of infants has reduced the HCC incidence (116). HBV vaccination was the first vaccination programme for preventing cancer.
Aims of the study

The aims of this thesis were:

- To describe the clinical expression of acute intermittent porphyria (AIP) in women in northern Sweden, their experiences of hormonal contraception, of hormone replacement therapies and of pregnancies.

- To describe benefits and side effects of gonadotropin-releasing hormone (GnRH)-agonist treatment in the prevention of menstrual-cycle-related porphyria attacks.

- To measure serum concentrations of progesterone, estradiol, allopregnanolone and pregnanolone in the follicular and the luteal phases of the menstrual cycle in women in fertile age with manifest and latent AIP, and to compare with healthy controls.

- To evaluate the benefit of screening for hepatocellular carcinoma (HCC) in AIP gene carriers >55 years of age and to estimate the annual incidence of HCC in this patient group.
Materials and methods

All studies were conducted at the Department of Clinical Science, Obstetrics and Gynecology, and at the Department of Public Health and Clinical Medicine, Family Medicine, Umeå University, Umeå, Sweden. The studies were approved by the Research Ethics Committee, Umeå University, Sweden. For a detailed account of material and methods the reader is referred to the individual papers.

Study subjects and methods

Paper I

Subjects All women (n=190) ≥18 years in northern Sweden with DNA-diagnosed AIP in 1995 were invited to the study, and 166 women participated (87%). Manifest AIP was seen in 91 women (55%), mean age 52 years (19–76). The latent AIP group consisted of 75 women, with a mean age of 40 years (19–80).

Methods This is a retrospective population-based study, with questionnaires containing closed and open questions. All women were recruited from the previous Norrland study including the four northernmost counties in Sweden (17). The main topics focused on description of AIP symptoms, experience of AIP attacks, pregnancies, miscarriages, use of hormonal contraceptives, and use of hormone replacement therapy for climacteric symptoms.

Paper II

Subjects A total of 16 women with DNA-diagnosed acute porphyria and frequent menstrual-cycle-related porphyria attacks were invited to participate in this retrospective follow-up. They had all been receiving GnRH-agonist treatment initiated at the University Hospital in Umeå during the years 1984–2000. Fourteen women participated, of whom 13 women had AIP and one woman had VP.

Methods This is an explorative follow-up study of the experience of GnRH-agonist treatment in acute porphyria for women with severe or very severe menstrual-cycle-related porphyria attacks. The follow-up was based on questionnaires, interviews and medical records. The follow-up period was 3–16 years (mean 8 years).

The main focus was on porphyria symptoms before and during GnRH-agonist treatment, use of hormonal add-back therapy and the subjective experience of GnRH-agonist treatment and add-back therapies. One woman was not alive at the time of follow-up, but data from case records were sufficient to keep her included.
GnRH-agonist treatment was initiated on the first day of menstrual bleeding. Routes of administration were intranasal, subcutaneous or intramuscular depot injections. Most of the women had intranasal buserelin acetate in a daily dose of 900 µg with an intra-individual dose range of 25 µg once every second day to 1350 µg daily. Four women changed therapy from intranasal to intramuscular or subcutaneous injections. Three women received intramuscular depot injections of triptorelin (3.75 mg monthly) and one woman received subcutaneous buserelin injections.

Estradiol add-back was mostly administered in low-dose patches (25–50 µg/24 hours, but occasionally 100 µg/24 hours). Less often estradiol was administered by the oral route. The progesterone add-back regimes varied and different regimes were tested. Mostly, natural progesterone by the vaginal route was used. Vaginal progesterone was given in a dose of 200 mg daily for 14 days every third month in four women and in a fifth woman a dose of 400 mg was used. Transdermal administration of norethisterone acetate (NETA 62.5–250 µg/24 hours) or oral administration of medroxyprogesterone acetate (MPA 5–10 mg 10 days every month) were tried in four women. In two women, the progesterone add-back was given either as a hormonal intra-uterine device (levonorgestrel 20 µg/24 hours) or as an intramuscular depot injection of MPA (25 mg as a single dose).

**Paper III**

**Subjects** Forty-seven women from the north of Sweden with DNA-diagnosed AIP, who had participated in study 1, were asked to give repeated blood samples for measuring serum levels of progesterone, estradiol, allopregnanolone and pregnanolone during a full menstrual cycle. In total, 32 women with AIP participated (14 with MAIP, 18 with LAIP), mean age 37 years (27–48). For comparison, a control group of 20 healthy women was recruited, mean age 34 years (25–40). The study was performed in the years 1995–2000.

**Methods** The AIP group and the controls registered menstrual cycle days prospectively using a menstrual calendar in 1–2 consecutive menstrual cycles. Oral and written instructions for blood sampling were given to the AIP women and the control group. For the AIP group, blood samples were drawn at the local health centre, according to written recommendations for routine sampling at the chemistry laboratory of the University Hospital in Umeå, Sweden. Serum-estradiol and serum-progesterone were sent to this accredited laboratory for analyses. Serum-allopregnanolone and serum-pregnanolone were frozen and sent to Umeå Neuroendocrine Research Centre for analysis.

In the control group, blood samples (s-progesterone, s-estradiol, s-allopregnanolone and s-pregnanolone), were drawn at Umeå Neuroendocrine Research Centre and all analyses were performed in this research laboratory.

In the AIP group, blood samples (s-progesterone, s-estradiol, s-allopregnanolone and s-pregnanolone) were drawn in the morning twice in the follicular phase (days 5–12 in the menstrual cycle), and three times in the
luteal phase (days 18–26). Day one in this schedule was the first day of menstrual bleeding. Ovulation during ongoing menstrual cycle was established with s-progesterone levels ≥15 nmol/L in the luteal phase. For one woman with a late sample in the luteal phase, an s-progesterone of 8.7 nmol/L the day before the onset of the next menstruation was accepted as an ovulatory value. For all AIP women, s-progesterone and s-estradiol concentrations according to sample day were used for dating ovulation (117, 118). In the control group, blood samples for analyses of s-progesterone, s-estradiol, s-allopregnanolone and s-pregnanolone were drawn in the morning on six occasions during the menstrual cycle. Blood samples were scheduled to coincidence with the follicular phase (days 4±1–12±1), and the luteal phase sampling was scheduled according to a positive LH assay (postovulatory day 4±1–12±1). In the control group, ovulation was confirmed using a urinary testing with Clearplan (Unipath, Bedford, UK) which predicts the pre-ovulatory rise in LH concentrations, and an ovulatory sample was taken on that day, or the day after a positive urinary LH test, e.g. when the estradiol levels have already declined.

Serum estradiol and progesterone for both the AIP and the control group were analysed according to manufacturer’s instructions, using commercially obtained kits at an accredited chemistry laboratory at Norrland University Hospital in Umeå, Sweden.

Analysis of serum concentrations of allopregnanolone and pregnanolone in both the AIP and the control group were performed at our research laboratory, Umeå Neuroendocrine Research Centre. In brief, the samples (0.4 ml) were extracted with diethylether (Merck). Allopregnanolone and pregnanolone were separated from cross-reacting steroids with celite column chromatography. Allopregnanolone was measured by radio immunoassay (RIA) using a polyclonal rabbit antiserum raised against 3α-hydroxy-20-oxo-5α-pregnan-11-yl carboxymethyl ether coupled to bovine serum albumin, made by R. H. Purdy (The Scripps Research Institute, La Jolla, CA, USA) (58, 119). The sensitivity of the assay is 25 pg with an intra-assay coefficient of variation for allopregnanolone of 6.5% and an inter-assay coefficient of variation of 8.5%.

For pregnanolone measured by RIA the antiserum was raised against 3α-21-dihydroxy-5β-pregnan-20-one-21-hemisuccinate coupled to bovine serum albumin in a rabbit by Dr Robert H. Purdy (The Scripps Research Institute, La Jolla, CA, USA). The antibody was used at a dilution 1:2300 and the solution was prepared using [11,12-3H]pregnanolone custom-synthesized by NEN (New England Nuclear, Boston, MA, USA). The recovery of pregnanolone was 93%. The sensitivity of the assay is 25 pg, with an intra-assay coefficient of variation of 5.2% and inter-assay coefficient of variation of 7.8%. The results were compensated for recovery (120).

These methods are described in detail in previous reports from our laboratory (58, 120).
Paper IV

Subjects A mean 62 of AIP gene carriers (76%) >55 years of age in the county of Norrbotten (n=81) participated in this regular screening programme for HCC during the years 1994–2009. They comprised one third of the AIP gene carriers in the four northern counties in Sweden in the same age group (n=180).

Methods This is a prospective study with case-control approach carried out in northern Sweden over 15 years. All HCC diagnoses in the four northernmost counties in Sweden during the study period were registered using the Swedish cancer registry and cross-referenced to the AIP patients in this region.

Two study groups were defined; group A consisted of AIP patients who had had repeated screening within <2-year intervals, and the control group (group B) consisted of AIP patients not screened within <2-year intervals.

The control group contained three subgroups; B1 consisted of patients who have never been screened, B2 consisted of patients with HCC diagnosed on their first screening occasion, and group B3 consisted of patients with screening intervals ≥2 years or dropouts.

The screening included radiological examination of the liver by contrast-enhanced computed tomography (CT), ultrasonography or magnetic resonance imaging (MRI), relevant blood tests for liver function and urinary porphyrin precursors.

We compared group A with the control group with respect to sex, LAIP or MAIP, age at diagnosis, levels of U-ALA, U-PBG and serum alpha-fetoprotein (AFP) at diagnosis, tumour burden at diagnosis, kind of treatment, and survival time.

A questionnaire was used on each screening occasion. The questionnaire focused on liver diseases, alcohol consumption and occupations with special focus on exposure to liver-toxic substances.

We also estimated the annual incidence of HCC in the AIP group aged >55 years in the northern region, and calculated the incidence rate ratio by comparing the annual incidence of HCC in the AIP group aged >55 years with the normal population in the area at the same time and age.
Statistics

In paper I, the chi-squared test and Fisher’s exact probability test were used in statistical analyses of discrete variables.

In paper II, the sample was a small heterogeneous population. Where applicable, medians and proportions are presented and the chi-squared test and Fisher’s exact probability test were used for nominal variables.

In paper III, mean ± standard error of the mean (SEM) were used in the figures to illustrate how menstrual cycle patterns of estradiol, progesterone, allopregnanolone and pregnanolone differed between groups. In the statistical testing mean follicular and luteal phase values were calculated for each individual. Differences in serum levels of progesterone, estradiol, allopregnanolone and pregnanolone between the groups were tested with Kruskal-Wallis test. The SPSS statistical package (version 18) and Microsoft Office Excel 2003, SP3 were used for analysis. For analysing differences between menstrual-cycle phases, related-samples, Wilcoxon signed ranks test was used and ANOVA tests were used to analyse SEM and p-values within the AIP and the control groups.

In paper IV the chi-squared test and Fisher’s exact probability test were used in statistical analyses for nominal variables. Significant difference in survival time was calculated by Kaplan Meier log-rank test. The SPSS statistical package (version 11) for Mac OSX was used.

In all studies, a p-value<0.05 was considered statistically significant.
Results

Paper I

Experience of attacks. The most frequent symptom of AIP was abdominal pain (99%). Other symptoms were constipation (57%), fatigue (54%), vomiting (51%), pain in the limbs (43%) and muscle weakness (35%). Pain in the back, depression, sensory impairment, confusion, anxiety and paralysis were also seen. Other symptoms such as palpitations (6%), insomnia (3%), and diarrhoea (2%) were also reported. The mean age of the first attack was 25 years (range 11–49) and 42% of the women suffered the first attack in the range 20–29 years. One third had their first attack before the age of 20 years, 40% of the women reported >20 attacks, and 41% reported only 1–3 attacks. Severe porphyria attacks were reported in 83% of MAIP. In MAIP, menstrual-cycle-related attacks were seen in 39%. Reduction of AIP symptoms in the climacteric transition was reported by about half of the postmenopausal women, while the remainder reported status quo. Hospital admission during porphyria attacks was reported by 80% of the women. Chronic AIP symptoms were seen in about 20%.

Pregnancy and miscarriages. Pregnancy was reported by 85% in the AIP study group (n=140). In the MAIP group, pregnancy was reported by 78 women and in the LAIP group by 62 women. In total there were 257 pregnancies in the MAIP group and 142 in the LAIP group. In the manifest group the mean number of pregnancies was 3.3 (range 1–10) and 2.4 (range 1–6) in the latent group. No change of the porphyria frequency or severity was reported in 67% of the women with MAIP, in 23% there was amelioration of symptoms, and impairment was reported by only 10% in this group. Miscarriages were more common in the MAIP group, i.e. in 65 of 257 pregnancies (25%) compared with 22 of 142 pregnancies (15%) in the LAIP group.

Hormonal contraceptives. Oral contraceptives were used by 58% of the AIP gene carriers (n=94) and another 10% (n=16) had experience of other hormonal contraceptives such as a hormonal intra-uterine device (levonorgestrel 20 µg/24 hours) or depot injections of medroxyprogesterone acetate (MPA). Mean age when therapy started was 22 years (range 14–47). The majority had used oral contraceptives containing a combination of ethinyl estradiol and progestin. In the MAIP group mean treatment duration was 3.9 years (0.1–14 years) and in the LAIP group 4.2 years (0.1–20 years). Two-thirds continued to use oral contraceptives for more than one year.

In the MAIP group, 12 of the 50 women who had used oral contraceptives (24%) associated oral contraceptives with AIP attacks and in nine of these women the use of oral contraceptives precipitated their first attack. The 12 women were severely stricken by their AIP, i.e. with frequent and severe attacks. Three women reported exacerbation of AIP attacks when using oral contraceptives. One woman experienced improvement in AIP symptoms during treatment with combined oral contraceptives.
Nine women used hormonal contraception with only progestins. Treatment duration was 1–4 years in both the MAIP and the LAIP group. In two women attacks were provoked. Ten women were using a hormonal intra-uterine device. One of these women reported improvement of AIP symptoms. Treatment duration was 1–4 years. Four women were using depot injections of MPA and in one of them AIP attacks were provoked.

**Hormonal replacement therapy to reduce menopausal symptoms.** Systemic hormonal replacement therapies (HRT) were used by 25% of women ≥45 years of age (n=22). The treatment time was ≤1 year in one third and ≥5 years in another third of these women. In the MAIP group (n=15), 12 reported their premenopausal AIP-attacks as severe or very severe. No porphyrin attacks were associated with the use of HRT in these women. In HRT the transdermal route (patches) was most frequently used and attacks were not provoked, see Table 4. In six women in whom oral hormonal contraceptives in their earlier ages had precipitated attacks, HRT for menopausal symptoms was later used without provoking porphyrin attacks.

<table>
<thead>
<tr>
<th>Type of AIP</th>
<th>AIP total (%)</th>
<th>Latent AIP (%)</th>
<th>Manifest AIP (%)</th>
<th>Attack provocation (%)</th>
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<tr>
<td>Number of gene carriers</td>
<td>166</td>
<td>75</td>
<td>91</td>
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<tr>
<td>Oral contraceptives</td>
<td>94 (58)</td>
<td>44 (59)</td>
<td>50 (57)</td>
<td>12 (13)</td>
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<td>Combination contraceptives</td>
<td>85</td>
<td>39</td>
<td>46</td>
<td>10</td>
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<td>Progesterone contraceptives</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>2</td>
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<tr>
<td>Other hormonal contraceptives</td>
<td>16 (10)</td>
<td>11 (15)</td>
<td>5 (6)</td>
<td>1</td>
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<tr>
<td>Intra-uterine device (Levonova®)</td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>0</td>
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<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Not recalled by the patient</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>HRT for climacteric symptomsa</td>
<td>22 (25)</td>
<td>7 (30)</td>
<td>15 (23)</td>
<td>0b</td>
</tr>
<tr>
<td>Patch (estradiol/norethisterone)</td>
<td>12</td>
<td>2</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Patch (estradiol) + tabl progesterone</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Oral (estradiol + progesterone)</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0b</td>
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<tr>
<td>Not recalled by the patient</td>
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<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>HRT to treat vaginal dryness</td>
<td>26 (28)</td>
<td>7 (30)</td>
<td>19 (29)</td>
<td>0c</td>
</tr>
<tr>
<td>Local administration of estrogen</td>
<td>20</td>
<td>6</td>
<td>14</td>
<td>0c</td>
</tr>
<tr>
<td>Oral (estriol)</td>
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<td>5</td>
<td>0</td>
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<tr>
<td>Not recalled by the patient</td>
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<td>0</td>
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</tr>
</tbody>
</table>

HRT, hormonal replacement therapy.

aPercentage of women who have used HRT refers to age-group ≥ 45 years (n = 89, latent AIP: manifest AIP, 23 : 66).

bSlight muscle pain and weakness during 1-month treatment reported by a 52-year-old woman, but not precipitating an AIP attack.

cSlight abdominal pain reported by two women, aged 42 and 63 years, but not precipitating an AIP attack.
**Hormonal treatment to remedy vaginal dryness.** In 26 AIP gene carriers treatment to remedy vaginal dryness were used (MAIP=19, LAIP=7). Local vaginal administration of estradiol or estriol was most often used (n=20), and in five women oral estriol. Treatment had been used by 28% of the women aged ≥45 years (n=26). The treatment time was ≤1 year in 46% and >5 years in 8% of the women. In both groups the mean duration of treatment was 2.5 years. No acute attack was provoked by the treatment, see Table 4.

**Paper II**

We evaluated benefits and adverse effects of GnRH-agonist treatment in 14 women with acute porphyria. Their median age at the first attack was 26 years (14–43), and the number of porphyria attacks prior to the GnRH-agonist treatment was 10–100. They had suffered from porphyria attacks six years (1–17), before GnRH-agonist treatment started and their age at the follow-up was 43 years (22–55). Overall, 11 women of the 14 benefited from GnRH-agonist therapy, see Figure 8. Among the women benefiting from treatment, four were almost relieved of attacks and in seven women attacks came sparsely and/or with reduced symptoms. In one woman there was only an initial symptom relief from GnRH-agonist therapy and in two women no effect was seen.

![Figure 8](http://example.com/figure8.png)

**Figure 8.** GnRH-agonist treatment effect in 14 women with menstrual-cycle related acute intermittent porphyria attacks.

When GnRH-agonist therapy was interrupted, four women reported worsening of porphyria symptoms and three resumed therapy after which porphyria symptoms declined. One woman was almost free of porphyria symptoms during GnRH-agonist therapy for two years but stopped treatment when she travelled abroad and had no access to medication. One month later at the age of 31 years she died from a severe AIP attack.

Four women ended GnRH-agonist treatment within a year, and they comprised all women who did not receive add-back. In six women, treatment
duration was more than four years and in four of these women the treatment was ongoing at the time of this evaluation.

Eleven women received hormonal add-back therapy. In 10 of these women, either solely estradiol add-back therapy or a combination of estradiol and progesterone add-back was used. One woman received only progesterone add-back.

Porphyria attacks were triggered in two women after estradiol add-back and in 5 out of 9 women after progesterone add-back usage. In total 9 women received progesterone add-back.

In one woman hormonal add-back was finished due to attack provocation. In this woman a low dosage of GnRH-agonist was used instead.

**Paper III**

The main result in this study is that serum concentrations of the 5α-metabolite allopregnanolone are significantly lower in the whole AIP group than in controls. This was seen in both phases of the menstrual cycle but was most prominent in the luteal phase, \( p<0.001 \), and in the follicular phase \( p=0.021 \); see Table 5 and Figure 9.

Secondly, s-pregnanolone levels increased significantly in both controls and the whole AIP group from the follicular to the luteal phase \( p=0.003 \) and \( p=0.018 \), respectively, see Figure 9. No significant differences were seen in either of the two separate menstrual-cycle phases between the groups (the AIP group and the control group), see Table 5 and Figure 9.

Thirdly, follicle phase serum-estradiol and progesterone did not differ between AIP and controls but, in the luteal phase levels were significantly higher in the AIP group, see Table 5 and Figure 10.
### Table 5. Sex steroid levels for subjects with manifest acute intermittent porphyria (MAIP) and latent acute intermittent porphyria (LAIP) compared with controls in the follicular and the luteal phase of the menstrual cycle.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Variables</th>
<th>P nmol/L</th>
<th>Allopregnanolone nmol/L</th>
<th>Pregnanolone nmol/L</th>
<th>E2 pmol/L</th>
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</thead>
<tbody>
<tr>
<td><strong>Follicular phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAIP</td>
<td>No. of subjects/samples</td>
<td>14/31</td>
<td>8/13</td>
<td>8/13</td>
<td>14/31</td>
</tr>
<tr>
<td></td>
<td>Mean ±SEM</td>
<td>2.1 ± 0.2</td>
<td>0.48 ± 0.05</td>
<td>0.44 ± 0.05</td>
<td>590 ± 98</td>
</tr>
<tr>
<td>LAIP</td>
<td>No. of subjects/samples</td>
<td>18/42</td>
<td>12/29</td>
<td>12/29</td>
<td>18/43</td>
</tr>
<tr>
<td></td>
<td>Mean ±SEM</td>
<td>2.3 ± 0.2</td>
<td>0.43 ± 0.03</td>
<td>0.38 ± 0.03</td>
<td>470 ± 63</td>
</tr>
<tr>
<td>Control group</td>
<td>No. of subjects/samples</td>
<td>20/50</td>
<td>20/50</td>
<td>19/48</td>
<td>20/50</td>
</tr>
<tr>
<td></td>
<td>Mean ±SEM</td>
<td>1.9 ± 0.4</td>
<td>0.61 ± 0.04</td>
<td>0.37 ± 0.02</td>
<td>340 ± 45</td>
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<tr>
<td>p-value MAIP,</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LAIP vs. control</td>
<td>0.021</td>
<td></td>
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<td><strong>Luteal phase</strong></td>
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<tr>
<td>MAIP</td>
<td>No. of subjects/samples</td>
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<td>7/15</td>
<td>7/15</td>
<td>14/38</td>
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<tr>
<td></td>
<td>Mean ±SEM</td>
<td>28.7 ± 3.4</td>
<td>0.77 ± 0.06</td>
<td>0.61 ± 0.05</td>
<td>490 ± 47</td>
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<td>LAIP</td>
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<td>12/27</td>
<td>12/27</td>
<td>18/46</td>
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<tr>
<td></td>
<td>Mean ±SEM</td>
<td>29 ± 2.1</td>
<td>0.75 ± 0.06</td>
<td>0.59 ± 0.04</td>
<td>430 ± 34</td>
</tr>
<tr>
<td>Control group</td>
<td>No. of subjects/samples</td>
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<td>20/54</td>
<td>20/54</td>
<td>20/54</td>
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<td>Mean ±SEM</td>
<td>21.2 ± 1.8</td>
<td>1.86 ± 0.09</td>
<td>0.70 ± 0.04</td>
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<td>p-value MAIP,</td>
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<td>&lt;0.001</td>
<td></td>
<td>ns</td>
<td>&lt;0.01</td>
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<tr>
<td>LAIP vs. control</td>
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</table>

Mean concentrations and standard error of the mean of progesterone (P), pregnanolone, allopregnanolone and estradiol (E2) are presented in follicular and luteal phase. Number of subjects and number of samples per study group are presented, along with p-values. Differences between groups were tested with Kruskal-Wallis test. Non-significant values are denoted ns.
Figure 9. Top panel. Mean (SEM) allopregnanolone levels in the menstrual cycle for the AIP group and controls in nmol/l. Blood samples are centered on the calculated day of ovulation in AIP patients and in relation to the day of the preovulatory LH surge in controls. As all females did not give blood in all phases, the number of subjects differs between the 6 time points, see Table 5. In the AIP group the periovulatory value is calculated using a method described elsewhere (117). The periovulatory samples in the AIP group were taken just before ovulation and the LH surge while in the control group after the LH surge and ovulation.

Bottom panel. Mean (SEM) levels of pregnanolone in the menstrual cycle for AIP and controls measured in nmol/l, for more sampling details, see Figure 9 top panel.
Figure 10. Top panel. Mean (SEM) levels of estradiol in the menstrual cycle for AIP and controls measured in pmol/l. Blood samples are centred on the calculated day of ovulation in AIP patients and in relation to the day of LH surge in controls. As all females did not give blood in all phases, the number of subjects differs between the 6 time points, see Table 5 for information. In the AIP group the periovulatory value is calculated using a method described elsewhere (117). The periovulatory samples in the AIP group were taken just before ovulation and the LH surge while in the control group after the LH surge and ovulation.

Bottom panel. Mean (SEM) for s-progesterone levels in the menstrual cycle for the AIP group and controls measured in nmol/l; for more sampling details, see Figure 10 top panel.
**Paper IV**

HCC was diagnosed in 22 AIP gene carriers (m:f 12:10) in the four northern counties in Sweden during the study period. This indicates a 64-fold higher HCC risk in subjects with AIP than in the general population at the same age (>50 years) in this region during the same time period. The increase in risk of HCC in AIP gene carriers was 93-fold for female and 53-fold for male AIP gene carriers.

The most common mutation was the high-penetrance AIP W198X, present in 19 subjects. Another mutation with high penetrance, the R173W mutation, was seen in one woman with MAIP and the remaining mutations were R167W and I113T.

In the 22 HCC cases, the mean age was 69 years (59–82 years). Sixteen subjects (73%) had MAIP (m:f 8:8) and 6 subjects (27%) with HCC had never experienced an AIP attack, see Table 6.

All but two patients had elevated levels of U-ALA and U-PBG.

None of the patients in group A had elevated serum alpha-fetoprotein levels.

No patient in group A or in the control group had hepatitis or alcohol abuse. Four patients had liver cirrhosis.

Liver resection was an option in 7/8 patients in group A and in 4/14 patients in the control group (p=0.024). The tumour burden was ≤7 cm in 7/8 of the patients in group A, and in 4/14 in the control group. See Table 6 for further details.
Table 6.
Patient data, screening interval, treatment and survival data from 22 patients with hepatocellular carcinoma and acute intermittent porphyria.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Case</th>
<th>Sex</th>
<th>LAIP Age at</th>
<th>U-PBG</th>
<th>LA</th>
<th>Age at</th>
<th>U-ALA</th>
<th>AFP</th>
<th>Previous Tumour Treatment</th>
<th>Survival months (yrs)</th>
<th>Recurrence</th>
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</table>

Sex: m = men, w = women. LAIP = latent AIP, MAIP = manifest AIP. U-ALA, U-PBG. N = within reference levels, + = above reference levels. Ref. levels; U-ALA < 3.1 mmol/mol creatinine or < 45 µmol/L. U-PBG < 1.2 mmol/mol creatinine or < 11 µmol/L. One missing value. AFP. Alpha-fetoprotein, N = within reference levels, < 20 ng/mL. RFA = Radiofrequency ablation. Survival months. ++ indicates patient alive. * Deceased from pulmonary carcinoma.
The 3-year survival was significantly higher in the regularly screened group (group A, 5 alive 0 deceased) than in the control group (group B, 3 alive 11 deceased), p=0.005. The 5-year survival was also significantly higher for group A (4 alive 1 deceased of 5 eligible) compared to controls (group B, 3 alive 11 deceased), p=0.038. These differences between the two groups are also seen in Figure 11 in the survival analysis.

Figure 11. Survival time for 22 AIP patients with HCC. Dotted line, Group A, repeated screening within 1–2 years. Full line, control group, never screened, first round screened, screening intervals >2 years, dropouts. Case 17 excluded from Group A (deceased from pulmonary carcinoma). Log Rank (Mantel-Cox) p=0.004.
Discussion

Women in fertile age are frequently and severely stricken by AIP attacks. It is well known from case reports and studies that menstrual-cycle-related porphyria attacks are seen especially in the luteal phase (1, 20-24, 30). Inhibition of corpus luteum formation would thus be a treatment for the luteal phase attacks.

**Paper I**

The new and most interesting result in the present study is that 24% of women with manifest AIP (n=12) had experienced porphyria attack provocation when using oral hormonal contraceptives. Furthermore, in 9 of these cases oral hormonal contraceptives were also connected with their first attack and manifestation of the AIP. Attack provocation in this frequency by the use of oral hormonal contraceptives among female AIP gene carriers has not previously been reported. Attack provocation by the use of oral hormonal contraceptives was too high, especially because the prognosis of manifest AIP is worse than LAIP.

**Hormonal contraceptives**

In our follow-up low-dose oral contraceptive with the 19-nor-progestine derivative noretisterone acetate (NETA) was predominantly used (NETA 0.5 mg–1 mg, and ethinyl estradiol 35 µg). One woman with severe AIP reported relief of porphyria symptoms when using combined oral contraceptives. In another woman a hormonal intrauterine device with levonorgestrel (LNG 20µg/24h) was beneficial in preventing attacks. In one woman progesterone depot injections medroxyprogesterone acetate (MPA) improved AIP and in another impaired porphyria.

According to our experience it is not wise to use hormonal contraceptives in AIP gene carriers because of the risk of precipitating porphyria attacks. This is also supported by the drug database in Europe (80), and the USA (26). However, if a woman insists on hormonal contraceptive treatment, a porphyria specialist should be consulted (85). Levels of U-ALA and U-PBG should be monitored before treatment and every week during the first treatment month. If levels increase the treatment should be stopped and a porphyria attack can hopefully be prevented.

**Hormone replacement therapy**

In the present study we found that no AIP attacks were provoked by HRT treatment, neither for women treated for menopausal symptoms nor for women treated with low-dose estrogens to remedy vaginal dryness. The women were all ≥45 years of age.

Interestingly, HRT was used without problems in these postmenopausal women above 45 years of age, although some of them in their earlier, fertile ages had experienced both attack provocation during periods of oral
hormonal contraceptive use and also in some cases had suffered from menstrual-cycle-related attacks. The MAIP group with HRT for alleviating menopausal symptoms (12 of 15 women in our follow-up study) had had severe or very severe porphyria attacks but they did not experience porphyria attack provocation by the HRT.

This indicates that the low sex steroid doses in HRT were not attack-provoking in postmenopausal women. One important factor is that postmenopausal women have lower endogenous steroid hormone concentrations needing metabolism with less demand on increased heme production as a result.

On the other hand, in paper II, in which HRT was given to 11 women as hormone add-back during ongoing GnRH-agonist treatment, 5 of 11 experienced adverse effects of HRT. This could be explained by the fact that this selected group with menstrual-cycle-related porphyria attacks was extremely sensible to sex hormones.

In one study of estrogen-treated postmenopausal women it was advocated that progestins should be taken for at least 10 days per month to reduce the risk of endometrial hyperplasia (121). As the only reason for progestin in HRT is endometrial protection, the aim is to reduce progestin exposure. One approved recommendation for endometrial protection is 20 mg of MPA daily for 14 days every third month (122). This may, however, be difficult to tolerate for patients with AIP.

According to a Cochrane Collaboration review the recommendation is that hormone therapy is given at the lowest effective dose and that treatment should be reviewed regularly (52).

In this review of HRT for postmenopausal women in 2009, the lowest “safe” dose of progesterone or progestins in various regimes added to estrogens was evaluated. The aim of the review was to identify the minimum dose(s) of progestin required to be added to estrogen so that the rate of endometrial hyperplasia was not increased compared to placebo (45 trials were included in the review) (52).

The study with the lowest dosage of progesterone was a randomized double-blind placebo controlled study, in which bone mineral density and risk of endometrial hyperplasia during treatment was investigated. In this study, sequential ultra-low doses of estradiol (orally 0.25 mg/d) and progesterone (orally 100 mg /15 days every 6th month) were used without endometrial pathology (treatment duration of 3 years, n=167) (123).

There is another randomized double-blind placebo controlled study (a 2-year follow-up, n=417), which investigated safety and effectiveness in preventing bone loss of unopposed, very low-dose transdermal estradiol (transdermal estradiol 0.014 mg/d) for women aged 60–80. In this study bone mineral density improved without increasing the rate of endometrial hyperplasia (124).
If deviation from an approved HRT regime is needed, closer monitoring of the endometrial growth and potential symptoms such as irregular bleedings must be observed. If intervals between the progesterone treatment periods are more than three months, more thorough monitoring is recommended. Vaginal ultrasonography for measuring endometrial thickness may be used. Endometrial biopsy is another way to monitor the endometrium.

**Pregnancy**

Pregnancy can exacerbate attacks but is usually well tolerated (1, 22). In the present study we showed that pregnancy was not a major problem for patients with AIP. Pregnancy contributed to attacks in 10%, and in 23% of the women amelioration of porphyria symptoms was reported. Although sex-hormone levels are extremely high during pregnancy, it does not provoke attacks. This may be due to the altered origin of steroids during pregnancy, since steroids are chiefly of placental origin, in contrast to progesterone in the luteal phase which are mainly from the ovary. Large amounts of progesterone are produced only in corpus luteum or the placenta (50). The placenta has a huge steroid production but also a huge metabolizing capacity (38, 39). Nevertheless, when pregnancy is diagnosed, patients with AIP are advised to contact a porphyria specialist.

**AIP attacks**

In our study AIP attacks in most women were reported as severe. Attacks were also reported to be menstrual-cycle-related in 40% of the women. In 20% the first attack appeared before 20 years of age. The numbers of attacks were reduced after menopause among half of the women. In the remainder, status quo was reported. This finding indicates the key role of female sex hormones in AIP.

**Paper II**

The most important result of this follow-up study was that GnRH-agonist treatment reduced and even eliminated acute porphyria attacks in 11 of 14 women with severe or very severe menstrual-cycle-related porphyria attacks. In addition, the study confirmed that the GnRH-agonist treatment can proceed for several years. Because of the long GnRH-agonist treatment periods in our study (six women were treated more than four years with a maximum of nine years), it is necessary to find safe hormonal add-back regimes for preserving well-being and to avoid osteoporosis.

Today recurrent menstrual-cycle-related porphyria attacks are preferably treated with GnRH-agonist therapies and low-dose estrogen patches as hormonal add-back. There are recommendations for gynecological examinations every six months but there are no guidelines for endometrial problems that might occur during therapy or how to manage the increased risk of endometrial hyperplasia (26). The GnRH-agonist therapy in which
ovulation is highly suppressed increases the demands for an appropriate hormonal add-back therapy. Most treatment studies include GnRH-agonists without add-back, and side effects such as menopausal symptoms and bone demineralization are reported. These menopausal adverse effects can be avoided through only partly interrupted ovulation (low-dose GnRH).

**GnRH therapy**

We find it easier to give high doses to achieve relief of menstrual-cycle-related attacks. We started GnRH-agonist therapy with full (high) dosage (busereline 900 µg/day intranasally). When using GnRH-agonist in lower doses there are problems with irregular bleedings and also recurrence of menstrual-cycle-related porphyria symptoms.

Intranasal administration of GnRH-agonists makes individualized doses possible. An advantage of GnRH-agonist treatment is that it is reversible, e.g. when intranasal treatment is interrupted ovulation returns within 4 weeks (125). However, in our experience GnRH-agonist injections were easier to handle than intranasal therapies, with more reliable administration of GnRH. The suppressed ovulation is more stable with this therapy. There are recommendations to reassess the need for treatment after 1–3 years because there are observations that suggest that susceptibility to cyclical attacks may vary over time (26, 33).

**Estrogen add-back**

When a high GnRH dose is given, both menopausal symptoms and the risk of developing osteoporosis is a problem. However, a low dose of GnRH might be a treatment option if steroid add-back provokes porphyria attacks. Porphyria symptoms and menopausal symptoms can both be ameliorated by this strategy because progesterone levels remain suppressed while estrogen levels are incompletely suppressed. This strategy is more difficult to administer and the risk of endometrial hyperplasia cannot be excluded. There is also a risk of recurrence of premenstrual attacks if menstruations return during therapy. An increased GnRH dose is then necessary to restore the therapeautic effect.

In women with acute porphyria and GnRH-agonist treatment for menstrual-cycle-related attacks, menopausal side effects can in most cases be safely prevented by transdermal estrogen patches in low doses (26).

Our experience from this follow-up is that estrogen add-back was easier to administer than progesterone add-back. Transdermal estrogen therapy was preferably chosen for ten women who received GnRH-agonist treatment; one of them also had experience of oral estradiol as add-back. We prefer transdermal treatment with solely estradiol for the first 4–6 months. At treatment start we use a low dose of estradiol; doses can be increased if menopausal symptoms are not relieved and no AIP attacks are provoked. A
review of treatment recommendations for the acute porphyrias also reported a similar strategy, but the estrogen dosage was not discussed (26).

In our experience a low dose (6.25 µg/24 hours), twice a week of solely transdermal estradiol can be used at treatment start. After one month, the dose can be increased to 12.5 µg/24 hours twice a week, if climacteric symptoms persist and no AIP attacks are triggered. The dosages can if needed be further increased. The estrogen add-back-therapy dose was individualized in this follow-up; most commonly 50 µg/24 hours (25–100 µg) was used.

Two women ended estrogen add-back due to attack provocation. In one of these GnRH-therapy ended, but she restarted treatment because of impairment in AIP. To avoid hormonal add-back intranasal GnRH in extremely low doses (buserelin 25 µg every second day) was used with good outcome.

There are data corroborating that reduction of estrogen to menopausal levels is not necessary to achieve prevention of menstrual-cycle-related porphyria attacks (33).

**Progesterone, progestin add-back**

For endometrial protection it is necessary to find safe regimes either for progesterone add-back or regimes to avoid progesterone.

Unopposed estrogen add-back therapies for long periods may cause endometrial proliferation, and risk of endometrial pathology (39, 52, 75, 76).

The increased risk of endometrial hyperplasia has to be considered. If the endometrium is ≤5 mm (with both endometrial layers included at ultrasonography investigation of the uterus), endometrial hyperplasia and carcinoma can be ruled out with good certainty (51). Under these circumstances we are of the opinion that addition of progesterone can be avoided.

There are two strategies for endometrial protection. Firstly by adding progesterone or progestins at regular intervals if no attacks are provoked. In estrogen-only therapies, another way is by ultrasonography controls at regular intervals to evaluate endometrial proliferation and if there are indications of hyperplasia or pathology also adding endometrial biopsies.

To reduce unwanted side effects of progesterone in general, natural progesterone in low doses by vaginal admission is preferable though hepatic first pass is avoided (126).

In a study by Ross et al, estrogen-treated healthy postmenopausal women were receiving sequential vaginal progesterone gel (45 mg, n=15 or 90 mg, n=16) every second day on seven occasions on day 17–27 in a treatment cycle. In the group with 90 mg, none of the women developed endometrial hyperplasia, and in all a secretory transformation of the estrogenized
endometrium was seen. In one woman in the 45 mg group a proliferative endometrium was seen.

Side effects of progesterone/progestin add-back in porphyria are a problem during GnRH treatment. During GnRH-agonist therapy half of the women who were given progesterone/progestin add-back reported attack provocation by this medication. Despite this, we believe that adding progesterone/progestin, seldom and in small doses, preferably as natural vaginal progesterone, is a complement to long-term estrogen therapies in order to protect the endometrial mucosa from hyperplasia.

The treatment regime mostly used in this follow-up was vaginally administered micronized natural progesterone in individualized doses and intervals, in dosages of 200–400 mg/14 days at about three-month intervals. In two women extremely sensitive to progesterone we avoided progesterone add-back, and ultrasonography controls and endometrial biopsies were performed as endometrial check-up.

However, for patients with porphyria, finding the optimal threshold level of progesterone/progestin add-back therapy is a problem. In our opinion micronized progesterone does not provoke porphyria attack as often as progestins in equipotent doses. This is in our opinion because progestins are more difficult to metabolize than endogenous progesterone. We prefer vaginal administration of progesterone to avoid first-passage metabolism in the liver. Furthermore, dosages can be reduced with maintenance of endometrial protection. In porphyria it is valuable to avoid metabolism by the liver in order to reduce the strain on the hepatic cytochrome P450 enzymes.

In two cases with menstrual-cycle-related porphyria hormone add-back was difficult to tolerate, and in these subjects oophorectomy and hysterectomy were performed. The indication was in one case uterine fibroids and 5 years of solely estrogen add-back. The strategy was to continue a low endogenous hormone production to prevent menstrual-cycle-related porphyria attacks, with solely estrogens as add-back. In the other case GnRH treatment was successful in preventing menstrual-cycle-related porphyria attacks, but progesterone add-back provoked severe attacks. Surgery was a strategy in order to avoid progesterone and continue with solely estrogens as HRT.

**Therapy in relation to age**

Women in postmenopausal ages do not seem to be as sensitive to estrogens and progestins as women in fertile age are.

Among postmenopausal women with HRT therapies containing estradiol, porphyria attacks were not provoked. Estradiol (in HRT therapies) most likely provokes fewer porphyria attacks than the more potent ethinyl-estradiol (in oral contraceptives). However, in the group with GnRH-agonist therapy and menstrual-cycle-related porphyria, two younger women experienced attacks by estradiol add-back.
There are in our opinion several possible additive reasons for the increased sensitivity to sex steroids in women during fertile years compared to postmenopausal ages. Firstly, endogenous estrogens are not of the same origin or molecular structure in these different ages. Secondly, estrogens produced after menopause are less potent than those produced in fertile ages. Levels of the most potent estrogen, e.g. 17β-estradiol, are also much lower. Thirdly, postmenopausal serum progesterone levels are low compared to the levels in the luteal phase. Finally, there might be a reduced demand for sex-steroid metabolism in the liver after menopause.

**Summary of GnRH-agonist therapy and hormonal add-back therapy**

Menstrual-cycle-related porphyria attacks are preferably treated with GnRH-agonist therapies and low-dose estrogen patches as hormonal add-back to prevent menopausal adverse effects. Progesterone add-back must be considered for endometrial protection.

In acute porphyria attacks can be triggered by sex steroids, especially progestin and progesterone, but also estrogens, and therefore it is important to find strategies for safe add-back regimes. The dosages of GnRH-agonist therapies and hormonal add-back therapies are intricate. Further studies are needed to evaluate optimal doses of hormonal add-back during GnRH-agonist treatment, to maximize the endometrial protection and to minimize adverse effects.

**Paper III**

In this study, sex-steroid levels during a full menstrual cycle in AIP and controls were investigated, which has not been done before.

Our results indicate that 5α-reductase in the AIP group is deficient. No difference was found between the LAIP and the MAIP group. Allopregnanolone levels are different and reduced in both menstrual phases in AIP compared to controls. However, pregnanolone levels were similar in controls and in the AIP group, suggesting that the 5β-reductase activity was intact.

There are several in vitro studies of heme synthesis that show an increased ALAS activity caused by progesterone and its 5β-reduced metabolite, pregnanolone (19, 127, 128). Many 5β-steroid and 5α-steroid metabolites are ALAS-inducing in experiments. However, there are divergent opinions as to whether the β-metabolites are more potent in the induction of ALAS than the α-metabolites are (64).

It is therefore of interest to study the progesterone metabolites in AIP patients.

Earlier studies of steroid metabolite levels in porphyria have included both sexes and most commonly subjects with manifest porphyria. Levels of steroid metabolites between the different phases of the menstrual cycle are
not compared. The subjects in these studies are both in fertile ages and in older ages.

In earlier literature a deficient \(5\alpha\)-reduced steroid metabolism of injected \(14C\)-labelled steroids, without increased production of \(5\beta\)-metabolites, has been reported in AIP patients. Levels of \(5\beta\)-reduced steroid metabolites in urine did not differ between AIP and controls (62). These data support our idea that there is a deficient \(5\alpha\)-reductase in AIP, and that there are no changes in the \(5\beta\)-reductases between AIP and controls.

In 1972 Kappas et al. investigated MAIP patients of both sexes, both asymptomatic and with chronic symptoms. Intravenous injections of \(14C\)-labelled testosterone were given, and \(5\alpha\)- and \(5\beta\)-reductase activity was estimated by measuring excreted urinary \(14C\)-labelled testosterone and dehydroisoandrosterone metabolites. The results showed decreased levels of \(5\alpha\)-reduced metabolites.

Fractions between different endogenous and \(14C\)-labelled-steroid \(5\beta\)- and \(5\alpha\)-steroid metabolites were compared in each study group. The next comparison by the authors concerned the \(5\beta/5\alpha\) ratio in the study groups, which was higher in the MAIP group, and might have lead the authors to believe that there is increased production of \(5\beta\)-metabolites among the MAIP patients (60).

In two other studies by this research group, both acute and cutaneous porphyria (AIP and PCT) were investigated. The researchers suggest a shunting mechanism towards \(5\beta\)-metabolites (61), but their results show decreased levels of \(5\alpha\)-metabolites in AIP without enhanced production of \(5\beta\)-metabolites. The \(5\beta/5\alpha\) ratio shows a difference between AIP and controls due to decreased level of \(5\alpha\)-metabolite and not to increased levels of \(5\beta\) metabolites.

In reality the study thus supports our interpretation of a reduced \(5\alpha\)-steroid metabolite level in AIP but we cannot agree with the theory of a shunting mechanism towards \(5\beta\)-metabolites.

In one study comparing MAIP and LAIP, reduced levels of \(5\alpha\)-reduced steroid metabolites were found in MAIP, but not in the LAIP group (63).

In our study we found a significantly reduced level of allopregnanolone during the menstrual cycle in the AIP group, but levels of the \(\beta\)-metabolite pregnanolone did not differ between AIP and controls. The results of our study with similar levels of pregnanolone in healthy controls and AIP women are new findings.

The results in this paper are clear-cut. We found a deficient \(5\alpha\)-reduced steroid metabolite level in both LAIP and MAIP in both the follicular and the luteal phase of the menstrual cycle. The \(5\beta\)-reduced steroid metabolite levels were unchanged. This has not been shown earlier.

The gene map locus for the three different \(5\alpha\)-reductases is not at the same chromosome as the AIP gene; in our opinion this may be a sign of a multi-genetic link in AIP (65-68, 129).
Paper IV

This is the first study on the benefit of screening for HCC in AIP gene carriers. We found that radiological screening for HCC in AIP gene carriers enables early diagnosis and a choice of potentially curative treatments and improves the prognosis. However, liver function tests and AFP were not useful in screening for HCC. We also found that gene carriers for AIP aged ≥55 in Northern Sweden constitute a high-risk group for developing HCC. The globally common risk factors for HCC such as male sex, alcoholism, liver cirrhosis and hepatitis B or C, were not applicable in this study.

Our study is the first prospective investigation to estimate the incidence of HCC in AIP in a population-based study and to estimate the benefit of screening this patient group. The study is based on a well-characterized AIP population covering almost every adult AIP gene carrier in the northern region of Sweden, and it is probably the largest AIP population described (17). As the number of undiagnosed AIP gene carriers in our catchment area is small, the risk of overestimating the incidence of HCC in the AIP group is minimal.

A weakness of our study could be that the founder mutation W198X of the PBGD gene is present in 90% of our AIP gene carriers, and this mutation is reported as severe with high penetrance (10). This might reduce the possibility to generalize our results. Another problem connected to surveillance studies is the length bias, i.e. that slow-growing tumours could be overrepresented in surveillance programmes, and lead-time bias, i.e. surveillance does detect the disease at an earlier stage. Only RCT studies can eliminate the bias. Only one RCT study on HCC has been published. It was carried out in China on subjects with chronic HBV infections and showed that screening with ultrasonography twice a year reduced the 5-year mortality by 37% (130). Such studies are nowadays considered impossible to perform from an ethical point of view.

In our study we found a great variation of tumour growth when comparing group A (regularly screened subjects within 2 years) with Group B the control group (screening intervals exceeding 2 years). For example, in one patient in Group A the tumour size increased from not detectable to 12 cm within 1.5 years, and in another patient the tumour growth during the same time was only 2 cm. The same pattern was seen in the subgroup Group B3; in one patient the tumour growth was from not detectable to >10 cm within 2.8 years, and in another patient it was only 4 cm during 3 years. Disease-free survival is nowadays considered the best outcome (115). In our study three of the six patients in the regularly screened group were disease-free after surgery and in the control group B recurrence was seen in all three liver-resected patients.
The annual incidence of HCC in our study was 0.8% for AIP gene carriers aged >50 years, thus the number needed to screen was only 125. The annual incidence of HCC in acute porphyrias was 0.16% in the French study and 0.3% in a study from Switzerland. In a retrospective study from Stockholm covering 22 years, the annual risk was calculated as nearly 1% and a relative risk of 66 compared to an age-matched population in the Stockholm area. A summary of nine larger series, including this study, on acute porphyria and HCC, 104 cases, 97 AIP, 5 VP and 2 hereditary coproporphyria (HCP) (99-103, 110), see Table 7.
Table 7. Summary of 9 larger series on acute porphyria (AIP, VP, HCP) and HCC comprising 104 cases (97 AIP, 5 VP and 2 HCP).

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study details</th>
<th>No. of HCC</th>
<th>Proportion HCC &gt;50 yrs (%)</th>
<th>M:W</th>
<th>Age (range)</th>
<th>Manifest: Latent</th>
<th>Increased U-ALA, U-PBG</th>
<th>Circrhosis</th>
<th>Hepatitis</th>
<th>Alcohol abuse</th>
<th>Cirrhosis</th>
<th>Increased U-ALA, U-PBG</th>
<th>Screening result and remarks</th>
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<tbody>
<tr>
<td>Bengtsson, et al. 1986</td>
<td>Acute porphyria in AIP family study, Sweden</td>
<td>5/9 (55%)</td>
<td>3:2</td>
<td>68 (66-70)</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Re-exam of biopsies + mutation test for hotspots</td>
<td></td>
</tr>
<tr>
<td>Kauppinen et al. 1988</td>
<td>Acute porphyria in Finland 1929-1985</td>
<td>7/82 (9%)</td>
<td>5:2</td>
<td>66 (55-82)</td>
<td>2/5</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Re-exam of biopsies + mutation test for hotspots</td>
<td></td>
</tr>
<tr>
<td>Bjersing, Andersson et al 1996</td>
<td>Acute porphyria in Sweden 1994-2009</td>
<td>17/208 (8%)</td>
<td>7:10</td>
<td>68 (59-80)</td>
<td>14:3</td>
<td>4:3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Re-exam of biopsies + mutation test for hotspots</td>
<td></td>
</tr>
<tr>
<td>Andant, Puy et al. 2000</td>
<td>Acute porphyria in France 1989-1996</td>
<td>7/650 (1%)</td>
<td>3:4</td>
<td>50 (37-65)</td>
<td>4:3</td>
<td>7/7 (100%)</td>
<td>2/7</td>
<td>2/7</td>
<td>0</td>
<td>0</td>
<td>0.16%</td>
<td>Screening for alpha-feto protein</td>
<td></td>
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<tr>
<td>Schneider-Yin et al. 2009</td>
<td>Acute porphyria in France 2000</td>
<td>4/640 (0.6%)</td>
<td>0:4</td>
<td>80-82</td>
<td>1:1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3%</td>
<td>Retrospective study</td>
<td></td>
</tr>
<tr>
<td>Sardh et al. Manuscript 2010</td>
<td>Acute porphyria in Sweden 1987-2009</td>
<td>22/2080 (1%)</td>
<td>5:17</td>
<td>68 (53-82)</td>
<td>12:10</td>
<td>8/12</td>
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<td>1%</td>
<td>Retrospective screening study</td>
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<tr>
<td>Andersson Innala Submitted 2010</td>
<td>Acute porphyria in Sweden 1994-2009</td>
<td>22/1800 (1.2%)</td>
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<td>69 (59-82)</td>
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<td>0</td>
<td>0.8%</td>
<td>Prospective screening study</td>
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Summary: 104 HCC cases from 9 studies (97 AIP, 5 VP, 2 HCP). 90% of HCC cases were in women. Women at highest risk. screened HCC in patients with acute porphyria improved prognosis, i.e. 3- and 5-yr survival.
The higher annual incidence in Sweden could to some extent be explained by the presence of the W198X mutation in the PBGD gene with high penetrance (10). However, a great variety of mutations in the PBGD gene has been found in the larger series of HCC in AIP subjects (102, 110). Thus, specific AIP mutations seem only partly to explain the variations in annual incidence of HCC in acute porphyrias in different countries.

The relative risk or standardized incident rate ratio (SIR) for HCC in AIP gene carriers has been estimated in several studies, with a great variation. The French study reported SIR 36 (SIR m:f 19:110) and our study 64 (SIR m:f 52:93). The lower figure from France could be explained by the lower annual incidence of HCC reported. Interestingly, the risk estimates for women are at the same high level in the two studies. The relative risk reported from the first AIP-HCC study was 83 (103), and a 61-fold risk was calculated (m:f 64:55-fold) in the Finnish study (101).

The general pattern from the studies so far is that women with acute porphyria are at a higher relative risk of HCC than men are. In the nine larger series there was a female predominance with 60% women, see Table 7. In most series the age at diagnosis was above 50–55, with a mean 68–69 years. However, the French study reported 50 years as the mean age (37–65 years). Hepatitis was only found in the French study (2 of 7 HCC cases, but not in the youngest with HCC). This might to some extent explain the lower mean age at the HCC diagnosis in the French AIP population. Interestingly, the presence of cirrhosis was low in all series (around 30% ± 10%) contrary to the fact that liver cirrhosis is present in 80–90% of HCC patients in general (107). Thus the globally common risk factors for HCC are not applicable in acute porphyrias. A porphyria-specific risk factor is plausible but still obscure. Interestingly, when patients with porphyria cutanea tarda, who are at high risk of developing HCC, were treated with phlebotomy or chloroquine, the levels of porphyrins decreased and the risk of HCC was reduced (131).

Evidence of a porphyria-specific factor should be considered since the proportion of MAIP was 68%, calculated from pooled data, see Table 7 summary. Furthermore, women are more severely stricken by AIP than men, and the proportion of manifest AIP is higher in women. This corresponds to the female predominance in HCC, a reversal of the global sex ratio of liver cancer. Increased levels of U-ALA and U-PBG are more common in subjects with manifest AIP than in those with latent AIP (17), and the increased levels of porphyrin precursors might be involved in the risk of HCC in AIP. Various hypotheses have been suggested to explain the high prevalence of HCC in acute porphyrias (100-103, 132). Through autooxidation of ALA, chronic increased levels of ALA in the liver could lead to the generation of free radicals and subsequently to carcinogenesis over time.
Furthermore, in porphyrias, a reduced free heme pool could adversely affect cytochrome P450 and important antioxidants, leading to an increased mutation rate. The model strongly predicts the high risk of HCC in patients with acute porphyrias (132). Our results with increased levels of porphyrin precursors in almost all AIP patients with HCC are in accordance with the model.

The screening methods should have high sensitivity and specificity for detection of early stages of HCC. Alpha-fetoprotein was of no use as a screening tool in our study and should be discouraged according to other studies or guidelines (133, 134). Abdominal ultrasound is nowadays considered the most appropriate screening technique, with a sensitivity of 60–80% and over 90% specificity in early HCC detection (135). It should be supplemented with contrast-enhanced ultrasonography to characterize a suspected tumour. A vascular pattern, with intense contrast uptake in the arterial phase, followed by rapid washout in the portal phase, has proved to be specific to HCC. The method is not expensive and there is no irradiation risk, which could be the case in repeated CT used as a general screening method.

The ideal screening interval is not known. An interval of 6–12 months has been proposed. The recommended screening interval of 6 months is mainly based on the speed of tumour growth, but data are limited (133). The survival time was not different in cirrhotic patients screened at 6- or 12-monthly intervals (136).

After diagnosis of HCC, various staging systems are used for the treatment decision. In initial stages treatment options with curative intention are available, i.e. liver resection, radiofrequency ablation or percutaneous injection of alcohol and liver transplantation. For the intermediate stage of HCC only palliative treatment options are available, i.e. transarterial chemoembolization.

Resection is the first option in single tumours in a non-cirrhotic liver. This is the situation in most cases of HCC in AIP patients. These patients will tolerate major resections with low morbidity, since the risk of liver failure is low.

The prognosis, i.e. the 5-year survival rate, for treatment of small tumours (stage A in the BCLC system) is 50–75%, using resection, ablation or liver transplantation (133). Life expectancy has also been calculated relating to tumour burden as “the rule of seven”. Hence, in tumours up to 7 cm or the sum of tumours of 7 cm, 5-year survival after liver transplantation is 50% (vascular invasion) and 70% (non-vascular invasion) (113). Five-year survival rates in large patient series, after resection, for HCC ≤2 cm, 2–5 cm and >5 cm were 66%, 52% and 37%, respectively, as reported from Japan (137). However the good outcomes from early detection of HCC are hampered by
high incidence of recurrence: tumour recurrence complicates 70% of cases at 5 years (113). The recurrence rate is lower after liver transplantation.

In AIP gene carriers with HCC, liver transplantation could be an important option since it is also a cure for AIP per se (93, 94).

Since HCC in AIP patients is usually well differentiated, in non-cirrhotic liver with normal liver function – the prognosis after early detection could be expected as good.

Surveillance is recommended for groups of patients at high risk of HCC, i.e. certain groups of HBV carriers, HCV carriers, alcoholic cirrhosis, haemochromatosis, and primary biliary cirrhosis. The recommendations are based on an annual incidence. Surveillance for Asian men is recommended from age 40 onwards, with an annual incidence exceeding 0.2% (115). It could be noted that the annual incidence in Sweden of HCC is not known for haemochromatosis, which is on the at-risk list (115). Other factors to consider in the decision to enter a patient group into a screening programme should be its ability to increase longevity and its cost-effectiveness.

AIP should be added to the at-risk population where screening for HCC is recommended since the reported annual incidence is high, the costs of annual ultrasound screening of this small patient group are low and our results indicate improved prognosis.

AIP gene carriers aged >50 years fulfil the Wilson-Junger ethical principles and practice of screening for disease, i.e. it is an important health problem, the screening population can be defined, facilities for diagnosis and treatment are available and the treatment confers benefit (138).

We recommend annual ultrasound screening for HCC in AIP gene carriers from the age of 50 and as long as therapeutic options are applicable regarding concomitant disease and age.
General conclusions

- Female AIP gene carriers in northern Sweden show a high clinical expression. Pregnancy was not a big problem in AIP.
- Hormonal contraceptives can provoke AIP attacks. Hormonal replacement therapies in treatment of either menopausal symptoms or genitourinary atrophy symptoms did not provoke AIP attacks.
- GnRH-agonist treatment can ameliorate and even eliminate menstrual-cycle-related acute porphyria attacks.
- GnRH-agonist treatment induces hypoestrogenic side effects which demand hormonal add-back therapy.
- Progesterone and progestins in hormonal add-back therapies can be attack-provoking.
- The progesterone metabolism in AIP gene carriers differs from healthy controls. Levels of allopregnanolone, but not pregnanolone, are significantly lower, indicating a deficiency in the 5α-reductive pathway of progesterone metabolism.
- Levels of progesterone, estradiol, allopregnanolone and pregnanolone during the menstrual cycle show the same cyclicity in women with latent compared to manifest AIP.
- AIP gene carriers in northern Sweden are at high risk of developing HCC. The annual incidence is 0.8% from the age of 50. The global risk factors seen in HCC are not applicable in the AIP group.
- Radiologic screening for HCC in gene carriers of AIP enables early diagnosis, potentiates curative treatment and improves the prognosis. We thus recommend annual screening using liver imaging for AIP gene carriers >50 years of age.
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